

**A retrospective study to determine the bacterial causes of bloody  
diarrhea and their antimicrobial susceptibility patterns at Tabitha  
medical clinic in Kibera, Kenya**

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**A thesis submitted in partial fulfillment for the degree of Master of  
Science in Public Health in the Jomo Kenyatta University of  
Agriculture and Technology**

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## DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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## **DEDICATION**

I dedicate this thesis to my husband Antony who has been a constant source of support, has inspired me to do my best and always gave me the encouragement I needed to accomplish my goals. I would also like to extend this dedication to my lovely daughters Sylvia and Shanice for their love. It is due to the support of these amazing people that I have been able to accomplish my goals.

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## LIST OF ABBREVIATIONS

<b>AIDS</b>	Acquired immunodeficiency virus
<b>CDC</b>	Centers for Disease Control and prevention.
<b>DRC</b>	Democratic Republic of Congo
<b>EHEC</b>	Enterohemorrhagic <i>E. coli</i>
<b>HIV</b>	Human immunodeficiency virus
<b>HUS</b>	Hemolytic Uremic syndrome
<b>ITROMID</b>	Institute of Tropical Medicine and Infectious Diseases
<b>JKUAT</b>	Jomo Kenyatta University of Agriculture and Technology
<b>IQR</b>	InterQuartile Range
<b>KEMRI</b>	Kenya Medical Research Foundation
<b>MAC</b>	Mycobacterium Avium Complex
<b>MOH</b>	Ministry of Health
<b>0157:H7</b>	Entero hemorrhagic <i>E.coli</i>
<b>ORS</b>	Oral Rehydration Salts
<b>PCR</b>	Polymerase Chain Reaction
<b>SD</b>	Standard Deviation
<b>SMAC</b>	Sorbitol- MacConkey Agar
<b>SPSS</b>	Statistical Package for Social Scientists
<b>SSC</b>	Scientific Steering Committee
<b>TD</b>	Travellers' Diarrhea
<b>WHO</b>	World Health Organization

## ABSTRACT

Bloody diarrhea causes substantial morbidity and mortality in Africa, but data on the epidemiology and antimicrobial susceptibility to the common causative pathogens is limited. Amongst the well-recognized bacterial agents of diarrheal diseases, *Shigella* species, non-typhi *Salmonella*, *Campylobacter* and *Enterohaemorrhagic E. Coli* are the important causes of morbidity in developing countries. Antibiotics are recommended for treating bloody diarrhea to shorten the duration of illness, decrease morbidity and mortality and reduce the duration of bacteria shedding. Resistance to commonly used antimicrobial agents has been reported worldwide. Treatment of dysentery with antibiotics to which the etiologic agent is resistant may prolong illness and increase the rate of transmission to other individuals. The main aim of this study was to determine the bacterial causes of bloody diarrhea in selected areas of Kibera and establish their antimicrobial susceptibility pattern. The study was conducted at Tabitha Medical Clinic, located within Kibera slums. Kibera, one of the largest slums in Africa lacks basic services like sanitation, sewage and adequate water supply. For these reasons diarrheal diseases pose a major threat to the health of people living in Kibera. This was a retrospective study whereby the clinic/ laboratory records of 189 patients who were treated for bloody/ mucoid diarrhea at Tabitha medical clinic within the period of October 2006 to January 2008 were evaluated. Data entry, cleaning, validation and analysis were undertaken using Statistical Package for Social Scientists (SPSS) version 12.0. The main outcome variables were the kind of bacteria grown on culture and the antimicrobial sensitivity pattern.

About half of the records (49%) were of adult patients and 51% were of patients in the pediatric age group (< 12 years). Of those in the pediatric age group, children under five years of age (38%) were most affected. The records showed that culture of stool specimens from 86 (45.5%) patients yielded 88 bacterial pathogens: 74 *Shigella* (43 *S. flexneri*, 8 *S. dysenteriae* type non-1, 4 *S. boydii*, 9 *S. sonnei*, 10 unidentified *Shigella* species), 7 *Campylobacter*, 5 non-typhoidal *Salmonella*, and 2 *Salmonella typhi*. No EHEC was isolated. Majority of the isolates came from samples that had been described as bloody (58.8%) or mucoid (32.6%) on laboratory physical examination. More than 90% of the isolates (excluding *Campylobacter*) were resistant to trimethoprim-sulfamethoxazole, streptomycin and sulfisoxazole; 82.5 % to tetracycline and 62.5% were resistant to ampicillin. No pathogen was resistant to ciprofloxacin and gentamycin. From this study, it can be concluded that *Shigella*, especially *S. Flexneri* was the predominant cause of bloody diarrhea in Kibera, with *Campylobacter* being mainly a disease of children under five years of age. There was significant resistance to the commonly used antibiotics. Ciprofloxacin was determined to be the drug of choice for treatment of bloody diarrhea in adults and nalidixic acid the drug of choice in children. Continuous surveillance for bloody diarrhea is needed in order to regularly advice on the most effective modes of therapy. Strategies to improve prescription practices that use surveillance data to rationally guide more judicious use of antibiotics should be considered.

# CHAPTER 1

## INTRODUCTION

### 1.1 BACKGROUND

Diarrhea causes substantial illness in sub-Saharan Africa. Visibly bloody diarrhea (dysentery) causes proportionally greater morbidity and mortality (Ronsmans *et al.*, 1988). Bacterial infections are by far the most common causes of bloody diarrhea. Infective diarrhea is predominantly a disease of poverty, overcrowding and environmental contamination (Wittenberg, 2006).

Kibera one of the largest slums in Africa lacks basic services like sanitation, sewage and adequate water supply (Bapat *et al.*, 2003); moreover bad odor attracts flies which spread diseases through food and water contamination (Unger, 2008). For these reasons diarrheal diseases pose a major threat to the health of people living in Kibera slums (WHO 2008). Poor environmental conditions, poor methods of excreta disposal and high poverty levels expose the community to diarrhoeal diseases (Kungu *et.al*, 2002).

Soweto and Gatwikera are the two most underserved, densely populated villages of Kibera; associated with high incidence of diseases linked to poor sanitation and contaminated water supply.

The bacteria that cause bloody diarrhea include *Shigella*, *Campylobacter*, enterohaemorrhagic *Escherichia coli* (0157:H7/ EHEC) and non-typhoid *Salmonella*

species of bacteria. The prevalence of each pathogen varies considerably in different regions of the world; for example *Shigella* which causes shigellosis, is most common in Latin America while *Campylobacter* that is responsible for campylobacteriosis is the dominant bacteria in Southeast Asia. Most of the cases of bloody diarrhea in developing countries are due to shigellosis. Typically more than 50 % of bloody diarrhea of young children can be characterized as shigellosis (Huilan *et al.*, 1991; Ronsmans *et al.*, 1988). Episodes of bloody diarrhea caused by other bacterial pathogens occur less frequently than shigellosis and are less serious. Despite the fact that amoebiasis, caused by *Entamoeba histolytica* also causes bloody diarrhea, treatment for amoebiasis should only be done when typical trophozoites containing red blood cells are seen in the stool or there is no response to antimicrobial therapy for shigellosis. Hence routine treatment for bloody diarrhea in developing countries should be treatment for shigellosis (Guerrant *et al.*, 2001).

Antibiotics are recommended for treating bloody diarrhea to shorten the duration of illness, decrease morbidity and mortality and reduce the duration of bacterial shedding (Guerrant *et al.*, 2001; WHO, 1995b). Antimicrobial resistance among the major bacterial causes of bloody diarrhea is increasing worldwide (Sack *et al.*, 2001). Several authors have reported high rates of resistance to commonly used inexpensive oral antimicrobial agents such as ampicillin, tetracycline and trimethoprim-sulphamethoxazole (Mikhail *et al.*, 1990). Treatment of dysentery with antibiotics to



which the agent is resistant may prolong illness and increase the risk of hemolytic uremic syndrome and death (Butler *et al.*, 1987; Legros *et al.*, 1999).

Transmission of most of the bacteria causing bloody diarrhea is faeco-oral; mainly through direct ingestion of bacteria from stools. The incidence of bloody diarrhea is highest in densely populated areas with inadequate sanitation. Shigellosis occurs worldwide but is endemic in developing countries where there is poor sanitation and overcrowding (Guerrant *et al.*, 1999; Warrell *et al.*, 2003).

## **1.2 STATEMENT OF THE PROBLEM**

Bloody diarrhea is endemic in most parts of Africa especially the low-income areas associated with poor sanitation. The catchment area of the study clinic is underserved and densely populated, likely to be associated with high levels of unemployment and high incidence of diseases that are linked to poor sanitation and contaminated water supply.

Bloody diarrhea is a common presentation in the study clinic and other clinics in the study area. Most laboratories in the slum setting are not adequately equipped to conduct culture and examination tests. Bacteria causing bloody diarrhea require special culture media, unusual growth conditions or diagnostic antiserum that are often unavailable in low-income settings like Kibera.

Majority of the clinicians in this area treat patients with bloody diarrhea empirically with the available antimicrobial agents without considering whether they are sensitive. Treatment of dysentery with antibiotics to which the etiologic agent is resistant may prolong illness, increase rate of transmission and even lead to emergence of multi- drug resistant strains.

### **1.3 JUSTIFICATION OF STUDY**

Diarrhea is a commonly reported cause of morbidity and mortality worldwide. Bloody diarrhea is endemic in most parts of Africa but data on the epidemiology and antimicrobial susceptibility to the common causative pathogens is limited.

Kibera lacks basic services like sanitation, sewage and adequate water supply. For these reasons diarrheal diseases pose a major threat to the health of people living in Kibera slums. Soweto and Gatwikera are two of the most underserved and densely populated areas of Kibera associated with high levels of unemployment and high incidence of diseases linked to poor sanitation and contaminated water supplies. Additionally, the two villages border the Motoine River that is heavily polluted and thought to be a major vector for disease.

Despite the WHO recommendation on use of a number of antibiotics in the treatment of dysentery resistance has been reported in a number of settings. Knowledge of the common antimicrobial sensitivity pattern will aid in a more accurate empirical treatment.

Most of the bacteria causing bloody diarrhea require special culture media, unusual growth conditions, or diagnostic antisera that are often unavailable in most hospitals in Kenya. Attempts to isolate *Shigella* may fail unless the specimen is inoculated immediately and properly transported to the laboratory. Moreover, the results of culture

are available only after two or three days, whereas treatment may be needed when the patient is first seen, especially if it is a child. Hence knowledge of appropriate empirical therapy is very important. This will aid in promptly treating all the cases with an effective oral antimicrobial.

According to the WHO the antimicrobial susceptibility of local *Shigella* strains should be monitored regularly and results used to develop or modify national treatment guidelines.

## **1.4 HYPOTHESIS**

Specific bacteria are important agents in the etiology of bloody diarrhea in Kibera slums. There is significant resistance of these agents to the commonly used antimicrobials.

## **1.5 OBJECTIVES**

### **1.5.1 General Objective**

To establish the major etiologies of bloody diarrhea in selected areas of Kibera and evaluate their antimicrobial susceptibility patterns.

### **1.5.2 Specific Objectives**

1. To establish the annual incidence of shigellosis in selected areas of Kibera.
2. To determine the pathogens causing dysentery in selected areas of Kibera slums.
3. To determine the susceptibility patterns of the isolated pathogens to available antimicrobial agents.
4. To establish the relationship between age and the occurrence/ etiology of dysentery.

## CHAPTER 2

### LITERATURE REVIEW

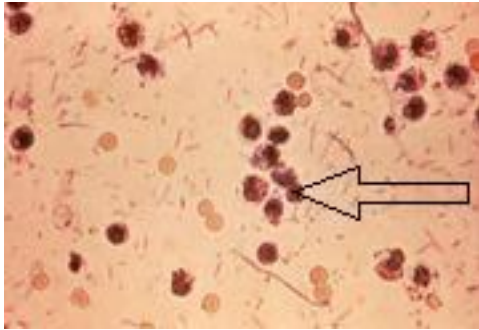
#### 2.1 Definition

Bloody diarrhea refers to any loose stool that is tinged with blood; more often than not it may also have some mucous. Bloody diarrhea most commonly occurs as a result of infection by bacteria. The most important include *Shigella*, invasive strains of *E.coli*, *Campylobacter* and *non-typhoid Salmonella* species. Most of these bacteria present with similar symptomatology including bloody/ mucoid stools, abdominal cramps, fever, tenesmus, nausea and vomiting. Numerous studies have revealed that the most common cause of dysentery in developing countries is shigellosis (Huilan *et al.*, 1991; Ronsmans *et al.*, 1988). In fact the recommended treatment for bloody diarrhea in developing countries is treatment for *Shigella*.

#### 2.2 Shigellosis

Shigellosis is caused by one of the several types of *Shigella* bacteria, and is common in developing countries especially where there is overcrowding and poor sanitation. *Shigella* bacterium was named after Kiyoshi-shiga who discovered it in 1898 (Kibari, 2002; Trofa *et al.*, 1999). According to recent estimations, shigellosis kills between 600,000- 1,000,000 people throughout the world especially children under five years old in developing countries (WHO, 2003).

*Shigella* are Gram negative, non-motile, facultative anaerobic non-spore forming rods (Figure 1). Organisms of the genus *Shigella* belong to the tribe *Escherichiae* in the family *Enterobacteriaceae*.



**Figure 1: Photomicrograph of *Shigella* species in a stool specimen**

Source: Baron *et al.*, 1996.

The genus *Shigella* is subdivided into four serogroups with multiple serotypes which include; Group A – *Shigella dysenteriae* (12 serotypes), Group B – *Shigella flexneri* (6 serotypes), Group C – *Shigella boydii* (18 serotypes) and Group D – *Shigella sonnei* (1 serotype). Serotypes A, B and C are similar physiologically while *S. sonnei* can be differentiated from the other serogroups by positive beta-D-galactosidase and ornithine decarboxylase biochemical reactions (Bennish *et al.*, 1992). Almost all fatal cases of shigellosis occur in developing countries and most deaths from shigellosis result from endemic disease especially that caused by *Shigella flexneri* (Wojtyniak and Bennish, 1991).

### **2.2.1 Epidemiology**

Humans are the primary reservoir of *Shigella* species with captive sub-human primates as accidental hosts (Warrell *et al.*, 2003). Transmission of *Shigella* bacteria is faeco-oral mainly through direct ingestion of bacteria from stools for example in cases of poor hygiene or through contamination of food and water. Flies may contribute to spread from faeces to food (Unger, 2008).

The incidence of shigellosis is highest in densely populated areas with inadequate sanitation. It occurs worldwide but is endemic in developing countries where there is poor sanitation and overcrowding. Developed countries commonly experience sporadic food/water borne outbreaks. A report from the Centres for Disease Control (CDC, 2004) showed that 300,000 cases of shigellosis are reported annually in the United States of America (USA). Shigellosis also presents a significant risk to travellers from developed countries when visiting endemic areas (Black, 1990). This is commonly referred to as travellers' diarrhea (TD), a term which encompasses diarrheal diseases in persons travelling to endemic regions, mostly developing countries. Among the microorganisms responsible for TD are *Shigella* species, *Escherichia coli*, *Campylobacter* and *Salmonella* species.

Typically, 10-20 % of enteric disease and 50 % of bloody diarrhea in young children can be characterized as shigellosis (Ronsmans *et al.*, 1988; Huilan *et al.*, 1991). A separate



study showed the frequency of isolation of *Shigella* from bloody diarrhea to be 34 % (Urio *et al.*, 2001).

*Shigella dysenteriae* and *S. flexneri* are the most virulent types. The most virulent serotype, *S. dysenteriae* type 1 has been responsible for large dysentery epidemics in India (Pal, 1984), Guatemala and other parts of Central America, Zaire (now DRC), Kenya (Iijima *et al.*, 1995), Bangladesh (Bennish *et al.*, 1992) and recently West Africa (Guerin *et al.*, 2003). In USA, *S. sonnei* causes more than two-thirds shigellosis and *S. flexneri* causes the rest. In a Gaborone city clinic, *S. boydii* was the most frequently encountered species followed by *S. flexneri* and *S. sonnei* (Urio *et al.*, 2001).

Shigellosis is an acute infection with onset of symptoms usually occurring within 24-48 hours of ingestion of etiologic agent. An infective dose as low as 10 cells can lead to the infection depending on the age and immune status of the host. Volunteer challenge studies show that shigellosis can be evoked by extremely small inoculums (10-100 micro-organisms) and the time of onset of symptoms is somewhat influenced by the size of the challenge. The natural course of shigellosis is 2-7 days with an average duration of 3 days. The bacteria remain active during the illness and for a week or 2 after the host recovers. Some people may not develop the illness but still transmit *Shigella*. In a study conducted by Urio *et al.*, 2001, two of the 100 stool specimens from children without diarrhea had *S. boydii*.

### **2.2.2 Pathogenesis**

Infection is initiated by ingestion of shigellae usually via faeco-oral contamination. This is through direct contact with the bacteria in stool or bacterial contamination of food and water. Diarrhea is usually an early symptom initiated by enterotoxin and/or cytotoxin and it occurs as the organism passes through the small intestines. The hallmark of shigellosis is bacterial invasion of the colonic epithelium and inflammatory colitis (Perdomo *et al.*, 1994). The resulting colitis and ulceration of the mucosa with concomitant malabsorption, results in the characteristic sign of shigellosis; scanty, unformed stools tinged with blood and mucous.

### **2.2.3 Symptoms**

The hallmark symptom of shigellosis is bloodstained stools that are commonly mucoid. Diarrhea may be non-blood stained in the initial stages of the infection that cannot be distinguished clinically from other bacterial, viral, or protozoal causes of diarrhea. Stools in shigellosis are usually stained with fresh bright red blood as compared to amoebic stools that are brownish in appearance (Apel, 2003). Other symptoms of shigellosis include fever, abdominal cramps, tenesmus, nausea, vomiting and dehydration.

### **2.2.4 Complications**

Shigellosis usually clears on treatment with no sequelae but in a few cases especially young children some complications may occur. These include seizures (its unclear

whether these are due to the fever or *Shigella* itself), proctitis (inflammation of the rectal mucosa), rectal prolapse, hemolytic uremic syndrome, toxic mega colon and reiter's syndrome (Hill and Lillicrap, 2003).

### **2.2.5 Diagnosis**

Bloody stools can result from a number of diseases hence confirming shigellosis is important in diagnosis (Escheverria *et al.*, 1991). This is by laboratory examination of stools, which are commonly blood stained and/or mucoid on physical examination and show the presence of neutrophils on microscopy of fecal smears. Culture of the stool to isolate *Shigella* or any of the other bacteria is the confirmatory test.

A new diagnostic test using Polymerase Chain Reaction (PCR) that is unaffordable for most hospitals in Kenya is promising to be even more reliable (Sethabutr *et al.*, 1994). PCR method increased the diagnosis of *Shigella* and *EHEC* infections by 45% among patients with dysentery when compared to bacteriological methods. PCR method was also found to be sensitive and useful in a study in Bangladesh (Islam *et al.*, 1998). PCR assay was not only highly sensitive but could provide a result on the same day that the specimen was submitted for evaluation. It was concluded that the PCR method was highly sensitive, specific, rapid, simple and convenient.

### **2.2.6 Treatment**

Though shigellosis may be self-limiting, antibiotics are recommended for treating bloody diarrhea to shorten the duration of illness, decrease morbidity and mortality and reduce the duration of bacterial shedding (Guerrant *et al.*, 2001; WHO, 1995a). Antibiotics also shorten the length of the illness and reduce spread to others in a group living status. As shigellosis in children often leads to growth retardation, anemia and other sequelae, early detection and treatment with appropriate antibiotics is recommended (WHO, 1999).

The commonly used antibiotics include trimethoprim-sulphamethoxazole, ampicillin, azithromycin and 4-fluoroquinolones that include nalidixic acid, norfloxacin and ciprofloxacin. Antidiarrheals are not recommended in treatment of shigellosis; in fact they have been established to prolong the course of the disease and make it worse (Blaser *et al.*, 1984). Replacement of the lost fluids is also an important aspect of management. This could be by taking a lot of oral fluids; oral rehydration salts (ORS) or intravenous fluids for those who are severely dehydrated. Resistance to some of the antibiotics and even multi-drug resistance has been noted in some settings.

### **2.2.7 Prevention**

Prevention of shigellosis could be through maintenance of proper hygiene and sanitation practices that include proper hand washing and disposal of excreta. According to one

study, azithromycin prophylaxis was established to prevent dysentery epidemics (Shanks *et al.*, 1999).

### **2.2.8 Vaccine**

A candidate vaccine against shigellosis, developed by the institute Pasteur is currently being clinically tested in humans (Levine, 2000). Safety, immunogenicity and efficacy of *S. sonnei*, *Salmonella typhi* bivalent attenuated vaccine (strain 5076-IC) is also under evaluation. Two lots of this vaccine prepared at Forest Glen provided highly significant protection against challenge with pathogenic *S. sonnei* in study volunteers. Studies are underway to determine the factors that must be present to assure potency of this promising vaccine and to eliminate lot-to-lot variability in efficacy. A trial is being conducted (phase 1 and 2) of live attenuated *S. dysenteriae* type 1: HgR oral vaccine SCSGG in healthy human adult volunteers (Launay *et al.*, 2009).

## **2.3 Antimicrobial Resistance**

### **2.3.1 Global Picture**

Multidrug resistant strains of *S. dysenteriae* type 1 were implicated in 3 outbreaks and sporadic cases of dysentery in eastern India in 2002 and 2003 (Gururaja *et al.*, 2004). This study also identified new clones of *S. dysenteriae* type-1 which were resistant even to the newer antimicrobials like norfloxacin and ciprofloxacin.

At the Dhaka diarrheal centre; out of the 113 strains of *S. dysenteriae* isolated, 60% of the strains were sensitive to commonly used antibiotics, only 6% (n=7) strains were resistant to nalidixic acid and none of the strains were resistant to ciprofloxacin (Talukder *et al.*, 2006).

In India, 3500 stool samples from patients with diarrhea were evaluated in a period of 4 years (Dutta *et al.*, 2003).. The isolated strains included *S. dysenteriae*, *S. flexeneri*, *S. boydii* and *S. sonnei*. Among the *Shigella* species, very few strains showed resistance to routinely used antimicrobial agents like nalidixic acid, gentamicin, chloramphenicol and norfloxacin. Amikacin showed 100% sensitivity. The emergence of resistance to ciprofloxacin was a matter of concern and it was thought that limiting its use to multi-drug resistant strains of *Shigella*, rather than indiscriminately using it for all patients with bloody diarrhea could check it.

### **2.3.2 Kenya**

Resistant *S. dysenteriae type1* strain was reported along the coastal area of Kenya. During a clinic based surveillance in rural Nyanza province, the most frequent bacterial pathogens isolated were *Shigella* (44%) followed by *Campylobacter* (30%) *Vibrio cholera* (18%) and *Salmonella non typhi* (14%) (Shapiro *et al.*, 2001).

In a study conducted in western Kenya where bloody stool samples from 451 patients were collected for a period of 4 years, cultures of 231 (51%) specimens yielded 247

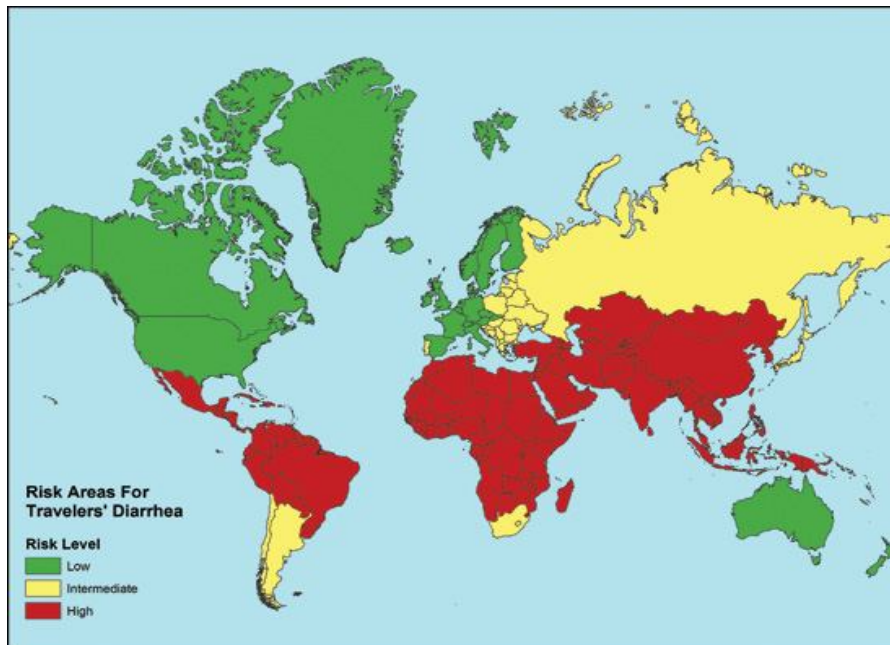
bacterial pathogens: 198 *Shigella* (97 *S. flexneri*, 41 *S. dysenteriae type 1*, 39 *S. dysenteriae type non 1*, 13 *S. boydii*, 8 *S. sonnei*), 33 *Campylobacter*, 15 non typhoidal *Salmonella*, and 1 *Vibrio cholerae* 01. More than 90% of the isolates (excluding *Campylobacter*) were resistant to trimethoprim-sulfamethoxazole and tetracycline and more than 80% were resistant to ampicillin. Seventy four percent of the people treated received medication to which their isolate was resistant (Brooks *et al.*, 2003a).

## 2.4 Travellers' Diarrhea

Travellers' diarrhea (TD) is a term which encompasses diarrheal disease in persons travelling to endemic regions, mostly developing countries (Black, 1990). Among the microorganisms responsible are *Shigella species*, *Escherichia coli*, *Campylobacter* and *Salmonella species*. The low infectious dose of *Shigella* makes it one of the more commonly reported bacteria associated with TD. The risk of acquiring infection with *Campylobacter* appears to vary by destination, with travel to Asia posing a higher risk in most studies. Although non-typhoidal *Salmonella* infections are frequently associated with food borne outbreaks in industrialized countries, they are an infrequent cause of TD worldwide.

In Figure 2, the red areas represent areas at high risk for travellers' diarrhea, that is areas endemic for bacterial diarrhea (CDC 2008).





**Figure 2: Areas of risk for travellers' diarrhea.**

Source: CDC, 2008

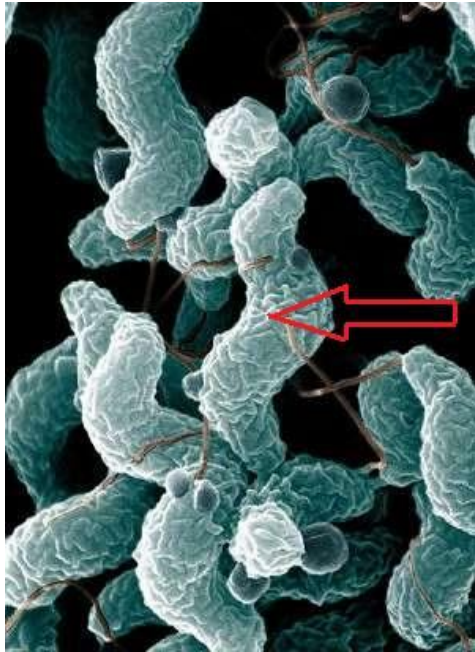
Travellers' diarrhea occurs equally in males and females and is more common in young adults than in older people. In short-term travellers, bouts of TD do not appear to protect against future attacks and more than one episode of TD may occur during a single trip. On average 30%-50% of travellers to high-risk areas will develop TD during a 1-2 week stay. Based on the annual figure of 50 million travellers to developing countries, this estimate translates to approximately 50,000 cases of TD each day (Black, 1990). In more temperate regions, there may be seasonal variations in diarrhea risk. In South Asia for example, during the hot months preceding the monsoon much higher TD attack rates are reported.

## **2.5 Campylobacteriosis**

Campylobacteriosis is an infectious disease caused by bacteria of the genus *Campylobacter* that includes 18 species; the main ones pathogenic to humans being *C. jejuni* and *C. fetus*. Other less common species may be present in conditions of reduced immunity for instance HIV/AIDS. In a study conducted by Jenkin and Tee, 1998, *Campylobacter upsaliensis* was isolated from the faeces of 20 HIV-infected patients with diarrhea over a 67-month period, representing 18.5% of fecal *Campylobacter* isolates from the HIV-seropositive patients.

### **2.5.1 Transmission**

*Campylobacter* pathogens are small, curved, motile, microaerophilic, gram-negative rods that vary in width from 0.2-0.9  $\mu\text{m}$  and vary in length from 0.5-5.0  $\mu\text{m}$ . Figure 3 shows a scanned electron microscope image of *C. jejuni* illustrating its cork screw appearance and bipolar flagella.



**Figure 3: Scanning electron microscope image of *Campylobacter* bacteria**

Source: Altekruze *et al.*, (1999).

Campylobacteriosis affects humans and animals. The animal reservoir is the gastrointestinal tract of dogs, cats and other pets. Transmission of *C. jejuni* to humans occurs by ingestion of contaminated food or water, including unpasteurized milk and undercooked poultry, or by direct contact with faecal material from an infected human or animal. Exposure to bacteria is often more common during travel to endemic zones and therefore campylobacteriosis is a common form of travellers' diarrhea.

### **2.5.2 Epidemiology**

Most estimates of incidence in developing countries are from laboratory-based surveillance of pathogens responsible for diarrhea. *Campylobacter* isolation rates from

developing countries range from 5-20% (Oberhelman *et al.*, 2000). Despite lack of incidence data from national surveys, case control community based studies have provided estimates of 40,000-60,000/ 100,000 for children under 5 years of age (Oberhelman *et al.*, 2000). In contrast, the figure for developed countries is 300/ 100,000 (Tauxe *et al.*, 1992). Estimates in the general population in developing and developed countries are very similar, approximately 90/ 100,000 (Tauxe *et al.*, 1992; Taylor *et al.*, 1991), confirming the observation that campylobacteriosis is often a pediatric disease in developing countries. This is likely due to the development of protective immunity secondary to a high level of exposure to the organism early in life.

### **2.5.3 Pathophysiology**

Factors responsible for the disease caused by *C. jejuni* are not well known. Based on the clinical illness, 3 mechanisms have been postulated (Murray, 1986). The first mechanism is adherence and production of heat-labile enterotoxins inducing secretory diarrhea. Secondly, invasion and proliferation within the intestinal epithelium, leading to cell damage and inflammatory response. The third mechanism is translocation of the organism into the intestinal mucosa and proliferation in the lamina propria and mesenteric lymph nodes, leading to extra-intestinal infections such as meningitis, cholecystitis, urinary tract infection and mesenteric adenitis (Wassenaar and Blaser, 2003).

#### **2.5.4 Symptoms**

The symptoms of campylobacteriosis include diarrhea, cramping, abdominal pain, tenesmus and fever within two to five days after exposure to the organism (Wassenaar and Blaser, 2003). The diarrhea may be bloody and can be accompanied by nausea and vomiting. The abdomen is frequently tender upon palpation and rarely splenomegally may be present. The illness typically lasts one week. Some infected persons do not have any symptoms. Most symptoms are indistinguishable from those caused by *Shigella* organisms, *Escherichia coli* and *Salmonella* species.

#### **2.5.5 Diagnosis**

*Campylobacter* organisms can be detected on Gram stain of stool with high specificity and sensitivity of approximately 60%, but are most often diagnosed by stool culture (Wassenaar and Blaser, 2003). Fecal leukocytes may be present and indicate an inflammatory diarrhea.

#### **2.5.6 Treatment**

The infection is usually self-limiting and in most cases, symptomatic treatment by reposition of liquid and electrolyte replacement is enough. The use of antibiotics, on the other hand, is controversial. Antimotility agents such as loperamide can lead to prolonged illness or intestinal perforation in any invasive diarrhea and should be avoided. Antibiotic treatment has only a marginal benefit (1.32 days) on the duration of symptoms and should not be used routinely (Ternhag *et al.*, 2007). Erythromycin can be

used in children and tetracycline in adults. However, some studies show that erythromycin rapidly eliminates *Campylobacter* from the stool without affecting the duration of illness (Saenz *et al*, 2000). Nevertheless children with dysentery due to *C. jejuni* benefit from early treatment with erythromycin. Treatment with antibiotics therefore depends on the severity of symptoms.

*Campylobacter upsaliensis* was isolated from the faeces of 20 HIV-infected patients, all tested isolates were susceptible to erythromycin and doxycycline, but three isolates from two patients were resistant to ciprofloxacin (Jenkin and Tee, 1998).

### **2.5.7 Complications**

Some (less than 1 in 1000 cases) individuals develop Guillain Barre syndrome in which the nerves that join the spinal cord and brain to the rest of the body are damaged, sometimes permanently. This occurs only with infection of *C. jejuni* and *C. upsaliensis* (Murray, 1986). Other complications include toxic megacolon, dehydration and sepsis. Such complications generally occur in young children (< 1 year of age) and immunocompromised people. Chronic course of the disease is possible but such form of the process is likely to develop without a distinct acute phase. Chronic campylobacteriosis features long period of sub-febrile temperature and asthenia; eye damage, arthritis and endocarditis may develop if infection is untreated. Occasional deaths occur in young, previously healthy individuals because of volume depletion and in persons who are elderly or immunocompromised.

### **2.5.8 Prevention**

Prevention can be achieved by pasteurization of milk and chlorination of drinking water to destroy the organism. Treatment with antibiotics can reduce fecal excretion. Infected health care workers should not provide direct patient care. Separate cutting boards should be used for foods of animal origin and other foods. After preparing raw food of animal origin, all cutting boards and countertops should be carefully cleaned with soap and hot water (Wassenaar and Blaser, 2003).

## **2.6 Enterohemorrhagic *Escherichia Coli***

*Enterohemorrhagic E.coli* are gram negative rod shaped bacterium in the *Escherichia* group commonly referred to as O157:H7; the 'O' refers to the somatic antigen number, whereas the 'H' refers to the flagella antigen. While most pathogens in the *Escherichia* group are harmless and normally found in the intestines of mammals, *E. coli serotype O157:H7 (Enterohemorrhagic E.coli)* usually produces shiga-like toxins which cause severe illness (Strockbine *et al.*, 1986). Among the factors increasing its virulence are periplasmic catalase and the shiga-like toxins. Shiga-like toxins are functionally identical to toxins produced by virulent *Shigella* species (Calderwood *et al.*, 1987). The periplasmic catalase is encoded on the pO157 plasmid and is believed to be involved in virulence by providing additional oxidative protection when infecting the host (Brunner *et al.*, 1996).

### **2.6.1 Transmission**

A major source of infection is consumption of undercooked meat, unpasteurized milk and contaminated vegetable (Karch *et al.*, 2005). Water borne transmission can also occur through swimming in contaminated lakes or pools and drinking inadequately treated water. Meat can be contaminated during slaughter and bacteria may even be present on the cows' udders or on the milking equipment hence get into the raw milk.

### **2.6.2 Signs and Symptoms**

*E. Coli O157:H7* infection causes severe acute bloody diarrhea and abdominal cramps. Usually little or no fever is present and illness resolves in 5 to 10 days (Karch *et al.*, 2005). In about 2-7% of cases especially children under 5 years and elderly individuals, the infection may cause haemolytic uremic syndrome in which haemolysis occurs and acute renal failure may occur (Riley *et al.*, 1983).

### **2.6.3 Diagnosis**

A stool culture can be done to detect the bacterium; usually the sample is cultured on sorbitol-MacConkey agar (SMAC) or variant cefeximine potassium tellurite sorbitol-MacConkey agar (CT-SMAC). CHROM agar has also been used to culture *E.coli* O157:H7, which is shown in darker color in Figure 4. Newer methods like PCR techniques can also be used though expensive and not commonly available (Wells *et al.*, 1983).





**Figure 4: *Escherichia coli* in CHROM agar.**

Source: Bettelheim, 2002.

#### **2.6.4 Prevention**

Following strict hygiene and good culinary practices can prevent *Enterohaemorrhagic E.coli*. These practices include cooking meat thoroughly, proper storage of meat, good hand washing practices and avoidance of un-pasteurized milk or juices.

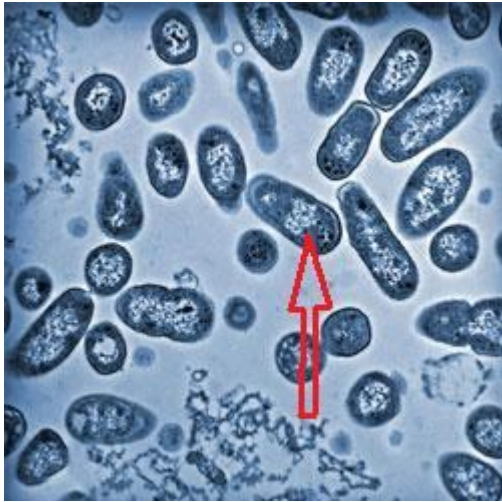
In January 2007, Canadian bio-pharmaceutical company Bioniche announced it had developed a bovine vaccine capable of reducing O157:H7 in cattle by over 99% (Canadian research collaboration, 2007).

## **2.7 Non-typhi *Salmonella***

Infections with *non-typhi Salmonella* are a significant cause of illness and death worldwide (Shimoni *et al.*, 1999). They are of particular importance in developing countries. For example *non-typhi Salmonella* is the most frequent cause of septicemia in Rwanda (Lepage *et al.*, 1987) and Zaire (now DRC); (Green and Tillotson, 1997). Each year, an estimated 1.4 million persons are infected with *non-typhi Salmonella enterica* in the United States, resulting in 15,000 hospitalizations and 400 deaths (Voetsch *et al.*, 2004).

### **2.7.1 Transmission**

*Salmonella*, which are rod-shaped motile bacteria that possess flagella (Figure 5), live in the intestinal tracts of humans and other animals including birds. *Salmonella* are usually transmitted to humans by eating foods contaminated with animal feces. Contaminated foods are often of animal origin for example beef, poultry, milk, or eggs, but any food, including vegetables, may become contaminated. Thorough cooking kills *Salmonella*. Food handlers could also contaminate food if they don't observe hand-washing practices adequately. Children are the most likely to get salmonellosis. The rate of diagnosed infections in children less than five years old is about five times higher than the rate in all other persons. Young children, the elderly, and the immunocompromised are the most likely to have severe infections (Hohmann, 2001).



**Figure 5: *Salmonella* under the microscope of proteomics**

Source: Buchwald and Blaser, 1984.

### **2.7.2 Symptoms**

Most persons infected with *Salmonella* develop diarrhea, fever and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days and most individuals recover without treatment. However, in some the diarrhea may be so severe that the patient may need to be hospitalized (Graham *et al.*, 2000).

### **2.7.3 Diagnosis**

Culture of a stool sample is necessary to identify the bacterium (Graham *et al.*, 2000).

#### **2.7.4 Treatment**

*Salmonella* infections usually resolve in 4-7 days and often do not require treatment other than oral fluids. Persons with severe diarrhea may require rehydration with intravenous fluids. Antimicrobial agents are not essential for the treatment of most *Salmonella* infections but may be lifesaving for patients with severe infections. Antibiotics such as ampicillin, trimethoprim-sulfamethoxazole or ciprofloxacin may be used (Marimon *et al.*, 2003; Reid, 2006).

The prevalence of resistance among *Salmonella* to several antimicrobial agents including ampicillin and trimethoprim-sulfamethoxazole has increased in recent decades (CDC, 2004). Fluoroquinolones including ciprofloxacin are the most commonly used antimicrobial agents in the United States for the treatment of *Salmonella* infections in adults. In many tropical countries, ampicillin, chloramphenicol or cotrimoxazole are the drugs of choice in treatment of *non-typhi salmonella*. In a study conducted in Kenya (Kariuki *et al.*, 1996), only 31 (16%) of *non-typhi Salmonella* were sensitive to all the antibiotics tested. Thirty-eight (20%) were resistant to one agent (usually streptomycin or tetracycline) and 33 (17%) were resistant to 2 agents usually streptomycin and tetracycline or tetracycline and ampicillin. The remainder were resistant to 3 or more antibiotics.

### **2.7.5 Complications**

Persons with diarrhea usually recover completely, although it may be several months before their bowel habits are entirely normal. A small number of persons with *Salmonella* develop pain in their joints, irritation of the eyes and painful urination. This is called Reiter's syndrome. It can last for months or years and can lead to chronic arthritis, which is difficult to treat. Antibiotic treatment does not make a difference in whether or not the person develops arthritis (Hohmann, 2001).

### **2.7.6 Prevention**

Like most of the other diarrheal diseases, safe hygiene and cooking practices are mandatory in the prevention of non-typhi *Salmonella*. Keeping uncooked meats separate from vegetables, cooked foods and ready-to-eat foods; thoroughly washing utensils after contact with uncooked foods; proper hand washing before handling foods and even in between handling different food items is very important in the prevention of salmonellosis (Hohmann, 2001).

People who have salmonellosis should not prepare food until their diarrhea has resolved. Washing hands immediately after touching any poultry as well as cooking poultry, beef and eggs thoroughly is strongly advocated for in the prevention of non-typhi *Salmonella*. It is also advisable not to eat or drink foods containing raw eggs or raw (unpasteurized) milk; particular care should be taken when preparing food for infants, the elderly and the

immunocompromised. Mother's milk is the safest food for young infants; breastfeeding prevents salmonellosis and many other health problems (Rowe *et al.*, 2004).

## CHAPTER 3

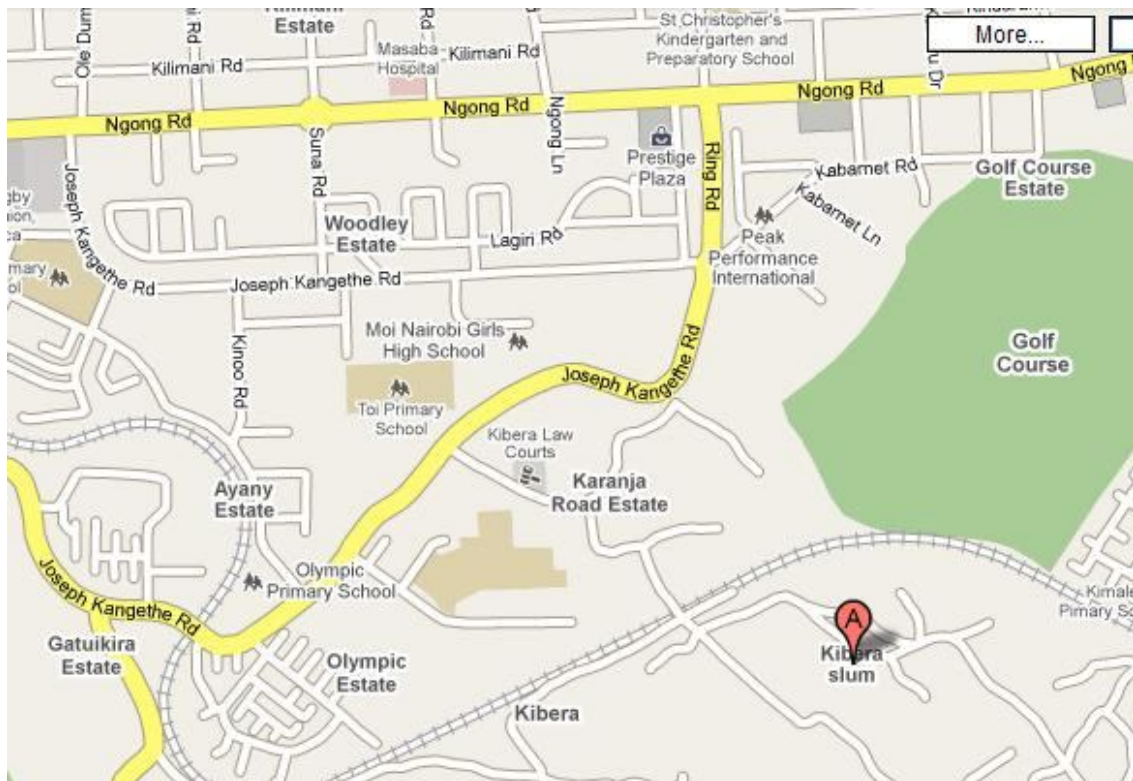
### MATERIALS AND METHODS

#### 3.1 Study site

This study took place at Tabitha Medical clinic, located in the heart of Kibera slums (Appendix 2), Nairobi. Tabitha Medical clinic is a study clinic; managed by CDC-KEMRI whereby families of up to 35,000 individuals participating in the demographic surveillance survey can access free medical care for any acute illnesses. The clinic mainly serves patients from Soweto and Gatwikera villages of Kibera (Appendix 3) at the rate of approximately 150 patients each day and is run by two medical officers, three clinical officers, six nurses, two pharmacists and two laboratory technicians.

Kibera slum, one of the largest in Africa is located approximately 7km to the western side of Nairobi city. Estimates of Kibera's population vary widely; official government statistics projected a 2004 population of 355,471 while other sources indicate the population may be as high as 1.2 Million ((Ngongo *et al.*, 2008; Schwartz-Barcott -oral communication). The informal settlement has grown considerably in recent years with influx of people from rural locations looking for work in the city of Nairobi. The slum is composed of numerous single rooms made up of mud walls; tin roofs and some have a small window for ventilation. The average size of each room is approximately 10x10 feet. Approximately five members inhabit each room. Although there is no demographic surveillance system in Kibera and data from other sources is scarce, it is understood that

people from multiple ethnic groups live within Gatwikera and Soweto. These are two of the most underserved and densely populated areas of Kibera (Schwartz-Barcott - oral communication), likely associated with high levels of unemployment and high incidence of diseases linked to poor sanitation and contaminated water supplies. Additionally, the two villages border the Motoine River that is heavily polluted and thought to be a major source of disease.



**Figure 6: Map of study area**

Source: Google maps



## 3.2 Study design

This was a retrospective clinic based study.

Data was extracted from clinical and laboratory records of patients who were treated for bloody and/ or mucoid diarrhea between the months of October 2006 to January 2008.

### 3.2.1 Inclusion criteria

Records of patients who presented to the study clinic with blood stained / mucoid stools from October 2006 to January 2008.

### 3.2.2 Exclusion criteria

Records from patients who presented to the clinic with malaena stools or diarrhea that was not described as mucoid or bloody.

### 3.2.3 Sample size

In this study, considering the proportion of interest i.e., cases of bloody/ mucoid diarrhea to be 40% (P1) and clinical variations from previous studies to be 15% (P2), the sample size was calculated using the formula of Lameshow *et al.*, (1996)

$$n = \frac{\{Z_{1-\alpha/2} \sqrt{[2P(1-P)]} + Z_{1-\beta} \sqrt{[(P_1(1-P_1) + P_2(1-P_2))]\}}^2}{(P_1 - P_2)^2}$$

Where;

At 95% confidence,  $Z_{1-\alpha/2} = 1.96$

At 90% power,  $Z_{1-\beta} = 1.28$

$P_1$  = Prevalence for group 1 = 40% i.e. 0.4

$P_2$  = Prevalence for group 2 = 15% i.e. 0.15

$P = (P_1 + P_2)/2 = (40\% + 15\%)/2 = 27.5\%$  i.e. 0.275

$$n = \frac{\{1.96 \sqrt{[2*0.275 (1-0.275)]} + 1.28 \sqrt{[(0.4 (1-0.4) + 0.15 (1-0.15))]} \}^2}{(0.4-0.15)^2}$$

$$n = \frac{\{1.238 + 0.776\}^2}{0.25^2}$$

$$n = 65$$

For a two-sided 5% test, a sample size of 65 subjects per group or a total of 130 subjects were required to achieve a 90% power. To cater for unexpected data incompleteness, 43% of all the calculated total subjects were added. The adjusted total was 186.

### **3.3 Data Management**

#### **3.3.1 Data collection**

The variables of interest extracted from the patients records were tabulated into an excel format (Appendix 4). The variables included patient demographics, nature of stool (bloody or mucoid), bacteria grown on culture of stool and the antimicrobial susceptibility pattern.

Data about the existing treatment of bloody diarrhea in the study clinic, and two other clinics in Kibera area (Ushirika and Kibera medical clinics) was acquired by interviewing the medical and clinical officers and compared with the sensitivity patterns from the laboratory results.

#### **3.3.2 Data entry and storage**

Data was double entered and stored in a database Microsoft access. After data entry, hard copies of laboratory result forms were stored in locked long-term storage units, which were restricted to relevant staff.

#### **3.3.3 Confidentiality of data**

Every effort was made to ensure data confidentiality. Hard copies of the patient records were stored in a lockable cabinet. Access to these files was limited to relevant persons (Investigator/ supervisors). An electronic copy of the data was kept in the above-mentioned database, which was password protected and only accessible to the relevant parties.

### **3.3.4 Data analysis**

Data cleaning, validation and analysis was undertaken using Statistical Package for Social Scientists (SPSS). The main outcome variables were the kind of bacteria grown on culture and the antimicrobial sensitivity pattern. The values of variables were counted and summarized in tables of frequency. Exploratory Data analysis was performed to show the structure of data. Frequency distribution and cross tabulation techniques were used to show distribution of various pathogens and their susceptibility patterns. Pearson chi-square was used to test for relationship between pathogen and age/ susceptibility patterns.

### **3.4 Ethical Consideration**

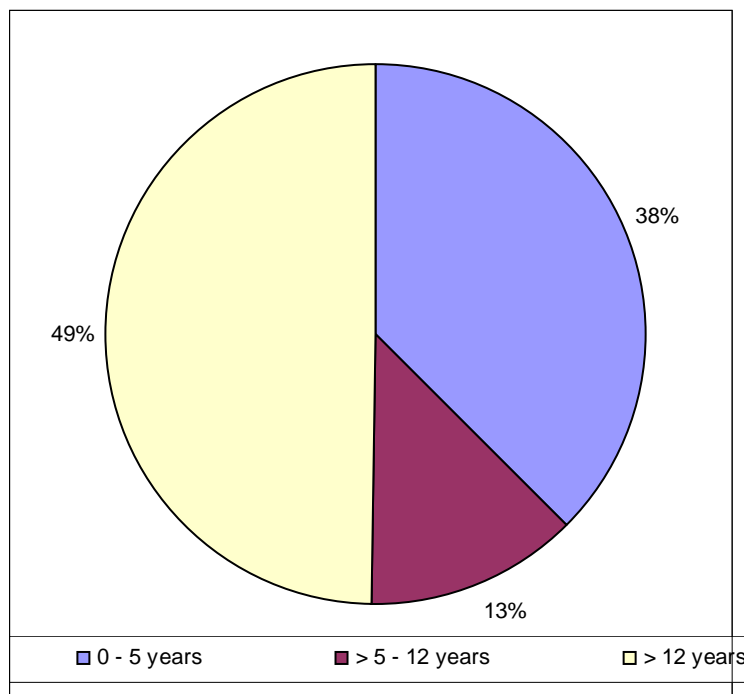
This study sought clearance from the KEMRI Scientific Steering Committee (SSC) and the KEMRI/ National Ethical Review Board. Being a clinic based study, approval was sought from the study clinic (Tabitha Medical Clinic) to use the data collected for this study (Appendix 5). Implementation of all the aspects of the project were in agreement with international ethical guidelines for research involving human subjects as stated in the latest version of the Helsinki declaration.

## CHAPTER 4

### RESULTS

#### 4.1 Demographics

Between October 2006 to January 2008, 189 patients with bloody and mucoid diarrhea had their stool analyzed (Figure 6). About half of the participants (49%) were adults ( $\geq 12$ ) and 51% were of pediatric age group ( $< 12$  years). Most of those in the pediatric age group (38%) were children under five years old. The median age of all the patients who provided a sample was 12.00 with an IQR of 25.50 (Table 1). Male/ Female participants were 49% and 51% respectively (Table 2).



**Figure 7. Percentage distribution of participants by age.**

**Table 1. Distribution of age of participants in years**

Mean	16.75
SD	15.17
Minimum age	0.06
Lower quartile	3.00
Median	12.00
Upper quartile	28.50
Maximum age	66.00
IQR	25.50

**Table 2. Gender of study participants**

	Male	Female	Total
<b>Number of participants (n)</b>	93	96	189
<b>Percentage distribution (%)</b>	49	51	100

#### **4.2 Physical appearance of stool**

Over 80% of the stool samples were identified to be either bloody or mucoid in the laboratory (Table 3). Sixty three percent of the samples were described as bloody; 24.3% as mucoid, 2.6% were described as loose, 0.5% as watery and 18 samples (9.5%) were not classified on laboratory physical examination.

**Table 3. Distribution of stool by physical appearance**

<b>Stool appearance</b>	<b>n</b>	<b>%</b>
Bloody	119	63.0
Mucoid	46	24.3
Loose	5	2.6
Watery	1	0.5
Unidentified	18	9.5
<b>Total</b>	<b>189</b>	<b>100</b>

Forty-six percent (46%) of the samples had specific bacteria isolated (Table 4). Majority of the isolates came from samples that were described as bloody (58.8%) or mucoid (32.6%) on laboratory physical examination. The samples that were described as watery or loose on laboratory physical examination had no pathogens isolated.

**Table 4. Distribution of isolate by stool appearance**

<b>Sample analysis</b>	<b>Stool appearance</b>										<b>Total</b>	
	<b>Bloody</b>		<b>Mucoid</b>		<b>Loose</b>		<b>Watery</b>		<b>Unidentified</b>		<b>n</b>	<b>%</b>
	<b>n</b>	<b>%</b>	<b>n</b>	<b>%</b>	<b>n</b>	<b>%</b>	<b>n</b>	<b>%</b>	<b>N</b>	<b>%</b>		
Isolates	70	58.8	15	32.6	0	0	0	0	2	11.1	87	46.0
Normal flora	49	41.2	31	67.4	5	100	1	100	16	88.9	102	54.0
<b>Total</b>	<b>119</b>	<b>100</b>	<b>46</b>	<b>100</b>	<b>5</b>	<b>100</b>	<b>1</b>	<b>100</b>	<b>18</b>	<b>100</b>	<b>189</b>	<b>100</b>

Using Pearson chi-square test of significance, there was significant relationship between stool appearance and sample analysis outcome ( $P < 0.001$ ), the samples that were described as bloody or mucoid had pathogens isolated as opposed to the loose and watery stools.

### **4.3 Pathogen isolation**

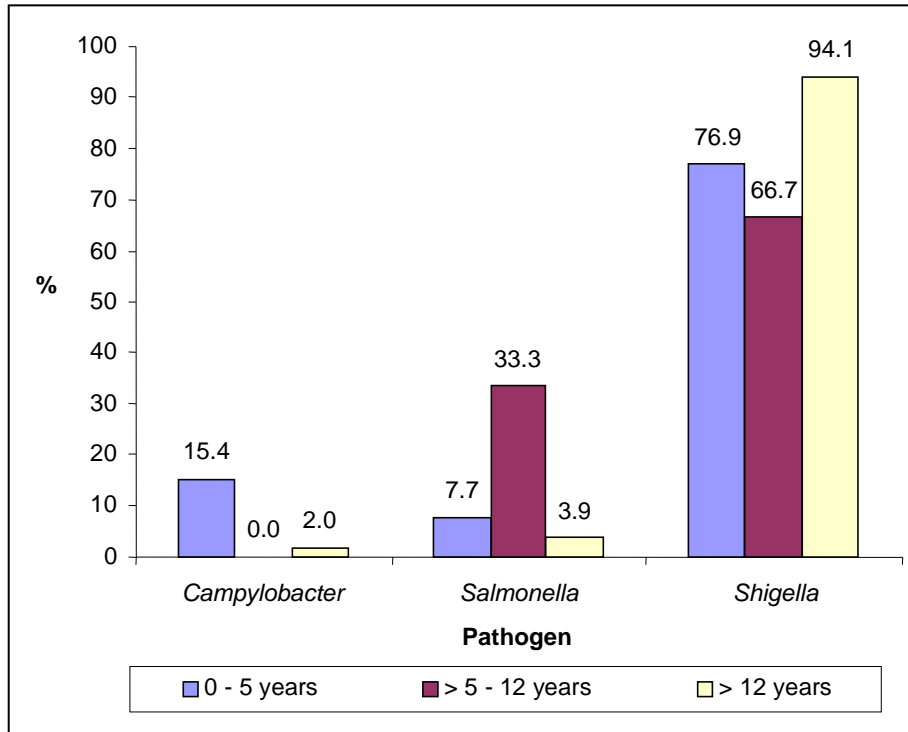
At least 1 pathogen was isolated in 83 samples (45.5%); 2 pathogens in 3 (1.6%) samples and no pathogen was isolated in 103 (54%) samples (Table 5). *Shigella* was the most common and accounted for 74 (39.1%) isolates the most prevalent being *Shigella flexneri* 41 (21.7%). Of the pathogens that had 2 isolates, *Campylobacter* species was always the second isolate. The other pathogens isolated included *Salmonella* group B, *Salmonella* group C1, *Salmonella* group D, *Salmonella typhi* and *Campylobacter* species. None of the patient records showed isolation of *Escherichia Coli*.



**Table 5. Distribution of isolated pathogens by type**

<b>Isolated pathogens</b>	<b>N</b>	<b>%</b>
Normal flora	103	54.5
<i>Campylobacter</i> species	5	2.6
<i>Salmonella</i> GP B	1	0.5
<i>Salmonella</i> GP C1	1	0.5
<i>Salmonella</i> GP D	3	1.6
<i>Salmonella typhi</i>	2	1.1
<i>Shigella boydii</i>	4	2.1
<i>Shigella dysenteriae</i> non-type1	7	3.7
<i>Shigella dysenteriae</i> non-type1/ <i>Campylobacter</i>	1	0.5
<i>Shigella flexeneri</i>	41	21.7
<i>Shigella flexeneri</i> / <i>Campylobacter</i> species	2	1.1
<i>Shigella sonnei</i>	9	4.7
<i>Shigella species</i>	10	5.3
<b>Total</b>	<b>189</b>	<b>100</b>

There was a significant relationship between age and pathogen isolated (Figure 7). Of the pediatric age group, children under 5 years old were most affected by bloody diarrhea. *Shigella* was a common isolate in all the age groups being present in 48 isolates in adults over 12 years, 20 isolates in children under 5 years and 6 isolates in the middle age group. *Campylobacter* species was established to be predominantly a disease of children under 5 years; *Salmonella* was predominant in children aged between 5- 12 years and *Shigella* was commonest in adults (Pearson chi-square value = 15.1, P=0.005).

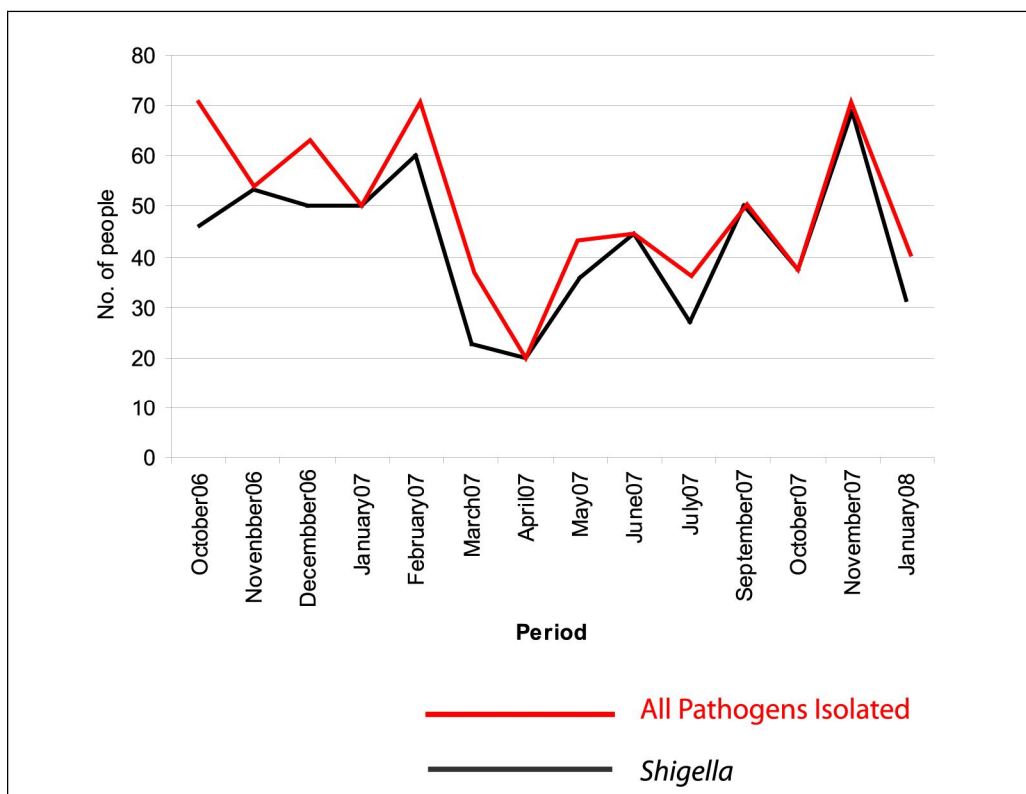


**Figure 8. Distribution of pathogens according to age of participants.**

#### 4.4 Seasonal variations

The samples were collected from 1<sup>st</sup> of October 2006 to 31<sup>st</sup> January 2008 with some months having more causative pathogens isolated compared to others (Figure 8). Despite some confounding factors, the dry months of November to February saw a higher incidence of the total pathogens isolated compared to the wet months of March to June. No consistency was observed in the proportion of samples with pathogens isolated for similar months of different years; for instance October 2006 had 70% isolates while

October 2007 had 40% isolates, November 2006 had 53% isolates while November 2007 had 70% isolates and January 2006 had 50% isolates while January 2007 had 40% isolates. Because of some technical difficulties the months of August and December 2007 had no stool sample delivered to the laboratory for analysis.



**Figure 9: Proportion of stool specimens with pathogens isolated by month and year**

The trend of *Shigella* isolates also followed a similar picture of higher levels in the dry months as compared to the wetter months of the year.

#### 4.5 Antimicrobial susceptibility patterns

Excluding *Campylobacter*; more than 90% of the isolates were resistant to trimethoprim-sulfamethoxazole, sulfisoxazole and streptomycin; 82.5% were resistant to Tetracycline, 62.5% to ampicillin and 52.5% to chloramphenicol (Table 6). Less than 5 % were resistant to ceftriaxone, nalidixic acid and kanamycin. Remarkably, no pathogen showed resistance to Ciprofloxacin and Gentamycin.

**Table 6. Susceptibility patterns for different antimicrobial agents against the bacteria isolates**

Antimicrobial agents	Resistant (R)		Sensitive (S)	
	n	%	n	%
Chloramphenicol	42	52.5	38	47.5
Trimethoprim sulfa	77	96.3	3	3.8
Tetracycline	66	82.5	14	17.5
Ciprofloxacin	-	-	80	100.0
Sulfisoxazole	77	96.3	2	2.5
Nalidixic acid	1	1.3	79	98.8
Ampicillin	50	62.5	30	37.5
Streptomycin	74	92.5	5	6.3
kanamycin	1	1.3	77	96.3
Gentamycin	-	-	80	100.0
Ceftriaxone	1	1.3	79	98.8
Amox cluvanic acid	26	32.5	43	53.8

The antimicrobial resistance pattern among 81 bacteria isolates tested from the study participants is summarized in Table 7. *Shigella* showed most resistance. Considering individual agents where *Shigella* showed resistance, *S. flexeneri* was associated with over 50% of the resistance. The other *Shigella* serotypes isolated also showed quite high levels of resistance with *S. boydii* and *S. dysenteriae* showing 100% resistance to over 2 first-line antimicrobial agents. Nalidixic acid, ciprofloxacin and gentamycin did not encounter any resistance with *Shigella* species. Among *Shigella* species multi-agent antimicrobial resistance was common. *S. dysenteriae type 1* and *S. flexeneri* isolates were resistant to over 5 first-line antimicrobial agents; namely chloramphenicol, trimethoprim sulfamethoxazole, tetracycline, sulfisoxazole, ampicillin, amoxiclavullin and streptomycin. Some agents in the *Salmonella* group also showed over 50% resistance to more than five first-line antimicrobial agents; *Salmonella typhi* notoriously showed 100% resistance to 6 commonly prescribed antimicrobials, namely chloramphenicol, trimethoprim-sulphamethoxazole, tetracycline, sulfisoxazole, ampicillin and streptomycin.

**Table 7: Antimicrobial resistance patterns among 81 bacteria isolates tested from persons with bloody diarrhea.**

Pathogen(number tested)	Percent resistant isolates											
	chl	Tri	Tetr	Cip	Sulf	Nal	Amp	Stre	Kan	Ge nt	Ceft	x
All isolates (81)	52.5	96.3	82.5	0	96.3	1.3	62.5	92.5	1.3	0	1.3	32.5
<i>Shigella</i> spp (74)	52.1	97.3	83.6	0	97.3	0	61.6	94.5	1.4	0	1.4	35.6
<i>S. boydii</i> (4)	25	100	100	0	100	0	25	100	0	0	0	25
<i>S.dysenteriae</i> (8)	37.5	100	50	0	100	0	50	100	0	0	0	0
<i>S. flexeneri</i> (43)	67.4	95.3	86	0	95.3	0	79.1	93	0	0	0	53.5
<i>S. sonnei</i> (9)	0	100	88.9	0	100	0	11.1	88.9	0	0	2.3	0
<i>S. unidentified</i> (10)	55.6	100	88.9	0	100	0	55.6	100	11.1	0	0	22.2
<i>Salmonella non typhii</i> (5)	40	80	60	0	80	20	60	60	0	0	0	0
<i>Sal. Gp B</i> (1)	0	100	0	0	100	0	100	100	0	0	0	0
<i>Sal. Gp C1</i> (1)	0	100	100	0	100	100	0	0	0	0	0	0
<i>Sal. Gp D</i> (3)	66.7	66.7	66.7	0	66.7	0	66.7	66.7	0	0	0	0
<i>Salmonella typhii</i> (2)	100	100	100	0	100	0	100	100	0	0	0	0

**Key:**

Chl – Chloramphenical  
Tet – Tetracycline  
Sulf – Sulfoxazole  
Gen- Gentamycin

Amp – Ampicillin  
Stre - Streptomycin  
Nal – Nalidixic acid

Cip – Ciprofloxacin  
Tri – Trimethoprim  
Kan – Kanamycin

**4.6 Treatment of bloody diarrhea in clinics in the study area.**

The three clinics in the study area were assessed to evaluate the standard modes of therapy for bloody diarrhea (Table 8). All the clinics used trimethoprim/

sulfamethoxazole in the treatment of bloody diarrhea in children while two of the clinics (Ushirika and Kibera) also used it in treatment of the adults. Tabitha medical clinic used either ciprofloxacin or amoxiclavullinic acid in treatment of adult bloody diarrhea and nalidixic acid or trimethoprim/sulphamethoxazole in children. Ampicillin and streptomycin were also used in the other two clinics. Tetracycline and metronidazole were used in treatment of adults and children at Ushirika and Kibera clinics respectively.

**Table 8. Treatment protocols for bloody diarrhea in clinics within the study area**

Clinic	Mode of therapy	
	Adults	Children
Tabitha Clinic	Ciprofloxacin, Amoxiclavulin	Trimethoprim, Nalidixic acid
Ushirika clinic	Trimethoprim, streptomycin, Tetracycline	Trimethoprim, Ampicilin
Kibera Clinic	Ampicillin, Trimethoprim, Streptomycin	Trimethoprim, Metronidazole

## CHAPTER 5

### 5.1 DISCUSSION

At least one pathogen was isolated from 45.5% of the stool samples collected at Tabitha medical clinic in Kibera between October 2006 to January 2008. This observation is similar to that in previous studies in developing countries (Taylor *et al.*, 1991; Brooks *et al.*, 2003b). The Brooks *et al.*, (2003b) study conducted in Western Kenya showed an overall isolation rate of 49.2% from samples of bloody diarrhea. A separate study in rural Nyanza showed an isolation of 33% from samples of bloody and or mucoid stools (Shapiro *et al.*, 2001). In a study conducted in Bolivia, a bacterial pathogen was identified in 41% of the specimens (Townes *et al.*, 1997). The differences may be due to certain factors such as the use of antimicrobial therapy before sample collection, which is known to reduce the percentage of bacterial pathogens isolation. Prior antibiotic use was not established in this study because the patients, who had prior medication, could not establish whether the medicine they took was an antibiotic, antidiarrheal or analgesic.

Some studies have documented low sensitivity of the traditional culture methods; some of the reasons for the low sensitivity include competition from other commensal microorganisms and inappropriate changes in ambient temperature and pH during specimen transport (Taylor and Schelhart, 1975). The use of PCR assay based on the amplification of the invasion plasmid antigen H (*ipaH*) gene sequence can overcome some of the shortcomings of culture methods, but the method itself has not yet received



global acceptance due to the high costs of implementation which are unaffordable for developing countries (Cheng *et al.*, 2005).

Majority of the isolates came from samples, which appeared, bloody (58.5%) or mucoid (32.6%) on laboratory physical examination. The samples that were described as watery or loose on laboratory physical examination significantly had no pathogens isolated. This is an indication that bloody diarrhea as opposed to the watery type is very likely to be of bacterial origin hence should be treated with an appropriate antimicrobial agent. Bloody diarrhea is associated with rupture of intestinal epithelial barrier, followed by the invasion and destruction of the intestinal mucosa resulting in the proliferation of the pathogens faster than that occurring in patients with a milder disease (Sansone *et al.*, 1999). A direct relationship between bacterial load, detection by culture and disease severity has also been reported (Thiem *et al.*, 2004).

This study confirmed that *Shigella* was a major cause of bloody diarrhea in Kibera (39.1%). This is similar to other studies conducted in Western province, which showed *Shigella* isolation of 40% (Brooks *et al.*, 2003a) and more or less similar proportions in other developing countries (Shapiro *et al.*, 2001, Urrio *et al.*, 2001). Townes *et al.*, (1997) found *Shigella* organisms in 29% of the samples in the study conducted in Bolivian children. No outbreak of *Shigella dysenteriae* type 1 was noted in the period of study; this is different from other studies conducted earlier in Western Kenya whereby epidemics of *S. Dysenteriae* 1 were noted (Brooks *et al.*, 2003b; Iversen *et al.*, 1992). This is likely to

be due to improvement in mothers' education, better primary health care and presumably the increased and uncontrolled use of antibiotics.

Campylobacteriosis was established to be predominantly a disease of children under 5 years. *Campylobacter* was isolated almost 25% as frequently as *Shigella* in children under 5 years of age. This is unlike the Brooks *et al.*, (2003a) study whereby *Campylobacter* and *Shigella* were isolated in equal frequencies in children under 5 years of age. In a different study *Shigella* was observed to cause 50% of the bloody diarrhea in children less than 5 years of age (Ronsmans *et al.*, 1988). Although *Campylobacter* infections are a common cause of childhood diarrhea in developing countries, young children in these circumstances also exhibit high rates of asymptomatic *Campylobacter* carriage (Coker *et al.*, 2002, Oberhelman *et al.*, 2000). Since this study did not have stool from healthy children cultured, it is not possible to quantify *Campylobacter's* contribution to bloody diarrhea. Previously published data has shown asymptomatic carriage rates for *Campylobacter*, *Shigella* and *Salmonella* among 100 persons (>18 years) to be 8%, 3% and 2% respectively (Shapiro *et al.*, 2001).

In this study the contribution of enteroparasites in the etiology of bloody and mucoid diarrhea was not established. This has been established previously in a study conducted in Mozambique, which showed that 9.3% and 2.5% of diarrhea in children under the age of five was due to *Ascaris lumbricoides* and *Giardia lamblia* respectively (Inacio *et al.*, 2007).

Infection with HIV may be an important co-morbidity for bloody diarrhea in the Kibera community where an estimated 15% of young adults are infected with HIV/ AIDS (Medicens San Frontiers, 2008). According to Sande and Volberding, (1997) diarrhea is experienced by over 50% of AIDS patients at some time during the course of their illness and it can be an important cause of morbidity and mortality in up to a quarter of all patients. The most common bacterial causes of diarrhea are *Salmonella*, *Clostridium difficile*, *MAC*, *Shigella*, and *Campylobacter*. The overall incidence of diarrhea has been reduced by the widespread use of trimethoprim/sulfamethoxazole for *Pneumocystis* prophylaxis. In a study by Batchelor *et al.*, (1996) in Kenya, the most commonly isolated organisms in stools were *Shigella flexneri* (49 for HIV-positive cases and 9 in HIV-negative cases), *S. typhimurium* (40 in HIV-positive cases and 3 in HIV-negative cases), and *Cryptosporidium parvum* (45 in HIV-positive cases and 0 in HIV-negative cases). This work did not ascertain the HIV status of the participants and therefore cannot compare the burden of disease and its etiologies between HIV infected and uninfected individuals.

Most of the isolates from bloody diarrhea samples were in the dry months of November to February as compared to the wet months of March to June. The picture was similar for *Shigella* and *Campylobacter*, which appeared to be more prevalent in the drier months. This could be due to scarcity of water seen in the study area within these periods, usually associated with higher levels of contaminated water use, which may contribute to a higher incidence of diarrheal diseases. This compares well with a study

conducted in Mumias, Kenya whereby there was an epidemic of dysentery (bloody diarrhea) that began in December 1994 and peaked in February 1995, coinciding with the very dry season (Iversen *et al.*, 1992). In a study by Victoria *et al.*, (1985) the incidence of diarrheal deaths was almost 4 times higher during the summer months (January-February) than during the winter months (July-October). In a study conducted in New Zealand to evaluate seasonal variation of campylobacteriosis, differences were noted between the August (winter) and February (summer) serotypes, with the most frequently isolated serotypes in February being completely absent in August (Hudson *et al.*, 1999). In the Kibera study only *Campylobacter jejuni* serotype was isolated mainly in the dry months. A separate study established that *Campylobacter* infections occur to a greater extent in late springtime, affecting mainly children (Alcalde and Javier, 2007). On the contrary a separate study conducted in Mozambique showed that the incidence of cases of diarrhea was higher during the rainy season than during the dry season though when taking into account all enteropathogens, no seasonal difference in pathogen isolation was observed (Inacio *et al.*, 2007). This shows that diarrhea in general gives a different type of seasonal variation as opposed to bloody diarrhea, which is usually of bacterial origin. In a study conducted in Tanzania, patients presented mainly with watery diarrhea in both the dry (70.68%) and the rainy seasons (72.81%), while only 18.10% and 5.82%, respectively presented with dysentery. Dehydration was detected in 11 patients (10.68%) in the rainy season and in 40 patients (11.49%) in the dry season (Wargas *et al.*, 2004). The seasonal variation of various bacterial causes of diarrhea should be further evaluated in future studies in Kenya.

There was a high level of resistance to first line antibiotics (ampicillin, chloramphenicol, tetracycline, streptomycin and trimethoprim/sulfamethoxazole). Most clinics in the study area prescribed trimethoprim/sulfamethoxazole, streptomycin and ampicillin for treatment of bloody diarrhea. These antibiotics showed high levels of resistance, that is 96.3%, 92.5% and 62.5 % respectively. Antimicrobial-resistant bacterial diarrhea has been described as a significant public health problem throughout the developing world. The steady increase in antibiotic resistance makes the emergence of massive epidemics a possible scenario, particularly in socially unstable areas (O'Brien, 1997; Ries *et al.*, 1994).

There was no resistance to ciprofloxacin and very minimal resistance to nalidixic acid (1.3%). Other studies have also found little resistance to nalidixic acid and/ or no resistance to ciprofloxacin among *Shigella* in East Africa (Kruse *et al.*, 1992; Legros *et al.*, 1998; Materu *et al.*, 1997) and other African countries (Adeleye and Adetosoye, 1993; Pitman *et al.*, 1996). Despite nalidixic acid being a preferred treatment for bloody diarrhea, it was noted that in areas where nalidixic acid has been introduced as a drug of choice to treat shigellosis empirically, a marked increase in corresponding resistance has been observed (Karch *et al.*, 1993; Ries *et al.*, 1994). Thus although nalidixic acid is a good option for treatment of bloody diarrhea where antimicrobial resistance limits other options, it should be used ideally only for illnesses most likely caused by *Shigella* or where *Shigella* infection could result into greater morbidity and increased risk of death for instance in persons with AIDS. Nalidixic acid is the preferred choice in children

below 12 years in whom ciprofloxacin induced arthropathy has been reported (Dagan, 1995; Warren, 1997). Chromosomal resistance can easily be transferred between nalidixic acid and ciprofloxacin; moreover experience in other parts of the world has confirmed that resistance to these agents arises rapidly once selective pressure is exerted through intensive use of fluoroquinolones (Acar and Goldstein, 1997; Frost *et al.*, 1985).

Recent WHO guidelines recommend fluoroquinolones for instance ciprofloxacin, in the treatment of shigellosis, which is a major cause of bloody diarrhea in developing countries (WHO 1995b). Hence judicious, controlled use of fluoroquinolones for adults with clinically significant bloody diarrhea is recommendable. For children, nalidixic acid is a safer yet equally effective empiric choice. In this study there was only 1.3 % resistance to nalidixic acid, which was actually in one adult (over 12 years). The challenge still remains that fluoroquinolones are expensive and of limited supply within a low socioeconomic area like Kibera.

Apart from the study clinic the other clinics in the study area treated bloody diarrhea with antimicrobial agents that were more than 75% ineffective. Ushirika clinic used trimethoprim/sulfamethoxazole, tetracycline and streptomycin, which had 96.3%, 82.5% and 92.5% resistance respectively. Ampicillin, which was also used in children at Ushirika clinic, had 62.5% resistance. Kibera clinic used trimethoprim, ampicillin, streptomycin and metronidazole. The first three antimicrobials showed high levels of resistance at the rate of 96.3%, 62.5% and 92.5% respectively. Metronidazole, an anti-

protozoal agent with no effect on the bacteria causing bloody diarrhea was also used in pediatric patients at Kibera clinic. Similarly a separate study in Western Kenya established that more than half of all pathogens isolated were not susceptible to empiric therapy that was used (Brooks *et al.*, 2003b). Ineffective treatment has multiple consequences including delay of time until effective treatment is offered to the patients; it creates a false sense of security for both patients and health care providers; it adds significantly to the cost of treating diarrhea diseases and contributes to development of resistance of both enteric and non-enteric bacterial pathogens. The development of antimicrobial resistance limits treatment options for potentially fatal epidemics. Given the magnitude of the problem, strategies to improve prescription practices that use surveillance data to rationally guide more judicious antibiotic should be considered.

In summary shigellosis especially that due to *Shigella flexineri* was the most common cause of bloody diarrhea in Kibera although *Campylobacter* mainly caused bloody diarrhea in children under the age of five. There is significant resistance among *Shigella* bacteriae to antimicrobial agents commonly used for treatment of bloody diarrhea and many persons receive medication to which the infecting bacterium is resistant. Thus antimicrobial surveillance should be continuous to monitor multi-drug resistant strains.

## **5.2 STUDY LIMITATIONS**

1. Being a clinic rather than a community based study, the incidence of bloody diarrhea established in this study may be an under estimate. This is because it only captured those who came to seek medical treatment at the clinic. In addition some of the eligible children and even a few adults were unable to produce a stool sample at the time of the clinic visit.
2. Over the counter dispensing of antibiotics is a common phenomenon in Kibera area. The use of some antibiotics prior to presentation to the clinic with a history of bloody diarrhea might have interfered with the stool culture results.
3. The clinic is located in an area with very poor infrastructure; in periods of rainfall the roads are inaccessible hence some patients would opt to remain at home and not seek care from the clinic. This factor may act as a confounding factor in the seasonal distribution of bloody diarrhea presentations at the clinic.
4. Being an out patient clinic, Tabitha medical clinic operates from Monday to Friday 8.00 am to 5.00 pm. Because of the operating hours not all the clients in this area were captured when they had bloody diarrhea.
5. Tabitha medical clinic is a CDC/KEMRI research clinic run by qualified medical and clinical officers. On the other hand, the other 2 health centers, Ushirika and



Kibera clinic are operated by enrolled community nurses hence comparison of the treatment protocols may not be entirely practical.

## CHAPTER 6

### CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 CONCLUSIONS

- The findings from this study confirm that *Shigella* especially that due to *S. flexneri* is the predominant cause of bloody diarrhea in Tabitha Medical Clinic in Kibera. However, *Campylobacter* contributes to a good proportion of bloody diarrhea in children under the age of five in this area.
- Bloody diarrhea, especially that caused by shigellosis is more common in the dry months as compared to the rainy periods of the year. This is thought to be due to scarcity of clean water in these dry seasons.
- Diarrhea described by a patient as bloody or mucoid is very likely to be of bacterial origin as opposed to the watery type. Hence clinicians should treat the affected patients with an appropriate antibiotic.
- There are high levels of resistance of the pathogens causing bloody diarrhea to the first line antimicrobial agents illustrating the effect of longstanding unregulated antimicrobial use.
- Most patients presenting with bloody diarrhea in the study area receive medication to which the causative bacteria is resistant. This leads to

recurrence of the problem and even an increased rate of transmission to other individuals.

## **6.2 RECOMMENDATIONS**

- Strategies to improve prescription practices that use surveillance data to rationally guide more judicious antibiotic should be considered. For instance, regular training of health care workers in low income settings like Kibera on the best modes of therapy for diarrhea and other infectious diseases.
  
- Judicious use of antimicrobial therapy requires the education of health care workers and patients, adequate laboratory diagnostic capabilities even in the low-income settings and government regulation.
  
- There is need for updated epidemiological data; hence continuous surveillance for bloody diarrhea is important in order to regularly advice on the most effective modes of therapy.
  
- Trimethoprim/sulfomethaxazole should not be used for treatment of bloody diarrhea for any age group. This is because of the very high levels of resistance reported in this and similar studies in developing countries.

- Ciprofloxacin is the drug of choice in treatment of bloody diarrhea in adults and nalidixic acid is safe and effective in treatment of bloody diarrhea in the pediatric age group (below 12 years). Nevertheless, clinicians should use it judiciously to avoid development of undue resistance.
- The government of Kenya through the Ministry of Health should facilitate cost effective availability of quinolones and flouroquinolones in the local clinics in Kibera and other remote parts of the country.
- There is need for tighter restrictions on the availability of antimicrobial agents, standardized treatment among clinical officers and physicians and programs aimed at the primary prevention of diarrheal diseases for instance the provision of safe drinking water and improved sanitation.
- More studies are recommended to evaluate the role of hand washing, use of drinking water sterilizers and routine deworming of under fives in reducing the burden of diarrheal diseases in Kibera. The relationship between the burden of HIV/ AIDS and the prevalence of diarrheal diseases should also be evaluated. The seasonal variation of various bacterial causes of diarrhea in Kenya should be further evaluated in future studies.

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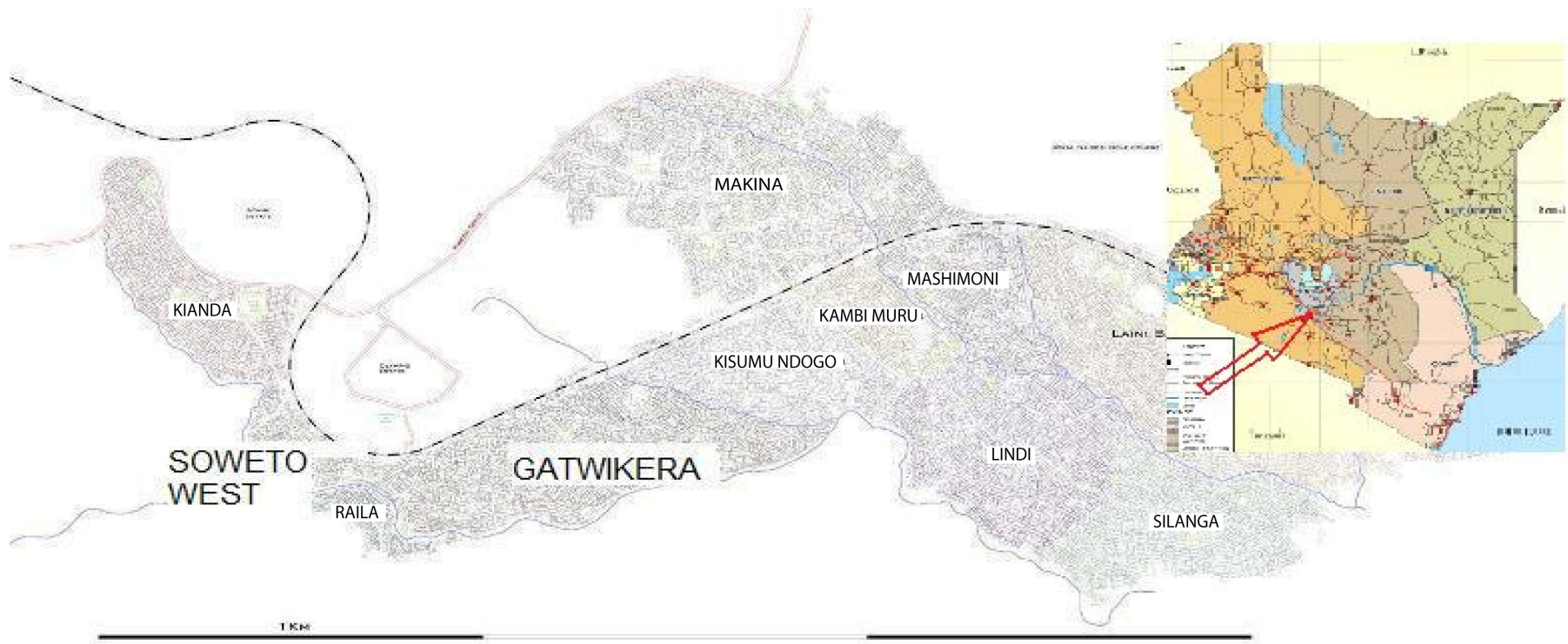
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## APPENDICES

### APPENDIX 1: Map of Kibera (Kenya inset)



BY SUBMITTED TO: KAROLINA FOR KIBERA / TECHNICAL UNIVERSITY OF KENYA / 2013 BY: 2013 KAROLINA FOR KIBERA



**APPENDIX 2: Picture of selected areas of Soweto and Gatwikera**



### APPENDIX 3: Data entry form

The following variables were entered into a Microsoft excel sheet.

Date	Age	Gender	Stool appearance	Culture Results	ANTIMICROBIAL AGENTS											
					Chl	Amox-Cl	Tri	Tet	Cip	Nal	Amp	Sul	Str	Kan	Gen	
10.5.06	1.4yrs	M	Bloody	Normal flora												
10.5.06	36yrs	F	Bloody	Normal flora												
10.5.06	3yrs	F	Bloody	Normal flora												
10.5.06	7months	M	Bloody	Normal flora												
10.5.06	6yrs	F	Bloody	Normal flora												
10.5.06	4yrs	M	Bloody	<i>Shigella boydii</i>	S		R	R	S	S	S	R	R	S	S	
10.6.06	2.10yrs	M	-	Normal flora												
10.13.06	19yrs	M	Bloody	<i>Shigella dysenteriae</i> non-type1	R		R	R	S	S	R	R	R	R	S	
10.13.06	14yrs	M	Bloody	Normal flora												
10.13.06	3.8yrs	F	Bloody	<i>Campylobacter</i> spp												
10.13.06	35yrs	M	Bloody	<i>Shigella flexneri</i>	R		R	R	S	S	R	R	R	S	S	
10.17.06	30yrs	F	Mucoid	Normal flora												
10.18.06	24yrs	F	Mucoid	<i>Shigella sonnei</i>	S		R	R	S	S	S	R	R	S	S	
10.18.06	66yrs	M	Bloody	Normal flora												
10.18.06	23yrs	F	Mucoid	<i>Shigella boydii</i>	S		R	R	S	S	S	R	R	S	S	

**KEY:**

Chl – Chloramphenicol    Amp - Ampicillin    Cip – Ciprofloxacin    Tet – Tetracycline    Str- Streptomycin    AmoxCl-Amoxclav  
 Tri – Trimethoprim    Sul – Sulfisoxazole    Nal – Nalidixic acid    Kan – Kanamycin    Gen- Gentamycin



## **APPENDIX 4: Informed Consent Form**

### **Title of the Study**

A retrospective study to evaluate the bacterial causes of bloody diarrhea and their antimicrobial susceptibility patterns at Tabitha medical clinic in Kibera, Kenya.

### **Investigators and institutions**

		Institution	Contact
Principal Researcher	Salome O. Gitari	Jkuat-ITROMID	0722-319746
Supervisors	Dr Yeri Kombe	KEMRI	0734-257864
	Mr. Charles Mbakaya	KEMRI	0722-846964
	Dr. Venny Nyambati	JKUAT	0720-414081

### **Introduction**

Bloody diarrhea refers to the presence of blood in stool. This condition is referred to as dysentery whereby someone passes stool that is blood stained and could also contain mucous. This study proposes to determine the main causes of dysentery in selected areas of Kibera and also establish which antibiotics work best against those germs. This study will also determine how much of this problem is in Kibera. This will help the clinicians in this area to know the best drugs to use in treatment of bloody/ mucoid diarrhea.

Tabitha Medical clinic has been collecting stool samples from patients having bloody and mucoid stools since August 2006 to date. After collection the stool samples are

usually taken to the CDC laboratory, analyzed and culture and sensitivity results reported and delivered to Tabitha medical clinic.

We would like to seek approval from the Tabitha medical clinic manager to use the laboratory achieves of all the stool samples collected for the period between November 2006 to January 2008.

Name: Hillary Omalla

Clinic manager, Tabitha medical clinic.

Signature..... Date.....

**Purpose of the study**

- The purpose of the study is to determine the main germs that cause dysentery in this area and also establish which antibiotics work best against those germs. In this study we will also know how much of this problem is in Kibera.

**Risks and /or discomforts**

We do not anticipate any risks or discomforts to you during this study. We will make every effort to protect your privacy and confidentiality of all the reports used in analysis for this study.

## **Benefits**

Results from this study will be of importance to Tabitha medical clinic and other clinics in Kibera. They will aid the clinicians to revise the treatment of bloody and mucoid diarrhea, in order to use the most effective therapy.

## **Records Privacy and Confidentiality**

Permission to access the patient's records sought from the Tabitha medical clinic manager. Every effort will be made to keep the information obtained from the records private and confidential. Personal information from records will not be released. The laboratory results will be stripped of all identifiers; no names will be used in the data tallying, analysis and reporting. Identity numbers, in chronological order will be assigned as the records are entered into an excel sheet.

The information obtained may be reviewed by:

- Study investigators
- Ethics Committee at KEMRI.

If you ever have questions about this study you should contact: The principal Investigator, Dr Salome Okutoyi Gitari on mobile 0722-319746 or email [sallygitari@yahoo.co.uk](mailto:sallygitari@yahoo.co.uk). You can also mail using the address given below:

P.O Box 3630-00506 Nairobi.

The research proposal has been reviewed and approved by the Kenya National Ethical Review Committee, and KEMRI's Scientific Steering Committee. These committees have reviewed this study in order to protect the patient's records privacy and confidentiality.

If you have any questions you may contact: The secretary, KEMRI/ National Ethical Review Committee, P.O Box 54840-00200 NAIROBI, Kenya.

Tel 2722541, 0722-205901, 0733-400003.