# ENTERIC PATHOGENS AND POTENTIAL RISK FACTORS FOR ACUTE BLOODY DIARRHOEA IN NAIROBI WEST AND KILIFI SUB COUNTIES. A CASE CONTROL STUDY

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# Enteric Pathogens and Potential Risk Factors for Acute Bloody Diarrhoea in Nairobi West and Kilifi Sub Counties.

## A Case Control Study

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**A Thesis Submitted in Partial Fulfillment for the Degree of Doctor of Philosophy in Epidemiology 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 beloved parents, the Late Mzee Jacob Njuguna Kimani and Hannah Muthoni Njuguna. I salute my late father, the fallen hero and a legend for the great legacy he left behind for me. My dear loving mum, for your resilience in withstanding the many shocks of life for our sake. Although both of you did not go to school, you sacrificed a lot, did everything possible within your power and means to send us to school. My mum, i remember your great desire to read and write when you were the chairlady of Umoja Women Group. Despite the many responsibilities at home, you attended the evening adult learning classes popularly known as "*ngumbaro"* at Kahuho tea buying centre. This was a great inspiration for me and by God's grace i have made you proud by going beyond your limits. Thank you for ploughing the fallow ground (*gitira*) for me. May the Lord God bless you and preserve you.

I also dedicate this thesis to my wife Catherine Murugi Kuria, who offered me unconditional love and support throughout the course and the research period. Without your support, and gentle prodding, i would not have managed. Finally, I dedicate this thesis to my beloved children Sharon Muthoni Kuria, Timothy Njuguna Kuria and Teddy Munene Kuria who persevered throughout the course of this thesis. As your beloved father, i have shown you the way, follow my footprints, this is your heritage and the Lord God will shower you with his abundant life.

Above all, I will forever remain grateful to the almighty God for his sufficient grace as i tried to balance between my studies, work, family and church leadership. It was not easy but he saw me through it all. To him be the glory now and forever more Amen!

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## ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| **AFRO** | Africa Regional Office |
| **AIDS** | Acquired Immunodeficiency Syndrome |
| **AST** | Antimicrobial susceptibility testing |
| **ATCC** | American Type Culture Collection |
| **BMI** | Body Mass Index |
| **cAMP** | Cyclic Adenosine Monophosphate |
| **CMR** | Centre for Microbiology Research |
| **CDC** | Centers for Disease Control and Prevention |
| **CLSI** | Clinical and Laboratory Standards Institute |
| **DAEC** | Diffusely Adhering *Escherichia coli* |
| **DDSR** | Division of Disease Surveillance & Response |
| **DNA** | Deoxyribonucleic Acid |
| **EAEC** | Enteroaggregative *Escherichia coli* |
| **EHEC** | Enterohemorrhagic *Escherichia coli* |
| **EIEC** | Enteroinvasive *Escherichia coli* |
| **EPEC** | Enteropathogenic *Escherichia coli* |
| **EPI INFO** | Epidemiological information statistical Software |
| **EPR** | Epidemic Preparedness and Response |
| **ERC** | Ethical review Committee |
| **ETEC** | Enterotoxigenic *Escherichia coli* |
| **GoK** | Government of Kenya |

| | |
|---|---|
| **HIV** | Human Immunodeficiency Virus |
| **HMIS** | Health Management Information System |
| **IDSR** | Integrated Disease Surveillance and Response |
| **IHR** | International Health Regulations |
| **ITROMID** | Institute of Tropical Medicine and Infectious Diseases |
| **JKUAT** | Jomo Kenyatta University of Agriculture and Technology |
| **KEMRI** | Kenya Medical Research Institute |
| **MCH** | Maternal Child Health |
| **MOH** | Ministry of Health |
| **MOPHS** | Ministry of Public Health & Sanitation |
| **MOMS** | Ministry of Medical Services |
| **MIC** | Minimum Inhibitory Concentration |
| **MUAC** | Mid Upper Arm Circumference |
| **NGO** | Non-Governmental Organization |
| **ORS** | Oral Rehydration Solution |
| **OOP** | Office of the President |
| **P** | Probability Value |
| **PCR** | Polymerase Chain Reaction |
| **PMNs** | Polymorphonuclear |
| **QC** | Quality Control |
| **SAM** | Service Availability Mapping |
| **Sd1** | *Shigella dysenteriae type I* |
| **SPSS** | Statistical Package for Social sciences |

| | |
|---|---|
| **SSC** | Scientific Steering Committee |
| **SSS** | Salt and Sugar Solution |
| **STI** | Sexual transmitted Infection |
| **US** | United States |
| **WHA** | Word Health Assembly |
| **WHO** | World Health Organization |

## DEFINITION OF TERMS

The following definitions apply to terms used in this document.

**Acute diarrhoea:** Diarrhoea lasting less than 14 days (< 14 days)

**A clinician:** Any Medical officer, Clinical officer or a Nurse who is registered by the Medical Practitioners and Dentist Board, Clinical Officers Council and Nursing Council of Kenya respectively in the Republic of Kenya.

**Bloody diarrhoea:** Any number of stools containing blood in a 24-hour period, either suspected by history from patient, or confirmed by inspection of stool, and not attributable to local anorectal bleeding.

**Body temperature:** The body temperature (axillary) recorded during attendance in the outpatient department or casualty unit.

**Bacillary dysentery:** Bloody diarrhoea caused by *Shigella*.

**Coma:** Any patient with scores of 3-8 on the Glasgow coma scale (appendices 7).

**Diarrhoea:** Passage of loose or watery stool ≥ 3 times in a 24 hour period

**Duration of diarrhoea (days):** The duration of diarrhoea from onset (1$^{st}$ episode) to the end (last episode). The duration will be in days (0–24 hours =1 day; 24–48 hours =2 days, etc)

**Duration of abdominal pain (days):** The duration of abdominal pain from onset to the end. The duration will be in days (0–24 hours =1 day; 24–48 hours =2 days, etc)

**Duration of vomiting (days):** The duration of vomiting from onset to the end. The duration will be in days (0–24 hours =1 day; 24–48 hours =2 days, etc)

**Invasive diarrhoea:** Diarrhoea caused by bacterial pathogens, including *Shigella*, and some *Salmonella, E. coli* and *Campylobacter jejuni*, that invade the bowel mucosa, causing inflammation and tissue damage.

**Multidrug resistance:** Resistance to three or more antimicrobial agents among the World Health Organization ranked antimicrobials (important, highly important and critically important) in human medicine i.e. amphenicols, penicillins & aminopenicillins, sulphonamides, tetracyclines, quinolones, cephalosporins (third generation).

**Persistent diarrhoea:** Diarrhoea that that starts acutely but lasts for 14 days or more (≥14days)

**Sanitary hygiene:** Sanitary hygiene practices includes washing hands with water *and* soap, using a pit latrine and keeping water stored in the home sealed and/or covered.

**Severe Diarrhoea**: Passage of loose or watery stool for >6 times in a 24 hour period

## ABSTRACT

Globally, three million people die due to diarrhoeal diseases every year. Shigellosis is a major cause of diarrhoea-related morbidity and mortality, especially in developing countries, with an estimated annual incidence of 165 million cases and 1 million deaths. Kenya experienced a significant increase in acute bloody diarrhoea cases in Coast, Western, Nyanza and Nairobi regions in 2009 (48,272 cases) and 2010 (64,107 cases). Therefore, it was necessary to determine the epidemiological, clinical and laboratory characteristics of acute bloody diarrhoea cases occurring in the urban and rural populations in Kenya. The study enrolled 805 participants (284 cases and 521 controls) into a hospital based matched case control study between the period of January and December 2012. The main presenting clinical features for bloody diarrhoea cases were: blood in stool (100%) abdominal pain (69%), mucous in stool (61%), loose stools (54%) and anorexia (50%). Pathogen isolation rate from stool was 40.5% (115) with bacterial and protozoal pathogens accounting for 28.2% and 12.3%, respectively. The isolation rate among the rural population (Kilifi) was 24.7% while among the urban population (Nairobi) it was 65.5%. *Shigella* was the most prevalent bacterial pathogen isolated in 22.8% of the cases while *Entamoeba hystolytica* was the most prevalent protozoal pathogen isolated in 10.2% of the cases. A total of 86.3% of the bacterial pathogens were resistant to sulfamethoxazole-trimethoprim, 73.8% to tetracycline, 63.8% to ampicillin, 21.3% to chloramphenicol, 3.8% to nalidixic acid, 2.5% to ciprofloxacin and none was resistant to ceftriaxone. High levels of multidrug resistance to three or more antimicrobial agents were observed 68.8% of all bacterial pathogens with resistance in *Shigella* being 53.7%. There was a serious

disconnect between clinical guidelines and clinical practice, clinicians in Nairobi West and Kilifi prescribed to patients 67.6%, 47.7% respectively antimicrobial drugs that were within the high resistance zone (>20%). On binary logistic regression, two factors in rural and three in urban setting remained independently and significantly associated with acute bloody diarrhoea at 5% significance level. In rural setting the factors were: condition of toilet clean and poor general compound cleanliness while in urban setting; other diarrhoea cases in household in previous 2 weeks, drinking water stored in a super drum and hand washing after last defecation were associated with acute bloody diarrhoea transmission. Binary logistic regression for merged rural and urban showed; storage of drinking water separate from water for other use, washing hands after last defecation and presence of coliform in main source water remained significantly associated with acute bloody diarrhoea at 5% significance level. Detection of coliform bacteria in drinking water was used as markers of faecal contamination. A total of 302 water samples {rural (171); urban (131)} were collected and analysed from both settings. In the rural setting, 40.9% of the household water contained total coliforms and 21.5 % faecal coliforms whereas 38.6% of the main source contained total coliforms and 11.9% faecal coliforms. In the urban setting, 10.7% of the household water tested contained total coliforms and 6.2 % faecal coliforms whereas 8.2% of the main source contained total coliforms and 6.2% faecal coliforms. There was a positive correlation between bloody diarrhoea and long term (1971-2013) mean rainfall both in rural (Pearson's $r$=0.55) and urban (Pearson's $r$=0.85) populations. There was also a positive correlation between bloody diarrhoea and long term (1974-2013) mean minimum and maximum Temp but the correlation with minimum Temp was stronger in rural (Pearson's r=0.42) and urban (Pearson's r=0.76).

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background

Diarrhoea is an alteration of normal bowel movement characterized by an increase in the water content, volume, or frequency of stools. A decrease in consistency (i.e. soft or liquid) and an increase in frequency of bowel movements to > 3 stools per day have often been used as a definition of diarrhoea for epidemiological investigations (Baldi, Bianco, Nardone, Pilotto, & Zamparo, 2009). Depending on the pathogenetic mechanism, infectious bacterial diarrhoeas can be divided into cytotonic (pathogens stimulate secretory function by activating intracellular enzymes without damaging the epithelial layer e.g. *Vibrio cholerae*, some strains of *Escherichia coli*, *Bacillus cereus* and cytotoxic (pathogens damage directly epithelial cell e.g. *Shigella*, *Clostridium perfringens*, *Clostridium difficile*, *Staphylococcus aureus*, *Salmonella* and *Campylobacter*) (Baldi *et al.*, 2009). Clinical classification of diarrhoea and an understanding of its main pathogenic mechanisms are fundamental for a diagnostic and therapeutic approach (Baldi *et al.*, 2009).

Infectious diarrhoeal diseases are of great concern throughout the world, as they are responsible for considerable morbidity and mortality, visibly bloody diarrhoea causes proportionally greater morbidity and mortality especially in developing countries (Jafari, Shokrzadeh, Hamidian, Salmanzadeh-Ahrabi, & Zali, 2008). It has been reported that diarrhoeal diseases cause approximately three million deaths worldwide

per year (Jafari *et al.*, 2008). The major causes of diarrhoea differ significantly in developed and developing countries. In the developed world, the most common causes of infectious diarrhoea are viruses, whereas in developing countries, bacterial agents such as enterotoxigenic *Escherichia coli* (ETEC), *Campylobacter*, *Shigella*, and *Salmonella* spp account for most diarrhoeal infections (Jafari *et al*., 2008).

Symptoms such as fever and bloody diarrhoea are strongly suggestive of the presence of an invasive bacterium like *Shigella* spp, *Salmonella* spp, *Campylobacter jejuni* and *Clostridium difficile* (Baldi *et al.*, 2009). Shigellosis is a major cause of diarrhoea-related morbidity and mortality, especially in developing countries, with an estimated annual incidence of 165 million cases and 1 million deaths (Baldi *et al.*, 2009; Jafari *et al.*, 2008). Ninety-nine percent of infections caused by *Shigella* occur in developing countries (WHO, 2005). The majority of cases (~70%), and of deaths (~60%), occur among children less than five years of age (Sire *et al*., 2008). Enteric viruses are not known to cause bloody diarrhoea (J. T. Brooks, Shapiro, Kumar, Wells, Phillips-Howard, Shi, *et al*., 2003).

Appropriate antimicrobial treatment can shorten the duration and severity of illness, decrease morbidity and mortality, and reduce the duration of bacterial shedding ( Brooks *et al*., 2003; Folster *et al.*, 2011). Resistance causes people to be sick for longer, increases the risk of death, increases the cost of health care due to lengthier stays in hospital and requirement for more intensive care (WHO, 2014). The World Health Organisation's first global report on antimicrobial resistance, focusing on antibiotic resistance, provides the most comprehensive picture of resistance to date, with data

provided by 114 countries (WHO, 2014). It reveals that resistance to important antibiotics for treating common life-threatening infections has spread to all regions of the world. The report also shows that key tools to tackle antibiotic resistance such as basic systems to track and monitor the problem have major gaps or do not exist in many countries at all. The emergence of multidrug resistance has exacerbated the problem and made the availability of effective antimicrobial therapy more difficult (Folster *et al*., 2011)

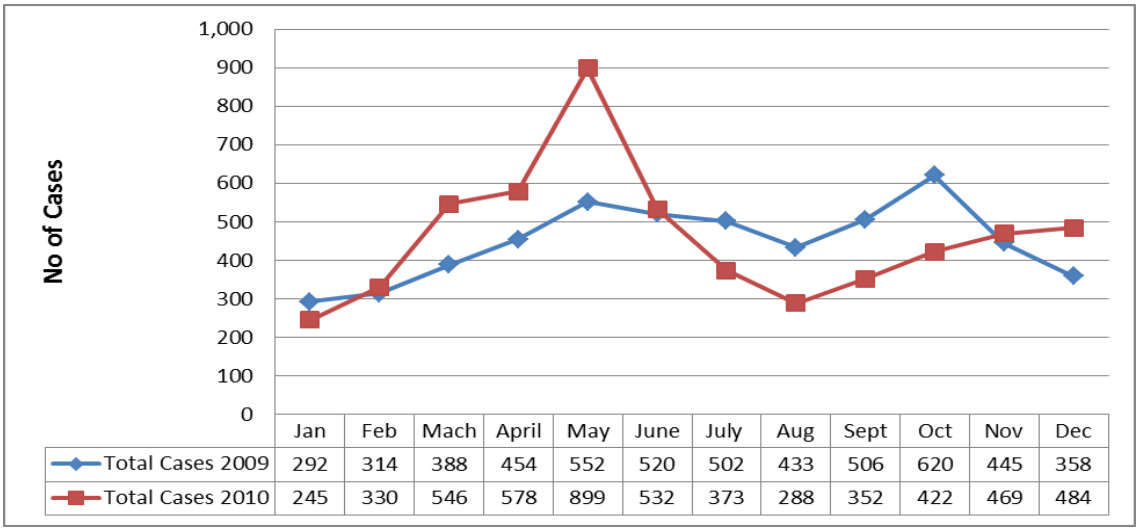
### 1.2 Statement of the Problem

Kenya experienced an increase in acute bloody diarrhoea cases in some parts of Coast, Nyanza, Western and Nairobi provinces. A total of 48,272 cases and 20 deaths were reported through the weekly IDSR system in 2009 compared to 64,107 cases in 2010. The national completeness rate (number of health facilities reporting) improved from 66% to 68% and the average national reporting rate (number of subcounty reporting) improved from 77% to 89% between 2009 and 2010. While there was an increase of 2% and 12% for the completeness and national reporting rates respectively, there was a 32.8% increase in the number of reported acute bloody diarrhoea cases. The data reveal a worrying trend in the occurrence and distribution of acute bloody diarrhoea cases.

The national incidence (attack rate) for acute bloody diarrhoea in 2010 was 166 cases per 100, 0000. The distribution of cases varied from region to region with both the urban and rural populations being affected. Coast Province was the most affected province with an incidence of 377/100,000; Western province 275/100,000; Nyanza

province 236/100,000; Rift Valley province 168/100,000 and Nairobi province 149/100,000. The top ten affected sub counties in 2010 were Loitokitok 4972 cases; Kilifi 3828 cases; Suba 3251 cases; Malindi 3205 cases; Nairobi West 2913 cases; Bungoma West 2530 cases; Busia 2134 cases; Bondo 2059 cases; Kaloleni 1852 cases and Nairobi North 1736 cases.

Surveillance of acute bloody diarrhoea at the sub county level is still sub-optimal. The graph below indicates an unusual increase in cases between March & June 2010 in Kilifi County. However, such unusual increase was not detected by the surveillance system and as a result an opportunity to investigate and determine the causative agent was missed. Such missed opportunities may persist in parts of the country and are largely attributed to the weakness in the health care system surveillance.




| | Jan | Feb | Mach | April | May | June | July | Aug | Sept | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total Cases 2009 | 292 | 314 | 388 | 454 | 552 | 520 | 502 | 433 | 506 | 620 | 445 | 358 |
| Total Cases 2010 | 245 | 330 | 546 | 578 | 899 | 532 | 373 | 288 | 352 | 422 | 469 | 484 |

Source: Ministry of Health, Kilifi County Hospital 2011

**Figure 1.1: Trends of dysentery in Kilifi County 2009-2010**

The Kenya health care system has a major gap in the laboratory network and capacity. Laboratory based surveillance which should guide health authorities in planning, formulating strategies and critical decision making is either weak or none existent. As a result, little is known about the etiologic epidemiology of pathogens other than epidemic *Vibrio cholerae*. When such infections do occur, they are often treated empirically based on clinical judgment, even though very little is known about the etiologic agents in our population. In many cases, they prompt self-medication with antibiotics, which are often available without prescription. If this trend is not checked, the country will be at increased risk of multi drug resistant strains.

The selection of antimicrobial treatment should be based on recent susceptibility testing of the strains from the area. In developing a treatment policy, the antimicrobial agent chosen should be effective against at least 80% of local strains, be available for oral administration, be affordable and available locally (CDC, 1999). While antimicrobial susceptibility patterns differ by geographic area and changes over time, the country lacks an elaborate mechanism for monitoring the antimicrobial susceptibility. For this reason, it is essential that antimicrobial susceptibility be monitored periodically to ensure correct and effective use of treatment against locally isolated pathogens. If the cases are not diagnosed and treated with appropriate antibiotic, there will be a high possibility of persistence of resistant bacteria and subsequent transmission and spread in the community of emerging resistant bacteria.

## 1.3 Justification

Information on prevalence and characteristics of acute bloody diarrhoea in Kenya is scarce. Findings from a study on epidemiology of sporadic bloody diarrhoea in rural western Kenya reported 51% growth of bacterial pathogens in stool cultured (Brooks e*t al.*, 2003) while a 6 years surveillance of bacterial diarrhoea reported 46% isolation rate among the bloody diarrhoea cases (Brooks *et al*., 2006). However, both studies were limited in scope; contribution of infection like protozoans (*Entamoeba histolytica, Giardia lamblia, Schistosoma mansoni*) and parasites (*Trichuris trichiura*) were not determined. Other limitations included; both studies were done in one geographical setting in Asembo Bay which is a rural community bordering Lake Victoria in the Nyanza region, Kenya; the quality of drinking water was not ascertained despite linking drinking water from Lake Victoria with increased risk and failure to exclude the possibility of controls as healthy carriers. In a study of acute bloody diarrhoea in a comparable population of adults in Malawi, 8% of specimens yielded *Schistosoma mansoni* as the sole pathogen (Pitman *et al*., 1996). In a study of bloody diarrhoea specimens collected during an *S. dysenteriae type 1* outbreak in Burundi, 6% of the samples without *S. dysenteriae type 1* yielded *E. histolytica* (Ries *et al*., 1994).

In Kenya, the role of the laboratory in confirmatory diagnosis has been limited due to its weak capacity, and as a result full antimicrobial treatment of most patients is based on clinical diagnosis upon first contact in the health facility. The recent Clinical Management guidelines for Level 4-6 Hospitals developed by the Ministries of Health (2009), recommends the use of trimethoprim-sulfamethoxazole (cotrimoxazole),

amoxicillin or ciprofloxacin for management of *Shigella* dysentery (GOK/MOMS/MOPHS, 2009). However, studies have revealed widespread drug resistance of *Shigella* spp. to ampicillin, trimethoprim-sulfamethoxazole (cotrimoxazole), chloramphenicol and tetracycline (CDC, 1999; Tjaniadi *et al*., 2003). *E. coli* isolated from cases of diarrhea have exhibited resistance to ampicillin (85%), cotrimoxazole (79%), tetracycline (65%), and nalidixic acid (28%) (Ochoa *et al*., 2009).

The antimicrobial susceptibility of enteric pathogens differs by geographic region and also changes over time. For this reason it is essential that antimicrobial susceptibility be monitored periodically so that recommended treatment is effective against locally isolated pathogens. Since it is difficult to confirm the causative organism and antimicrobial susceptibility for each case presenting in a health facility, one method of overcoming these shortcomings is to carry out a multisite study to determine the organisms causing acute bloody diarrhoea and their susceptibility patterns, use the information to guide the health authority to choose an appropriate antimicrobial agent for treatment, then develop a treatment policy based on the syndrome. This method will conserve resources and improve the case management of acute bloody diarrhoea.

Identifying the risk factors associated with the increase of acute bloody diarrhoea cases will provide a platform for putting in place mitigation measures that will reduce the morbidity and mortality rates attributed to acute bloody diarrhoea. The findings from this study will provide an opportunity for initiation of a laboratory based surveillance system for enteric pathogens in the country, re-appraisal of the current guidelines for management of acute bloody diarrhoea and/or revision of the existing prevention and

control strategies for acute bloody diarrhoea. Additionally, this will contribute to strengthening surveillance and reporting of outbreaks due to acute bloody diarrhoea in accordance with International Health Regulations (2005) and building local capacities for data collection and analysis and encompassing information on crucial determinants such as water sources, sanitation coverage, environmental conditions and cultural practices.

## 1.4 Research Questions

1.4.1 What are the presenting clinical features and the etiologic agents of acute bloody diarrhoea responsible in each population in Nairobi West and Kilifi Sub counties, Kenya?

1.4.2 Is there a difference in the epidemiological, clinical and laboratory characteristics of acute bloody diarrhoea cases occurring in urban and rural populations in Kenya?

1.4.3 Is there a variation between socio-demographic characteristics as well as seasonality for acute bloody diarrhoea cases occurring among the urban and rural populations.

1.4.4 Which socio-demographic, socio-cultural and environmental characteristics are risk factors for acute bloody diarrhoea?

## 1.5 Null Hypotheses

1.5.1 There is no difference in the presenting clinical features and the etiologic agents of acute bloody diarrhoea responsible in each population in Nairobi West and Kilifi sub counties, Kenya.

1.5.2 There is no difference in the epidemiological, clinical and laboratory characteristics of acute bloody diarrhoea cases occurring in urban and rural populations in Kenya

1.5.3 There is no variation between socio-demographic characteristics as well as seasonality for acute bloody diarrhoea cases occurring among the urban and rural populations.

1.5.4 Socio-demographic, socio-cultural and environmental characteristics are not risk factors for acute bloody diarrhoea

## 1.6 Study Objectives

### 1.6.1 Main objective

To determine the epidemiological, clinical and laboratory characteristics of acute bloody diarrhoea cases in selected urban and rural populations in Kenya and provide scientific reference for prevention and control of enteric infectious diseases.

### 1.6.2 Specific Objectives

1.6.2.1 To identify the presenting clinical features and the etiologic agents of acute bloody diarrhoea responsible in each population in Nairobi West and Kilifi sub counties, Kenya.

1.6.2.2 To identify differences in the epidemiological, clinical and laboratory characteristics of acute bloody diarrhoea cases occurring in urban and rural populations in Kenya.

1.6.2.3 To demonstrate variation between socio-demographic characteristics as well as seasonality for acute bloody diarrhoea cases occurring among the urban and rural populations.

1.6.2.4 To determine the socio-demographic, socio-cultural and environmental factors associated with acute bloody diarrhoea.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Diarrhoea Diseases

Diarrhoeal diseases are a major cause of disease burden worldwide (Murray *et al.*, 2013). The Global Burden of Disease Study 2010 (GBD 2010) ranked diarrhoeal diseases as the fourth largest disease burden, accounting for 3.6% of the total disease burden globally. Diarrhoeal diseases accounted for an even higher proportion (5%) of the total disease burden in children <5 years of age (Murray *et al*., 2013). Diarrhoeal diseases have a substantially higher impact in low-income countries and regions with poor water quality, sanitation and food safety.

Diarrhoeal diseases are caused by a variety of bacteria, viruses, and parasites, many of which are commonly transmitted through food (Tauxe, Doyle, Kuchenmüller, Schlundt, & Stein, 2010). Abstracted data from systematic reviews and, depending on the overall mortality rates of the country was used to estimate the aetiology-specific incidence and mortality of diarrhoeal diseases, by age and region. The nine diarrhoeal diseases assessed caused an estimated 4.6 billion cases and 1.6 million deaths worldwide in 2010 (Pires *et al.*, 2015). The largest numbers of cases were caused by norovirus (677 million), Enterotoxigenic *Escherichia coli* (ETEC) (233 million), *Shigella* spp. (188 million) and *Giardia lamblia* (179 million). The largest numbers of deaths were caused by norovirus (213,515), Enteropathogenic *Escherichia coli* (EPEC) (121,455), Enterotoxigenic *Escherichia coli* (73,041) and *Shigella* spp. (64,993). Nearly 40% of

cases and 43% of disease deaths caused by these nine pathogens occurred in children <5 years of age (Pires *et al*., 2015) .

Enteric diseases cause considerable morbidity and mortality worldwide, especially among children in developing countries. Shigellosis (bacillary dysentery), typhoid fever, and cholera are severe diseases caused by the pathogens *Shigella* spp., *Salmonella* Typhi, and *Vibrio cholera* respectively (Bhan, Bahl, & Bhatnagar, 2005; Crump, Luby, & Mintz, 2004; Kindhauser, 2003; Lanata, Mendoza, & Black, 2002). The causative microbes are environmentally determined, with transmission occurring through faecal contamination of food or water or by person-to-person contact. Infection rates are highest where general standards of living, water supply, and sanitary conditions are low or inadequate (Kelly-Hope *et al*., 2007).

### 2.1.1 *Shigella* spp

*Shigella* spp are Gram-negative, non-spore forming, non-motile bacilli belonging to the family *Enterobacteriacae*. The genus *Shigella* includes four species (serogroups): *Shigella dysenteriae, Shigella flexneri, Shigella boydii* and *Shigella sonnei*, also designated groups A, B, C and D, respectively. The first three species include multiple serotypes, *Shigella dysenteriae* (12 serotypes), *Shigella flexneri* (6 serotypes), *Shigella boydii* (23 serotypes). *Shigella sonnei* and *Shigella boydii* usually cause relatively mild illness in which diarrhoea may be watery or bloody. *Shigella flexneri* is the chief cause of endemic shigellosis in developing countries (WHO, 2005). Immunity is serotype specific. *Shigella* spp cause acute bloody diarrhoea by invading and causing patchy

destruction of the colonic epithelium. This leads to the formation of micro-ulcers and inflammatory exudates, and results in inflammatory cells (polymorphonuclear leucocytes, PMNs) and blood appearing in the stool. Diarrhoeal stool may contain 106-108 *Shigella* bacteria per gram. Once excreted, the organism is very sensitive to environmental stress and dies within a short time, especially when in dry environment or exposed to direct sunlight (WHO, 2005).

#### 2.1.1.1 *Endemic shigellosis*

Shigellosis is an ongoing global public health problem. The overwhelming burden of shigellosis is found in resource-poor seetings with inadequate sanitation due to the fecal-oral transmission route of the organisms (Miller, Sentz, Rabaa, & Mintz, 2008; Ram, Crump, Gupta, Miller, & Mintz, 2008a). Shigellosis is a major cause of diarrhoea-related morbidity and mortality, especially in developing countries where it is endemic, with an estimated annual incidence of 160-188 million cases and mortality in excess of 1 million deaths globally per year (Baldi *et al*., 2009; Njuguna *et al*., 2013; Pires *et al*., 2015; L von Seidlein *et al*., 2006; WHO, 2005). Ninety-nine percent of infections caused by *Shigella* spp occur in developing countries, and the majority of cases (~70%), and of deaths (~60%), occur among children less than five years of age. Probably less than one percent of cases are treated in hospital (WHO, 2005).

With an estimated number of episodes exceeding 90 million per annum in Asia alone, Shiegellosis represents a significant proportion of the total number of bacterial gastrointestinal infections worldwide (Vinh *et al.*, 2009). A prospective population-

based study in six Asian countries to gain a better understanding of the burden of shigellosis in Asia, over 600,000 persons of all ages residing in Bangladesh, China, Pakistan, Indonesia, Vietnam, and Thailand were included in the surveillance. *Shigella* was isolated from 2,927 (5%) of 56,958 diarrhoea episodes detected between 2000 and 2004. *Shigella flexneri* was the most frequently isolated *Shigella* species (1,976/2,927 [68%]) in all sites except in Thailand, where *Shigella sonnei* was most frequently detected (124/146 [85%]) (Lorenz von Seidlein *et al*., 2006).

In a study examining causes of diarrhoea in Tehran, Iran; a total of 369 (45.6%) bacterial pathogens were recovered from 808 patients presenting with bloody diarrhoea as follows: *Shigella* spp., 155 (45.6%); diarrhoeagenic *Escherichia coli* 143 (38.8%); *Salmonella* spp., 51 (13.8%); and *Campylobacter* spp., 20 (5.4%). Among *Shigella* spp. Isolates, 69 (44.5%) *Shigella flexneri* were predominant. The study revealed a high prevalence of *Shigella* as one of the predominant causes of bacterial diarrhoea in that region of the world (Jafari *et al.*, 2008). In Bangladesh, shigellosis is a major public health problem. In a study to determine the prevalence and distribution of different *Shigella* species over 10 years (2002-2011), a total of 10,827 *Shigella* isolates from patients were analysed. *S.flexneri* was the predominant species isolated throughout the period. However, the prevalence of *S. flexneri* decreased from 65.7% in 2001 to 47% in 2011, whereas the prevalence of *S. sonnei* increased from 7.2% in 2001 to 25% in 2011. *S. boydii* and *S. dysenteriae* accounted for 17.3% and 7.7% of the isolates respectively throughout the period (Ud-Din *et al.*, 2013).

No population-based studies of *Shigella* burden have been conducted in sub-Saharan Africa or in low human development countries (Ram, Crump, Gupta, Miller, & Mintz, 2008b). In a study done in Dakar Senegal to determine antimicrobial suspectibility of *Shigella* spp; among the 165 non-duplicate strains collected, 81 (49%) were identified as *Shigella flexneri*, 75 (45%) as *Shigella sonnei*, 5 (3%) as *Shigella boydii*, and 4 (2%) as *Shigella dysenteriae* (Sire *et al.*, 2008). In a study conducted in Mwanza, Tanzania to determine frequency and pattern of antimicrobial susceptibility of *Shigella* species isolated from stool specimens collected from patients presenting with bloody diarrhoea. *Shigella* species were isolated from 14% (69/489) of the stool specimens collected. Of the sixty nine species of *Shigella* spp isolated, 62 (90%) were *S. flexneri* and 7 (10%) were *S. dysenteriae* (Temu *et al.,* 2007).

In Kenya, a six year surveillance of bacterial diarrhoea was done in rural Nyanza region; among stool specimens from 3445 persons, 1092 (32%) yielded at least 1 bacterial pathogen. *Shigella* species was most commonly isolated and was responsible for 16% of all illnesses with 54% of isolates being *Shigella flexneri* (Brooks *et al.*, 2006). In another study done to investigated the epidemiology of shigellosis and drug susceptibility patterns within a densely populated urban settlement in Nairobi, Kenya through population-based surveillance, *Shigella* species were isolated from 224 (23%) of 976 stool specimens. Isolates were: *Shigella* flexneri (64%), *Shigella* dysenteriae (11%), *Shigella* sonnei (9%), and *Shigella* boydii (5%) (Njuguna *et al.*, 2013).

#### 2.1.1.2 Epidemics caused by *Shigella dysenteriae* serotype 1

Epidemic dysentery in developing countries is usually caused by *Shigella dysenteriae* serotype 1 (sd1). Sd1 is unusually virulent enteric pathogen that causes endemic or epidemic dysentery with high death rates (CDC, 1999). Outbreaks of bloody diarrhoea due to Sd1 are most common in overcrowded, impoverished areas with poor sanitation, inadequate hygiene practices, and unsafe water supplies. Refugees and internally displaced persons are at high risk. In the past two decades major outbreaks have occurred in Africa, South Asia and Central America. Between 1993 and 1995, outbreaks were reported in several central and southern African countries (Kerneis, Guerin, von Seidlein, Legros, & Grais, 2009). In 1994, an explosive outbreak among Rwandan refugees in Democratic Republic of Congo caused approximately 20,000 deaths during the first month alone. Between 1999 and 2003, outbreaks were reported in Sierra Leone, Liberia, Guinea, Senegal, Angola, the Central African Republic and the Democratic Republic of Congo (Guerin *et al*., 2003; Bercion *et al.*, 2006). In 2000, outbreaks of bloody diarrhoea due to Sd1 that were resistant to fluoroquinolones occurred in India and Bangladesh. In Central America, the largest epidemic lasted from 1969 to 1973 and was responsible for more than 500,000 cases and 20,000 deaths. (WHO, 2005).

#### 2.1.1.3 Mode of transmission for *Shigella*

Transmission usually occurs via contaminated food and water or through person-to person contact. Flies may also transmit the organism. The low infective dose, as few as

200 viable organisms, facilitates person to-person spread. Humans and a few primates are the only reservoir of *Shigella* (Baldi *et al*., 2009; WHO, 2005).

#### 2.1.1.4 Clinical presentation of Shigellosis.

*Shigella* bacteria multiply within colonic epithelial cells causing inflammation, mucosal ulceration, and bleeding. After an incubation period of one to four days, patients typically present with diarrhoea and/or dysentery with frequent mucoid bloody stools, abdominal cramps and tenesmus (unproductive, painful straining). Fever and anorexia are also common, but are not specific (Baldi *et al*., 2009; WHO, 2005).

The severity of the clinical picture is directly related to the infecting strain; *S. sonnei* causes mild diarrhoea, whereas *S. dysenteriae* and *S. flexneri* usually cause mucoid bloody diarrhoea (Baldi *et al*., 2009). Patients may, however, present only with acute watery diarrhoea without visible blood or mucus, and without the other symptoms described above, especially at the beginning of their illness. If dehydration occurs, it is usually moderate in degree. Although most patients recover uneventfully within seven to ten days, serious complications may occur, including: metabolic abnormalities, sepsis, convulsions, rectal prolapse, toxic megacolon, intestinal perforation and haemolytic-uraemic syndrome (WHO, 2005).

The epidemiologic and clinical characteristics of 412 patients infected with *Shigella* from a systematic sample of approximately 100,000 patients attending Dacca Hospital, International Centre for Diarrhoeal Disease Research, Bangladesh, were reviewed. *Shigella* was isolated from 11.6% of the 3,550 patients in the sample and was the

second most common isolate in patients over two years old. Two clinical presentations of shigellosis were found: (I) Watery diarrhoea occurring in younger children and associated with a shorter duration of illness and with more vomiting and dehydration and (2) Dysentery with stool blood and abdominal pain. These different presentations may reflect two mechanisms in the pathogenesis of shigellosis or different stages of the disease. The most useful signs and symptoms for the diagnosis of shigellosis were stool with blood and abdominal pain in all patients and the absence of watery diarrhoea and vomiting in patients over one year old. Simple visual inspection of stool for blood correctly identified 44% of all patients infected with *Shigella*. (Stoll, 1999).

### 2.1.2 *Escherichia coli*

*Escherichia coli (E. coli)* is a bacterium that is commonly found in the gut of humans and warm-blooded animals. Most strains of *E. coli* are harmless. Some strains however, such as enterohaemorrhagic *E. coli* (EHEC), can cause severe foodborne disease. It is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products, raw milk and contaminated raw vegetables and sprouts (WHO, 2011b).

Pathogenic variants of Diarrhoeagenic *Escherichia coli* (pathovars or pathotypes) are important cause of endemic and epidemic diarrhoea worldwide (Croxen *et al*., 2013), (Jafari *et al*., 2008). These organisms are currently classified in six categories as follows: enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *Escherichia coli* (ETEC), enteroinvasive *Escherichia coli* (EIEC), diffusely adhering *Escherichia coli*

(DAEC), enteroaggregative *Escherichia coli* (EAEC), and enterohemorrhagic *Escherichia coli* (EHEC) (Jafari *et al.*, 2008).

Entero-haemorrhagic *Escherichia coli* may cause epidemics of bloody diarrhoea. Outbreaks due to *Escherichia coli* O157:H7 have been reported in Swaziland in 1992, as well as in Cameroon in 1997-1998. Culture-proven cases of bloody diarrhoea due to *Escherichia coli* O157:H7 have been reported in Central African Republic, Côte d'Ivoire, Kenya and Nigeria, demonstrating the circulation of this pathogen in Africa. Identification of *Escherichia coli* O157:H7 requires culture and serotyping in a qualified laboratory. (WHO, 2005).

Diarrhoeagenic *Escherichia coli* was recovered from 143 (38.8%) of the 808 patients presenting with bloody diarrhoea in Tehran, Iran. Most of the diarrhoeagenic *E. coli* were Shiga toxin-producing *E. coli*, with 64 (44.7%) isolates, followed by 47 (32.9%) enterotoxigenic *E. coli* isolates. The study revealed a high prevalence of *Shigella* and diarrhoeagenic *E. coli* as the predominant causes of bacterial diarrhoea in that region of the world (Jafari *et al*., 2008).

Diarrhoeagenic *Escherichia coli* pathotypes have been associated with diarrheal disease in different parts of Africa, particularly among young children, HIV-positive individuals, and visitors from abroad. All known diarrheagenic *E. coli* pathotypes have been reported from diverse locations within Africa but the true burden from these pathogens is unknown because very few studies seeking these organisms with discriminatory methodology have been performed. Recent reports implicate ETEC and

EAEC in a considerable proportion of childhood and travellers' diarrheas and suggest that some sub-types of these categories may have greater epidemiological significance than others. The significant contribution of EHEC to bloody diarrhea and hemolytic uremic syndrome is underappreciated because diagnostic capacity for this pathotype is generally inferior to that for confounders such as *Shigella* and *Entamoeba* (Okeke, 2009).

In Kenya, surveillance for bacterial diarrhea in rural Nyanza region of Kenya was done between 1997–2003. Diarrheal stool samples from persons presenting to 4 sentinel health centers were cultured by standard techniques for routine bacterial enteric pathogens. A random subset of 705 specimens (20% of the total) was also evaluated for diarrheagenic *Escherichia coli*. The examination identified 25 isolates as enteroaggregative, 18 as enterotoxigenic, 12 as entero-pathogenic, 4 as enteroinvasive, and 1 as non-O157 Shiga toxin-producing *E. coli*. For 43 (72%) of these specimens, diarrheagenic *E. coli* was the sole pathogen identified hence probing for diarrheagenic *E. coli* increased the overall specimen yield by 6% (Brooks *et al.*, 2006).

### 2.1.3 *Salmonella* spp

Salmonellosis is one of the most common and widely distributed foodborne diseases and is caused by the bacteria *Salmonella* (WHO, 2013b). It is estimated that 93.8 million cases of gastroenteritis due to *Salmonella* species occur globally each year, with

155,000 deaths (Majowicz *et al*., 2010). *Salmonella* infection represents a considerable burden in both developing and developed countries.

*Salmonella* species are Gram-negative aerobic/anaerobic bacilli that cause substantial morbidity, mortality and burden of disease globally. *Salmonella* can colonize both the small bowel and colon causing different clinical pictures. Typhoid fever (*S. typhi* and *S. parathyphi*) and enteritis (*S. enteritidis* and *S. typhimurium*) are the most common disease syndromes. Typhoid fever is particularly frequent in under-developed countries. In year 2000, a total of 21.6million cases occurred with more than 216 000 deaths (Baldi *et al*., 2009).

A population-based prospective surveillance design was used to study the burden of typhoid fever in five Asian countries (China, India, Indonesia, Pakistan and Viet Nam). A total of 21 874 episodes of fever were detected. *Salmonella* Typhi was isolated from 475 (2%) blood cultures, 57% (273/475) of which were from 5–15 year-olds (Ochiai *et al.*, 2008). In a study examining causes of diarrhoea in Tehran, Iran; a total of 369 (45.6%) bacterial pathogens were recovered from 808 patients presenting with bloody diarrhoea. *Salmonella* spp., 51 (13.8%) (Jafari *et al*., 2008).

As part of population-based surveillance among 55,000 persons in malaria-endemic, rural and malaria-nonendemic, urban Kenya from 2006–2009, blood cultures were obtained from patients presenting to referral clinics with fever ≥38.0°C or severe acute respiratory infection. Non-Typhi *Salmonella* accounted for 60/155 (39%) of blood culture isolates in the rural and 7/230 (3%) in the urban sites (Tabu *et al.*, 2012). During

6 years of surveillance in Nyanza region of Kenya, Nontyphoidal *Salmonella* was recovered from 173 isolates (5%) and *Salmonella enterica* serotype Typhi from 8 isolates (<1%) (Brooks *et al*., 2006)

### 2.1.4 *Campylobacter* spp

*Campylobacter* bacteria are a major cause of foodborne diarrhoeal illness in humans and are the most common bacteria that cause gastroenteritis worldwide. In developed and developing countries, they cause more cases of diarrhoea than foodborne *Salmonella*. The high incidence of *Campylobacter* diarrhoea, as well as its duration and possible sequelae, makes it highly important from a socio-economic perspective. In developing countries, *Campylobacter* infections in children under the age of two years are especially frequent, sometimes resulting in death (WHO, 2011a).

The true incidence of gastroenteritis due to *Campylobacter* spp. is poorly known, particularly in Low and middle income countries; studies in high-income countries have estimated the annual incidence at between 4.4 and 9.3 per 1000 population (WHO, 2013a)).

A total of 369 (45.6%) bacterial pathogens were recovered from 808 patients presenting with bloody diarrhoea in Tehran, Iran. *Campylobacter* spp., accounted for 20 (5.4%). (Jafari *et al*., 2008). The level of resistance to the most commonly used antibacterial agents in the developing world is increasing in *Campylobacter* spp., the increase in the resistance to quinolones being of special concern. A study done in Spain to determine the levels of resistance to nine antimicrobial agents in clinical isolates of

*Campylobacter* spp. causing diarrhea during the period from 1993 to 2003; high levels of resistance to four out of nine antimicrobial agents tested were detected: ampicillin (66.3%), nalidixic acid (52.2%), ciprofloxacin (46.7%), and tetracycline (42.4%) were reported (Ruiz, Marco, Oliveira, Vila, & GascON, 2007).

In Kenya, *Campylobacter* species was recovered from 294 isolates (8%) in a surveillance done over 6 years. Speciation of a random subset of 119 *Campylobacter* isolates identified 97 (82%) as *Campylobacter jejuni* and 22 (18%) as *Campylobacter coli* (Brooks *et al*., 2006)

### 2.1.5 Amoebiasis

Amoebiasis is the second leading cause of death from parasitic disease worldwide (Baldi *et al*., 2009). The causative protozoan parasite, *Entamoeba histolytica*, is a potent pathogen infecting about 50 million people and resulting in 40 000 deaths per year. Infections are prevalent in developing countries, particularly India, Africa, Mexico and South America. People at risk of infection include immigrants, travellers returning from countries of high endemicity, and men who have sex with men. Clinical manifestations range from asymptomatic carriage to invasive disease (bloody diarrhoea), to extra-intestinal disease with liver abscess (Baldi *et al*., 2009). Other amoebae infecting humans include; *Dientamoeba fragilis*, *Entamoeba dispar*, *Entamoeba hartmanni*, *Entamoeba coli*, *Entamoeba moshkovskii*, *Endolimax nana* and *Iodamoeba butschlii*. Except for *Dientamoeba* which causes Dientamoebiasis, the parasites above are not thought to cause disease ((Berger & Marr, 2006).

### 2.1.6 Schistosomiasis

Schistosomiasis or bilharzia is a tropical disease caused by worms of the genus *Schistosoma*. The transmission cycle requires contamination of surface water by excreta, specific freshwater snails as intermediate hosts, and human water contact. The main disease-causing species are *S haematobium, S mansoni,* and *S japonicum*. According to WHO, 200 million people are infected worldwide, 97% of which are on the African continent, leading to the loss of 1·53 million disability-adjusted life years (Gryseels, Polman, Clerinx, & Kestens, 2006), (Steinmann, Keiser, Bos, Tanner, & Utzinger, 2006).

*Schistosoma mansoni*, the intestinal type, is responsible for blood in stool in an estimated 4.4 million individuals, and 8.5 million were estimated to have hepatomegaly because of the infection (van der Werf, Bosompem, & de Vlas, 2003; van der Werf, de Vlas, Landoure, Bosompem, & Habbema, 2004). The disease is especially important in poor, rural areas, where attempts to alleviate poverty also promote small-to-large scale water-related development projects that may increase transmission (Danso-Appiah, De Vlas, Bosompem, & Habbema, 2004). Intestinal schistosomiasis (caused by *S. mansoni*) gives rise to blood in stool or (bloody) diarrhoea and abdominal pain. (Danso-Appiah *et al*., 2004).

A survey of 1,246 children 10–12 years old in 32 primary schools in Kenya near Lake Victoria was conducted to determine prevalence and distribution of schistosome and geohelminth infections. Stool and urine samples were collected and examined for eggs

of *Schistosoma mansoni*, *S. haematobium*, and intestinal helminths. The mean school prevalence of *S. mansoni* infection was 16.3% (Handzel *et al.*, 2003)

**2.2 Antimicrobial drugs sensitivity pattern**

*Shigella* is becoming an increasing public health problem due to development of multiple antimicrobial resistance, frequently resulting in treatment failure. A systematic review was conducted based on a literature search of computerised databases. In the area of Asia–Africa, resistance rates to nalidixic acid and ciprofloxacin were 33.6% and 5.0% respectively, 10.5 and 16.7 times those of Europe–America. Moreover, resistance to nalidixic acid and ciprofloxacin in Asia–Africa progressively increased each year, reaching 64.5% and 29.1% respectively, in 2007–2009, whilst isolates in Europe–America remained at low levels of resistance (<5.0% and <1.0%, respectively). All *Shigella flexneri* strains showed higher resistance than *Shigella sonnei* in Europe–America: overall, 3.5% vs. 2.6% resistant to nalidixic acid and 1.0% vs. 0.1% resistant to ciprofloxacin. In Asia–Africa, a similar trend was found for ciprofloxacin (3.0% vs. 0.5%), whereas the trend was reversed for nalidixic acid (32.6% vs. 44.3%) (Gu *et al.*, 2012). The study concluded that quinolone resistance in *Shigella* has increased at an alarming speed, reinforcing the importance of continuous monitoring of antimicrobial resistance in *Shigella*.

It is well established that *Shigella flexneri* is the most commonly isolated species in developing countries, and its presence has been associated with inadequate sanitation; in contrast, *Shigella sonnei* predominates in developed countries (Tjaniadi *et al.,* 2003). A

study on antimicrobial susceptibility of *Shigella* spp done in Dakar Senegal, among the 165 non-duplicate strains collected, 81 (49%) were identified as *Shigella flexneri*, 75 (45%) as *Shigella sonnei*, 5 (3%) as *Shigella boydii*, and 4 (2%) as *Shigella dysenteriae* (Sire *et al.*, 2008). Majority of isolates were resistant to sulphonamides, trimethoprim-sulfamethoxazole, streptomycin, and tetracycline (respective overall resistance rates: 90, 90, 96, and 94%). More than half of the *S. flexneri* isolates were resistant to amoxicillin, amoxicillin-clavulanic acid, and chloramphenicol (respective resistance rates: 59, 58, and 52%), and almost all of the *S. sonnei* isolates were susceptible to these antimicrobials (respective resistance rates: 4, 1, and 4%). Only one isolate (belonging to the species *S. sonnei*) was resistant to nalidixic acid and displayed reduced susceptibility to ciprofloxacin, all strains were susceptible to cefotaxime. One hundred and fifty-five isolates (94%) were resistant to three or more antimicrobial agents determinative of characteristic multidrug-resistance profiles. Parenteral third-generation cephalosporins such as cefotaxime or ceftriaxone remain highly effective, representing possible treatment options for severe infections, especially among hospitalized patients. The widespread use of nalidixic acid as a first-line drug for shigellosis in many countries has resulted in the emergence of resistant strains. To date, this antimicrobial agent is no longer recommended in the international guidelines, and the use of ciprofloxacin is currently encouraged as a first-line treatment instead (Sire *et al*., 2008).

In our neigbouring country Tanzania, a study to determine frequency and pattern of antimicrobial susceptibility of *Shigella* species isolated from stool specimens collected from patients presenting with bloody diarrhoea in Mwanza. *Shigella* species were

isolated from 14% (69/489) of the stool specimens collected. Of the sixty nine species of *Shigella* spp isolated, 62 (90%) were *S. flexneri* and 7 (10%) were *S. dysenteriae* (Temu *et al*., 2007). All *Shigella* species isolated showed high resistance to ampicillin, tetracycline, trimethoprim-sulphamethoxazole and chloramphenicol, drugs commonly used for management of shigellosis in Tanzania. However all isolates were fully susceptible to ciprofloxacin, nalidixic acid, erythromycin, cefuroxime and gentamycin. *Shigella flexneri* showed resistance to amoxy-clavulanic acid and azithromycin in 5% and 2% of isolates, respectively. None of the *Shigella dysenteriae* isolates were resistant to these two drugs. *Entamoeba histolytica, Giardia lamblia* and *Schistosoma mansoni* were microscopically detected in 16.5%, 4.4% and 5.3% of patients, respectively. These findings suggest that there is a need to carry out extensive susceptibility studies in different parts of the country with view of re-appraising the current guidelines for management of bloody diarrhoea in Tanzania (Temu *et al.*, 2007).

In a study by Sang *et al*., 2011, antibiotic susceptibility testing was done using the E-test strips containing Tetracycline, Gentamicin, Chloramphenicol, Fosfomycin, Amoxicillin/Clavulanic acid, Trimethoprim/Sulphamethoxazole, Ticarcillin/Clavulanic acid and Ciprofloxacin. The resistance frequencies did not differ significantly between other *E. coli* and Shiga toxigenic *E. coli*, respectively; Gentamicin (3% vs. 3%), Chloramphenicol, (24% vs. 23%) and ampicillin (25% vs. 23%), Tetracycline (63% vs. 68%), Fosfomycin (44% vs. 54%) and Trimethoprim/Sulphamethoxazole (84% vs. 84%). Overall antibiotic resistance levels were at much lower levels than those reported

from the rest of Kenya, possibly due to the lower levels of exposure and usage of antimicrobials among the Maasai community (Sang, 2011).

During 6 years of surveillance in Nyanza region of Kenya, With the exception of *Campylobacter* species, susceptibility to the antimicrobials used most widely in the community was low: <40% for all isolates tested and <25% for *Shigella* species. Most persons were treated with an antimicrobial to which their isolate was resistant. Susceptibility to specific antimicrobials was inversely proportional to the frequency with which they were prescribed (Brooks *et al*., 2006).

In a study to investigated the drug susceptibility patterns within a densely populated urban settlement in Nairobi, Kenya through population-based surveillance. Over 90% of all *Shigella* isolates were resistant to trimethoprim-sulfamethoxazole and sulfisoxazole. Additional resistance included nalidixic acid (3%), ciprofloxacin (1%) and ceftriaxone (1%) (Njuguna *et al.*, 2013).

**2.3 Risk factors for acute bloody diarrhoea**

A population-based case control study of risk factors for acute infectious diarrhoea in rural children in central Wisconsin. Rural residents may be exposed to enteric pathogens from farms and other sources not found in an urban environment. Children 1 to 18 years old with acute diarrhoea (≥ 3 loose stools/day) were enrolled when seeking medical care from Feb 1997 to Sept 1998. Among 131 stool specimens, the most commonly identified pathogens were Cryptosporidium (n=16), *Campylobacter* (n=12), rotavirus

(n=11), *Salmonella* (n=7) and adenovirus 40/41 (n=7). No pathogen was identified in 76 children. In multivariate analyses, bacterial infections were associated with entry into a calf pen (odds ratio 5.8; p=.004); protozoal infections were associated with contact with livestock drinking water (odds ratio 7.5; p=.004). Viral infections were associated with recent diarrhoeal illness in other household members (odds ratio 4.0; p=.01) and inversely associated with age (odds ratio .67; p=.02). The study concluded that direct and indirect livestock exposure is an important source of bacterial and protozoal enteric infections for children living in rural Wisconsin (Borchardt, Belongia, Chyou, & Devries, 1999).

In a population-based surveillance matched case-control study to assess the potential risk factors for shigellosis in Thailand found out; Hygiene behaviors such as regular hand washing ($p < 0.05$), a clean environment surrounding the household ($p < 0.001$), and the availability of water to flush the toilet ($p = 0.08$) were associated with a reduced risk for shigellosis in the multivariate model. In contrast factors indicating a lower than average socioeconomic status, such as having to rent instead of owning one's housing ($p < 0.001$) and a low family income ($p < 0.01$) were associated with an increased risk for shigellosis. For children, breastfeeding showed a strong protective effect in reducing the risk of shigellosis ($p < 0.01$). Prior to adjustment for environmental factors, fly density in the kitchen area was associated with an increased risk of shigellosis ($p < 0.01$) (Chompook *et al*., 2006).

A study among 254 children aged 12-24 months in rural South Africa and Zimbabwe assessed risk factors for child dysentery and watery diarrhoea in households where

drinking water was collected from communal sources (Gundry *et al*., 2009). For dysentery, drinking water from sources other than standpipes had a relative risk ratio of 3.8 (95% CI 1.5-9.8). Poor source water quality, as indicated by *Escherichia coli* counts of 10 or more colony-forming units 100 ml-1, increased risk by 2.9 (1.5-5.7). There were no other significant risk factors for dysentery and none for watery diarrhoea. Endemic dysentery is associated only with faecal contamination of source water. Sources other than standpipes, including improved groundwater, are of greater risk (Gundry *et al*., 2009).

In Kenya, a laboratory-based surveillance and a case-control study to characterize the epidemiology of bloody diarrhoea in Asembo Bay area of Siaya sub county, Western Kenya was done. Stool from 451 was collected from persons with bloody diarrhoea presenting to four rural clinics. Drinking Lake Victoria water and sharing latrines between multiple households increased risk of bloody diarrhoea. Washing hands after defecating was protective. Providing safe drinking water, more latrines, and promoting hand washing could reduce the burden of illness from bloody diarrhoea while limiting injudicious antimicrobial use (Brooks *et al*., 2003).

**2.4 Effect of climate on enteric diseases**

Cholera outbreaks exhibit strong seasonality, tending to occur after increased rainfall and warm temperatures (Reyburn *et al*., 2011). A 1°C increase in temperature at 4 months lag resulted in a 2-fold increase of cholera cases, and an increase of 200 mm of rainfall at 2 months lag resulted in a 1.6-fold increase of cholera cases. Temperature and

rainfall interaction yielded a significantly positive association ($P < 0.04$) with cholera at a 1-month lag. This study provides reassuring evidence that rainfall and temperature, among various climate and ocean environmental variables are the key drivers of cholera outbreak, consistent with results from other studies.

Shigellosis/dysentery, typhoid fever, and cholera have different geographical patterns in Vietnam, and their prevalence and distribution may be associated with a combination of different ecological factors (Kelly-Hope *et al.*, 2007). Overall, shigellosis/dysentery was the most reported disease with ~435,000 cases from 1991 to 2001, compared with 187,000 typhoid fever and 17,000 cholera cases. Statistical analyses suggested that high rainfall and urban poverty were the most significant risk factors of shigellosis/dysentery.

## 2.5 Public Health Surveillance

### 2.5.1 Public Health Surveillance in African Region

The surveillance of infectious diseases has recently assumed greater importance because of emerging and re-emerging infectious diseases, and because strains of pathogens causing TB, malaria, cholera, dysentery, and pneumonia have developed resistance to antibiotics. But in Africa, where infectious diseases continue to be a major health problem, many of the national surveillance systems ensure neither timely detection nor an effective response to these diseases (Nsubuga *et al*., 2002).

Communicable diseases remain the most important health problems in Africa. The commonest causes of death and illness in the region are malaria, acute respiratory tract infections, diarrhoeal diseases, tuberculosis, HIV/AIDS, STIs, and vaccine preventable infections. Epidemic-prone diseases such as meningococcal meningitis, cholera, measles, and bacillary dysentery are also prominent health problems in the continent. An efficient and effective disease surveillance system is critical for priority setting, planning, resource mobilization and allocation, prediction and early detection of epidemics, as well as monitoring and evaluation of intervention programmes (WHO, 1999).

World Health Organization Regional Office for Africa (WHO/ AFRO) recognized that most of the existing disease surveillance systems in the continent were neither working effectively to measure the health impact of the major diseases nor adequately evaluating disease control programmes and detecting outbreaks for early investigation. In 1998, the 48$^{th}$ Regional Committee for Africa met in Harare and through resolution AFRO/RC48/R2, Member States adopted integrated disease surveillance and response (IDSR) as a regional strategy for early detection and efficacious response to priority communicable diseases for African region (WHO, 1999).

A functioning infectious disease surveillance system includes the following core activities: detection; confirmation and registration of cases; reporting; data analysis and interpretation; feedback; and dissemination. Core activities of the associated response capacity include immediate responses (e.g. outbreak investigations) and planned responses (e.g. community prevention activities). Health authorities support the

surveillance and response system by providing training, supervision and resources (Nsubuga *et al.*, 2002).

IDSR aims to improve the availability and use of surveillance and laboratory data for control of priority infectious diseases. The specific goals of IDSR are; to strengthen sub county-level surveillance and response for priority diseases; to integrate surveillance with laboratory support and to translate surveillance and laboratory data into specific public health actions (WHO, 2003). The IDSR strategy links community, health facility, county, regional and national levels with the overall objective of providing epidemiological evidence for use in making decisions and implementing public health interventions for the control and prevention of communicable diseases (Rumisha, Mboera, Senkoro, Gueye, & Mmbuji, 2007).

### 2.5.2 Public Health Surveillance in Kenya

In Kenya communicable diseases present a large threat to the well being of the Kenyan communities and accounts to 75% of the disease burden, there are well-known interventions that are available for controlling and preventing them (GOK/MOH, 2003). Kenya adopted Integrated Disease Surveillance and Response (IDSR) strategy in the year 2000. Through IDSR, data is collected from health facilities in an integrated manner and transmitted to the Sub County, County and National level. Surveillance of bloody diarrhoea (dysentery) remains part of the priority diseases under surveillance as per the IDSR Technical guidelines (GOK/MOH, 2012)

### 2.5.3 Surveillance for acute bloody diarrhoea

For surveillance and reporting purposes, the standard case definition of bloody diarrhoea or dysentery is "*diarrhoea with visible blood in the stool"* (WHO, 2005). Prompt detection and reporting of cases of acute bloody diarrhoea is the essential first step in the monitoring of endemic shigellosis and in the control of epidemic shigellosis, and also of outbreaks of dysentery caused by entero-haemorrhagic *Escherichia coli* O157:H7. The number of cases of bloody diarrhoea, and of deaths associated with bloody diarrhoea, should be determined and reported for two age groups: (i) under five years, and (ii) five years or older (WHO, 2005). Each health facility should designate a specific individual to be responsible for reporting all cases of, and deaths associated with, bloody diarrhoea. Reports should be provided each week to the county health officer responsible for monitoring the occurrence of cases and detecting outbreaks.

### 2.5.4 Alert and confirmation of an outbreak

An outbreak of shigellosis should be suspected, and a field investigation conducted, whenever the routine surveillance system reports cases of, or deaths due to, bloody diarrhoea that exceed the number expected for the location and the reporting period. An outbreak may also be suspected when a laboratory reports an increase in the number of bloody stool specimens received for culture. In communities and defined populations, such as refugee camps, where Sd1 has not been present a single isolation of Sd1 should raise concern about a possible impending outbreak (WHO, 2005).

When a field investigation is initiated, the urgent need is to confirm that there is an outbreak of dysentery, and to identify the causative organism and determine its antimicrobial susceptibility. The number of cases and deaths from bloody diarrhoea should be collected retrospectively from health facility registers to assess the magnitude of the outbreak. It is recommended that 10-20 stool specimens should be collected from untreated cases and delivered to the reference laboratory (WHO, 2005).

In a true outbreak of Sd1, a majority of dysenteric stool specimens will yield Sd1 when cultured, especially at the beginning of the outbreak. In contrast, isolation of Sd1 from a small proportion of specimens, together with frequent isolation of other enteric pathogens, may be encountered when shigellosis is endemic. The isolation rate for Sd1 will also be low, even during an outbreak, if techniques for specimen collection or transport are sub-optimal, or laboratory methods used to isolate or identify the *Shigella* are inadequate. If Sd1 are not identified from an outbreak of dysentery, stool samples should be cultured for entero-haemorrhagic *E. coli* O157:H7 either locally or by a regional WHO reference laboratory (WHO, 2005).

When an outbreak of shigellosis due to Sd1 is confirmed, local, provincial and national health authorities should be immediately informed so that appropriate control measures can be started. The report should state the number of patients affected, their ages, the date of onset of the outbreak, the locations affected, the number of stools cultured, the number that yielded Sd1, and the antimicrobial susceptibility of the Sd1 isolates. Reports of outbreaks should also be shared with neighbouring countries, as dysentery epidemics do not respect national borders. In addition, international notification of

epidemic bloody diarrhoea may be required in accordance with the International Health Regulations (IHR) of 2005 (WHO, 2005).

### 2.5.5 Laboratory based surveillance for acute bloody diarrhoea

At least one laboratory within the country should be able to isolate and identify *Shigella,* including Sd1, and perform antimicrobial susceptibility testing. This laboratory should train national and peripheral laboratory technicians in appropriate methods for collection and transport of stool samples, and for isolation, serotyping and antimicrobial susceptibility testing of *Shigella*. It should also assist in strengthening systems for monitoring the quality of the laboratory services throughout the country. It is preferable to have one well-equipped laboratory with suitably trained staff to which specimens can be quickly and safely transported, than to have several that are inadequately equipped or in which staff are not well trained (WHO, 2005).

If there is no competent national laboratory, collaboration should be established with an international reference laboratory to strengthen the national laboratory and to assist in isolation of *Shigella* from stool specimens. The antimicrobial susceptibility of *Shigella* differs by geographic area and also changes over time. For this reason it is essential that antimicrobial susceptibility be regularly monitored so that treatment can be recommended that is effective against locally isolated *Shigella*. Testing should not include antimicrobials that are known to be ineffective for the treatment of shigellosis. (WHO, 2005).

The national laboratory should establish a plan to collect and culture regularly stool specimens of untreated patients with bloody diarrhoea from representative areas of the country. If possible, samples should be from cases seen in the community as well as those treated in hospital. This is because antimicrobial resistance may be more frequent among *Shigella* isolated from patients treated in hospital, leading to over-estimation of the prevalence of resistance to some antimicrobials (WHO, 2005).

Fresh stool samples should reach the laboratory within two hours. If this is not possible, specimens should be placed in Cary Blair (or buffered glycerol saline) transport medium at +4°C and reach the laboratory within 48 hours. For seasonal epidemics, susceptibility testing should also be performed at the end of the epidemic season to determine the antibiotic policy for the following season. Unless there is evidence that entero-haemorrhagic strains of *E. coli* are circulating in a region, surveillance for this organism should be limited to outbreaks of bloody diarrhoea (WHO, 2005).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study Design

A case control study design was used to determine the risk factors for acute bloody diarrhoea. Quantitative data was collected from cases and control (primary source) through a semi structured questionnaire.

Stool specimens were collected from all cases in order to document the type of causative agents, distribution and the antimicrobial susceptibility of enteric pathogens isolated from diarrhoea with blood samples obtained from cases seeking medical treatment in selected six government health facilities in the two sub counties. After seeking informed consent from cases and controls, stool specimens were collected from consecutive patients with acute bloody diarrhoea who sought care at the outpatient department in the selected health facilities. Neighborhood controls were used since they were ideal for controlling issues such as socio-economic status and education. Stool specimens were collected from controls with an aim of controlling laboratory confounders. A case to control stool sample ratio of 2:1 was used.

The study period was for a period of 12 months with data collected from January 2012 to December 2012.

### 3.2 Study Site

The study was conducted in Kilifi Sub County in the Kilifi County and Nairobi West (Langata and Dagorreti Sub Counties) in Nairobi County. Kilifi County is largely composed of a rural population (www.opendata.go.ke) constituting 74.3% of the total county population (1,109,735). Kilifi County consists of Kilifi, Kaloleni and Malindi Sub counties. Nairobi West Sub County represent almost a 100% urban population. A Geographical Position System (GPS) was used for mapping households of cases and controls.

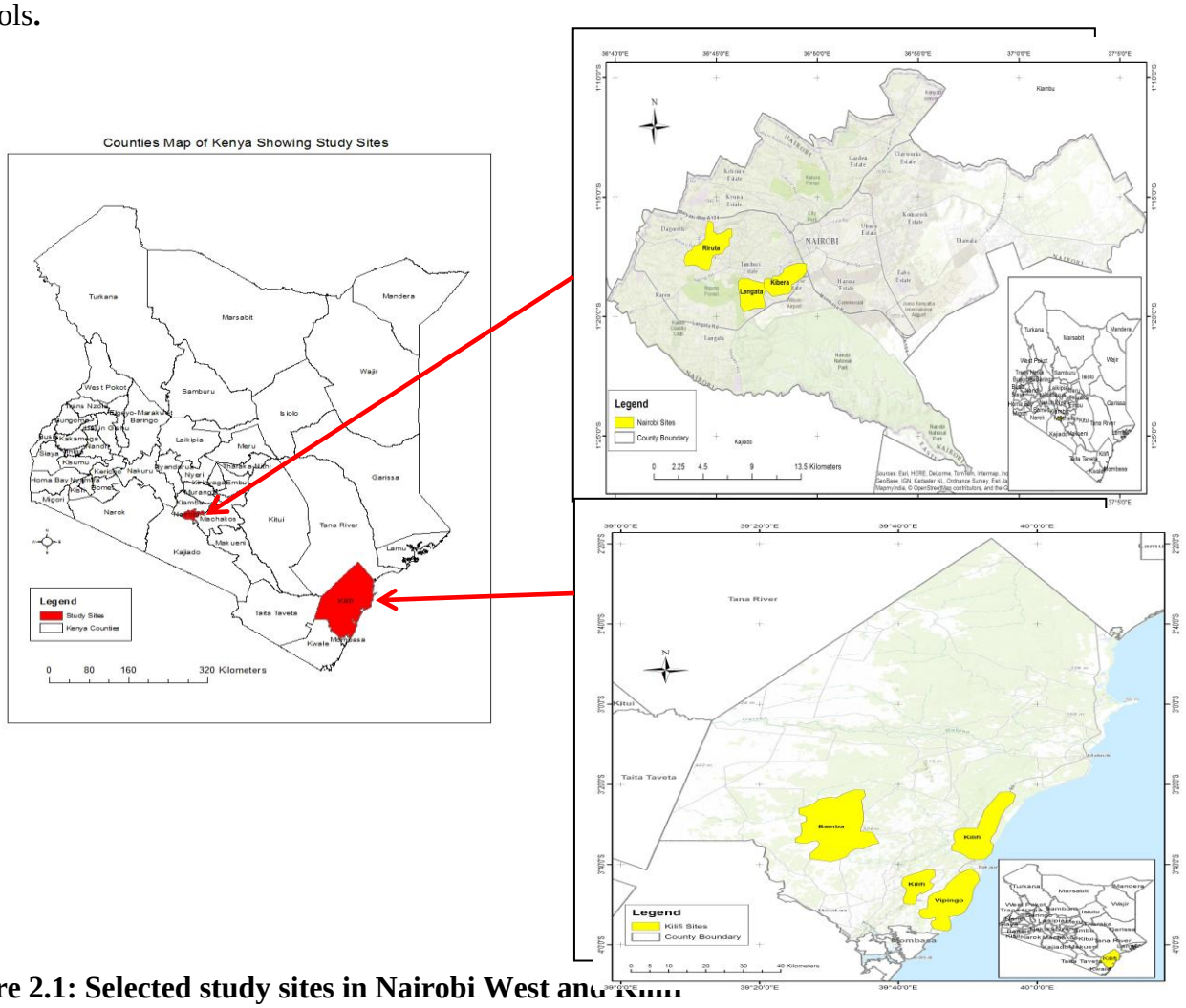



**Figure 2.1: Selected study sites in Nairobi West and Kilifi**

### 3.2.1 Kilifi Sub County Profile

*General Profile*

Kilifi Sub County formely known as Kilifi District borders Taita to the West, Malindi to the North West, Mombasa and Kwale to the South and Kaloleni to the West. The Sub County has an area of 44772.2 sq km with a 91.44Km shoreline that stretches from Mtwapa creek to Mida creek. (Kilifi District Strategic Plan 2005-2010)

*Demographic Profile*

The population of Kilifi Sub County in 2009 was 456,297 comprising 218,486 males and 237,811 females representing 47.9% and 52.1% of the population, respectively.

*Health Profile*

Kilifi Sub County has total of 73 health facilities distributed across the entire Sub County. Accessibility of health services is, however, low and 57% of the population live over 5kms to the nearest health facility. The doctor patient ratio stands at 1:100,000 which in itself a manifestation of staff shortages in the county (Kilifi District Strategic Plan 2005-2010)

Acute bloody diarrhoea (under 5 years) was ranked $10^{th}$ in the list of top ten causes of morbidity in Kilifi County Hospital in 2009 as indicated in appendices 10. (Kilifi District strategic plan 2005-2010).

*Socioeconomic profile*

Majority of the community members are small scale farmers with major economic activities being tourism, fishing, quarry, mixed farming, tapping, small scale business

and charcoal burning. The Sub County has relatively high illiteracy index (Kilifi District Strategic Plan 2005-2010).

*The Climate Profile*

The Sub County has two main rainfall seasons in a year. The long rains start from April to June, with a peak in May while the short rains fall from October to December. The two seasons are more prominent in the south. In the hinterlands or rangelands zone where rainfall is very unreliable, the seasonality is barely noticeable. In the period between October and December, when the coastal belt receives only 200 of the annual rainfall, the Nyika plateau and the rangeland get 400. The rainfall pattern is influenced by the county's proximity to the Indian Ocean, relatively low altitudes, temperatures and winds. The county has rainfall average of 1200mm along the coastal belt, and 600mm in the hinter lands. (Source: Kilifi District Long-Term Strategic Development Plan 2001 – 2015).

### *3.2.2 Nairobi County*

Nairobi County borders Kiambu County to the North and West, Kajiado to the South and Machakos to the East. Among the three neighbouring counties, Kiambu County shares the longest boundary with Nairobi County. The County has a total area of 696.1 $Km^2$ and lies at an altitude of 1,798 metres above sea level (Nairobi County Integrated Development Plan, 2014).

*Administrative sub-divisions*

The County is divided into nine sub-counties namely; Starehe, Kamukunji, Kasarani, Makadara, Embakasi, Njiru, Dagoretti, Langata and Westlands. The County has 27 divisions 64 locations and 135 sub-locations (Nairobi County Integrated Development Plan, 2014).

*Nairobi West Profile*

The former Nairobi West District was subdivided into Langata and Dagorreti Sub Counties. The population of Nairobi West in 2009 was 684,765 comprising of 352,227 males and 332,538 females representing 51% and 49% of the population, respectively. Nairobi West is one of the three administrative Sub County in Nairobi County. The Ministry of Health offers health services in conjunction with the Nairobi City Government. The number of health facilities is estimated at 422 of which 20% are public health facilities (USAID, 2003).

Nairobi is the capital of Kenya; it is the most populous city in East Africa, with a current estimated population of about 4 million, and hosting about 25 per cent of Kenya's urban population. The province has the highest number of informal settlements in the country. About 60% of the population lives in informal settlements which has multiple health risk factors such as; Poor water supply, Poor sanitation, Congestion, High level of poverty and Insecurity.

Nairobi West has Kibera slum which is one of the largest slums in Africa, where most residents live in extreme poverty, earning less than $1.00 per day. Unemployment rates are high. There are few schools, and most people cannot afford an education for their

children. Clean water is scarce and therefore diseases caused by related poor hygiene are prevalent. A great majority of people living in the slum lack access to healthcare.

*Nairobi Climate Profile*

Nairobi is situated quite close to the Equator, at an altitude of about 5,500 feet (1700 metres). The main features of the climate are the existence of definite wet and dry seasons, and the absence of any large seasonal change in temperature. The year can be subdivided into four seasons as follows;

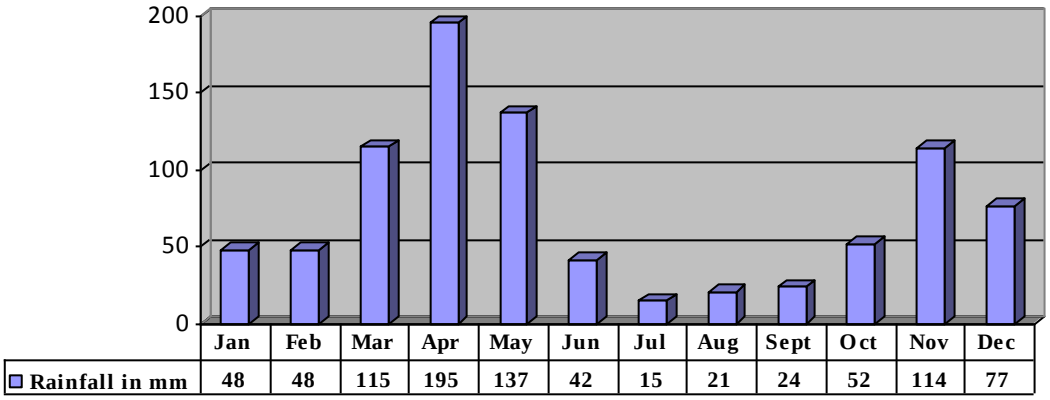
**Table 3.1: Weather seasons in Nairobi**

| **Period** | **Season** |
|---|---|
| Mid-December to Mid-March | Warm, sunny, dry |
| Mid- March to May | Main rainy season |
| June to Mid-October | Cool, rather cloudy (especially July-August), dry |
| Mid-October to Mid-December | Secondary rainy seasons |

Source: Kenya Meteorological Department (http://www.meteo.go.ke)

The County has a fairly cool climate resulting from its high altitude. Temperature ranges from a low of $10^0$C to a high of $29^0$C. It has a bi-modal rainfall pattern. The long rains season fall between March and May with a mean rainfall of 899 millimeters (mm) while the short rains season falls between October and December with a mean rainfall

of 638 mm. The mean annual rainfall is 786.5 mm (Nairobi County Integrated Development Plan, 2014).




Source: Kenya Meteorological Department (http://www.meteo.go.ke)

**Figure 3.2: The average rainfall (mm) in Nairobi, based on records for 50 years**

### 3.3 Study Population

The study population comprised persons of all age groups with acute diarrhoea with visible blood in the stools (cases) presenting in the outpatient department in health facilities in the selected study sites in two sub counties and any healthy persons of similar age group (controls).

#### 3.3.1 Inclusion Criteria

A case presenting in the general outpatient department and met the standard case definition criteria for acute bloody diarrhoea *"any person of any age with acute diarrhoea with visible blood in the stools"*

Healthy persons of similar age category and sex with the case and living in the neighbourhood of a case

Informed consent given by a case or control who is 18 years and above or an assent given by those above 7 years and below 18 years of age. Parents/guardians will give consent for the young children.

### 3.3.2 Exclusion Criteria

Any person recruited as a control who reported any form of diarrhoea or any other gastrointestinal symptoms within 14 days of enrolment was excluded.

Any case with persistent diarrhoea with visible blood (≥ 14 days) at the time of enrolment.

Any case or control who did not reside within the study area

Any case or control who declined to give consent or assent

Cases with concomitant infections were excluded from the study.

## 3.4 Sampling Methods

### 3.4.1 Sampling Procedure

A multistage sampling method was used to select two regions (former provinces), two Counties and six health facilities. Selection of regions was purposively done based on incidence rate of acute bloody diarrhoea in (2009-2010) rural and urban population. Coast region had the highest incidence rate in the country while Nairobi region had the highest incidence in an urban set up. Kilifi Sub County and Nairobi West (Langata and Dagoretti Sub Counties) had the highest incidence rates in their respective regions. Kilifi Sub County had about 75% of the population in the rural and Nairobi west had about 100% population in Urban. In each study area (Kilifi and Nairobi West), three high volume health facilities (sites) with the highest incidence of acute bloody diarrhoea

were purposively selected. In Nairobi West; Langata Health Centre, Riruta Health Centre and AMREF Kibera Health Centre were selected. In Kilifi; Kilifi County Hospital, Bamba Sub County Hospital and Vipingo Health Centre were selected. In each site, cases and controls were recruited in line with procedures explained below.

#### 3.4.1.1 Selection of Cases

A case of acute bloody diarrhoea was defined according to the World Health Organization (WHO) criteria as *"any person of any age with acute diarrhoea with visible blood in the stools"* (WHO IDSR 2010 Guidelines). A clinician diagnosed acute bloody diarrhoea on the basis of history of visible blood in stool for less than 4 days as recommended in WHO Surveillance standards. Cases who received antibiotic treatment in the week preceding sampling were included in the study. A study done in western Kenya indicated that the likelihood of isolating a pathogen did not differ significantly between persons who reported taking antibiotics before stool collection compared to those who did not (54% and 50%, respectively; p=0.55) (J. T. Brooks, Shapiro, Kumar, Wells, Phillips-Howard, & Shi, 2003).

Informed consent was sought from the patients in the consultation room, after which the clinician captured retrospective exposures over a period of one week; this is informed from the incubation period which is usually 1–3 days but may be up to 1 week for *S. dysenteriae* type 1. Stool specimens were collected from consecutive cases with acute bloody diarrhoea who sought care at the general outpatient department (OPD) in the selected health facilities (sites) in the two sub counties. Within 14 days after enrolment,

community health workers visited cases at home to conduct follow up interviews and collect observational data about the households.

#### 3.4.1.2 Selection of Neighbourhood Controls

A case was matched by age group and sex to two healthy controls (< 2 yrs, 2 – 4 yrs, 5-10 yrs, 11 – 17 yrs, 18-65 and >65 years) within two weeks of each case's presentation in order to control for seasonal variation. The neighbourhood controls were obtained using a systematic random sampling method. The home of a study case was identified as the starting point by the interviewers. Interviewers spun a bottle infront of the household until it came to a stop. They then walked in the direction the bottle pointed in and skipping the first household, randomly selected an eligible control living in that household. The next control was selected two households away from the first control in the same direction. Controls who met the criteria described above and who gave informed consent were included in the study.

### 3.4.2 Sample size determination

A minimum sample size of 596 was used. Selection of cases to controls was based on a 1:2 ratio with a minimum of 199 cases and 397 control subjects enrolled into the study from Kilifi Sub County and Nairobi West. The study assumed a 17% prevalence of risk factors among the cases. This was the estimated proportion of population without access to improved source of water. This was based on the WHO/UNICEF report- progress on Sanitation and drinking water (2013). The following formula was used to derive the sample size:

Where:

$$n = \frac{\left\{ Z_{(1-\alpha/2)} \sqrt{[2P(1-P)]} + Z_{(1-\beta)} \sqrt{[P_1 q_1 + P_0 q_0]} \right\}^2}{(P_1 - P_0)^2}$$

n = sample size of cases

$Z_{(1-\alpha/2)}$ = 1.96 is the value of the standard normal distribution corresponding to a significant level of α (alpha) for a 2-sided test at the 0.05 level

$Z_{(1-\beta)}$ = 0.84 is the value of the standard normal distribution corresponding to the desired level of power of 80%

$P_0$ = 0.10 was the estimated proportion of controls without access to safe water

$P_1$ = 0.17 was the estimated proportion of cases without access to safe water. The study assumed a 17% prevalence of risk factors among the cases (lack of improved drinking water source).

$q_0 = 1-P_0$

$q_1 = 1-P_1$

$P = ½(P_1 + P_0)$

**Table 3.2: Sample size computation**

| **SAMPLE SIZE COMPUTATION** | |
|---|---|
| $n = \frac{\{Z(1-\alpha/2) \sqrt{[2P(1-P)]} + Z(1-\beta)\sqrt{[P1q1 + P0q0]}\}^2}{(P1 - P0)^2}$ | |
| **Numerator** | |
| Z(1-α/2) | 1.96 |

| | |
|---|---|
| P | 0.135 |
| 2P | 0.27 |
| 1-P | 0.865 |
| 2P(1-P) | 0.23355 |
| √[2P(1-P)] | 0.48327 |
| (Z(1-β) | 0.84 |
| P1 | 0.17 |
| Q1 | 0.83 |
| Po | 0.1 |
| Qo | 0.9 |
| P1Q1 | 0.017 |
| Po-Qo | 0.8 |
| (P1Q1+Po-Qo) | 0.817 |
| √[P1q1 + P0q0] | 0.903880523 |
| Z(1-α/2) √[2P(1-P)] | 0.947209417 |
| Z(1-β)√[P1q1 + P0q0] | 75926 |
| {Z(1-α/2) √[2P(1-P)] +Z(1-β)√[P1q1 + P0q0]} | 1.70646906 |
| {Z(1-α/2) √[2P(1-P)] +Z(1-β)√[P1q1 + P0q0]}² | 2.91203664 |
| **Denominator** | |
| (P1 – P0) | 0.07 |
| (P1 – P0)² | 0.0049 |
| **Final computation** | |
| $\frac{\{Z(1-\alpha/2)\sqrt{[2P(1-P)]} + Z(1-\beta)\sqrt{[P1q1 + P0q0]}\}^2}{(P1 - P0)^2}$ | 594.29 |
| **Minimum Sample size (n)** | **594.29** |
| Case:control Ratio | (1:2) |
| **Minimum number of Cases** | **198.10** |
| **Minimum number of Control** | **396.20** |

### 3.5 Data Collection

#### 3.5.1 Collection and labelling of stool samples

Once consent was obtained from the participants enrolled into the study, fresh stool including portions with blood and/or mucus was collected from all cases presenting at the outpatient department. Within the same period, stool specimen from an age-matched control who did not have diarrhoea and who had no history of receiving antibiotic treatment for diarrhoea within the previous two months, according to the recollection of the mother in case of a child or from the control subject, was collected. The stool samples were placed in a leak proof sterile screw capped container. Precaution was taken not to allow the stool to dry out. In situations where the specimen could not reach the laboratory within two hours, a Cary-Blair transport medium was used. In case a patient was able to pass stool, a sample was collected with a sterile rectal swab and the swab placed in Cary-Blair transport medium. The tube was sealed to avoid leakage. All contaminated materials were disposed of safely according to standard operating procedures.

***Rectal swab***

A sterile cotton swab (Harwood products Company, Gilford Maine, USA) was moistened in sterile transport medium, inserted through the rectal sphincter 1-2cm, rotated twice and withdrawn. It was examined to ensure that there was some faecal material visible on the swab. The swab was immediately inserted into Carry-Blair transport medium. The specimen was labelled with subject's name, identification

number, date and time of collection using an indelible marker pen. The samples were then be placed in an insulated box with cool packs and processed within 6 hrs.

Surveillance form (Appendix 3) was used to record information on each subject. The home of these subjects was subsequently visited in order to collect water samples from the household and main source.

### 3.5.2 Collection and labelling of water samples

The homes of all cases and controls were visited in order to sample water. A total of two samples were collected, one from domestic sources e.g. borehole, river, stream, well, tap and the other sample from household container (drinking)[1]. Sterile screw-capped 200 ml bottles were used. The sample bottles were labelled with identification number, source of water and date and time of collection; a water sample collection form (Appendix 4) was filled for each sample. The water samples were transported to laboratory within 2 hours of collection.

***Collection of samples from river***

A sterile collection bottle was immersed, with its mouth closed by the stopper, a foot below the water surface. Facing the direction of the current, the bottle was filled with water by opening the lid, brought to the surface and the stopper replaced.

***Collection of samples from wells***

[1] **Collect a sample for microbiological water testing in plastic bottle containing thiosulfate and place on ice**

If a well was fitted with a hand pump or electric pump, alcohol was applied to the mouth of the pump and allowed to dry. Water was pumped to waste for 1 minute, before the sample was collected into a sterile bottle. This allowed time for flushing any stagnant water. Wells that had no pumping machinery, samples were collected directly from the well in a sterile bottle fitted with some weight at the bottom. Where it was possible to collect the sample directly into the bottle, samples were obtained by means of a metal jug/pot.

### 3.5.3 Transport and handling of samples

***Stool samples***

Specimens were refrigerated after collection until they reach the laboratory. If the laboratory was nearby, specimens were hand carried in an insulated box with ice packs. Specimens that could not be cultured within two hours of collection were placed in transport medium and refrigerated immediately at 4-8°C. Transportation using cool boxes was achieved for up to 36 hours by shipping in a well-insulated box with frozen refrigerant packs or wet ice.

*Water samples*

Immediately after collection, samples were placed in an insulated cold box for transport to the reference laboratory. Sample from Nairobi West were sent to National Public Health laboratories (NPHLS) while Kilifi Samples were sent to the Government Chemist Mombasa. Water samples were examined as soon as possible on arrival and usually within 6 hours of collection.

### 3.5.4 Direct microscopy and concentration technique for stool samples.

The testing and documentation was done according to the standard operating procedures (Appendix 14). The methods included microscopy for erythrocytes and parasites as well as culture for bacterial enteric pathogens. Stool specimens were examined macroscopically for gross blood and mucus. Wet mounts of fresh stool were made in normal saline and examined for parasites and erythrocytes. Direct microscopic examination of fresh stool was used to diagnose or rule out, infection with *Entamoeba histolytica, Schistosoma mansoni*, *Giardia lamblia* and *Trichuris trichiura*. However, finding cysts or non-haematophagous trophozoites of *Entamoeba histolytica* in a bloody stool did not indicate that it is the cause of illness.

Direct examination of wet mount may not have detected parasites in the stool specimens if the number was low, hence, concentration technique (Appendix 14) was used. Eggs, cysts and larvae are recovered after concentration procedures whereas trophozoites get destroyed during the procedure. This made direct wet mount examination obligatory as the initial phase of microscopic examination.

### 3.5.5 Biochemical identification for stool samples

Stool swabs were inoculated onto the surface of macConkey agar (MAC) and eosin methylene blue agars and streaked for colony isolation. Colonies arising after 24 h and 48 h of incubation at 37°C were streaked onto fresh plates and identified by conventional biochemical tests according to standard laboratory procedures. However, a definitive diagnosis of the type(s) of enteric infection was only made by isolating the organism from stool and serotyping the isolate. At the National reference laboratory, the

culture plates were incubated at 37°C for 24h, the non-lactose fermenting colonies (NLFs) were picked and subjected to a gram stain. Subsequently, all the Gram –ve colonies were picked from the respective plates and prepared for biochemical identification using the semi-automated bacterial identification systems, the Vitek 2®. The colonies were briefly emulsified into 0.45% normal saline solution for the system to attain a 0.5-0.63 McFarland strength. The Gram -ve identification cassette was inserted into the respective tubes and then into the system. After 18-24 h of incubation the biochemical reactions were obtained through a print-out from the machine.

### 3.5.6 Stool Culture

All stool specimens from Nairobi West were cultured for *Shigella, Escherichia coli, Salmonella*, *Vibrio*, *Campylobacter* and *Aeromonas* at the National Public Health laboratories (NPHLS) according to standard laboratory procedures (CMR/NPHLS) . Kilifi County laboratory did cultures and shared the isolates with NPHLS. Stocks of each isolate were maintained by cryopreservation. Enteric viruses are not known to cause bloody diarrhoea and the study did not examine for these agents (Brooks, Sha*et al*., 2003).

The turnaround time for negative result reporting was three days while that of positive reports was three to five days. The results of bacterial cultures and antimicrobial susceptibility testing were returned to Health facility staff at each surveillance site; these results were used to guide therapy. For every patient with positive stool culture results, a reassessment of their clinical condition, either by telephone contact or follow

up visit, was arranged. Any change in management was recorded in the case notes. This study retrospectively traced and identified every case in which management was subsequently changed (for example, institution or change of antibiotics) in the light of a positive stool culture result.

### 3.5.7 Bacteriological analysis for water samples

Paqualab system was used to test the quality of water at National Public Health Laboratories (NPHLS) and Kilifi County Hospital Laboratory. Government Chemist laboratory was used for quality control. Each water sample was thoroughly mixed by shaking the container several times. The samples (10 Mls) were diluted with double strength macConkey broth in universal bottle. One bottle was be incubated at 37$^{o}$C in order to isolate non-faecal coliforms, while the other was incubated at 44$^{o}$C in order to isolate thermotolerant (faecal) coliforms. At 44$^{o}$C the coliform isolates may be of human or environmental origin. However, in this study they were assumed to be human isolates. After 24 hrs incubation positive cultures (evidence of turbidity or gas production) were sub-cultured onto agar and macConkey agar plates. The bacterial isolates were further identified using their biochemical reactions on API -20E strips. The isolates were kept frozen on protect beads at -70ºC until used.

### 3.5.8 Real time Polymerise Chain Reaction (PCR)

Screening of the five categories of diarrhoeagenic *E.coli* was done using the real time PCR protocol and primers earlier described by Hardegen *et al.* (2010) (table 3.3). Briefly, the PCR mixture contained; 2× Quantitect probe PCR Mastermix, 0.3µl

forward primer (40 µM), 0.3 µl reverse primer (40 µM), 0.15 ul probe (40 µM) 12.25 µl nuclease free water. Target specific master mixes were made, 28 µl transferred into 0.1ml pcr tubes and then 2ul of the extracted DNA added into the specific tubes.

The thermocycling conditions consisted of cycles of hot start activation at 95°C for 15 min, amplification cycles of 95°C, 15s, 55 °C, 60sec. The amplification step was repeated for 45 cycles. The threshold was set to analyze the amplification curves. Any sample with a cycle threshold value (ct) of <37 was considered positive. Any sample with a threshold of > 37 were analyzed and considered as negative, positive or indeterminate based on the characteristics of the curve.

**Table 3.3: Primers used for diarrhoeagenic Escherichia coli species**

| **Pheno type** | **Forward primer** | **Reverse primer** | **Probe** |
|---|---|---|---|
| EPEC | GTT CTT GGC GAA CAG GCT TGT C | TTA AGC CAG CTA CCA TCC ACC C | AGT ACT GAC GTG CAG GTC GCC TGT TCG |
| EAEC | AGG TTT GAT ATT GAT GTC CTT GAG GA | TCA GCT AAT AAT GTA TAG AAA TCC GCT GTT | GTT CCT GAG AGT GCA ATC CCA GAC ATT AC |
| EIEC | GAA CTC AAA TCT TGC ACC ATT CA | CGT CCG TCC GAG AAC AAT TAA G | ATC CCC GAC ACC GTT TGT GAG TTT CAC T |
| ETEC | CTG GTT TTG ATT CAA ATG TTC GTG | TCC TGA GGG AAA GGT GAA AAA GAC | TTG ATT TCT TCA TAT TAC CTC CGG ACA TGG CA |
| EHEC | GAC GTG GAC CTC ACT CTG AAC TG | TCC CCA CTC TGA CAC CAT CC | TAC TCC GGA AGC ACA TTG CTG ATT CGC |

### 3.5.9 Antimicrobial drug susceptibility testing

#### 3.5.9.1 Disk diffusion Method

Antimicrobial susceptibility testing by disk diffusion on Muller Hinton agar was done using Kirby-Bauer technique (CDC, 1999). Antimicrobial susceptibility tests were performed using commercial discs following manufacturer's instructions (Becton, Dickson and company, Maryland USA). Susceptibility to penicillins antibiotics was tested using ampicillin (10 µg) while susceptibility to cephalosporin's was determined using ceftriaxone (30ug). Ciprofloxacin (5 µg), norfloxacin (10 µg), ofloxacin (5 µg) and nalidixic acid (30 µg) were used for testing susceptibility to the quinolones. Aminoglycoside used in susceptibility tests were streptomycin (30 µg). Tetracycline antibiotics included doxycycline (30 µg) and tetracycline (30 µg). Other antibiotics included were chloramphenicol (30µg), furazolidone (100 µg) and Sulphamethoxazole (23.75 µg)-trimethoprim (1.25 µg) and azithromycin (15 µg). Five colonies from a pure culture were selected and transferred with an inoculating needle to 5ml of Muller Hinton broth. The broth culture was then incubated at 37ºC for 3 hours to develop turbidity equivalent to 0.5 McFarland turbidity standards. The isolates in the broth were streaked onto Muller Hinton medium by dipping a sterile cotton swab to the inoculum and rotating it firmly several times against the wall of the tube to remove excess fluid. The emulsified colonies were streaked over the entire surface of the Muller Hinton agar plate three times turning the plate 60º between streaking to obtain an even inoculation. Disks were placed individually with sterile forceps onto the agar. The plates were incubated at 37ºC for 18 h. After incubation, the zones of complete inhibition were

measured and recorded in millimetres. Using the recorded inhibition zone diameters, the strains were classified as susceptible, intermediate or resistant to a particular antimicrobial agent using the Clinical and Laboratory Standards Institute guidelines (CLSI, 2011). *Escherichia coli* strain ATCC 25922 was used for quality control of growth and antibiotics potency.

### 3.5.9.2 Minimum Inhibitory Concentration

Antimicrobial susceptibility testing (AST) by Minimum Inhibitory Concentration was used to assess emerging bacterial resistance patterns especially to quinolones and cephalosporin. MIC was determined by broth dilution techniques as per the Clinical and Laboratory Standards Institute (CLSI, 2011). The broth dilution method was dependent on inoculation at a specific inoculum density of broth media (in tubes or microtitre plates) containing antibiotics at varying levels. Doubling dilutions was used and after incubation, turbidity was recorded either visually or with an automated reader, and the breakpoint concentration established. Microtitre plates or ready-to-use strips with antibiotics ready prepared in the wells were used. The agar dilution method was used, a small volume of suspension was inoculated onto agar containing a particular concentration of antibiotic, when the inoculum had dried the plate was incubated and again examined for zones of growth. A control plate containing no antimicrobial agent was also prepared for each test. *Escherichia coli* strains ATCC 25922 with known MIC values of each antimicrobial agent were included in each test as a control.

### 3.5.10 Risk Factors/Variables

Possible exposures were identified by administering a questionnaire to cases and controls. These exposures were broadly classified into socio-demographic, socio-cultural and environmental. The questionnaire had specific questions on water, food and hygiene related exposures among others. Water exposures included water source (e.g. riverbed, communal tap, shallow well, bottled water and water from street vendors) and water use (e.g. drinking, cooking, washing food related utensils and brewing alcoholic beverages). Data on water storage practices (e.g. type of container water stored in, length of storage) was also collected. Food exposures were gathered on food commonly eaten within the community and on location of consumption (e.g. home versus street vendor). Hygiene related exposures consisted of data pertaining to hand washing practices and latrine usage.

### 3.5.11 Meteorological data

Two sets of data on rainfall and temperature for Kilifi and Nairobi counties were obtained from the Kenya Meteorological Department; the mean monthly rainfall and temperature for 2012 and the long term mean rainfall and temperature (1971-2012). The mean monthly rainfall and temperature for the year 2012 (study period) were compared with the number of cases of acute bloody diarrhoea of each month. Pearson's correlation was used to establish the presence and strength of association.

### 3.6 Quality control of data

#### 3.6.1 Minimization of Errors and Biases

Data collection tools were pre-tested and necessary adjustments made. The study population was sampled prior the administration of the questionnaire. The supervisor's review of the questionnaire minimized some of the errors that were likely to occur. The interviewers were trained together to ensure that they have a common perception and understanding on the questions that increase the probability of similar question carrying the same meaning. Laboratory quality assurance was done. Specimens and Isolates were sampled randomly for analysis in reference laboratory regularly.

#### 3.6.2 Laboratory quality assurance

The steps involved in the accurate laboratory diagnosis of enteric pathogens included specimen collection and transport, the performance of laboratory procedures, and reporting. It was ensured that the correct specimen was collected in the correct volume, transported to the laboratory in the right condition, correct laboratory procedures were followed and reporting was accurate. These steps were monitored and correction took place where unacceptable performance was identified.

A quality control programme ensured that the information generated by laboratories was accurate, reliable and reproducible. This was accomplished by assessing the quality of specimens and monitoring the performance of test procedures, reagents, media, instruments, and personnel. The laboratory had internal quality control programme. A panel of reference isolates consisting of various isolated enteric pathogens was maintained. At periodic intervals (monthly), the laboratory supervisor submitted a

random selection of the reference isolates under code to laboratory technologists for evaluation. Quality control of the disc susceptibility test was done. The susceptibility zones for the reference strain against various antimicrobials had to be within the acceptable ranges. The results of these evaluations were entered in a quality control monitoring book. Appropriate measures were taken to solve any problems that were encountered. A national reference laboratory was used to confirm isolates in order to rule out any possibility of misidentification.

### 3.7 Data Management and analysis

#### 3.7.1 Data handling

All questionnaires were coded and validated daily during data collection. The various outcomes were coded for easy extraction as well as for ease of computer entry. Data cleaning was done to ensure errors were detected and addressed effectively. Data was validated by comparing it with the records at the sub county level. Double entry of collected data was done during the study to minimize entry errors and missing data.

#### 3.7.2 Data storage

Data was transferred from questionnaires to the computer using SPSS®. It was coded, stored, pass-word protected and backed-up on alternative secure storage media. Filled questionnaires are to be safely stored for at least 3 years.

#### 3.7.3 Data analysis

Data collected was analyzed using SPSS® and EPI Info® computer software. Descriptive analysis of frequencies and proportion was done. Odds ratio was used as a

measure of association between exposure and outcome. Any variable with $p< 0.05$ was considered statistically significant. All factors with a $p < 0.1$ in bivariate analysis were put in conditional logistic regression model with stepwise backward elimination, to come up with the final 'best-fit' model.

### 3.8 Ethical consideration

Ethical clearance of this study (SSC No. 2177) was approved by the Kenya Medical Research Institute (KEMRI), Centre for Microbiology Research (CMR), Scientific Steering Committee (SSC) and National Ethical Review Committee (ERC) (Appendices XIII and XII). Written informed consent was sought from the cases and controls before participation in the study using a standard consent form designed for the study (Appendices V). Subjects were informed of their right to opt out at any stage without any fear of retribution. Parents/guardians gave consent for the young children while assent was obtained from children above 7 years and below 18 years of age using a standard assent form designed for the study (Appendices VI).

Anonymity and confidentiality of the subjects was strictly upheld at all stages of the study. None invasive procedures were done, stool samples were collected from cases and controls for laboratory analysis. Approval from Ministries of Health was obtained to carry out the study in government Health facilities in the selected two sub counties.

Copies of the questionnaires are to be kept under lock and key for at least 3 years and will only be available to authorized persons.

# CHAPTER FOUR

# RESULTS

## 4.1 Introduction

Participants enrolled in the study were 805 (284 cases and 521 controls) into the study between January and December 2012 (Figure 4.1). The targeted case: control ratio of 1:2 was 45 controls short of being achieved which represented a 91% success rate.

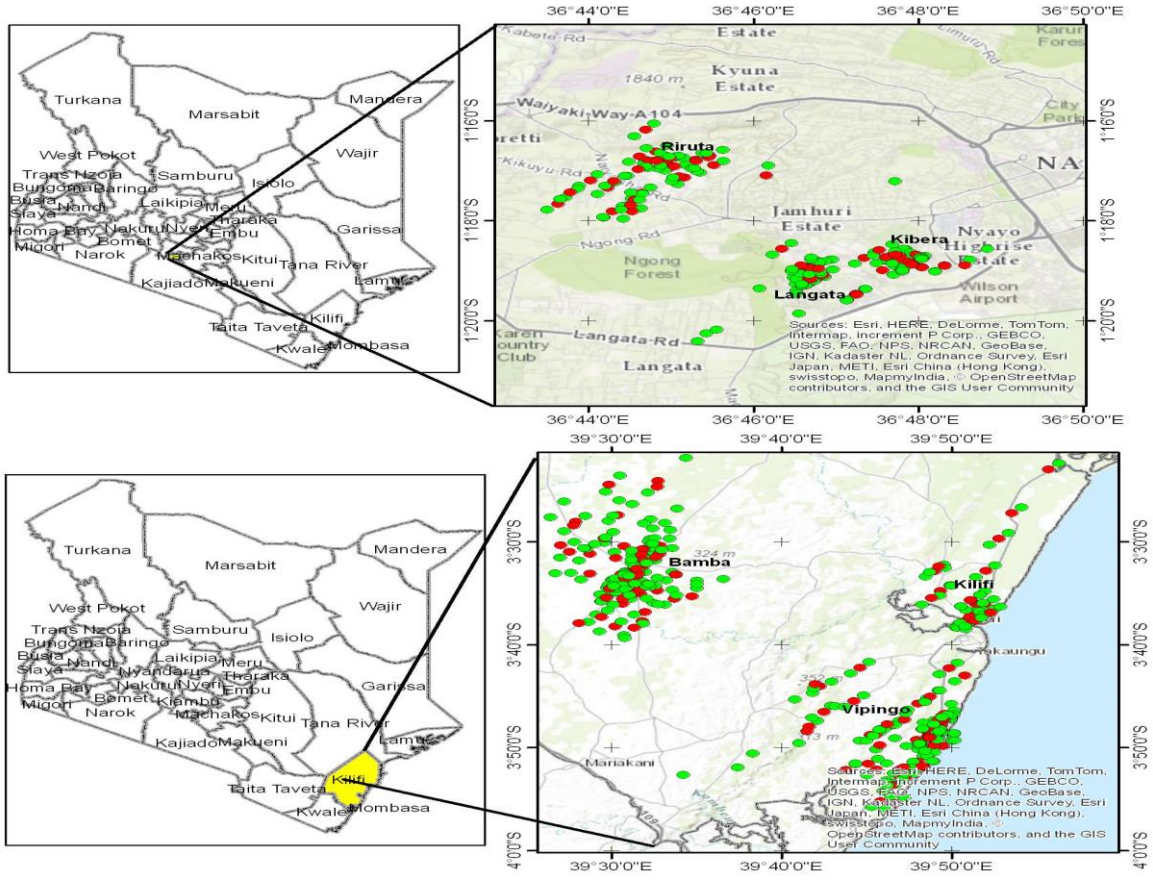



**Figure 4.1: Map of cases (red) and controls (green) in Kilifi and Nairobi West**

### 4.2 Demographic characteristics of study participants

The mean age of the cases was 24.4 years with a range of 1 month to 73 years while the mean age of controls was 27.5 years with a range of 3 months to 73 years. The proportion of females was 56% among cases and 67% among controls. About 61% of the cases and 63% of the controls resided in the rural areas (Table 4.1).

**Table 4.1: Demographic characteristics of the cases and controls**

| Variable | Cases<br>n (%) | Controls<br>n (%) | Total<br>n (%) |
|---|---|---|---|
| **Gender** | | | |
| Male | 124 (42) | 173 (33) | 297 (37) |
| Female | 160 (56) | 346 (67) | 506 (63) |
| **Age Group** | | | |
| <5 years | 63 (22) | 72 (14) | 135 (17) |
| 5-<11 years | 12 (4) | 23 (5) | 35 (4) |
| 11-<18 years | 33 (12) | 25 (5) | 58 (7) |
| 18-65 years | 166 (59) | 372 (74) | 538 (69) |
| >65 years | 7 (2) | 9 (2) | 16 (2) |
| **Age** | | | |
| Mean | 24.4 | 27.5 | 26.4 |
| Median | 22 | 26 | 25 |
| Standard deviation | 18.6 | 16.9 | 17.6 |
| **Residence** | | | |
| Kilifi | 174 (61) | 329 (63) | 503 (62) |
| Nairobi West | 110 (39) | 192 (37) | 302 (38) |

### 4.2.1. Social-demographic characteristics in rural and urban

Data was analysed according to this rural-urban classification, where Kilifi was the rural while Nairobi West was the urban setting. More than 60 % of cases in the urban setting had completed a secondary school education compared to 30% in the rural setting. Most of the cases (over 70%) from both rural and urban settings worked either as casual labourers, farmers, salaried workers or business people (Table 4.2). Over 80% of the cases from rural areas were in the less than KES. 5,000 income category while over 50% of those from urban areas had income levels of over KES. 5,000.

**Table 5.2: Social demographic characteristics of cases by residence**

| | **Residence** | | **Total** |
|---|---|---|---|
| | **Nairobi West (Urban) (%)** | **Kilifi (Rural) (%)** | |
| **Highest Level of Education** | **[n=105]** | **[n=140]** | |
| No formal education | 1.9% | 9.8% | 67 |
| Primary | 37.9% | 56.7% | 109 |
| Secondary | 46.6% | 30.8% | 65 |
| College or university | 13.6% | 2.8% | 4 |
| | | | |
| **Occupation** | **[n=102]** | **[n=138]** | |
| Casual laborer | 47.1% | 21.7% | 78 |
| Farming | 0% | 29.7% | 41 |
| Salaried worker | 12.7% | 14.5% | 33 |
| Business | 18.6% | 4.3% | 25 |
| Not working | 2.0% | 13.8% | 21 |
| Skilled laborer | 8.8% | 4.3% | 15 |
| Others | 3.9% | 5.1% | 11 |
| Fishing | 2.0% | 2.2% | 5 |
| Housewife | 2.9% | 1.4% | 5 |
| Student | 1.0% | 1.4% | 3 |
| Long distance driver | 1.0% | 0.7% | 2 |
| Quarry | 0% | 0.7% | 1 |

| | | | |
|---|---|---|---|
| **Income Level** | **[n=100]** | **[n=135]** | |
| <3000 | 35.0% | 60.7% | 117 |
| >3000-5000 | 15.0% | 20.7% | 43 |
| >5000-10,000 | 31.0% | 16.3% | 53 |
| >10,000-20,000 | 14.0% | 2.2% | 17 |
| >20,000-30,000 | 3.0% | 0% | 3 |
| >50,000 | 2.0% | 0% | 2 |
| | | | |
| **Drinking water access** | **[n=106]** | **[n=143]** | |
| Yes | 86.8% | 82.5% | 210 |
| No | 13.2% | 17.5% | 39 |
| | | | |
| **Source of drinking water** | **[n=106]** | **[n=142]** | |
| Piped water | 76.4% | 38.7% | 136 |
| Water pans | 0.9% | 32.4% | 43 |
| Common tank | 16.0% | 3.5% | 22 |
| Boreholes | 0.9% | 12.0% | 18 |
| Community well | 4.7% | 2.1% | 8 |
| Closed well | 0% | 4.9% | 7 |
| Open shallow well | 0% | 2.8% | 4 |
| Lake | 0% | 2.1% | 3 |
| Vendors | 0.9% | 0.7% | 2 |
| Dam | 0% | 0.7% | 1 |
| | | | |
| **Access to health facility** | **[n=105]** | **[n=143]** | |
| Walking | 83.8% | 56.6% | 169 |
| Bicycle | 0% | 2.8% | 4 |
| Motorcycle | 1.9% | 13.3% | 21 |
| Vehicle | 14.3% | 27.3% | 54 |
| | | | |
| **Household Members** | **[n=115]** | **[n=145]** | |
| 1-5 members | 71.8% | 50.7% | 150 |
| 6-10 members | 26.4% | 38.6% | 83 |
| 11-15 members | 1.8% | 9.3% | 15 |
| 16-20 members | 0% | 1.4% | 2 |

Piped water and common tank were the most common source of household drinking water among urban respondents while piped water, water pans and boreholes were the most prevalent among rural respondents. Over 97% of cases coming from urban areas had less than 10 members in their household; the proportion was lower among rural cases with over 10% of them having more than 10 members within their households.

### 4.3 Clinical features of cases presenting with acute bloody diarrhoea

#### 4.3.1 Signs and symptoms on clinical examination

The commonest presenting signs and symptoms were blood in stools (100%), abdominal pain (69%) and mucous in stool (61%) as shown in Table 6. The urban cases presented with higher proportions of the following signs and symptoms compared with the rural cases; anorexia, headache, chills, fever, watery stool, nausea, abdominal tenderness and dry mucous membrane. The rural cases had higher proportion of mucous in stool compared with urban (Table 4.3).

**Table 6.3: Signs and symptoms among cases in Kilifi and Nairobi West**

| Signs and Symptoms | Kilifi (n = 169) | Nairobi West (n= 87) | Total | Percentage (%) |
|---|---|---|---|---|
| Blood in stools | 169 (100%) | 87(100%) | 256 | 100 |
| Abdominal pain | 117(69.2%) | 58 (66.7%) | 175 | 69 |
| Mucous in stools | 111(65.7%) | 42 (48.3%) | 153 | 60 |
| Loose stools | 91(53.8%) | 47 (54%) | 138 | 54 |
| Anorexia (reduced appetite) | 75(44.4%) | 54 (62%) | 129 | 50 |
| General malaise | 72 (42.6%) | 41(47.1%) | 113 | 48 |
| Headache | 62 (36.7%) | 43 (49.4%) | 105 | 42 |
| Abdominal discomfort | 66(39%) | 29 (33.3%) | 95 | 37 |
| Chills | 48 (28.4%) | 42 (48.3%) | 90 | 36 |

| | | | | |
|---|---|---|---|---|
| Fever | 52 (30.8%) | 37(42.5%) | 89 | 34 |
| Watery Stools | 39 (23.1%) | 48(55.2%) | 87 | 34 |
| Nausea | 38 (22.5%) | 34(39%) | 72 | 28 |
| Dizziness | 43(25.4%) | 21(24.1%) | 64 | 25 |
| Vomiting | 42(24.9%) | 19(21.8%) | 61 | 24 |
| Cough | 29(17.2%) | 13(14.9%) | 42 | 17 |
| Painful defecation | 29(17.2%) | 12(13.7%) | 41 | 16 |
| Abdominal tenderness | 6(3.6%) | 32(36.8%) | 38 | 15 |
| Dry mucous membrane | 14(8.3%) | 23(26.4%) | 37 | 14 |
| Pallor | 6(3.6%) | 2(2.3%) | 8 | 3 |
| Weight loss | 2(1.2%) | 4(4.6%) | 6 | 2 |
| Respiratory distress | 1(0.6%) | 3(3.4%) | 4 | 2 |
| Rectal prolapse | 0(0%) | 3(3.4%) | 3 | 1 |
| Jaundice | 2(1.2%) | 1(1.1%) | 3 | 1 |
| Rice water stools | 0(0%) | 2(2.3%) | 2 | 0.8 |
| Altered consciousness | 0(0%) | 1(1.1%) | 1 | 0.4 |
| Oedema (lower limbs) | 1(0.6%) | 0(0%) | 1 | 0.4 |

### 4.3.2 Assessment of diarrhoea history

About 85% (n= 244) of the cases reported having diarrhoea that lasted one to three days. About 50% (n=122) reported the diarrhea to be bloody from onset. Dehydration was present in 18% (n=46) of the cases. The danger signs were; sunken eyes (7%), loss of skin turgor (3%), lethargy/unconsciousness (1%) and inability to drink fluids (<1%). Ninety six percent (n= 272) of the cases had between 2 and 10 incidences of stools in the preceding 24 hours before being interviewed while a majority (76%) of them indicated they had no incidences of vomiting. Twenty two percent (n=52) of the cases indicated they had previous history of bloody diarrhoea while 20% (n=49) of them also indicated that prior to onset of their diarrhoea, they had at least one household member who had bloody diarrhoea in the previous one month.

Among the 49 cases classified as children, 2% were exclusively breast fed, 40 % were artificially fed and 57% were on mixed feeding. Among the children aged >9 months to

< 5 years, 100% (n= 51) had been vaccinated against measles; 45% had an immunization card while for the remaining 55%, the vaccination status was through verbal confirmation by the caregiver/guardian.

### 4.3.3 Treatment received since onset of diarrhoeal episodes

The commonest self-medicating treatments used among cases was oral rehydration solution (17.5%), homemade salt and sugar solution (10.8%) and antibiotics (8%) as shown below (Figure 4.2).

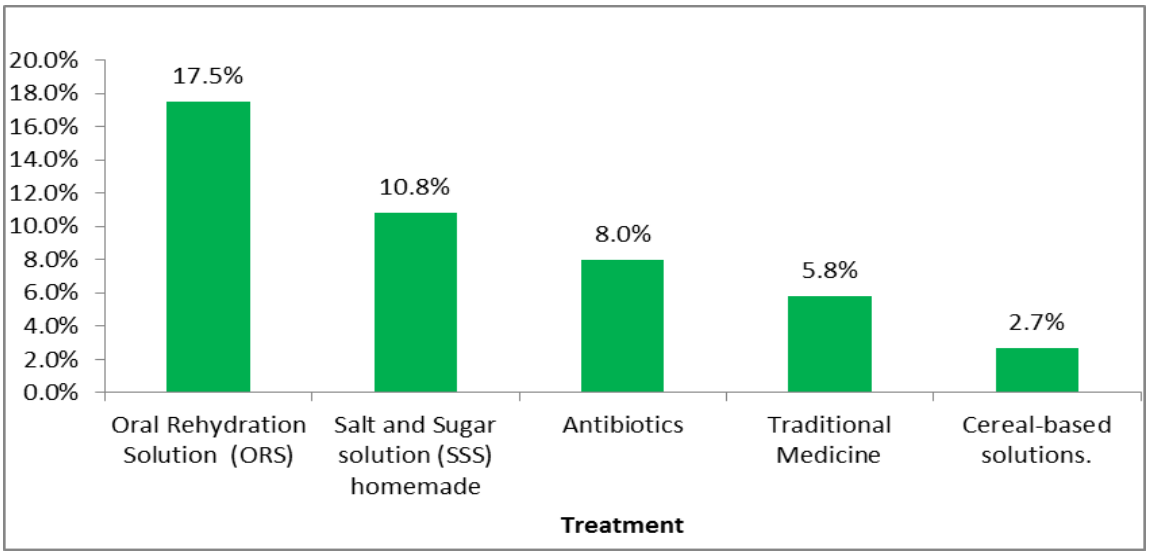



**Figure 5.2: Self-medication among Cases**

The most used antibiotics among the cases who specified they had taken an antibiotic as a mode of treatment were Doxycycline (26.7%), Amoxicillin (21.7%), and Cotrimoxazole (17.4%). Over 80% of these antibiotics were taken for between 1 and 4 days.

### 4.4 Clinical case management

#### 4.4.1 Prescribed antibiotics

About 86% (243/284) of the cases were given an antibiotic prescription. Ciprofloxacin was the most prescribed in Kilifi (46.3%) while Sulfamethoxazole-trimethoprim was the most prescribed in Nairobi West (33.3%). Doxycycline was prescribed to 16% of the cases in Nairobi West compared to 8.6% in Kilifi. All the 26 cholaramphenicol prescriptions were done in Kilifi while all the 9 Tetracycline prescriptions were done in Nairobi West (Table 4.4).

**Table 7.4: Prescribed antibiotics among cases in Kilifi and Nairobi West**

| Prescribed Antibiotic | Kilifi (n=162) | Nairobi West (n=81) | Total |
|---|---|---|---|
| Ciprofloxacin | 75 (46.3%) | 19 (23.5%) | 94 (38.7%) |
| Sulfamethoxazole-Trimethoprim | 26 (16%) | 27 (33.3%) | 53 (21.8%) |
| Doxycycline | 14 (8.6%) | 13 (16%) | 27 (11.1% |
| Chloramphenicol | 26 (16%) | 0 (0%) | 26 (10.7%) |
| Erythromycin | 6 (3.7%) | 7 (8.6%) | 13 (5.3%) |
| Tetracycline | 0 (0%) | 9 (11.1%) | 9 (3.7%) |
| Amoxicillin | 8 (4.9%) | 1 (1.2%) | 9 (3.7%) |
| Nalidixic acid | 5 (3.1%) | 2 (2.5%) | 7 (2.9%) |
| Norfloxacin | 0 (0%) | 3 (3.7%) | 3 (1.2%) |
| Amoxicillin-Clavullanic acid | 1 (0.6%) | 0 (0%) | 1 (0.4%) |
| Ceftriaxone | 1 (0.6%) | 0 (0%) | 1 (0.4%) |
| **Total** | **162** (100%) | **81** (100%) | **243** (100%) |

Metronidazole was the most (139/236) prescribed anti-amoebic drug for cases with bloody diarrhoea. About 70% (53/76) of cases in Nairobi West were given a prescription for compared to about 54% (86/160) in Kilifi (Table 4.5).

**Table 8.5: Prescribed anti-amoebic drugs in Kilifi and Nairobi West**

| Prescribed anti-amoebic drugs | Kilifi (n=160) | Nairobi West (n=76) | Total |
|---|---|---|---|
| Metronidazole (Flagyl) | 86 (53.8%) | 53 (69.7%) | 139 (58.9%) |
| Tinidazole | 41 (25.6%) | 18 (23.7%) | 59 (25%) |
| None | 33 (20.6%) | 5 (6.6%) | 38 (16.1%) |
| **Total** | **160 (100%)** | **76 (100%)** | **236 (100%)** |

### 4.4.2 Non-antibiotic Management

Oral rehydration solution (ORS) was the most (95%) prescribed fluid among the cases. Zinc and Paracetamol were the most used regime with more than 50% put on Zinc and Paracetamol individually or as a combination.

## 4.5 Etiologic agents associated with acute bloody diarrhoea

A total of 398 stool samples were tested (microscopy and culture); 284 from cases and 114 from controls. The expected case: control stool ratio of 2:1 was not achieved in Nairobi due to high refusal rate among the controls; the ratio was however achieved in Kilifi. Enteric pathogens known to cause bloody diarrhoea were isolated in 115 (40.5%) of the cases. The isolation rate among the rural population (Kilifi) was 24.7% while among the urban population (Nairobi) it was 65.5%.

### 4.5.1 Prevalence of protozoal pathogens in Kilifi and Nairobi West.

*Entamoeba histolytica* was the most prevalent protozoa {10.2% (29/284)} among the acute bloody diarrhoea cases in both setting (Table 4.6). *Entamoeba histolytica* was found to be proportionally higher {7.4% (21/284)} among cases in Nairobi West

compared to Kilifi {2.8% (8/284)}. *Giardia lamblia* {1.4% (4/284) and co-infections of *Entamoeba histolytica* & *Giardia lamblia* {0.7% (2/284)} were only found in Nairobi West. A total of 114 stool samples collected from controls in Kilifi (88) and Nairobi West (26). Only 2 (1.8%) were found to have trophozoites of *Entamoeba histolytica* (1) and *Giardia lamblia* (1) in Nairobi West.

On microscopic examination, Red Blood Cells (RBCs) were found in stool samples of 71.8% (125/174) of cases in Kilifi compared to 52.7% (58/110) of case in Nairobi West (table 9). Among the 114 samples from controls in both settings, about 3.5% (4/114) were found to have RBCs. On Proportional comparison, Nairobi West was higher with 7.7% (2/26) while Kilifi had 2.2% (2/88).

**Table 9.6: Prescribed anti-amoebic drugs in Kilifi and Nairobi West**

| | Kilifi Cases (%) | Nairobi West Cases (%) | Total (%) |
|---|---|---|---|
| **Trophozoites (n=284)** | | | |
| No trophozoites | 165 (58.1%) | 82 (28.9%) | 247 (87.0%) |
| *Entamoeba histolytica* | 8 (2.8%) | 21 (7.4%) | 29 (10.2%) |
| *Giardia lamblia* | 0 (0%) | 4 (1.4%) | 4 (1.4%) |
| *Trichomonas hominis* | 1 (0.4%) | 1 (0.4%) | 2 (0.7%) |
| *E. histolytica & G.lamblia* | 0 (0%) | 2 (0.7%) | 2 (0.7%) |
| **Cysts (n=284)** | | | |
| No Cysts | 143 (50.4%) | 90 (31.7%) | 233 (82%) |
| *Entamoeba histolytica* | 25 (8.8%) | 14 (4.9%) | 39 (13.7%) |
| *Escherichia coli* | 6 (2.1%) | 1 (0.4%) | 7 (2.5%) |
| *Giardia lamblia* | 0 (0%) | 4 (1.4%) | 4 (1.4%) |
| *G. lamblia & E. histolytica* | 0 (0%) | 1 (0.4%) | 1 (0.4%) |
| **Ova (n=284)** | | | |
| No Ova | 174 (61.3%) | 109 (38.4%) | 283 (99.6%) |
| *Trichuris Trichiura* | 0 (0%) | 0 (0%) | 0 (0%) |

| | | | |
|---|---|---|---|
| *Ascaris Lumbricoides* | 0 (0%) | 1 (0.4%) | 1 (0.4%) |
| **RBCs (n=284)** | | | |
| No RBCs | 49 (17.3%) | 52 (18.3%) | 101 (35.6%) |
| 1-5 | 65 (22.9%) | 9 (3.2%) | 74 (26.1%) |
| 6-10 | 3 (1.1%) | 3 (1.1%) | 6 (2.1%) |
| 11-15 | 11 (3.9%) | 4 (1.4%) | 15 (5.3%) |
| 16 -20 | 8 (2.8%) | 2 (0.7%) | 10 (3.5%) |
| > 20 | 38 (13.4%) | 40 (14.1%) | 78 (27.5) |

### 4.5.2 Prevalence bacterial pathogens associated with acute bloody diarrhoea

#### 4.5.2.1 Bacteria species isolated from culture growth

A total of 80 bacterial isolates were obtained from the specimens; 27.5% (78/284) were from cases and 1.8% (2/114) from controls. The bacterial isolation rate for cases in Kilifi was 20% (35/174) while for cases in Nairobi was 39% (43/110). Four different pathogens were isolated from the laboratory culture growth as shown below (Figure 4.3).

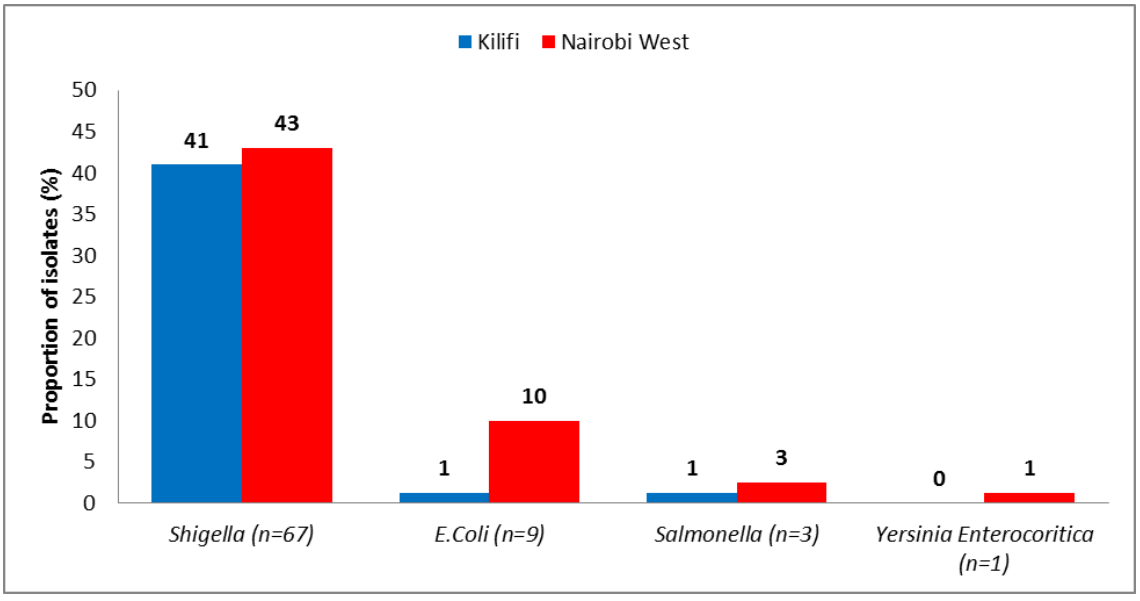



**Figure 6.3: Bacterial isolation in Kilifi and Nairobi West**

The *Shigella* isolation rate among cases in both setting was 22.8% (65/284). In rural (Kilifi), *Shigella* isolation rate was lower at 19% (33/174) compared to 29% (32/110) in Urban (Nairobi West). *Shigella* was the most prevalent bacteria accounting for 84% (n=67) of the 80 isolates. The proportion of *Shigella* isolated in Kilifi and Nairobi West were 41% and 43% respectively. Eight out of the nine *Escherichia coli* were isolated in Nairobi West. Nairobi West yielded 56.3% of the bacteria isolates while Kilifi had 45.7%.

#### 4.5.2.2 Distribution of bacteria species isolated in Kilifi and Nairobi West

*Shigella flexneri* was the most common bacterial pathogen accounting for 50% of the 80 isolated bacteria from the stool sample (Figure 4.4). There was no major difference between the prevalence of *Shigella flexneri* and *Shigella dysenteriae* in the two populations. However, *Shigella boydii* was more prevalent in Kilifi compared to Nairobi West and the vice-versa for *Shigella sonnei*. Enteroinvasive *Escherichia coli* species constituted 11% of the 80 bacterial isolates with 10% being from Nairobi West. *Salmonella* Typhi and *Yersinia enterocoritica* were 4% and 1% of the 80 isolates respectively.

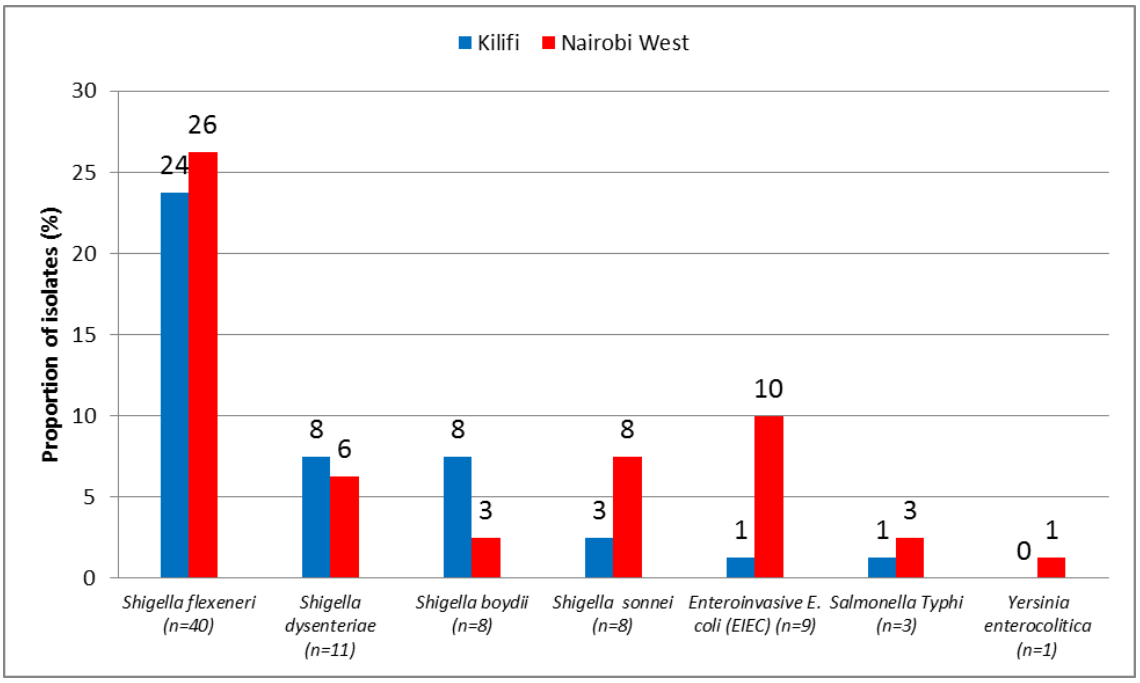



**Figure 7.4: Bacteria species isolation in Kilifi and Nairobi West**

***Co-infections among cases in Kilifi and Nairobi West***

Four of the stool samples were found to have both *Escherichia coli* and *Shigella* spp. Three cases were found to have a co-infection of *Shigella flexneri* and Enteroinvasive *Escherichia coli* (EIEC), two were from Nairobi West and one from Kilifi. One case in Nairobi West had a co-infection of *Shigella sonnei* and an Enteroinvasive *Escherichia coli* (EIEC).

## 4.6 Antimicrobial susceptibility pattern of bacterial pathogens

### 4.6.1 Drug susceptibility to all bacterial isolates in Kilifi and Nairobi West

Drug susceptibility of all bacterial isolates in Kilifi is shown below in Figure 4.5. Bacterial pathogens were most resistant to Sulfamethoxazole-Trimethoprim in Kilifi (94.3%). All bacterial pathogens were 100% sensitive to ciprofloxacin and ceftriaxone

in Kilifi. Bacterial resistance to ampicillin showed huge variation; Kilifi (85.7%) compared to Nairobi West (42.2%).

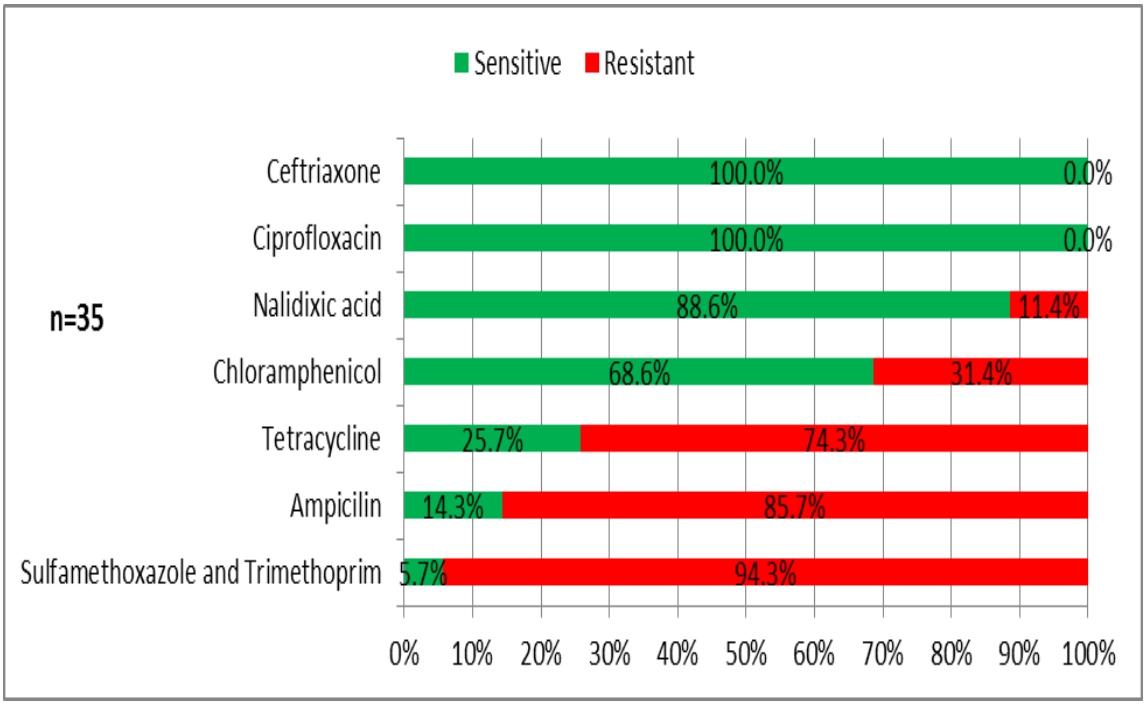



**Figure 8.5: Drug susceptibility to bacterial pathogens in Kilifi**

Drug susceptibility of all bacterial isolates in Nairobi West is shown below in Figure 4.6. Bacterial pathogens were most resistant to Sulfamethoxazole-Trimethoprim in Nairobi West (97.8%). Unlike Kilifi, resistance to ciprofloxacin (4.4%) and ceftriaxone (2.2%) were reported in Nairobi West.

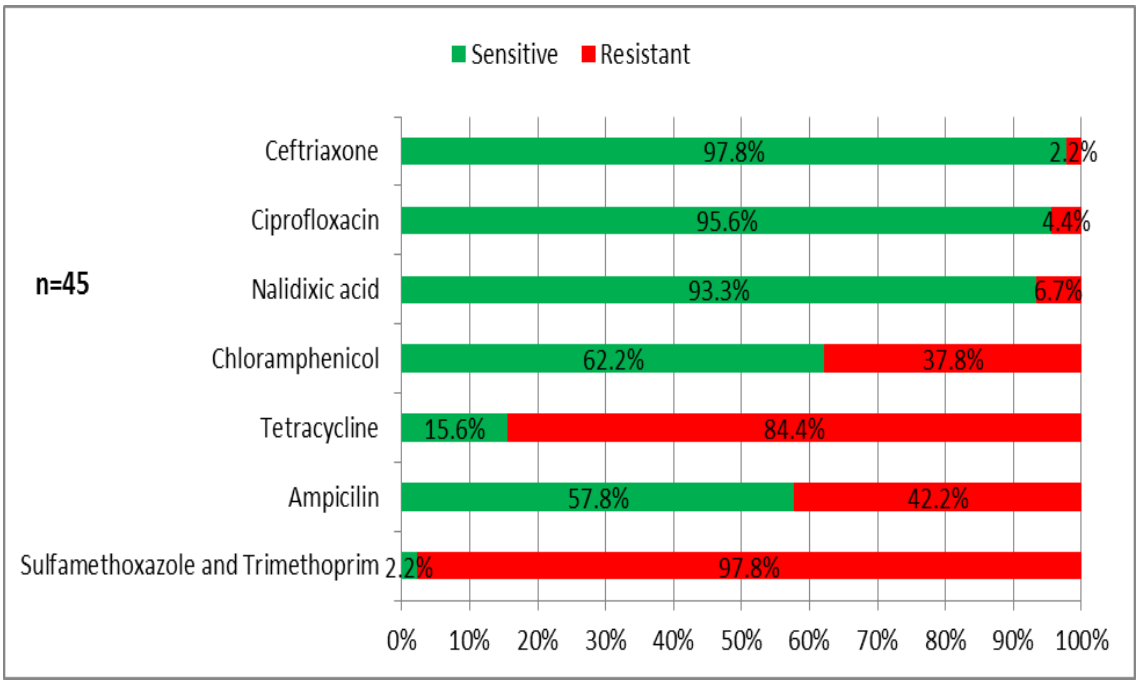



**Figure 9.6: Drug susceptibility to bacterial pathogens in Nairobi West**

### 4.6.2 Drug susceptibility to *Shigella* in Kilifi and Nairobi West

Sulfamethoxazole-trimethoprim showed high resistance to *Shigella* spp in Kilifi (94%) and Nairobi West (100%) followed by tetracycline, ampicillin and chloramphenicol. Ciprofloxacin showed low resistance in Nairobi West (5.9%) but no resistance in Kilifi (0%). Ceftriaxone was the only drug that had no resistance in both settings. There was significant difference in resistance to ampicillin in Kilifi (88%) and Nairobi West (32.4%). Similarly, resistance to nalidixic acid in Kilifi (33%) and Nairobi West (2.9%) showed significant difference (Figure 4.7).

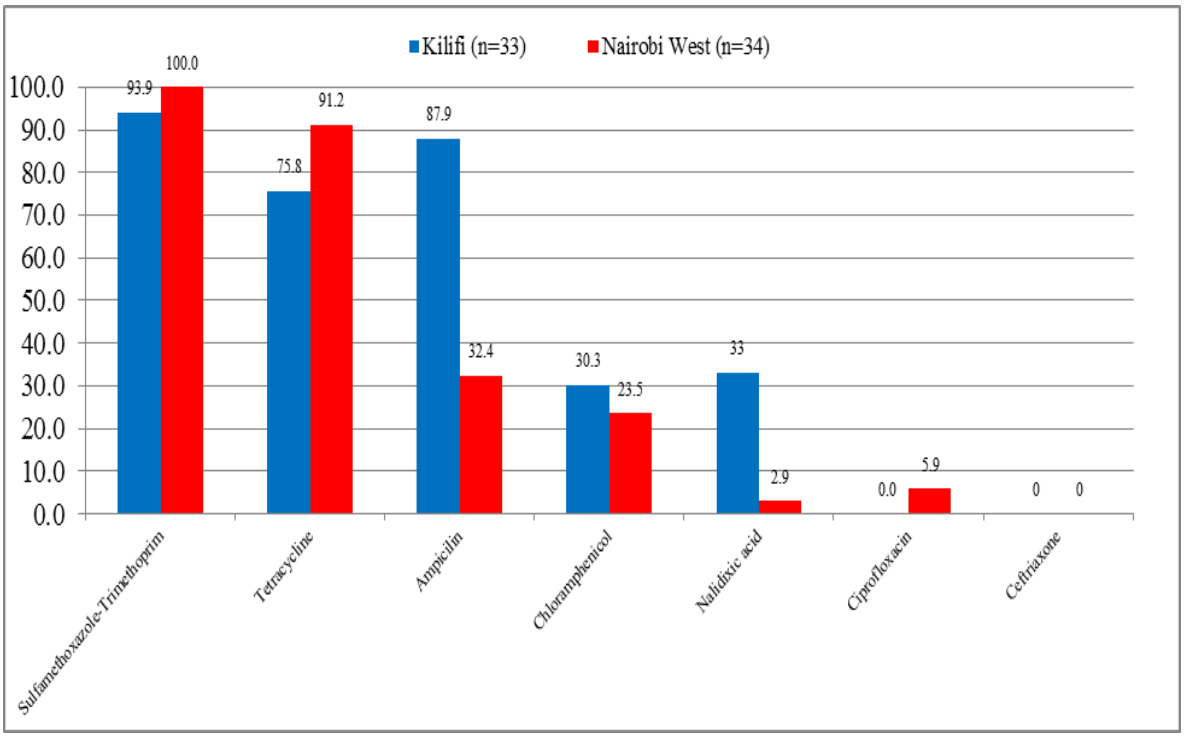



**Figure 10.7: Drug susceptibility to *Shigella* spp in Kilifi and Nairobi West**

### 4.6.2.1 Drug susceptibility among *Shigella* species in Kilifi and Nairobi West

Sulphamethoxazole-trimethoprim was 100% resistant to *Shigella flexneri, Shigella dysentriae, Shigella sonnei* and *Shigella boydii* in Nairobi west. In Kilifi, the drug was 100% resistant to *Shigella dysentriae, Shigella sonnei, Shigella boydii* and 89.5% resistant to *Shigella flexneri.* There was no resistance to ceftriaxone across all the four species in both settings. Emerging resistance to ciprofloxacin was observed in 9.5% of *Shigella flexneri* in Nairobi West. Resistance to ampicillin showed significant variation among *Shigella flexneri* in Kilifi (94.7%) and Nairobi West (23.8%). Tabulation of resistance by *Shigella* species is reflected below (Table 4.7).

**Table 10.7: Antimicrobial resistance among Shigella spp**

| ***Shigella* spp** | Rural/Urban | SXT | Tetracycline | Ampicilin | CAF | Nalidixic acid | Cipro | CFX |
|---|---|---|---|---|---|---|---|---|
| *Shigella flexeneri (n=40)* | Kilifi (n=19) | 89.5% | 73.7% | 94.7% | 36.8% | 15.8% | 0.0% | 0.0% |
| | Nairobi West (n=21) | 100.0% | 95.2% | 23.8% | 19.0% | 0.0% | 9.5% | 0.0% |
| *Shigella boydii (n=8)* | Kilifi (n=6) | 100.0% | 66.7% | 100.0% | 16.7% | 0.0% | 0.0% | 0.0% |
| | Nairobi West (n=2) | 100.0% | 100.0% | 50.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| *Shigella sonnei (n=8)* | Kilifi (n=2) | 100.0% | 100.0% | 50.0% | 50.0% | 0.0% | 0.0% | 0.0% |
| | Nairobi West (n=6) | 100.0% | 83.3% | 50.0% | 50.0% | 0.0% | 0.0% | 0.0% |
| *Shigella dysenteriae (n=11)* | Kilifi (n=6) | 100.0% | 83.3% | 66.7% | 16.7% | 0.0% | 0.0% | 0.0% |
| | Nairobi West (n=5) | 100.0% | 80.0% | 40.0% | 20.0% | 20.0% | 0.0% | 0.0% |

SXT=Sulfamethoxazole-Trimethoprim; CAF = Chloramphenicol; Cipro= Ciprofloxacin; CFX= Ceftriaxone

### 4.6.3 Drug susceptibility of the *Salmonella* Typhi

Three *Salmonella* Typhi were isolated, two from Nairobi West and one from Kilifi. All the three *Salmonella* Typhi isolates were highly resistant to; sulfamethaxazole-trimethoprim (100%), tetracycline (66.7%), chloramphenicol (66.7%) and ampicillin (66.7%) but 100% sensitive to ciprofloxacin, nalidixic acid and ceftriaxone.

### 4.6.4 Drug Susceptibility of the Enteroinvasive *Escherichia coli* pathotype

A total of nine Enteroinvasive *Escherichia coli* were isolated, eight from Nairobi West and one from Kilifi. High levels of antimicrobial resistance were observed among the Enteroinvasive *Escherichia coli*. All the nine Enteroinvasive *Escherichia coli* isolates

were highly resistant to; sulfamethaxazole- trimethoprim (88.9%), chloramphenicol (77.8%), ampicillin (66.7%), tetracycline (55.5%) and nalidixic acid (33.3%). The isolates were found to be 100% sensitive to ciprofloxacin but showed emerging resistance to ceftriaxone (11.1%) and amoxicillin-clavulanic acid (11.1%) (Figure 4.8).

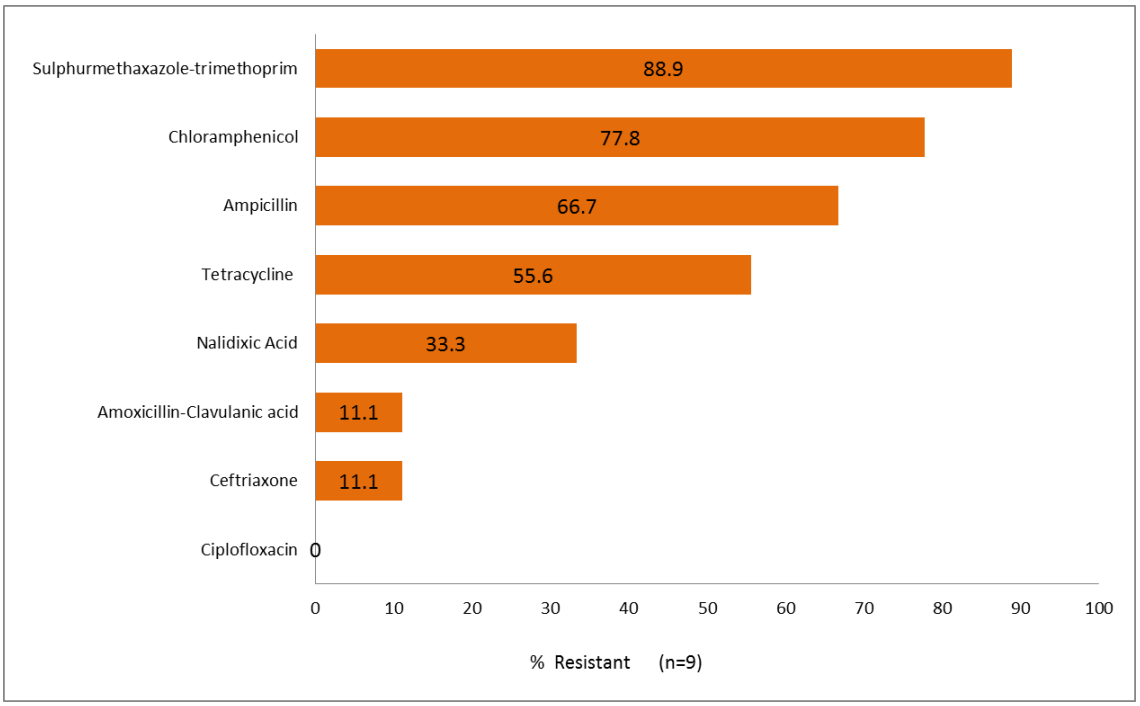



**Figure 11.8: Drug susceptibility to enteroinvasive *E. coli* pathotype**

### 4.7 Multidrug resistance

Resistance to 3 or more antimicrobial agents determinative of characteristic of multidrug resistance profile was observed as reflected below (Figure 4.9). The prevalence of multidrug resistance in Kilifi was 68.6% while in Nairobi West it was 55.5%. The overall prevalence of multidrug resistance for the bacterial pathogens in both settings was 61.25%.

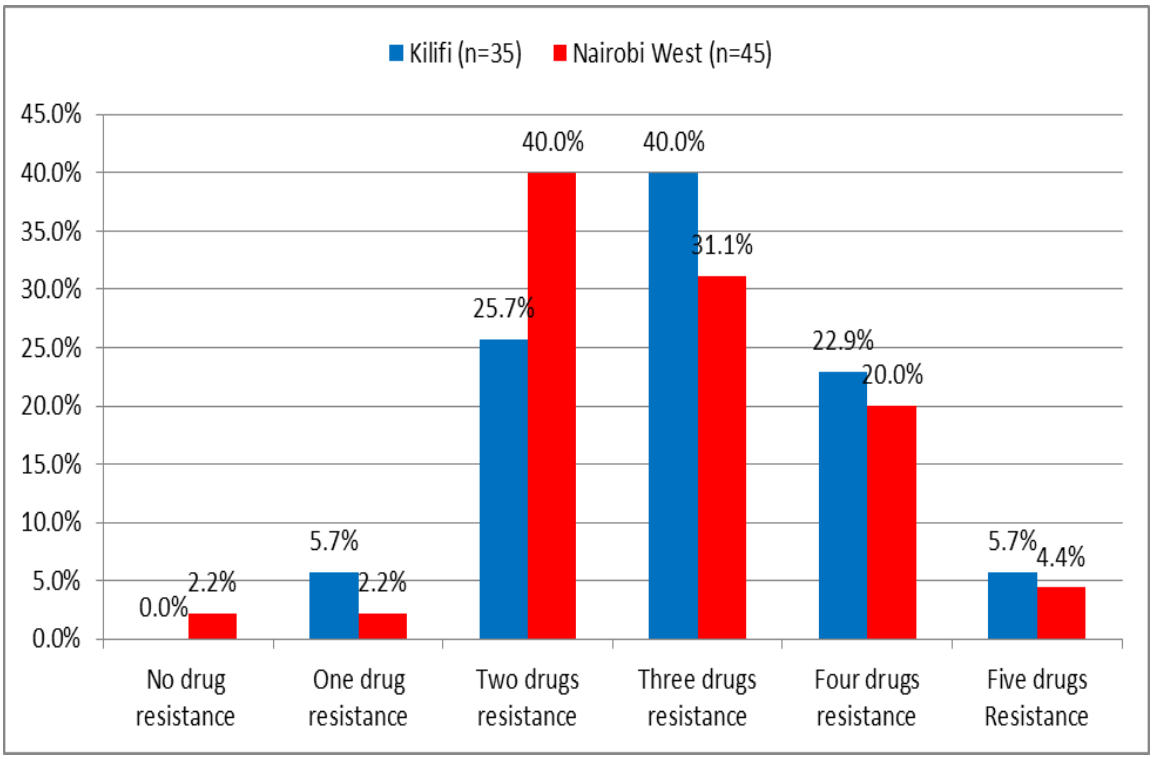



**Figure 12.9: Bacterial multidrug resistance in Kilifi and Nairobi West**

### 4.7.1 Multidrug resistance to *Shigella* species.

Multidrug resistance was highest among the *Shigella* species at 56.7% (n=38/67). The level of multidrug resistance varied among the four *Shigella* species. *Shigella boydii* had the highest multidrug resistance at 62.5% (n=5/8) followed by *Shigella flexneri* at 57.5% (23/40); *Shigella sonnei* had the least at 50.0% (n=4/ 8) (Figure 4.10).

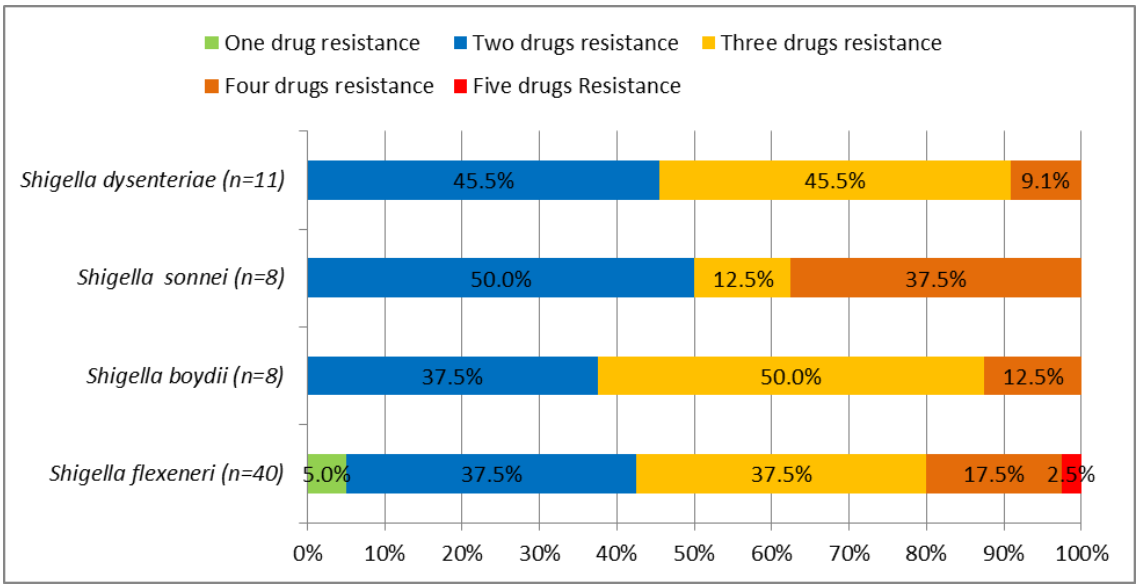



**Figure 13.10: Multidrug resistant *Shigella* species in Kilifi and Nairobi West**

Analysis of antibiotic resistance in Kilifi (Figure 4.11) and Nairobi West (Figure 4.12) showed significant difference in multidrug resistance. The multidrug resistance for all the four species was 69.7% in Kilifi compared to 44.1% in Nairobi West. The greatest variation was observed among the *Shigella flexneri* which had high multi drug resistance in Kilifi (78.9%) compared to Nairobi West (38.1%).

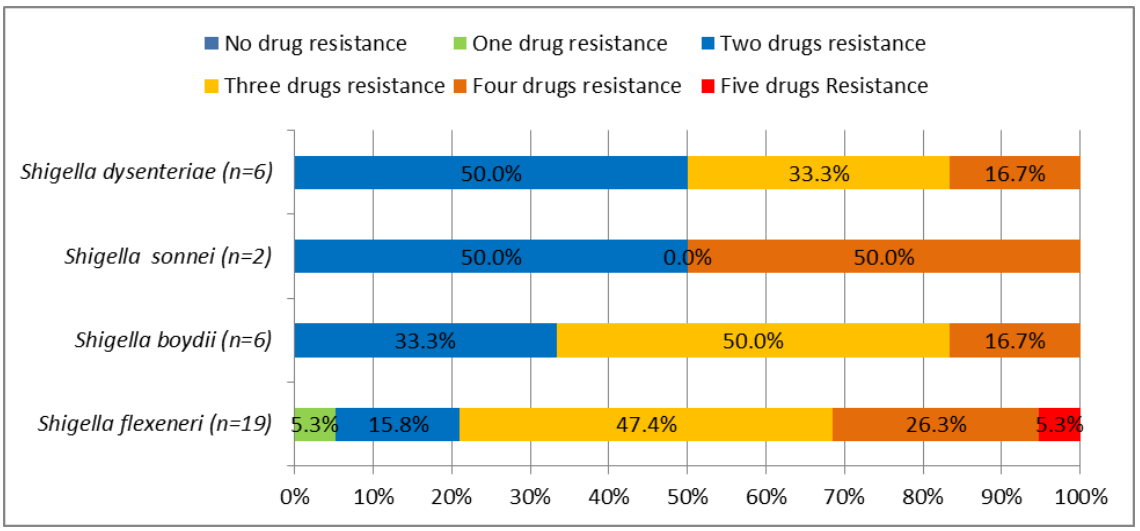



**Figure 14.11: Multidrug resistant Shigella species in Kilifi**

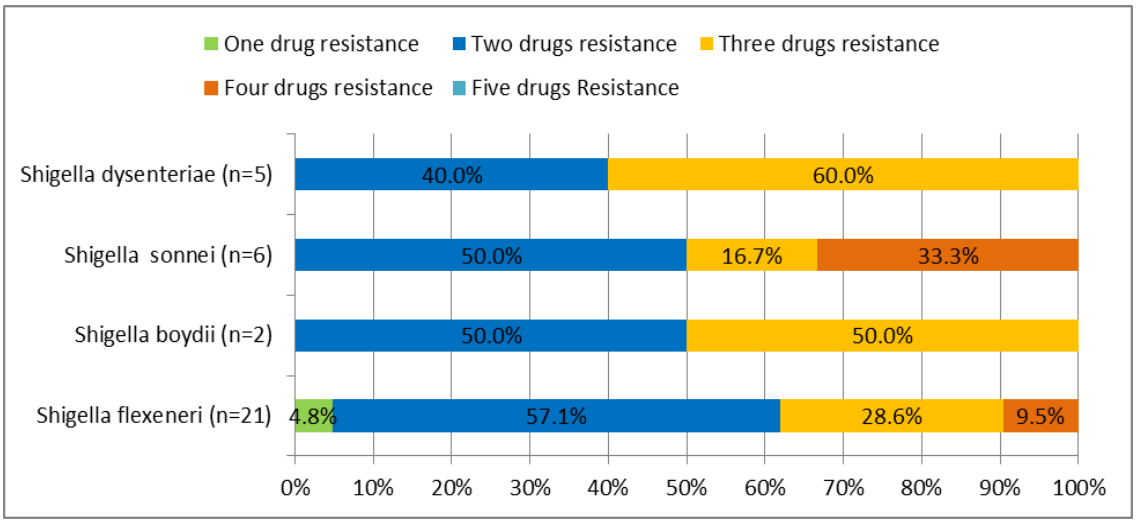



**Figure 15.12: Multidrug resistant *Shigella* species in Nairobi West**

### 4.7.2 Multidrug resistant *Salmonella* Typhi

Two of the three *Salmonella* Typhi isolates were resistant to four drugs while one was resistant to only one drug. Comparison between Kilifi and Nairobi West could not be done because of the low number of isolates.

### 4.7.3 Multidrug resistant Enteroinvasive *Escherichia coli* pathotype

About 89% (8/9) of the Enteroinvasive *Escherichia coli* pathotype isolates were resistant to three or more drugs (Figure 4.13). Only one *Escherichia coli* isolate was fully susceptible to all antibiotics tested while three isolates were resistant to three drugs, two isolates were resistant to four drugs while 3 isolates were resistant to five drugs. Comparison between Kilifi and Nairobi West could not be done because eight of the isolates were from Nairobi West and only one was from Kilifi.

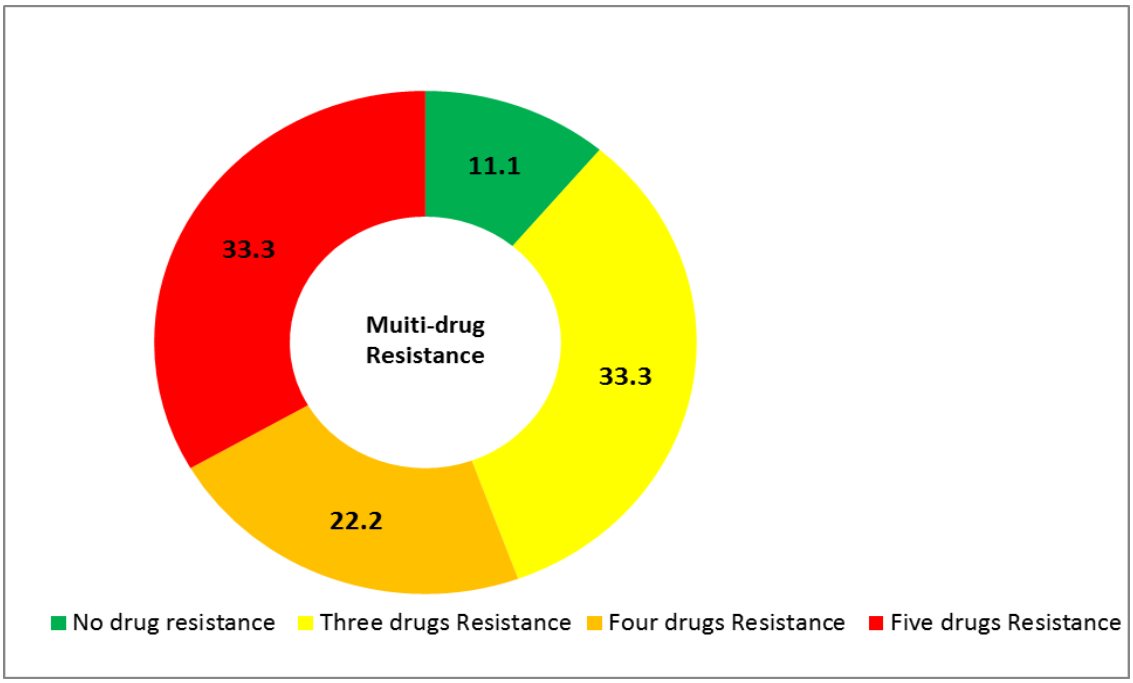



**Figure 16.13: Multidrug resistant Enteroinvasive *E. coli* pathotype**

### 4.8 Prescribed antibiotics and antimicrobial resistance levels

Antibiotic prescriptions were given to 93% (162/174) of the patients presenting with acute bloody diarrhoea in Kilifi and 73.6% (81/110) in Nairobi West. Antibiotic susceptibility was done for seven (7) which were prescribed to 95.7% (155/162) of the cases in Kilifi and 91.4% (74/81) in Nairobi West.

Comparing the clinical practice in the two settings (Figures 4.14 & 4.15), Nairobi West prescribed to 67.6% of the patients drugs that were within the high resistance zone (resistant to >20% bacterial pathogens) compared to 47.7% in Kilifi. These antibiotic were; sulfamethaxazole-trimethoprim, Tetracycline/doxycycline, amoxicillin/ampicillin and chloramphenicol.

Although bacterial pathogens were highly resistant to sulfamethaxazole-trimethoprim in Kilifi (94.3%) and Nairobi West (97.8%), it was the most prescribed antibiotic in Nairobi West (36.5%) and second highest in Kilifi (16.8%).

Statistically, there was insignificant negative (inverse) association between prescribed antibiotic and antibiotic resistance levels in Kilifi (correlation coefficient, $r$= -0.244).

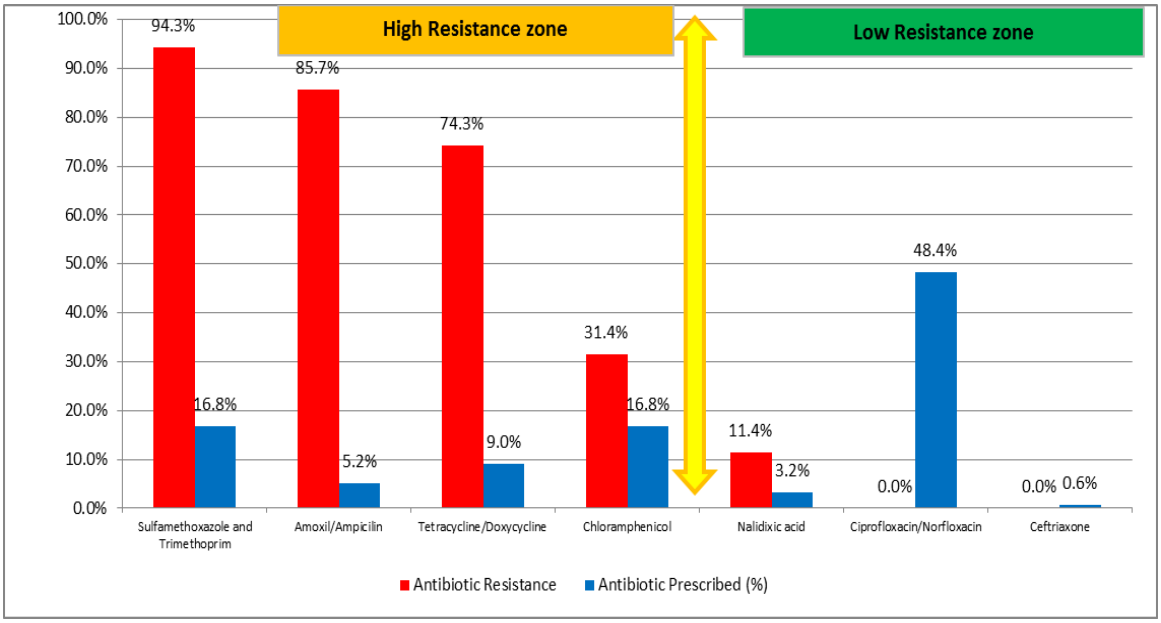



**Figure 17.14: Prescribed antibiotics and level of resistance in Kilifi**

Statistically, there was a significant positive association between prescribed antibiotic and antibiotic resistance levels in Nairobi West (correlation coefficient= 0.60).

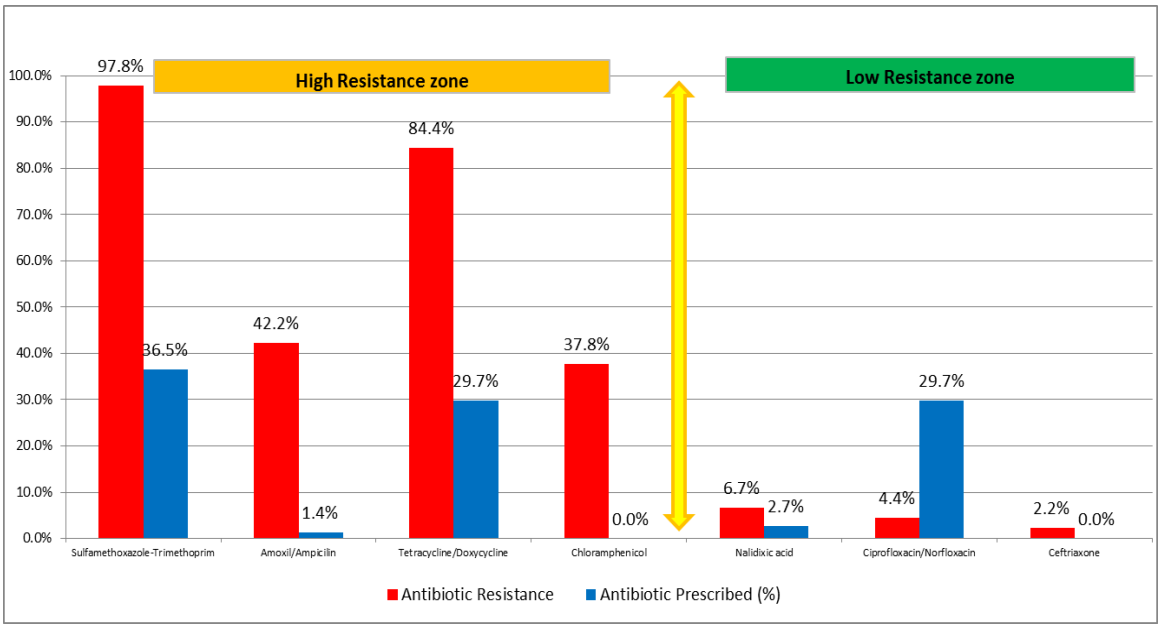



**Figure 18.15: Prescribed antibiotics and level of resistance in Nairobi West**

### 4.9 Seasonal variation of acute bloody diarrhoea cases in rural and urban

The climatic data used (rainfall and temperature) was obtained from the Kenya Meteorological department. Climatic conditions differed in the two areas of study. In Nairobi (urban), the average monthly rainfall during the study period was 123mm, this was higher than the long term mean 85.2mm for the period 1971- 2013. In Kilifi (rural), the average monthly rainfall for the study period was 86.6mm which was within the range of the long term mean of 86.9mm. In Nairobi, the mean monthly maximum and minimum temperatures over the study period were 24.4°C and 13.6°C respectively. These were both within the range of the long term mean maximum and minimum temperatures which were 24°C and 13.2°C. In Kilifi, the mean maximum temperature

for the study period was 29.9°C which was similar to the long term mean of 29.8°C. The mean minimum temperature for the study period was (24.0°C) which was higher than the long term mean (23.4°C). The seasonal patterns of acute bloody diarrhoea were the same in these two sites with two peaks in the year (April and October) (Figure 4.16 and 4.17).

There was a positive correlation between acute bloody diarrhoea and long term (1971-2013) mean rainfall both in rural and urban populations. In Kilifi (rural), the correlation was moderate, Pearson's r=0.55 (Figure 4.17) whereas in Nairobi West (urban), there was a strong correlation, Pearson's r=0.85 (Figure 4.16). There was also a positive correlation between acute bloody diarrhoea and long term (1974-2013, source: meteorological department) mean maximum and minimum temperatures but the correlation with minimum temperatures was stronger than that with maximum temperatures. In Kilifi (rural) the correlation was moderate, Pearson's r=0.42 for long term minimum temperatures and weak for maximum temperatures (Pearson's r=0.23). In Nairobi (urban), the correlation with long term minimum temperatures was strong, Pearson's r=0.76; the association with long time maximum temperatures was however weak, Pearson's r=0.27

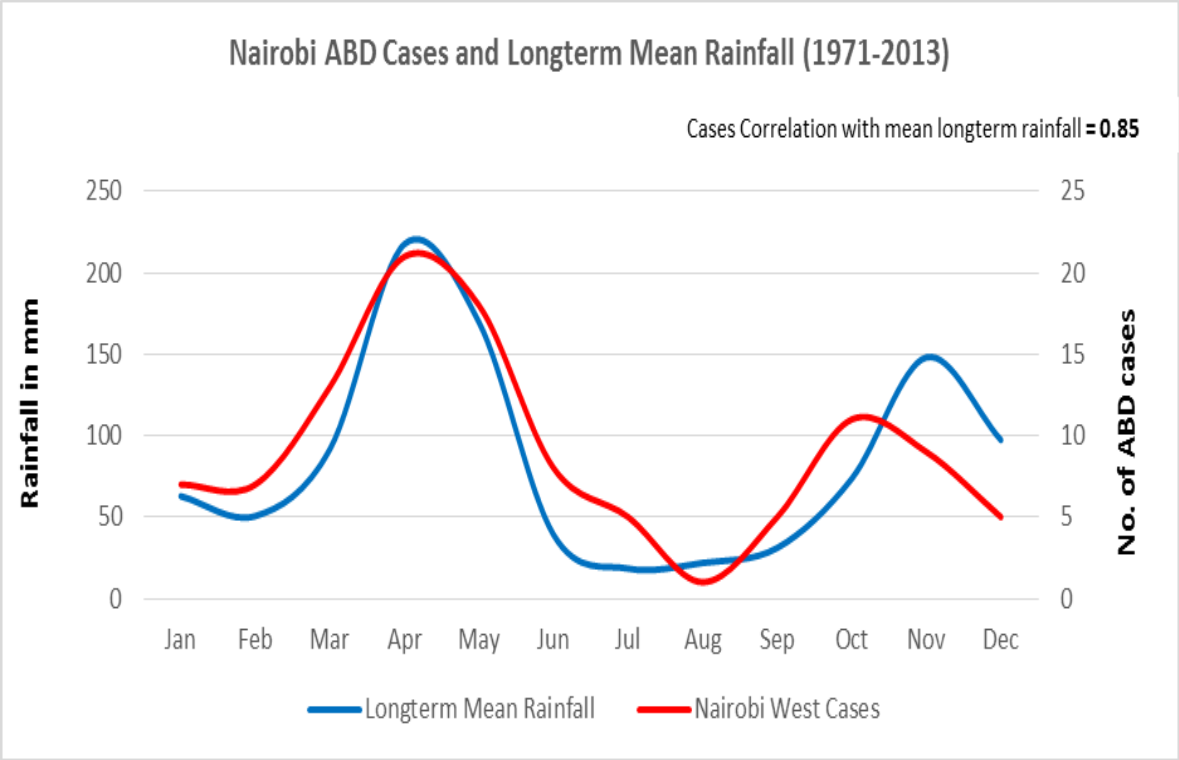



**Figure 19.16: Correlation between cases and long term mean rainfall in Nairobi**

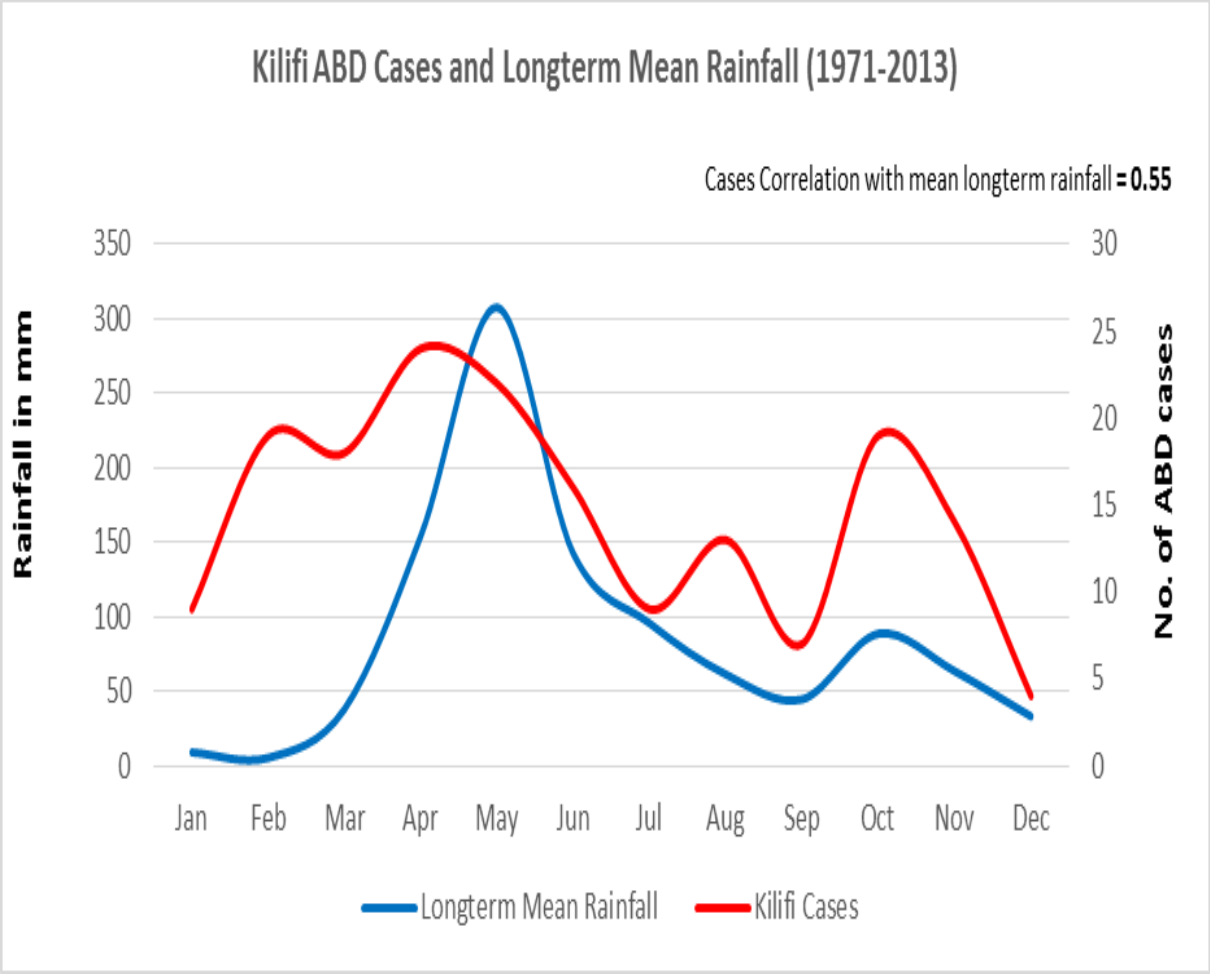



**Figure 20.17: Correlation between cases and long term mean rainfall in Kilifi**

### 4.10 Factors associated with acute bloody diarrhoea

#### 4.10.1 Significant factor associated with acute bloody diarrhoea in rural (Kilifi) and urban (Nairobi West) settings on bivariate analysis

About 70 potential factors associated with bloody diarrhoea were examined for any association (Appendix 18) in rural and urban settings. In the rural setting, a total of seven factors (6 protective and 1 risk factor) were statistically significant on bivariate

analysis; drinking water stored separately (OR= 0.66, 95% CI 0.453-0.974, p=0.036), condition of toilet clean (OR= 0.5, 95% CI 0.257-0.972, p=0.038), always washing hands after defecating (OR= 0.56, 95% CI 0.381-0.822, p=0.003), having washed hands after last defecation (OR= 0.49, 95% CI 0.0.309-0.772, p=0.002), always washing hands after disposing child's stool (OR= 0.61, 95% CI 0.384-0.958, p=0.032) and poor general compound cleanliness (OR= 3.1, 95% CI 1.656-5.736, p=0.000) (Table 4.8).

**Table 11.8: Significant factors on bivariate analysis in Kilifi**

| | **Cases** | | **Controls** | | | | |
|---|---|---|---|---|---|---|---|
| **Factors** | **Yes n(%)** | **No n(%)** | **Yes n(%)** | **No n(%)** | **Odds Ratio** | **95% CI** | ***P* value** |
| Drinking water stored separately | 66 (41) | 94 (59) | 166 (51) | 157 (49) | 0.66 | 0.45-0.97 | **0.036*** |
| Eating food cooked previous day | 55 (36) | 99 (64) | 143 (46) | 169 (54) | 0.66 | 0.44-0.98 | **0.038*** |
| Condition of toilet is clean | 155 (89) | 19 (11) | 310 (94) | 19 (6) | 0.50 | 0.26-0.97 | **0.038*** |
| Always hand-wash after defecating | 74 (47) | 85 (53) | 193 (61) | 124 (39) | 0.56 | 0.38-0.82 | **0.003*** |
| Washed hands after last defecation | 103 (69) | 46 (31) | 243 (82) | 53 (18) | 0.49 | 0.31-0.77 | **0.002*** |
| Always wash hands after disposing child's stool | 56 (50) | 55 (50) | 146 (63) | 87 (37) | 0.61 | 0.38-0.96 | **0.032*** |
| Poor compound cleanliness | 27 (17) | 136 (83) | 19 (6) | 295 (94) | 3.08 | 1.66-5.74 | **0.00023*** |

*statistically significant ($p<0.05$)

In the urban setting, a total of four factors (2 protective and 2 risk factors) were statistically significant on bivariate analysis; other diarrhoea cases in household in previous 2 weeks (OR=3.56, 95% CI 1.452-8.712, *P*=004), drinking water stored in a super drum (OR= 0.31, 95% CI 0.159-0.616, *p*=0.001), wide mouthed water storage container (OR= 1.82, 95% CI 1.033-3.204, *p*=0.037) and having washed hands after last defecation (OR= 0.38, 95% CI 0.161-0.879, *p*=0.020) (Table 4.9 ).

**Table 12.9: Significant factors on bivariate analysis in Nairobi**

| Factors | Cases | | Controls | | Odds Ratio | 95% CI | *P* value |
|---|---|---|---|---|---|---|---|
| | Yes n(%) | No n(%) | Yes n(%) | No n(%) | | | |
| Other diarrhoea cases in household in previous 2 weeks | 13 (16) | 67 (84) | 9 (5) | 165 (95) | 3.56 | 1.45-8.72 | **0.0036*** |
| Drinking water stored in super drum | 12 (11) | 98 (89) | 54 (28) | 138 (72) | 0.31 | 0.16-0.62 | **0.001*** |
| Wide mouthed water storage container | 64 (74) | 23 (26) | 104 (60) | 68 (40) | 1.82 | 1.03-3.20 | **0.037*** |
| Washed hands after last defecation | 71 (85) | 13 (15) | 160 (94) | 11 (6) | 0.38 | 0.16-0.88 | **0.02*** |

*statistically significant ($p<0.05$)

In both rural and urban settings, bivariate analysis identified eight (8) factors that were significantly associated with acute bloody diarrhoea, six protective and two risk factors (Table 4.10).

**Table 13.10: Significant factors on bivariate analysis in Kilifi and Nairobi**

| Factors | Cases | | Controls | | Odds Ratio | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| | Yes | No | Yes | No | | | |
| | n (%) | n (%) | n (%) | n (%) | | | |
| Drinking water stored in super drum | 29 (10.2) | 255 (89.8) | 90 (17.3) | 431 (82.7) | 0.55 | .035-0.85 | **0.007*** |
| Drinking water stored separately | 124(49.8) | 125(50.2) | 290(57.9) | 211(42.1) | 0.72 | 0.53-0.98 | **0.036*** |
| Coliform in main water source | 43 (31.4) | 94 (68.6) | 26 (18.8) | 112 (81.2) | 1.97 | 1.13-3.45 | **0.016*** |
| Main water source protected | 131 (78.0) | 37 (22.0) | 135 (86.5) | 21 (13.5) | 0.55 | 0.31-0.99 | **0.045*** |
| Always hand-wash after defecating | 133(53.6) | 115(46.4) | 324(65.2) | 173(34.8) | 0.62 | 0.45-0.84 | **0.002*** |
| Washed hands after last defecation | 174(74.7) | 59(25.3) | 403(86.3) | 64(13.7) | 0.47 | 0.32-0.70 | **0.0001*** |
| Always wash hands after disposing child's stool | 105 (56.8) | 80 (43.2) | 253 (65.4) | 134 (34.6) | 0.70 | 0.49-0.99 | **0.046*** |
| Poor compound cleanliness | 48(19.2) | 202(80.8) | 59(12.1) | 429(87.9) | 1.73 | 1.12-2.62 | **0.009*** |

*statistically significant ($p<0.05$)

### 4.10.2 Significant factor associated with acute bloody diarrhoea in rural (Kilifi) and urban (Nairobi West) settings on binary logistic regression analysis

Logistics regression was done separately for significant factors in each setting; two factors in rural and three factors in urban setting remained independently and significantly associated with acute bloody diarrhoea at 5% significance level. In rural setting the factors were; cleanliness of toilet (OR=0.38, 95% CI 0.15-0.95, *p*=0.038) and poor general compound cleanliness (OR=2.76, 95% CI 1.17-6.49, *p*=0.020) (Table 4.11)

**Table 14.11: Significant factors on binary logistic regression analysis in Kilifi**

| | | | 95% C.I. | |
|---|---|---|---|---|
| **Variable in the equation** | ***P* value** | **Odds Ratio** | **Lower** | **Upper** |
| Drinking water stored separately | 0.710 | 1.114 | 0.632 | 1.965 |
| Eating food cooked previous day | 0.249 | 0.740 | 0.444 | 1.234 |
| Toilet condition is clean | **0.038*** | 0.376 | 0.149 | 0.947 |
| Always hand-wash after defecating | 0.154 | 0.592 | 0.288 | 1.217 |
| Washed hands after last defecation | 0.869 | 0.939 | 0.443 | 1.989 |
| Always washes hands after disposing child's stool | 0.700 | 1.151 | 0.563 | 2.354 |
| Poor general compound cleanliness | **0.020*** | 2.756 | 1.170 | 6.490 |

* statistically Significant (*p*=<0.05)

In urban setting; other diarrhoea cases in household in previous 2 weeks (OR=3.64, 95% CI 1.407-9.406, *p*=0.008), drinking water stored in a super drum (OR=0.353, 95% CI 0.119-0.890, *p*=0.029) and having washed hands after last defecation (OR=0.326, 95% CI 0.138-0. 901, *p*=0.029) were associated with acute bloody diarrhoea transmission (Table 4.12).

**Table 15.12: Significant factors on binary logistic regression analysis in Nairobi**

| | | Odds | 95% C.I. | |
|---|---|---|---|---|
| **Variables in the equation** | ***p* value** | **Ratio** | **Lower** | **Upper** |
| Other diarrhoea cases in household in previous 2 weeks | **0.008*** | 3.638 | 1.407 | 9.406 |
| Drinking water stored in a super drum | **0.029*** | .326 | .119 | .890 |
| Wide mouthed water storage container | .833 | 1.091 | .485 | 2.454 |
| Hand washed after last defecation | **0.029*** | .353 | .138 | .901 |

*statistically Significant (*p*=<0.05)

In both rural and urban settings, after running binary logistic regression, Storage of drinking water separate from water for other use (OR=0.41, 95% CI 0.20-0.87, p=0.021), washing hands after last defecation (OR=0.24, 95% CI 0.08-.076, p=0.015) and presence of coliform in main source water (OR=2.56, CI 1.21-5.4, p=0.014) remained independently and significantly associated with acute bloody diarrhoea at 5% significance level (Table 4.13 )

**Table 16.13: Significant factors on binary logistic regression analysis in both settings**

| Factors | p-value | Odds ratio | 95% CI | |
|---|---|---|---|---|
| | | | Lower | Upper |
| Drinking water storage in super drum | 0.145 | 0.51 | 0.21 | 1.26 |
| Storage of drinking water separate from water for other use | **0.021*** | 0.41 | 0.20 | 0.87 |
| Hand-washed after defecating | 0.972 | 1.02 | 0.38 | 2.76 |
| Hand-washed after last defecation | **0.015*** | 0.24 | 0.08 | 0.76 |
| Hand-washed after child's stool disposal | 0.339 | 0.65 | 0.27 | 1.58 |
| Poor compound cleanliness | 0.97 | 1.02 | 0.35 | 2.95 |
| Main water source protected | 0.813 | 1.12 | 0.43 | 2.92 |
| Coliforms present in main source water | **0.014*** | 2.56 | 1.21 | 5.40 |

***statistically significant factors.** The reliability of the model was 66.1%

### 4.11 Comparison of water quality between rural (Kilifi) and urban (Nairobi West) settings

Detection of coliform bacteria and *Escherichia coli* in drinking water was used as markers of faecal contamination. Most probable number (MPN) test was done to detect the coliform in water samples collected from households and main water sources. Drinking water in the sampled communities was found to be of variable microbial safety and quality. The microbial indicators of faecal contamination (total coliforms and faecal coliforms) were detected in household water and main source. A total of 302 water samples were collected and analysed from both settings. In the rural setting, 40.9% of the household water tested (n=171) contained total coliforms and 21.5 % faecal coliforms whereas 38.6% of the main source contained total coliforms and 11.9% faecal coliforms (Figure 4.18). In the urban setting, 10.7% of the household water tested (n=131) contained total coliforms and 6.2 % faecal coliforms whereas 8.2% of the main source contained total coliforms and 6.2% faecal coliforms (Figure 4.18).

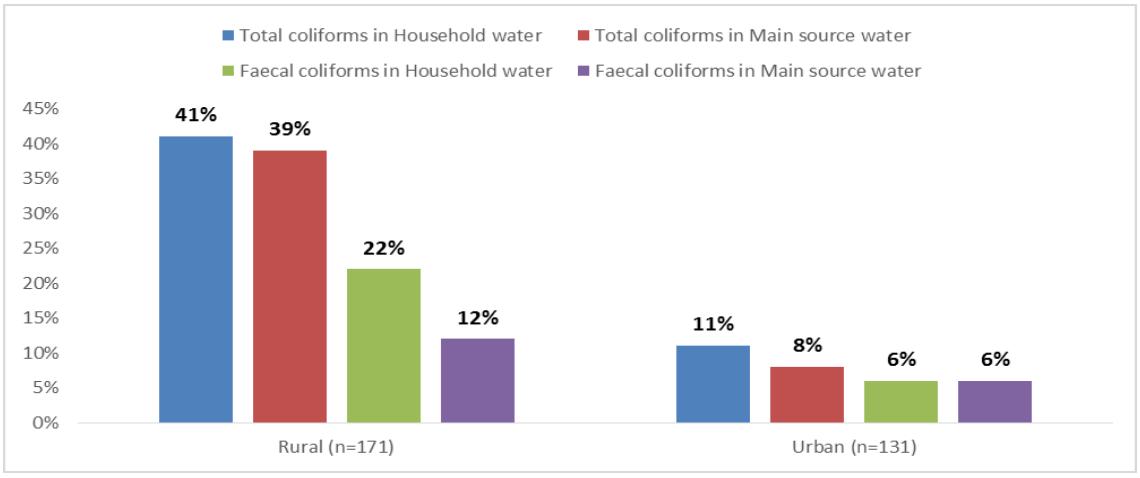



**Figure 21.18: Comparison of water quality in rural and urban**

# CHAPTER FIVE

# DISCUSSION

## 5.1 Presenting clinical features of acute bloody diarrhoea

The main signs and symptoms among patients associated with acute bloody diarrhoea in both settings were: blood in stool (100%), abdominal pain (69%), mucous in stool (60%), loose stools (54%) and anorexia (50%). Fever was present in 89 (34%) of the cases. There was no significant difference on the top 5 signs and symptoms in both setting but cases in Urban had on average a 5.5% higher proportion of signs and symptoms when the whole clinical spectrum was considered. This observation could be attributed to the fact that the isolation rate for enteric pathogens were significantly higher in urban (66%) compared to the rural (25%), this is an indicator of an ongoing active infection. Based on the socio-demographic data, the urban folks were better in many aspects including; education, income and access to health services. All these factors influence the health seeking behaviour of the populations directly or in-directly. The proportion of cases presenting with diverse clinical symptoms were lower in our study compared to the finding in a similar study in rural western part of Kenya where the main symptoms were reported as; abdominal cramping (78%), fever (76%), nausea (53%), vomiting (31%), coincident mucous diarrhoea (82%) and coincident watery diarrhoea (56%) (Brooks *et al*., 2003). There were no differences in the duration of diarrhoea among those with acute bloody diarrhoea due to bacterial and protozoal pathogens. The frequency of other clinical features were similar to a study in Turkey (Kuşkonmaz, Yurdakök, Yalçin, & Ozmert, 2009).

## 5.2 Prevalence of enteric pathogens

The main etiologic agents for acute bloody diarrhoea were *Shigella* and *E. histolytic*a with isolation rate of about 23% and 10.2% respectively among cases. Overall, 1092 specimens (31.7%) yielded at least 1 pathogen, Isolation rates were greater for specimens from patients with bloody diarrhoea (46%) than for specimens from patients with watery (29%), mucoid (25%), or uncharacterized diarrhoea (22%)

The *Shigella* isolation rate was approximately half of that seen (46%) in studies conducted in the western part of Kenya (Brooks *et al*., 2003; Brooks *et al*., 2006) but much higher compared with other studies done in Iran and Cameroon (Farshad, Sheikhi, Japoni, Basiri, & Alborzi, 2006; Njunda *et al*., 2012). In a study done in Cameroon, a total of 223 stool samples were cultured, 10 (4.5%) yielded *Shigella* species. However, the isolation rate was observed to be higher in rural areas (6.35%) (Njunda *et al.,* 2012). In our study, the *Shigella* isolation rate among cases in both settings was at 23% which was five times higher compared with the study done in Cameroon. In our study, the isolation rate was higher in the Urban site (29%) compared with the rural site (19%).

The proportions of the different species of *Shigella* isolated in our study showed a similar pattern to another study done in Kenya where *Shigella flexneri* was the commonest species followed by *Shigella dysenteriae, Shigella boydii and Shigella sonnei* in decreasing order for both studies (Brooks *et al*., 2003). This was however contrary to the findings of other studies where *Shigella Sonnei* was the most prevalent

species (Farshad *et al.*, 2006). The isolation rate of *E. histolytica* is similar to that in a study in Nigeria which had an isolation rate of 11% (Nyeke, Chukwujekwu, Stanley, & Awoibi, 2008).

### 5.3 Antimicrobial susceptibility pattern

Antibiotics misuse by both general public and health professional is rampant in low income countries where laboratory facilities are limited. High antimicrobial resistance to the commonly prescribed antibiotics like sulphamethoxazole-trimethoprim (86.3%), tetracycline (73.8%), ampicillin (63.8%) and chloramphenicol (21.3%) observed among enteric bacterial pathogens in our study is similar to that found in other studies done in Kenya (Brooks *et al.*, 2003; Sang, 2011). *Shigella* species represent one of the growing numbers of antimicrobial-resistant bacteria in developing countries. In our study, *Shigella* species exhibited high resistance to these antibiotics; sulphamethoxazole-trimethoprim (97%), tetracycline (83.6%), ampicillin (60.2%) and chloramphenicol (26.9%). These findings agree with studies done in other parts of the world which indicate that resistance to low cost antibiotics has reached unprecedented proportions.

A study done in India where *Shigella* species from different parts of India were analysed for antimicrobial susceptibility, much higher resistance levels to ampicillin (97%), tetracycline (95%) and chloramphenicol (94%) were reported (Pazhani *et al.*, 2008). Studies done in China, have also shown high degree of resistance among the *Shigella* species; tetracycline 74%-97%, ampicillin 85%-100%, sulphamethoxazole-trimethoprim 69%-97% and chloramphenicol 77% (Shen *et al.*, 2013; C.-L. Zhang *et*

*al*., 2014; W. Zhang *et al*., 2011). Originally, these antibiotics were effective in many developing and developed countries but *Shigella* species rapidly developed resistance to these agents. These are low-cost and widely available antibiotics that may be bought over the counter without prescription in many drug outlets in Kenya. The increase in resistance of *Shigella* species to sulphamethoxazole- trimethoprim and ampicillin has been reported worldwide (Bogaerts *et al.,* 1997; Saenz *et al.*, 2004; WHO, 2001).

The World Health Organization has developed and applied criteria to rank antimicrobials according to their relative importance in human medicine, the ranking has the critically important, very important and important antimicrobials (Collignon, Powers, Chiller, Aidara-Kane, & Aarestrup, 2009). A total of 12.4% resistance to the antimicrobial agents classified as critically important like the quinolone (nalidixic acid and ciprofloxacin) and third & fourth generation cephalosporin (ceftriaxone) was observed in our study. The widespread use of nalidixic acid as a first-line drug for dysentery in many countries has resulted in the emergence of resistant strains. To date, this antimicrobial agent is no longer recommended as a first-line drug in the international guidelines, and the use of ciprofloxacin is currently encouraged instead. Highly resistant *Shigella* isolates to nalidixic acid have been reported in China (75%-94%) and countries in South Asia region like India, Nepal and Bangladesh which exceeded 60% (Shen *et al*., 2013; Taneja, 2007; W. Zhang *et al*., 2011). A study done in Belgium for 18 years (1990-2007), *Shigella sonnei* resistance to nalidixic acid increased from 1998 to reach a peak of 12.8% in 2004 (Vrints, Mairiaux, Van Meervenne, Collard, & Bertrand, 2009). Our findings show resistance to nalidixic acid

of 9% among the bacterial pathogens isolated. The study revealed huge variation of Nalidixic acid resistance among the *Shigella* species in Kilifi (33%) and Nairobi West (2.9%). Our findings further alluded, variation of nalidixic acid sensitivity among the *Shigella* species exist, all *Shigella boydii* and *Shigella sonnie* were sensitive while *Shigella flexneri* in Kilifi and *Shigella dysentriae* in Nairobi showed resistance levels of 15.8% and 20% respectively. These findings do not corroborate with studies done in Madagascar, Senegal and United States where very limited nalidixic acid resistance in *Shigella* isolates was observed, 0.7%, 1% and 1% respectively (Randrianirina *et al*., 2014; Sire *et al*., 2008; Sivapalasingam *et al.*, 2006). A study done in the neighbouring country Tanzania and a published report on antibiotic use and resistance in Mozambique showed no resistance to nalidixic acid among the *Shigella* spp (GARP, 2015; Temu *et al*., 2007).

Emergence of ciprofloxacin resistant *Shigella flexneri* (9.5%) observed in our study (Nairobi west) was not reported from some studies done in Kenya, Tanzania, Senegal, Madagascar and Mozambique where all *Shigella* species were sensitive to ciprofloxacin (Brooks *et al.*, 2003; GARP, 2015; Randrianirina *et al*., 2014; Sire *et al*., 2008; Temu *et al*., 2007). High fluoroquinolones (ciprofloxacin) resistant *Shigella* species have been reported in India, the level of resistance range between 30% and 55% (Pazhani *et al*., 2008; Taneja, 2007). In the United States, very limited (<1%) ciprofloxacin resistant *Shigella* species have been reported (Sivapalasingam *et al*., 2006). Findings from the 18 years study in Belgium reported no ciprofloxacin resistance to *Shigella sonnei* isolates (Vrints *et al.*, 2009).

## 5.4 Multidrug resistant enteropathogens

Resistance to three or more antimicrobial agents was used to define multidrug resistance (MDR) profiles (Oundo, Kariuki, Boga, Muli, & Iijima, 2008). In this study, over half of the isolated *Shigella* species were MDR. The level of multidrug resistance varied among the four *Shigella* species. *Shigella boydii* had the highest multidrug resistance at 62.5% (5/8); *Shigella flexneri* had 57.5% (23/40); *Shigella dysentriae* had 54.6% (6/11), and *Shigella sonnei* had 50.0% (4/8). The most common resistance pattern detected in *Shigella* species combined sulphamethoxazole- trimethoprim, amoxicillin and tetracycline. Our findings on multidrug resistant *Shigella* corroborate with other studies done in some parts of Asia and Europe. In a study done in China, 78.3% of the *Shigella flexneri* and 74.3% of the *Shigella boydii* were resistant to at least three antibiotics (Shen *et al.,* 2013). In the Belgium study, multidrug resistance in the predominant *Shigella sonnei* emerged in 2007, with 82% of total isolates being MDR (Vrints *et al*., 2009). A study done in Iran reported all (100%) *Shigella* species being multidrug resistance to at least three classes of antimicrobial agent (Tajbakhsh *et al*., 2012). Currently, no licensed vaccines available against *Shigella* infection exist, both live and subunit parenteral vaccine candidates are under development (Kaminski & Oaks, 2009; Niyogi, 2005). While antibiotic treatment is recommended for dysentery, the emergence of multi-drug resistant strains of *Shigella* further complicates treatment, making prevention of infection critical.

This study demonstrated high levels of antibiotic resistance to *Escherichia coli* isolates to commonly used antimicrobials; sulfamethoxazole-trimethoprim, chloramphenicol,

ampicillin, tetracycline, and nalidixic acid. In the neighbouring country Tanzania, Diarrheagenic *Escherichia coli* as a group exhibited high levels of antimicrobial drug resistance in diarrhoea cases to sulfamethaxazole-trimethoprim (86.9%) , ampicillin (84.6%) , tetracycline (80%), and chloramphenicol (42.3%) but were highly susceptible to quinolones like nalidixic acid (1.5%) and ciprofloxacin (0%) (Vila *et al.*, 1999) . Similar findings were reported in a study done in Peru where diarrheagenic *E*. coli pathotypes exhibited high levels of antimicrobial drug resistance to ampicillin (85%), sulfamethaxazole- trimethoprim (79%), tetracycline (65%), and nalidixic acid (28%) (Ochoa *et al.*, 2009). Our study showed emerging resistance to third-generation cephalosporins (ceftriaxone) among the Enteroinvasive *Escherichia coli* pathotype, this finding corroborate with a study done in Costa Rica where *Escherichia coli* pathotypes showed low levels of resistance (Perez, Gomez-Duarte, & Arias, 2010).

Multidrug resistant *Salmonella* Typhi to four drugs (sulfamethoxazole -trimethoprim, tetracycline, chloramphenicol and ampicillin) was reported in all (100%) our three isolates. All the isolates were resistant to sulfamethoxazole-trimethoprim while 66.7% were resistant the other three drugs. These findings corroborate with results from a study done in Nairobi, Kenya (2004-2006) where the *Salmonella* Typhi was reported to be 70% multidrug resistant; sulfamethoxazole-trimethoprim (73%), tetracycline (62%), chloramphenicol (74%) and ampicillin (75%) (Mengo, Kariuki, Muigai, & Revathi, 2010). Similar findings on multidrug resistance *Salmonella* Typhi have been reported in the neighbouring country Uganda, where all isolates were found to be 76% resistant to five antibiotics including, ampicillin, tetracycline, sulfamethoxazole-trimethoprim. A

5% resistance to chloramphenicol was reported while no resistance to nalidixic acid and ciprofloxacin was reported *(*Neil *et al.,* 2012). No resistance to nalidixic acid, ciprofloxacin and ceftriaxone was reported in our study. Multidrug-resistant *Salmonella* Typhi has also been reported in Malawi and Mozambique (2009) where 100% (42) isolates tested were resistant to ampicillin, chloramphenicol, and sulfamethoxazole-trimethoprim while 4 (9.5%) were also resistant to nalidixic acid (Lutterloh *et al.*, 2012). The first-line antimicrobials should no longer be used as empirical treatment of suspected salmonellosis, instead parenteral third-generation cephalosporin such as ceftriaxone remain highly effective, representing possible treatment options for severe infections, especially among hospitalized patients.

Antibiotics may be unnecessarily prescribed by health care providers or unnecessarily purchased directly by consumers. About 51% of the acute bloody diarrhoea patients were given prescriptions of antibiotics which had high antimicrobial resistance. This finding corroborates with a surveillance done over 6 years in Western Kenya where 249 (53%) received only antimicrobials to which their isolate was not susceptible (Brooks *et al*., 2006). There was a significant positive association between prescribed antibiotic and antibiotic resistance levels in Nairobi West whereas there was an insignificant negative association between prescribed antibiotic and antibiotic resistance levels in Kilifi. The study could not determine the reason behind this phenomenal observation however there is need to underscore the importance of regular trainings for clinicians as part of continuous proffessional development. Empirical treatment of bloody diarrhoea cases with highly resistant antibiotics by clinicians exacerbates the

misuse of antibiotics. In spite of the seriousness of the issue, high antibiotic resistance is still not widely recognized as a problem, even among the health professionals. Raising awareness about resistance and educating health professionals, policy makers and the public can help to improve the rational antibiotic use.

## 5.5 Seasonal variation of acute bloody diarrhoea

The study showed strong seasonal variation of acute bloody diarrhoea with highest number of cases from the month of April –June followed by October –December which corresponds to the long and short rainy seasons in Kenya. These results agree with a study done in USA where the trend of shigellosis shows strong seasonal variations (Joh *et al*., 2013). Our study indicates that rainfall and temperature could be the key climatic indicators in the transmission of acute bloody diarrhoea disease. These results are consistent with other research on the effects of rainfall and temperature on enteric infections (Aklilu *et al*., 2015; Gao *et al*., 2014; Kelly-Hope *et al*., 2007; Li, Yang, & Wang, 2014; Z. Li *et al.*, 2013; Y. Zhang, Bi, Hiller, Sun, & Ryan, 2007). In Nairobi, we detected a strong positive associations between the incidence of acute bloody diarrhoea and two climatic variables (rainfall and minimum temperature) while moderate positive associations were observed in Kilifi. There was a weak positive association between the incidence of acute bloody diarrhoea and maximum temperature in both sites. These results agree with a study done by (Zhenjun Li *et al*., 2013; Y. Zhang *et al*., 2007) in China where the monthly incidence of dysentery was positively correlated with maximum temperature, minimum temperature and rainfall. The use of meteorological data for Malindi as a proxy for Kilifi could be a possible reason for the

variation on the strength of the associations; the two coastal towns are 60 Kms apart. In addition, Nairobi is 426 Kms flight distance from Kilifi and has an elevation of 1795m above the sea level compared to Kilifi which is at sea level. These variations affect humidity and air pressure which have been found to have positively and negatively correlated with incidence of dysentery respectively (Huang, Guan, Guo, Wang, & Zhou, 2008; Y. Zhang *et al*., 2007). A study done in China indicated that a 1°C increase in temperatures may cause more than a 12% increase in the incidence of bacillary dysentery (Gao *et al*., 2014).The impact of rainfall on diarrhoeal disease is however far from clear (Y. Zhang *et al.*, 2007). While the relationship between climate variation and diarrhoea diseases has received a great deal of attention recently (Guan, Huang, Guo, Wang, & Zhou, 2008; Kelly-Hope *et al*., 2007; Y. Zhang *et al.*, 2007), there are few papers examining the relationship in Africa, further studies are necessary in this area.

## 5.6 Factors associated with acute bloody diarrhoea

### *5.6.1 Personal hygiene (hand washing)*

The transmission of dysentery could be affected by many factors, including people's dietary pattern, hygiene behaviour, susceptibility to different pathogen strains, and sensitivity to the available drugs as well as local weather conditions (Huang *et al.*, 2008; Z. Li *et al*., 2013). In our study, washing hands after last defecation was found to be a significant protective factor against acute bloody diarrhoea at multivariate analysis. Our findings agree with other studies done in Kenya and Thailand where regular hand

washing was associated with a reduced risk of *shigella* infection (Chompook *et al*., 2005; Brooks *et al*., 2003).

Studies have indicated that hand washing, especially if soap is used, is effective in reducing substantially cases of dysentery, diarrhoeal diseases by 42-47% , secondary transmission and could save a million lives (Aiello, Coulborn, Perez, & Larson, 2008; Chiller *et al*., 2006; Curtis & Cairncross, 2003; Ejemot, Ehiri, Meremikwu, & Critchley, 2008). The risks of severe intestinal infections and of shigellosis were associated with reductions of 48% and 59%, respectively (Curtis, 2003). It has been estimated that the attributable risk for dysentery from not washing hands before preparing food in rural African communities is as high as 30% (Birmingham, Lee, Ntakibirora, Bizimana, & Deming, 1997).

A meta-analyses on the effect of hand hygiene on infectious diseases risk in the community setting established that, improvements in hand hygiene resulted in a 31% reductions in gastrointestinal illness (Aiello *et al.*, 2008). Interventions including promoting hand washing resulted in a 31% reduction in diarrhoea episodes in communities in low-middle income countries (Ejemot *et al*., 2008). In a study done in Karachi, Pakistan, households that received free soap and hand washing promotion for 9 months reported 51% less diarrhoea than controls (Chiller *et al.,* 2006). Promoting hand washing with soap and water can prevent the spread of diarrhoeal diseases in areas where comparatively costly interventions, such as supply of safe water and improved sanitation are not possible (Shahid, Greenough, Samadi, Huq, & Rahman, 1996). Significant reduction in diarrhoeal incidences was observed in all age groups for all pathogens and was comparable to the effect of providing clean water in low-income

areas (Ejemot *et al.*, 2008). However, more and better-designed trials are needed to measure the impact of washing hands on acute bloody diarrhoea in developing countries.

### *5.6.2 Water Safety*

Safe water storage and hand hygiene have been shown to reduce faecal contamination and improve health. In our study, separating drinking water and water for others uses at household level and safe storage of water in super drum were found to be protective factors while presence of coliform in water was found to be a risk factor. This study revealed that the level of water contamination was much higher at household level in both rural (40.9%) and urban (10.7%) compared with the main source which was 38.6% and 8.2% respectively. This finding corroborates with the United Nations indicators for 2015, the proportion of Kenya's population using an improved drinking water source in rural and urban Kenya is 57% and 82% respectively (Nations, 2015). A study done in Peru revealed that post-source contamination increased successively through the step is of usage from source water to the point of consumption where source water was microbiologically clean, but 28% of samples from water stored for cooking had faecal contamination (Oswald *et al.*, 2007). Testing for evidence of water contamination has been traditionally accomplished by the detection or enumeration of total and faecal coliforms. Coliforms should not be detectable in treated water supplies, they can be used as an indicator of treatment effectiveness and to assess the cleanliness and integrity of distribution systems and the potential presence of biofilms (Nikaeen, Pejhan, & Jalali, 2009; G. WHO, 2011). Communities continue to face challenges of accessing

clean and safe water for domestic use. Consuming water contaminated with faecal coliforms increases the attributable risk for household members contracting water borne disease like dysentery, cholera and salmonellosis. A study done in rural South Africa and Zimbabwe assessed risk factors for child dysentery and watery diarrhoea in households where drinking water was collected from communal sources. The study found that drinking water from sources other than standpipes had a relative risk ratio of 3.8 for dysentery (Gundry *et al.*, 2009).

In the past decade, further evidence has emerged that supports the beneficial outcomes of water, sanitation, and hygiene interventions in developing countries (Montgomery & Elimelech, 2007). A meta-analysis on the impact of such interventions concluded that increasing water quantity reduced the occurrence of diarrheal diseases by 25%, whereas point-of-use (POU) household water treatment and improved sanitation led to reductions in diarrheal diseases of 35% and 32%, respectively (Fewtrell *et al*., 2005). Sanitation and POU interventions may have resulted in greater reductions because they directly block pathways of exposure (Fewtrell *et al*., 2005). Other studies done in Kenya, Guatemala, and India have demonstrated that use of POU treatments leads to a reduction in diarrhoea by 40% for PUR and solar disinfection and by up to 85% for chlorine (Chiller *et al*., 2006; Mintz, Bartram, Lochery, & Wegelin, 2001; Rose *et al*., 2006). Use of chlorine may lead to a greater reduction in diarrhoea because of its advantages relating to low cost, ease of use, and its ability to be manufactured locally. However, continuous promotion on use of POU treatments is required among communities in order to realize the desired optimal levels for prevention of diarrhoea diseases. In a chlorine-disinfection and safe-storage project in rural Kenya, only 33% of

households had chlorine residual six months after implementation of the intervention, this was evidence of use of POU treatments (Makutsa *et al.*, 2001).

Use of an improved drinking water source is a proxy for measuring access to safe drinking water. Improved drinking water sources are more likely to be protected from external contaminants than unimproved sources either by intervention or through their design and construction. Greater access to improved drinking water sources is important as it contributes to lowering the incidence of diarrhoeal diseases in developing countries (Nations, 2015). An improved drinking-water source is defined as one that, by nature of its construction or through active intervention, is protected from outside contamination; in particular from contamination with faecal matter while an improved sanitation facility is defined as one that hygienically separates human excreta from human contact.

### ***5.6.3 Environmental hygiene***

Adequate sanitation, together with good hygiene and safe water, are fundamental to good health and to social and economic development(Mara, Lane, Scott, & Trouba, 2010). Improving domestic hygiene practices, good sanitation and hand washing with soap are potentially the most effective primary barriers to reducing the global burden of diarrheal diseases (Curtis & Cairncross, 2003; Curtis, Cairncross, & Yonli, 2000). If proper mechanisms for faecal disposal are not in place or if hand washing is not optimal, then enteric agents can contaminate the environment. At this point secondary barrier behaviors such as washing hands before preparing food, reheating of food, controlling flies, water treatment or boiling become important. In our separate multivariable logistic regression models, the incidence of acute bloody diarrhoea in

rural setting was related to poor general compound cleanliness (hygiene). Our findings agree with a risk factor study done in Thailand where a clean environment surrounding the household was associated with a reduced risk for shigellosis in the multivariate model (Chompook *et al*., 2005).

Of all human excreta, faeces are the most dangerous to health. One gram of fresh faeces from an infected person can contain around $10^6$ viral pathogens, $10^6$–$10^8$ bacterial pathogens, $10^4$ protozoan cysts or oocysts, and 10–$10^4$ helminth eggs (Mara *et al.,* 2010). In our study, good condition of toilet was found to be protective. The study shows that families in rural setting that do not have access to improved sanitation facilities have higher odds of contracting acute bloody diarrhoea. An improved sanitation corresponds to a simple pit latrine, a ventilated improved pit-latrine or a septic tank (Haller, Hutton, & Bartram, 2007). Proper excreta disposal will help in reducing the exposure from the domestic environment. A study done in a rural setting in western part of Kenya showed that sharing of latrines between multiple households increased risk of bloody diarrhoea (Brooks *et al*., 2003). Lack of a household improved latrine was associated with diarrhoea and under-five child mortality in Indonesia (Semba *et al.,* 2011) while availability of water to flush the toilet was associated with a reduced risk for shigellosis in Thailand (Chompook *et al.*, 2005). Systematic reviews suggest that improved sanitation can reduce rates of diarrhoeal diseases by 32%–37% (Fewtrell *et al.,* 2005).

## 5.7 Limitation of the study

The study had several limitations; first, the study was limited by our inability to culture *Campylobacter* spp at the time of the study. *Campylobacter* spp was isolated in 7% of patient's presentation with bloody diarrhoea in Kenya (Brooks *et al.*, 2003). Second, the study did not attempt to find out the real causes of the other bloody specimens. Further research is recommended. Third, infection with HIV/AIDS has been found to be an important co-morbidity for bloody diarrhoea (Baer *et al*., 1999; Sanchez *et al.*, 2005). We were unable to ascertain the true HIV status of the enrolled study participants. Fourth, the study was further limited in examining risk factors that may be unique for young children under 5 years who constituted about 17% of our enrolled study participants. Fifth, data was available on presence and condition of a household latrine and not actual monitoring of use of a household latrine. The field team members did visual inspection of each latrine in the 805 households visited in the study.

# CHAPTER SIX

# CONCLUSIONS AND RECOMMENDATIONS

## 6.1: CONCLUSIONS

The main etiologic agents for acute bloody diarrhoea among communities in Kilifi and Nairobi Counties were *Shigella* and *E. histolytica*. The main presenting clinical features in both setting were blood in stool and abdominal pains. The prevalence pattern of the four *Shigella* species has not changed significantly over the last decade and as a result this study concludes that *Shigella dysenteriae type 1* which is an epidemic strain is not the cause of increase in cases of acute bloody diarrhoea in Kenya. In both settings, majority of the cases were people in the low socio-economic strata like casual labourers who earn an average of <USD 2.0 per day. The level of education varied in both setting, majority of the cases in rural setting had attained primary level education while in urban setting majority had secondary level education.

There was variation in prevalence of enteric pathogens, antimicrobial susceptibility among the bacterial pathogens as well as across the *Shigella* species in rural and urban settings. In both settings, there were high levels of antibiotic resistance as well as multidrug resistance among the commonly prescribed antibiotics like sulphamethoxazole-trimethoprim, tetracycline, ampicillin and chloramphenicol.

There was lack of adherence to the clinical guidelines for the management of enteric bacterial pathogens in both settings; high proportions of antibiotic prescriptions issued were found to be in the high resistance zone. Statistically, there was a significant

positive association between prescribed antibiotics and antibiotic resistance in the urban setting (Nairobi West). There was wide variation in resistance to Ampicillin in rural and urban setting. Resistance to Nalidixic acid was > 10 times among the *Shigella* species in rural setting compared to urban. Emerging resistance to ciprofloxacin and ceftriaxone was only reported in the urban setting, this is likely to pose a serious public health threat in the management of enteric diseases in Kenya

Good personal hygiene practices such as washing hands after defecation, cleanliness of toilets and proper storage of drinking water were found to be the key protective factors for acute bloody diarrhoea while poor compound cleanliness around households, reported cases of diarrhoea in household in previous 2 weeks and presence of coliforms in main water source were found to be risk factors.

The prevailing local weather conditions (rainfall and temperature) were found to have a moderate-strong correlation with the occurrence of acute bloody diarrhoea in both setting.

## 6.2: RECOMMENDATIONS

The findings from the acute bloody diarrhoea study elucidates on need for a shift in public health interventions and policy formulation. The following are recommendations;

1. **Establish multi sectorial institutional capacity and capability to curb the rising antibiotic resistance levels:** The high rate of resistance to commonly used drugs call for policy change, reinforcement of the regulatory mechanism on the rational use of antibiotics and an all-round behaviour change. The emerging resistance to ciprofloxacin and ceftriaxone poses a serious public health threat in the management of enteric diseases in Kenya. Levels of resistance to ciprofloxacin and ceftriaxone should be constantly monitored to detect any shift in the quinolones, third and fourth generation cephalosporin resistance in order to measure the level of antibiotic pressure. A multi sectoral approach is required, this include;

   **Community members:** to tackle antibiotic resistance by using antibiotics only when prescribed by a certified health professional and taking the full prescription.

   **Health workers and pharmacists:** to help tackle resistance by only prescribing and dispensing antibiotics when they are truly needed and prescribing and dispensing the right antibiotic(s) to treat the illness.

   **Policymakers:** the policy makers can help tackle resistance by strengthening resistance tracking and laboratory capacity; strengthening infection control and

prevention, regulating and promoting appropriate use of medicines and promoting cooperation and information sharing among all stakeholders.

2. **Institute public health preventive measures to reduce the need for antibiotics.** Reducing the occurrence of acute bloody diarrhoea as well as secondary transmission in the rural and urban settings through promoting awareness of good personal hygiene (hand washing) and environmental cleanliness around the households, safe storage and treatment of water at the point of use and improved sanitation especially latrines.
3. **Strengthening the surveillance and response capacity for enteric pathogens that are a threat to public health security.**

   The current surveillance system for enteric pathogens is largely syndromic. The Ministry of Health through Disease Surveillance and Response Unit, National Public Health Laboratories should establish laboratory based surveillance sentinel sites for monitoring Enteric pathogens such as *Shigella dysenteriae* type 1, Multidrug resistance *Salmonella* Typhi, Enterohaemorrhagic *Escherichia coli* and Cholera which are a threat to public health security due to their ability to cause explosive epidemics with high morbidity and mortality rates.

4. **Establishment of water quality surveillance system.** There is need to establish water quality monitoring at County Level that will provide risk profiles and trigger action when certain threshold makers are surpassed.

5. **Strengthen evidence based data for appraising guidelines and policy formulation.** The country lacks adequate data on enteric diseases to appraise the existing guidelines and influencing policy change.

6. **Further research work:** Research is required to establish a scientific model for predicting the outbreaks of dysentery using climate variables and also looking at spatial pattern and seasonality of dysentery in the whole country.

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# APPENDICES

## Appendix 1: Case Report Form

### ENTERIC PATHOGENS AND POTENTIAL RISK FACTORS FOR ACUTE BLOODY DIARRHOEA IN KENYA. A CASE CONTROL STUDY

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**Instructions:** Please do not leave any question blank. Write text or tick when appropriate

**STANDARD CASE DEFINITIONS**

**Acute Bloody Diarrhoea:** ***A cases is*** *"any person of any age with acute diarrhoea with visible blood in the stools"*

| **1.0 General Information** |
|---|
| 1.1 Identification No: ☐☐☐☐<br>2 first letters of site' name + day (2 digits) and month (2 digits) numbers + Ranking number of the admission in the surveillance *(this number should be same for case and matching controls)* |
| 1.2 Date of Interview: _____/_____/_____( Day, month, year ) |
| 1.3 Clinician's Name (last, First): |
| 1.4 Patients Name (Last, Middle, First): |
| 1.5 Gender: Male☐ Female ☐ |
| 1.6 Age in years: Date of birth_____/_____/_____ |
| 1.7 Contact Person Name *(Last, First)* Cell phone:<br>*N/B: Required for follow-ups. If child less than age 15 years, the responsible adult will be the contact* |
| 1.8 District of residence: Dagoretti ☐ Langata ☐ Kilifi ☐ |
| 1.9 Division : |
| 1.10 Location |
| 1.11 Sub Location : |
| 1.12 Village/Estate : |

1.13 Patient seen at:

Mbagathi D.H ☐ Langata H/C ☐ Riruta H/C ☐

Kilifi D.H ☐ Bamba S.D.H ☐ Vipingo H/C ☐

1.14 Patients recruited at: Outpatient department ☐ Inpatient department ☐

MCH/Paediatric clinic ☐ Casualty room ☐

**2.0 Vital signs and recording**

2.1 Temperature recording (°c):

2.2 Pulse rate (per min):

2.3 Blood Pressure (BP) **(above 18 yrs):**

2.4 Weight in Kgs:

2.5 Height in cm:

2.6 Body Mass Index (BMI): (*skip, to be calculated automatically*)

2.7 Mid upper arm circumference (MUAC) in cms for <5yrs :

2.8 Date of onset (bloody diarrhoea): _____/_____/_____( Day, month, year)

**3.0 History of disease**

Check any of the following diseases the patient currently has:

3.1 Malaria* ***(microscopy or RDT)*:** Positive ☐ Negative ☐ Unknown ☐

3.2 HIV/AIDS status***(rapid test):*** Positive ☐ Negative ☐ Unknown ☐

3.3 Malnutrition: Yes ☐ No ☐ Unknown ☐

3.4 Tuberculosis: Yes ☐ No ☐ Unknown ☐

3.5 Diabetes : Yes ☐ No ☐ Unknown ☐

3.6 Hypertension: Yes ☐ No ☐ Unknown ☐

3.7 Cancer: Yes ☐ No ☐ Unknown ☐

3.8 Other Heart disease: Yes ☐ No ☐ Unknown ☐

3.9 Any other condition (specify):_____

***Malaria – presence of disease by laboratory confirmation and not clinical***

****HIV/AIDS status-Patients who have already been tested, use the rapid test results***

**4.0 Signs and symptoms (Clinical Examination) (***Tick appropriately***)**

4.1 Dry mucous membranes: Yes ☐ No ☐

| | | | |
|---|---|---|---|
| 4.2 Fever | Yes ☐ | No ☐ | |
| 4.3 Respiratory distress | Yes ☐ | No ☐ | |
| 4.4 Altered consciousness | Yes ☐ | No ☐ | |
| 4.5 Coma(*Glasgow coma scale*) | Yes ☐ | No ☐ | |
| 4.6 Convulsion | Yes ☐ | No ☐ | |
| 4.7 Loose stools | Yes ☐ | No ☐ | |
| 4.8 Watery stools | Yes ☐ | No ☐ | |
| 4.10 Rice water stools | Yes ☐ | No ☐ | |
| 4.11 Blood in stools | Yes ☐ | No ☐ | |
| 4.12 Mucous in stools | Yes ☐ | No ☐ | |
| 4.13 Nausea | Yes ☐ | No ☐ | |
| 4.14 Vomiting | Yes ☐ | No ☐ | |
| 4.15 Anorexia (reduced appetite) | Yes ☐ | No ☐ | |
| 4.16 Abdominal pain | Yes ☐ | No ☐ | |
| 4.17 Abdominal discomfort | Yes ☐ | No ☐ | |
| 4.18 Abdominal tenderness | Yes ☐ | No ☐ | if yes, location;_____ |
| 4.19 Chills | Yes ☐ | No ☐ | |
| 4.20 Headache | Yes ☐ | No ☐ | |
| 4.21 General Malaise | Yes ☐ | No ☐ | |
| 4.22 Dizziness | Yes ☐ | No ☐ | |
| 4.23 Rectal prolapse | Yes ☐ | No ☐ | |
| 4.24 weight Loss | Yes ☐ | No ☐ | |
| 4.25 Painful defecation | Yes ☐ | No ☐ | |
| 4.26 Cough | Yes ☐ | No ☐ | |
| 4.27 Pallor | Yes ☐ | No ☐ | |
| 4.28 Jaundice | Yes ☐ | No ☐ | |
| 4.29 Oedema (lower Limbs) | Yes ☐ | No ☐ | |
| 4.30 Any other sign/symptom:_____ | | | |

**5.0 Assessment of Dehydration and associated factors**

| | | | |
|---|---|---|---|
| 5.1 Is there dehydration? | Yes ☐ | No ☐ | Don't know ☐ |
| 5.2 If dehydrated, observe any of the following signs? | | | |
| Sunken eyes: | Yes ☐ | No ☐ | Don't know ☐ |
| Lethargy or unconsciousness: | Yes ☐ | No ☐ | Don't know ☐ |
| Loss of skin turgor: | Yes ☐ | No ☐ | Don't know ☐ |
| Inability to drink or drinking poorly | Yes ☐ | No ☐ | Don't know ☐ |
| 5.3 Assess for the degree of dehydration (*as per the protocol)* | | | |
| Severe dehydration ☐ | Mild/Moderate dehydration ☐ | No dehydration ☐ | |
| 5.4 Duration of diarrhoea in days: | | | |
| 5.5 Was the diarrhoea bloody at onset? Yes ☐ | No ☐ | Unknown ☐ | |
| 5.6 Number of stools in last 24 hours: | | | |
| 5.7 Number of episodes of vomiting in last 24 hours: | | | |
| 5.8 Have you/the case ever had bloody diarrhoea previously? Yes ☐ | No ☐ | | |
| 5.9 How many people (apart from you) in the household had diarrhoea in the last 1 month? Number:_____ | | | |
| 5.10Feeding since the onset of this condition: Reduced ☐ | Normal ☐ | More ☐ | |
| 5.11 If a child, is the child breastfeeding? Yes ☐ | No ☐ | | |
| 5.12 If a child, how is the child fed? | | | |
| Exclusive breastfeeding ☐ | Artificially fed ☐ | Mixed feeding (both) ☐ | |
| ***Artificially fed means: a child is being given other foods without breastfeeding*** | | | |
| 5.13 If a child (>9months and <5yrs), what is the status of measles vaccination? | | | |
| Vaccinated ☐ | Not vaccinated ☐ | Don't Know ☐ | |
| 5.14 If the child is vaccinated against measles, what was the basis? | | | |
| Immunization card ☐ | based on verbal report from caregiver/guardian ☐ | | |
| **6.0 Treatment received since current diarrhoeal episode began** | | | |
| 6.1 Oral rehydration Solution (ORS) | Yes ☐ | No ☐ | |
| 6.2 Salt and sugar solution (SSS) homemade | Yes ☐ | No ☐ | |
| 6.3 Cereal-based solutions. | Yes ☐ | No ☐ | |
| 6.4 Traditional medicine | Yes ☐ | No ☐ | |

| |
|---|
| 6.5 Antibiotics Yes ☐ No ☐ Unknown ☐ |
| 6.6 If taken antibiotic , name : _____Number of days taken_____ |
| **7.0 Clinician's Case Management (Prescription)** |
| **7.1 Antibiotics**: Prescribed☐ Not prescribed☐ |
| 7.2 If prescribed antibiotic, which one? |
| Nalidixic acid☐ Erythromycin☐ Cotrimoxazole☐ Doxycycline☐ |
| Tetracycline ☐ Amoxicillin☐ Ampicillin☐ Chloramphenicol☐ |
| Gentamycin☐ Amoxicillin-Clavulanic acid☐ Ciprofloxacin ☐ Ceftriaxone☐ |
| Other, specify, _____ |
| 7.3 **Antiamoeba:** Flagyl (metronidazole) ☐ Tinadazole ☐ None ☐ |
| 7.4 Any other drug(s) Specify: |
| 7.5 **Fluids :** IV fluids ☐ ORS ☐ |
| 7.6 Any other management: Yes ☐ No ☐ if yes, specify: |
| **8.0 Follow up of the case after outpatient?** |
| 8.1 Sent home with treatment: Yes ☐ No ☐ |
| 8.2 Hospitalized/admitted: Yes ☐ No ☐ if yes, date of admission: |
| 8.3 If admitted, outcome of illness: Survived☐ Died☐ |
| 8.4 If admitted and discharged later, date of discharge: |
| 8.5 Referred to another Health facility: Yes ☐ No ☐ |
| **9.0 Laboratory evaluation** |
| 9.1 Stools taken: Yes ☐ No ☐ if yes Date: |
| 9.2 Swab taken: Yes ☐ No ☐ if yes Date: |
| 9.3 Specimen delivered to laboratory: Yes ☐ No ☐ if yes Date: |

**Appendix 2:** Household Investigation Form

**ENTERIC PATHOGENS AND POTENTIAL RISK FACTORS FOR ACUTE BLOODY DIARRHOEA IN NAIROBI WEST AND KILIFI DISTRICTS. A CASE CONTROL STUDY**

**Instructions: Please do not leave any question blank. Write text or tick when appropriate**

| | |
|---|---|
| ***Definition of a case:*** *"any person of any age with acute diarrhoea with visible blood in the stools"*<br>***Definition of a Control: "****any* healthy person matched by age group (< 2 yrs, 2 – 4 yrs, 5-10 yrs, 11 – 17yrs, 18-65 and >65 years) within ONE week of each case's presentation" | |
| **Interviewers Name:** | **Interview date:** |
| **Name of contact person:** | **Cell phone:**<br>*N/B: The name of the contact person and cell phone number are required for follow-ups* |
| **1.0** | **IDENTIFICATION AND DEMOGRAPHIC INFORMATION** |
| 1.1 | Identification No. ☐☐☐☐<br>2 first letters of site' name + day (2 digits) and month (2 digits) numbers + Ranking number of the admission in the surveillance *(The identification number should be same for case and matching controls)* |
| 1.2 | **Status:** Case ☐ Neighbourhood Control **1** ☐ Neighbourhood Control **2** ☐ |
| 1.3 | Names (last, middle, first): |
| 1.4 | Age in years: Date of birth_____/_____/_____ |
| 1.5 | If the case or control is aged >9 months and <5years, is the child vaccinated against measles? Yes ☐ No ☐ Don't know ☐ Not applicable ☐ |
| 1.6 | Gender: Male ☐ Female ☐ |
| 1.7 | Religion: Christian ☐ Muslim ☐ Traditionalist ☐ Other ☐ specify_____ |
| 1.8 | District: Langata ☐ Dagoretti ☐ Kilifi ☐ |
| 1.9 | Division : |
| 1.10 | Location : |
| 1.11 | Sub Location : |

| | |
|---|---|
| 1.12 | Village/estate : |
| 1.13 | Is the case or control answering questions by self? Yes ☐ No ☐ |
| 1.14 | **If no**, what is the relationship of the person answering the questions to the case/control<br>Parent ☐ Sibling (Brother, sister)☐ Spouse ☐<br>Extended family(Aunt, uncle, grandparents) ☐ care giver/house girl☐<br>Friend ☐ Neighbour ☐ Other ☐, specify_____ |
| 1.15 | Why can't the case or control answer questions by him/herself? Is a child ☐<br>Case is too sick ☐ Not applicable☐ Other ☐ specify_____ |
| 1.16 | Name and age of the person providing the information: Name: Age: |
| 1.17 | Highest level of education completed (case or control):<br>no formal education ☐ some Primary ☐ completed Primary ☐<br>some secondary ☐ completed secondary ☐ college or university ☐ |
| 1.18 | What is the occupation of the case or control?<br>Business☐ Casual labourer ☐ Farming ☐<br>Fishing ☐ Hawker☐ Housewife ☐<br>Long distance driver☐ Quarry ☐ Not working ☐<br>Skilled labour (e.g. carpenter, tailor, jua kali) ☐ Salaried worker ☐<br>Pupil or student ☐ Still a child☐ Other ☐ (specify): _____ |
| 1.19 | What is the occupation of the head of the household? *(if he/she is not the case or control)*<br>Business☐ Casual labourer ☐ Farming ☐<br>Fishing ☐ Hawker☐ Housewife ☐<br>Long distance driver☐ Quarry ☐ Not working ☐<br>Skilled labour (e.g. carpenter, tailor, jua kali) ☐ Salaried worker ☐<br>Student ☐ Still a child☐ Other ☐ (specify): _____ |
| 1.20 | What is the highest formal education level of someone living in the household?<br>No formal education ☐ Some primary ☐ Completed primary ☐<br>Some secondary ☐ Completed secondary ☐ College or university ☐ |

| | |
|---|---|
| 1.21 | What is the residence of the case or control? Urban ☐ Slums (Urban) ☐<br>Semi-urban (shopping Centre) ☐ Rural ☐ |
| 1.22 | Case or control history of travel in the last 2 weeks? Yes ☐ No ☐ Don't know ☐ |
| 1.23 | Approximate distance from home to the health care facility (in Kms): |
| 1.24 | What means does the case or control use to reach the health facility?<br>Walking ☐ Bicycle ☐ motorcycle ☐ Vehicle ☐ |
| 1.25 | Approximate time take to reach the health facility (using the means selected): |
| 1.26 | Assess the main house's roof, walls and floor:<br>**Roof:** Thatched ☐ Mabati ☐ Clay tile ☐ Other *(specify)*_____<br>**Wall:** Mud ☐ Semi-permanent ☐ Permanent ☐ Other *(specify)* _____<br>**Floor:** Mud ☐ Wooden ☐ Cement ☐ Other *(specify)*_____ |
| 1.27 | How many rooms are there in your main house (not including closets and bathrooms):<br>One ☐ Two ☐ Three ☐ Four ☐ Five ☐ >Five ☐ |
| 1.28 | Total number of household members:_____ *(A person living in the household is defined as : either resident of the house or if not resident having slept in the house during the last 8 nights consecutively )* |
| 1.29 | How many household members are below 5 years? |
| 1.30 | Number of other diarrhoea cases in the same household in the previous 2 weeks ☐ |
| 1.31 | How much money ( Ksh) does your household earn on average every month?<br><3000 ☐ 3000-5000 ☐ >5000-10,000 ☐ >10,000-20,000 ☐<br>>20,000-30,000 ☐ >30,000-50,000 ☐ >50,000 ☐ |
| 1.32 | Does the household own any of the following?<br>Radio ☐ TV set ☐ Bicycle ☐ Motor cycle ☐ Vehicle ☐ |
| **2.0** | **WATER SAFETY** |
| 2.1 | Does the household access enough water for drinking as well as for all other domestic purposes such as cooking and washing?<br>Yes ☐ No ☐ Unknown ☐ |
| 2.2 | Where do you get your household drinking water (***main sources)?*** |

| | |
|---|---|
| | Tap inside house ☐ Piped water ☐ Common tank ☐<br>Vendors ☐ Borehole ☐ Rain water ☐<br>Open shallow well ☐ Closed well ☐ Community well ☐<br>River ☐ Lake ☐ Dam ☐ Ponds ☐<br>Trucked in ☐ Bottled ☐ Others ☐ specify_____ |
| 2.3 | Is the main water source treated? Yes ☐ No ☐ I don't know☐<br>**If yes,** how is the water treated? Chemically at source ☐ Chemically at home ☐<br>Boiling ☐ Other ☐ *specify*_____ |
| 2.4 | Do you have chlorine in your house? (If yes ask to be shown) Yes ☐ No ☐<br>I don't know☐ |
| 2.5 | What is the approximate distance from household to main water source?<br>Within homestead☐ Outside homestead < 500 metres ☐ 500metres-1Km☐<br>1-2 Kms ☐ 2-3Kms☐ 3-4Kms ☐ 4-5Kms ☐ >5kms☐ |
| 2.6 | What is the average queuing time at the main water source?<br><5min☐ 5-10Min☐ >10-15Min☐ >15-30Min☐<br>>30-60min☐ >1hour☐ Not applicable ☐ |
| 2.7 | What time is taken to fill a 20-litre container of water? <1min ☐ 1-3Min☐<br>3-5Min☐ 5-10Min☐ >10Min☐ |
| 2.8 | Average total time used to fetch water and get back home? <5min☐ 5-10Min☐<br>>10-20Min☐ > 20-30Min☐ >30-60min☐ >1hour☐ Not applicable ☐ |
| 2.9 | How much water (quantity) is used by the household per day?<br><20 Litres☐ 20-40Litres☐ 40-60Litres☐ 60-80litres☐<br>80-100litres☐ >100litres☐ *(1 jerican =20litres)* |
| 2.10 | Where do you get your household drinking water (***alternative sources)?***<br>Tap inside house ☐ Piped water ☐ Common tank ☐<br>Vendors ☐ Borehole ☐ Rain water ☐<br>Open shallow well ☐ Closed well ☐ Community well ☐<br>River ☐ Lake ☐ Dam ☐ Ponds ☐<br>Trucked in ☐ Bottled ☐ Others ☐ specify_____ |

| | |
|---|---|
| 2.11 | Is the alternative water source treated? Yes ☐ No ☐<br>**If yes**, how? Chemically at source ☐ Chemically at home ☐ Boiling ☐<br>Other ☐ *specify*_____ |
| 2.12 | What is the approximate distance from household to alternative water source?<br>Within homestead☐ Outside homestead < 500 metres ☐ 500metres-1Kms☐<br>1-2 Km ☐ 2-3Kms☐ 3-4Kms ☐ 4-5Kms ☐ >5kms☐ |
| 2.13 | How much time is used for queuing at the alternative water source?<br><5min☐ >5-10Min☐ >10-15Min☐ >15-30Min☐ >30-60min☐<br>>1hour☐ Not applicable ☐ |
| 2.14 | How much time is used to fill a 20-litre container of water at alternative source?<br><1min ☐ 1-3Min☐ 3-5Min☐ 5-10Min☐ >10Min☐ |
| 2.15 | How much time (total) is used to get water from alternative source and back home?<br><5min☐ >5-10Min☐ >10-20Min☐ > 20-30Min☐<br>>30-60min☐ >1hour☐ Not applicable ☐ |
| 2.16 | Which method do you use for storage of drinking water?<br>Jerry cans ☐ Bucket☐ Tank ☐ Drum ☐ Clay Pot ☐<br>Tap☐ water dispenser ☐ water not stored ☐ Other ☐ *(specify)* _____ |
| 2.17 | Is the storage container for drinking water covered? Yes ☐ No ☐ Not applicable ☐ |
| 2.18 | The storage container is made of? Plastic☐ metallic ☐ clay ☐ Other ☐, specify__ |
| 2.19 | Mouth of the storage Container? (observe)<br>Narrow-mouthed☐ wide-mouthed ☐ Unknown☐ Not applicable☐<br>(*Narrow-mouthed mean: opening can't allow the insertion of a hand by an adult)* |
| 2.20 | Which method is used for removing water from storage container?<br>a specific jug/cup ☐ any jug/cup ☐ don't use any jug/cup ☐<br>Pour water from the opening ☐ Unknown☐ Not applicable☐ |
| 2.21 | How often do you clean the storage container?<br>Every day☐ Every week☐ every month☐ every 3-4 months☐<br>twice a year☐ every year ☐ Don't know ☐ Not applicable☐ |

| | |
|---|---|
| | other ☐ specify_____ |
| 2.22 | Do you store water for bathing, washing and other purposes in separate container from drinking water? Yes ☐ No ☐ I don't know ☐ Not applicable ☐ |
| 2.23 | Did you drink water outside home in the last one week?<br>Yes ☐ No ☐ Don't know ☐<br>**If yes**, was the water treated by chemical or boiled?<br>Yes ☐ No ☐ don't know ☐ Not applicable ☐ |
| **3.0** | **FOOD SAFETY** |
| 3.1 | Do you eat raw foods like fruits and vegetables? Yes ☐ No ☐<br>**If yes**, do you wash the raw fruits and vegetables before eating?<br>Always ☐ Sometimes ☐ rarely ☐ Never ☐<br>Don't know ☐ Not applicable ☐ |
| 3.2 | Do you re-heat cold food before eating? (probe)<br>Always ☐ Sometimes ☐ rarely ☐ Never ☐ Not applicable ☐ |
| 3.3 | In the last 1 week, did you eat food cooked the previous day?<br>Yes ☐ No ☐ Don't know ☐ |
| 3.4 | Do you keep cooked food separately from uncooked food?<br>Yes ☐ No ☐ Don't Know ☐ Not applicable ☐ |
| 3.5 | Is the food protected from flies by means of fly screens?<br>Always ☐ Sometimes ☐ rarely ☐ Never ☐ Not applicable ☐ |
| 3.6 | In the last one week, did you eat food outside home?<br>Yes ☐ No ☐ Don't know ☐ Not applicable ☐<br>**If yes**, where?<br>Hotel ☐ street vendor ☐ another home ☐ others ☐, specify_____ |
| 3.7 | In the last one week, did you attend any social gathering where food/drinks were served?<br>Yes ☐ No ☐ don't know ☐<br>**If yes**, what kind of social gathering? Parties ☐ wedding ☐ funeral ☐ others, ☐<br>**If yes**, did you eat in the social gathering? Yes ☐ No ☐ Not applicable ☐<br>**If yes**, did you drink water in the social gathering? Yes ☐ No ☐ Not applicable ☐ |

| | |
|---|---|
| | **If yes**, did you drink local brew? Yes ☐ No ☐ Not applicable ☐ |
| **4.0** | **HYGIENE PRACTICE AND SANITATION** |
| 4.1 | Presence of a toilet at the home compound (observe); Yes ☐ No ☐ |
| 4.2 | What kind of toilet does the household have? Flush toilet ☐ VIP latrine ☐<br>Traditional pit latrine ☐ (VIP means ventilated improved pit) |
| 4.3 | What is the condition of the toilet? (observe) Clean ☐ Dirty ☐ Full ☐<br>Covered ☐ Not applicable ☐ |
| 4.4 | If there is no toilet facility in the homestead, how does the family dispose off faeces?<br>Use bush ☐ Use dug pits ☐ Communal latrine ☐ Neighbour's latrine ☐<br>Flying toilet ☐ Not applicable ☐ Other ☐, specify_____<br>*Flying toilet means: faeces are put in a polythene paper and throne away* |
| 4.5 | How often do you use a toilet? (probe) Always ☐ Sometimes ☐<br>rarely ☐ Never ☐ Not applicable ☐ |
| 4.6 | Do you allow other families or neighbours to use the compound toilet?<br>Yes ☐ No ☐ don't know ☐ Not applicable ☐ |
| 4.7 | What is the average number of people using one toilet?_____ |
| 4.8 | Do you wash your hands after defecating? (probe)<br>Always ☐ Sometimes ☐ rarely ☐ Never ☐<br>**If yes**, what do you use? water ☐ water and soap ☐ water and ash ☐<br>Not applicable ☐ other ☐, specify _____ |
| 4.9 | **If yes,** which method do you use for hand washing after defecation?<br>Tap water ☐ Water in basin ☐ leaky tin ☐ None ☐ |
| 4.10 | Did you wash your hands after defecating (last visit)? Yes ☐ No ☐ |
| 4.11 | Do you wash your hands after cleaning and disposing stools of a child who has defecated?<br>Always ☐ Sometimes ☐ rarely ☐ Never ☐ Not applicable ☐<br>**If yes**, what do you use?<br>water ☐ water and soap ☐ water and ash ☐ Not applicable ☐ |

| | |
|---|---|
| | other☐, specify _____ |
| 4.12 | Do you wash your hands before preparing food?<br>Always ☐ Sometimes ☐ rarely ☐ Never ☐ Not applicable ☐<br>**If yes**, what do you use?<br>water ☐ water and soap ☐ water and ash☐<br>Not applicable ☐ other☐, specify _____ |
| 4.13 | Do you wash your hands before eating food?<br>Always ☐ Sometimes ☐ rarely ☐ Never ☐<br>**If yes,** what do you use?<br>water ☐ water and soap ☐ water and ash☐<br>Not applicable ☐ other☐, specify _____ |
| 4.14 | **If yes**, describe how you wash your hands before eating?<br>Running water ☐ use water in basin alone ☐ use water in basin with others ☐<br>None ☐ other☐, specify _____ |
| 4.15 | Do you wash all cooking and serving utensils after use?<br>Always ☐ Sometimes ☐ rarely ☐ Never ☐ Not applicable ☐ |
| 4.16 | Do you dry all cooking and serving utensils after washing?<br>Always ☐ Sometimes ☐ rarely ☐ Never ☐ Not applicable ☐ |
| 4.17 | Do you keep clean utensils separately from unwashed utensils?<br>Yes ☐ No ☐ Don't Know ☐ Not applicable ☐ |
| 4.18 | Is there soap in the house for hand-washing? Yes ☐ No ☐ Don't know☐ |
| 4.19 | Approximate distance of toilets from dwellings. _____meters |
| 4.20 | Distance of toilet from the home compound's food preparation area, _____meters |
| 4.21 | Distance of toilet from the home compound's water source_____meters |
| 4.22 | Presence of human faeces in the home compound (observe)<br>Yes ☐ No ☐ |
| 4.23 | Presence of a refuse pit in the compound (observe) |

| | |
|---|---|
| | Yes ☐ No ☐<br>**If yes**, what is the distance from household to refuse pit. _____meters |
| 4.24 | Presence of a utensils drying rack in the compound (observe) Yes ☐ No ☐ |
| **5.0** | **ENVIRONMENT** |
| 5.1 | Number of separate buildings in the home compound: _____ |
| 5.2 | General cleanness of the compound? (observe) Good ☐ Fair ☐ poor ☐ |
| 5.3 | Do you have the following animal(s) in the compound?<br>**Sheep**: Yes ☐ No ☐ **Goat:** Yes ☐ No ☐<br>**Cow:** Yes ☐ No ☐ **Pigs:** Yes ☐ No ☐<br>**Chicken**: Yes ☐ No ☐ **Others** ☐**,** Specify_____ |
| 5.4 | Have you entered the calf/livestock pen in the previous 2 weeks?<br>Yes ☐ No ☐ Not Applicable ☐ |
| 5.5 | Did you have any contact with livestock drinking water in the previous 2 weeks?<br>Yes ☐ No ☐ Don't know☐ Not Applicable ☐ |
| 5.6 | Have you done any of the following in the last 2 weeks?<br>Walking in water? Yes ☐ No ☐ Don't know ☐<br>Swimming? Yes ☐ No ☐ Don't know ☐<br>Working in irrigation farm? Yes ☐ No ☐ Don't know ☐ |
| **6.0** | **KNOWLEDGE ON PREVENTION AND CONTROL** |
| 6.1 | Did you attend health education session on diarrhoea in the last 1 year?<br>Yes ☐ No ☐ Don't know ☐ |
| 6.2 | How severe do you think bloody diarrhoea is compared to other diseases?(probe)<br>Life threatening ☐ Severe but not life threatening ☐<br>A problem but not severe ☐ Only mild, not a problem at all ☐ |
| 6.3 | How do you think people get bloody diarrhoea? |
| 6.4 | What treatment do you use for acute bloody diarrhoea? |
| 6.5 | How can you prevent yourself from getting acute bloody diarrhoea?<br>Treat water with chemicals ☐ Boil water ☐ |

| | |
|---|---|
| | Wash hands with soap ☐ Cook foods well ☐<br>Dispose off human faeces well ☐ Other ☐ (specify)_____ |
| **7.0** | **GLOBAL POSITIONING SYSTEM (GPS) READING** |
| 7.1 | While outside the house of the case or control, use the GPS to mark waypoint/location.<br>GPs code (number): _____<br>Latitude (**south**) : S_____<br>Longitude (**East**) : E_____<br>Altitude (**Elevation**): _____meters |

Appendix 3: Laboratory Based Surveillance Form

| **ACUTE BLOODY DIARRHOEA CASES AND CONTROLS** |
|---|
| **1.1 Identification No**: ☐☐☐☐<br>2 first letters of site' name + day (2 digits) and month (2 digits) numbers + Ranking number of the admission in the surveillance ***(this number should be same for case and matching controls)*** |
| **1.2** Recruiting health facility: Mbagathi D.H ☐ Langata H/C ☐ Riruta H/C ☐<br>Kilifi D.H ☐ Bamba S.D.H ☐ Vipingo H/C☐ |
| **1.3** Name of the case/control (last, middle, first): |
| **1.4 Gender:** Male☐ Female☐ |
| **1.5 Age:** **data of birth** ____/_____/_____ |
| **1.6 Status:** Acute bloody diarrhoea case ☐ Neighbourhood control 1 ☐<br>Neighbourhood control **2**☐ |
| **1.7** Type of Sample: Stool ☐ Rectal Swab☐ |
| **1.8** Specimen collected at: Laboratory☐ Health facility☐ at home☐ |
| **1.9** Date specimen collected ____/_____/_____ Time specimen collected: |
| **1.10** Name of person who collected the specimen: |
| **1.11** If specimen collected outside the laboratory:<br>Date specimen received in lab ____/_____/_____ Time: |
| **1.12** Transport Media: Cary Blair Media☐ Filter paper☐ None☐ |
| **1.13** Specimen condition: Adequate ☐ Not adequate☐<br>If not adequate, explain_____ |
| **1.14** Taken antibiotics since the onset of symptoms(skip for controls): Yes☐ No☐<br>If yes, name of antibiotic_____Number of days taken_____ how many days ago _____ |
| **1.15** Taken Traditional Medicine Since the onset of symptoms(skip for controls):<br>Yes☐ No☐ if yes, number of days taken_____ how many days ago _____ |
| **1.16** Macroscopic examination done**: y**es☐ No☐ If no, why? _____ |
| **1.17** If macroscopic done, results: Formed☐ Loose ☐ Watery☐<br>Visible blood☐ Mucus ☐ maleana☐ Other, specify_____ |

**1.18** Presence of round worms, thread worms or tapeworm proglottids?

Yes☐ No☐ if yes, Specify_____

| **2.0 DIRECT EXAMINATION OF STOOL AND RECTAL SWAB SAMPLES** | | | |
|---|---|---|---|
| **Test Description** | **If done, indicate Results**<br>**If Not done, state why** | | |
| **2.1** Results on microscopic exam | Direct method<br>---<br>---<br>Concentration Method<br>---<br>--- | | |
| **2.2** Culture results (Description of cultural characteristics) | **Media Used** | | **Colony Morphology** |
| | 1. | | |
| | 2. | | |
| | 3. | | |
| | 4. | | |
| | 5. | | |
| | 6. | | |
| **2.3 Biochemical test** | **Results** | | **Additional comments** |
| | **Positive(+)** | **Negative(-)** | |
| Motility | | | |
| Oxidase test | | | |
| Urease test | | | |
| Indole | | | |
| String test | | | |
| TSI reaction | | | |
| | | | |

| | |
|---|---|
| **2.4** Indicate the conclusion on pathogen isolated | ---------- ---------- ---------- ---------- ---------- …… ……... |

| **3.0 SEROTYPING** | | | | | | |
|---|---|---|---|---|---|---|
| **Pathogen isolated** | **Type of test** | **Results** | | **Serotyping the isolate** | **Results** | |
| | | **(+) positive culture** | **(-) Negative culture** | | **(+) positive culture** | **(-) Negative culture** |
| **3.1** *Shigella* | Culture | + | - | Shigella Dysenteriae | + | - |
| | | | | Shigella boydii | + | - |
| | | | | *Shigella flexneri* | + | - |
| | | | | *Shigella sonnei* | + | - |
| **3.2** *Escherichia coli* | Culture | + | - | Enteropathogenic *E. coli* (EPEC) | + | - |
| | | | | Entrotoxigenic *E.coli* (ETEC) | + | - |
| | | | | Enteroinvasive *E. coli* (EIEC) | + | - |
| | | | | Diffusely adhering *E. coli* (DAEC) | + | - |
| | | | | Enteroaggregative *E. coli* (EAEC) | + | - |
| | | | | Enterohemorrhagic *E. coli* (EHEC) | + | - |
| **3.3** Vibrio | Culture | + | - | Inaba | + | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| Cholera | | | | Ogawa | + | - |
| **3.4** Salmonella | Culture | + | - | Salmonella *Typhi* | + | - |
| | | | | Salmonella *Paratyphi* | + | - |
| **3.5** *Camphylobacter Jejuni* | Culture | + | - | | | |
| **3.6** Aeromonas | Culture | + | - | | | |
| **3.7** Other, specify | Culture | + | - | | | |

**4.0 ANTIMICROBIAL SUSCEPTIBILITY TEST**

**4.1 Organism Isolated:** *Shigella* ☐ *Escherichia coli* ☐ *Vibrio Cholera*☐ *Salmonella*☐ *Camphylobacter jejuni*☐ *Aeromonas*☐ Other ☐, specify_____

*N/B: if more than one organism isolated, fill the results for each in a separate sheet*

| **4.2 Drug** | **Susceptible (S)** | **Intermediate (I)** | **Resistant ®** | **Additional comments** |
|---|---|---|---|---|
| Nalidixic acid | | | | |
| Erythromycin | | | | |
| Cotrimoxazole (Trimethoprim-Sulfamethoxazole) | | | | |
| Doxycycline | | | | |
| Tetracycline | | | | |
| Amoxicillin | | | | |
| Ampicillin | | | | |
| Chloramphenicol | | | | |
| Gentamycin | | | | |

| | | | | |
|---|---|---|---|---|
| Amoxicillin-Clavulanic acid | | | | |
| Ciprofloxacin | | | | |
| Ceftriaxone | | | | |

**5.0** Routine quality control (QC): Preserved isolate (Labelled) Yes☐ NO☐

**Lab Technologist _____ Sign_____ Date_____**

*N/B: all isolates will be sent to the National reference laboratory.*

**Appendix 4: Water Sample Collection Form**

## BACTERIOLOGICAL EXAMINATION OF WATER

| | |
|---|---|
| ***Instructions:*** *For every case of acute bloody diarrhoea, collect a total of 4 samples. Collect 2 samples (household and main source) from the case and 2 samples (household and main source) from the control which you collected a stool sample. Fill a form for each sample* | |
| **Identification No**: ☐☐☐☐<br>2 first letters of site' name + day (2 digits) and month (2 digits) numbers + Ranking number of the admission in the surveillance ***(this number should be same for case and matching controls)*** | |
| **Status** *(select appropriately)*<br>Case ☐<br>Neighbourhood control **1** ☐<br>Neighbourhood control **2** ☐ | **Sample taken from:**<br>Household ☐<br>Main source ☐ |
| Name of the client (last,middle, first) | |
| Time and date sample was taken | |
| Sample collected by | |
| Source of Sample (state if well, stream or public supply) | |
| Is the source protected? | |
| If protected, how? (is it completely covered or sides only) | |
| Is there a pump? | |
| If a pump is present, how long has it been in use? | |
| Has the Pump been overhauled recently? | |
| Specify the exact site sample was taken from (e.g. Kitchen tap, household container, storage tank, direct from Mains etc.) | |
| Are there any latrines or other source of pollution? | |
| If pollution exist, where? | |
| Is it chlorinated supply? | |

**BACTERIOLOGICAL EXAMINATION OF WATER**

**REPORT**

| | |
|---|---|
| Lab Ref. No: | |
| Time and date sample received | |
| Sample Quantity | Adequate ☐ Inadequate ☐ |
| Time and date Sample examined | |
| Total Coliforms Count (MPN/100mls) | |
| Faecal Coliforms (E.Coli) Count (MPN/100mls) | |
| Free Chlorine (mg/l) | |
| Total Chlorine (mg/l) | |

**Remarks**

_____

_____

_____

**Laboratory officer (name)**

**Appendix 5: Consent Form (English Version)**

**Identification No.** | | | | | **Interviewer:** _____

**Project title:** Enteric Pathogens and Potential Risk Factors for Acute Bloody Diarrhoea in Nairobi West and Kilifi Districts. A case control study

**Name of investigator:** Charles K. Njuguna

**Institution:** Institute of Tropical Medicine and Infectious Diseases, Jomo Kenyatta University of Agriculture and Technology

**Investigators Statements**

I am a Ph.D. student at JKUAT/ITROMID. Part of my study requirement is conducting research work. Therefore, i am kindly requesting you to take part in this research study. The purpose of this form is to make sure that you have all information about the study before you decide to join the study. Read the form very carefully, if you do not understand any part of it, I will be happy to make clarifications. You may ask questions about the risk, benefits and your rights as a volunteer or anything else about this research.

When you understand and feel satisfied with the answers to your questions, you can decide whether to join the study or not. Remember your participation is voluntary**.** The information you provide will be held in strict confidence and will be used for the purpose of this study.

**Purpose of the study**

This study involves research on Enteric Pathogens and Potential Risk Factors for Acute Bloody Diarrhoea in Nairobi West and Kilifi Districts. The aim of this study is to determine the epidemiological, clinical and laboratory characteristics of acute bloody diarrhoea cases occurring in the urban and rural populations in Kenya, so as to provide scientific reference for prevention and control of enteric infectious diseases.

**Request**

The investigator hereby requests your participation in this research study. For children, the details in this consent will be explained to the parent or guardian before the consent is obtained.

**Study procedures**

This study will involve interviews for all cases and control using a questionnaires. You will be asked questions relating to demographic (age, sex, education level, social economic status, social cultural issues etc.). You will also be asked about issues related to knowledge on prevention of diarrhoeal diseases, water, sanitation and personal hygiene. All cases will be asked to give some relevant clinical information; vital signs like Blood Pressure, Temperature and Pulse will be taken.

A stool samples or rectal swab will be taken from cases with acute bloody diarrhoea and control. You will be given a container to put your stool. If collection of a stool sample will be difficult, a rectal swab will be taken. Therefore, you will be made to lie down on a coach in a recovery position, a swab will be inserted in your rectum and this may cause some minimal discomfort. The stool or rectal swab will be taken to laboratory for analysis.

Water samples will be collected from your household storage container for drinking water and from your main source. The two samples will be taken to laboratory for analysis. A Global Positioning System (GPS) will be used to identify the location of your house for the purpose of mapping.

**RISK**

There are no risks whatsoever involved in the study. In case a rectal swab will be taken, it may cause minimal discomfort.

**BENEFITS**

The interview will help you personally to assess your hygiene status and whether you are taking the right measure towards prevention of diarrhoea infections. Your information will help in understanding the possible risk/protective factors related to acute bloody diarrhoea. The information will also help in developing interventions to improve the prevention and control of diarrhoea diseases. The stool samples will be analyzed in the laboratory free of charge, and the results will be used by your doctor in your clinical management should any infections be isolated and identified.

**CONFIDENTIALITY**

The investigator guarantees that all information given will be kept private and remains Confidential. Only the staff will have access to the information. Personal information will not be released to any one, only the investigator will review your study information. Questionnaires will be securely locked in cabinets. Specimens (isolates) will be archived securely. The results of the study may be published or disseminated without revealing your identity.

**RIGHTS**

Your participation in this study is voluntary. Your usual standard health care will not be affected if you decide not to be in the study. You may refuse or decline to answer any question that you don't want to answer. You are also free to leave interview at any time.

**COMPESATIONS**

After the completion of the study, participants will be informed of the findings of the research in general and individual subjects will be informed of any finding that relates to their particular health status. There will be no monetary compensation

**PARTICIPANT'S STATEMENT**

I have read the consent form. The details in this consent have been explained to me. The nature of the study has been explained to me and will involve interview, household investigation and having a stool or rectal swab taken. While the stool or rectal swab

results will remain the confidential property of the investigator, significant findings that may influence further management of any clinical condition detected will be made available to me. All my questions have been answered and I freely and voluntarily choose to be in the study. I understand that I have rights to refuse to answer any questions or withdraw from the study any moment, and this will not affect my future care or treatment in any way. I understand my rights and privacy will be maintained throughout.

I have been fully informed about the study including the benefits, risks and my rights as a participant. Consequently, I hereby wish to consent to be in the study.

Name (optional) _____Signature or thumbprint: _____Date:_____

**Investigator or Representative**

My signature certifies that I have explained the objectives and procedures for this study to the participant and that I have answered all the questions that the participant had about the study and that the participant has voluntarily agreed to take part in the research.

Name……Signature……. Date……..

**Note:** If you have any questions or concerns about this research study, please contact the investigator: Charles K. Njuguna, c/o ITROMID Director Telephone Number 067-52095; PO BOX 62000-00200 Nairobi. Cell phone 0722882214; Email: njugunack@gmail.com or The Secretary, KEMRI National Ethics Review Committee PO BOX 54840, 00200 Nairobi; Telephone Number 020-2722541 Cell phone 0722205901 or 0733400003.

**Appendix 6: Consent Form (Kiswahili Version)**

**Identification No.** | | | | | **Interviewer: _____**

***Anwani ya Utafiti:*** *Vimelea vya matombo na hatari wezekani za kuhara damu kunakokithiri katika wilaya za Nairobi West na Kilifi*. Somo la udhibiti.

***Jina la Mtafiti:*** *Charles K. Njuguna*

***Chuo****: Idara ya Utafiti wa Madawa na Magonjwa ya Kuambukiza katika Chuo Kikuu cha Kilimo na Teknolojia cha Jomo Kenyatta.*

**Taarifa ya Mtafiti**

*Mimi ni mwanafunzi wa shahada ya PHD katika chuo cha JKUAT/ITROMID. Kati ya mambo yanayohitajika katika somo/taaluma hii ni kufanya utafiti. Huu basi ni wito wa kukuomba ushiriki katika utafiti huu. Fomu hii ni ya kuhakikisha kwamba una habari zote kuhusiana na utafiti kabla ya kuchukua hatua ya kushiriki. Tafadhali isome fomu hii kwa makini na ikiwa kuna mahali usipoelewa niko tayari kutoa ufafanuzi. Unaweza kuuliza maswali kuhusu athari au tahadhari, manufaa ama haki zako kama anayejitolea ama mambo mengine yoyote yanayohusiana na utafiti huu.*

*Baada ya kuelewa na kutosheka na majibu ya maswali yako unaweza kufanya uamuzi wa kushiriki au kutoshiriki. Kumbuka kwamba kushiriki kwako ni kwa hiari. Habari utakazotoa zitawekwa kwa faragha na zitatumiwa kwa minajili ya utafiti huu pekee.*

**Kiini cha utafiti**

*Somo hili linahusisha utafiti juu ya vimelea vya matumbo na hatari wezekani za kuhara damu kunakokithiri katika wilaya za Nairobi West na Kilifi. Madhumuni ya somo hili ni kutambua hulka za kiepidemologia, kikliniki na za kimaabara za kuhara damu kunakokithiri, zinazowaathiri watu wa Mijini na Mashambani nchini Kenya, ili kutoa marejeleo yakisayansi ya kutoa kinga na kudhibiti magonjwa ambukizi ya matumbo.*

***Ombi***

*Mtafiti anaomba kushiriki kwako katika utafiti huu. Kwa watoto wazazi au walezi wao watapewa maelezo kabla kutoa idhini*

***Utaratibu wa Utafiti***

*Somo hili linahusisha mahojiano ya matukio yote na udhibiti kwa kutumia hojaji.Utaulizwa maswali ya kidemographia (umri, jinsia, viwango vya elimu, hali za kijamii na kiuchumi, kitamaduni na mengineo). Pia utaulizwa mambo yanayohusiano na ufahamu wako kuhusu udhibiti wa magonjwa ya kuhara, maji na usafi wa kibinafsi. Maswali yote yataulizwa ili kutoa habari husika za kikliniki; dokezo muhimu kama vile shinikizo la damu, Joto na kupiga moyo zitachukuliwa.*

*Sampuli ya choo ama makoleo yatokanayo na njia ya choo itachukuliwa kutoka matukio yote ya kuhara damu kunakokithiri na udhibiti. Utapewa chombo cha kutia choo chako. Kama haitawezekana kuchukua choo chako, makoleo ya njia ya choo itachukuliwa. Hivo basi utafanywa kulala chini katika kitanda kana kwamba unapumzika kisha utatiwa kidude katika njia ya choo, hii inaweza kukufanya uhisi usumbufu kidogo. Choo chako au koleo la kutoka njia ya choo itapelekwa kwa maabara kufanyiwa utafiti.*

*Sampuli ya maji itachukuliwa kutoka vyombo vya kuhifadhia maji ya kunywa na kutoka utoako maji. Sampuli mbili zitapelekwa kwa maabara kufanyiwa uchunguzi. Chombo cha Global Positioning System (GPS) kitatumiwa kutambulisha iliko nyumba yako kwa minajili ramani tambulizi.*

**HATARI/TAHADHARI**

*Hakuna hatari yoyote ile inayohusika na utafiti huu. Kama koleo la kutoka njia ya choo itachukuliwa  kuna uwezekano wa kuhisi usumbufu mdogo.*

***MANUFAA***

*Mahojiano yatakusaidia kibinafsi kupima hali ya usafi wako na kama unachukua hatua mwafaka kuzuia maambukizi ya kuhara. Habari utakazotoa zitatusaidia kuelewa hatari ama uzuiaji unaohusiana kuhara damu kunakokithiri. Habari hizi pia zitasaidia*

*kutengeneza njia za kusaidia kuzuia na kudhiti magonjwa ya kuhara. Sampuli ya choo chako itachunguzwa katika maabara bila malipo na matokeo yatatumiwa na daktari wako kutoa matibabu ikiwa utaonekana kuwa na maambukizi.*

***KUBANWA KWA UTAFITI***

*Mtafiti antoa hakikisho kuwa habari zitazotolewa zitawekwa bila kupeanwa na zitabaki kuwa siri. Ni wafanyikazi pekee watakaoruhusiwa kuzipata. Habari za kibinafsi hazitapeanwa kwa awaye yote, ni mtafiti pekee ataziangalia. Hojaji zitafungiwa katika makasha. Sampuli nazo zitafungiwa kwenye makavazi*

***HAKI ZAKO***

*Kushiriki kwako katika utafiti huu ni kwa hiari. Hali yako ya afya ya kawaida haitaathiriwa ikiwa utaamua kutokuwa katika utafiti huu. Unaweza kukataa kujibu swali lolote usilolipenda. Uko na uhuru wa kutoka mahojianoni wakati wowote.*

***FIDIA***

*Baada ya kukamilika kwa utafiti, washiriki watajulishwa matokeo yake kwa ujumla na kila mshiriki atajulishwa matokeo yanahusiana na hali zake za afya. Hakutakua na kulipwa kwa kushiriki ama fidia yeyote.*

***TAARIFA YA MSHIRIKI***

*Nimeisoma fomu hii ya kutoa idhini. Mambo yote yaliyo katika fomu hii yameelezwa kwangu. Hali zote kuhusiana na somo hili zimeelezwa na zitahusisha mahojiano, uchunuguzi wa jamaa ya nyumba yangu na kupeana sampuli ya choo changu au koleo kutokana na njia ya choo. Wakati uo huo sampuli ya choo changu au koleo kutokana na njia ya choo zinapochukuliwa habari hizi zitabaki kuwa siri ya mtafiti, huku matokeo muhimu yanayoweza kuathiri kumudu hali yeyote ya kimatibabu nitafahamishwa. Maswali yangu yote yamejibiwa na kwa hiari yangu najitolea kushiriki katika utafiti huu. Ninaelewa kwamba nina haki ya kukataa kujibu swali lolote ama kutoka kwa mahojiano wakati wowote ule na hii haitaathiri matibabu yangu ya siku zijazo kwa njia yoyote ile. Ninaelewa haki zangu na faragha yangu itaheshimiwa wakati wote.*

*Nimefahamishwa kuhusu utafiti huu, manufaa, yake, athari na haki zangu kama mshiriki. Hivyo basi, natoa idhini ya kushiriki katika utafiti huu.*

*Jina(Hiari)_____sahihi/Alama ya kidole gumba_____Tarehe_____*

***Mtafiti ama Mwakilishi***

*Sahihi yangu ni dhibitisho kuwa nimeeleza kiini na taratibu ya utafiti huu kwa mshiriki na nimeyajibu maswali yote ambayo mshiriki alikuwa nayo kuhusu utafiti huu na mshiriki kwa hiari yake amekubali kushiriki.*

***Dokezo***

*Ikiwa una swali ama shaka kuhusu utafiti huu tafathali wasiliana na mtafiti: Charles Njuguna, c/o ITROMID Director Telephone Number 067-52095; PO BOX 62000-00200 Nairobi. Cell phone 0722882214; Email:* njugunack@gmail.com *ama Katibu* KEMRI National Ethics Review Committee PO BOX 54840, 00200 Nairobi; Telephone Number 020-2722541 Cell phone 0722205901 or 0733400003.

**Appendix 7: Consent Form (Giriama Version)**

**Identification No.** | | | | | **Interviewer: _____**

***Anwani ya uyeyi:*** *Vimera vya ndani na hatari wezekani za kufyoka milatsa kahi za wilaya za Nairobi Magaribi na Chilifi*. Shomo ra uzibiti.

***Dzina ra muyeyi:****Charles K. Njuguna*

***Chuo****: Idara ya Uyeyi wa Mihaso na Manyonge ga Kubwizshana kahi za Chuo Chikulu cha Chirimo na Tekinolojia cha Jomo Chinyata.*

**Ujumbe wa Muyeyi**

*Mimi ni mwanafunzi wa shahada ya PHD kahi za chuo cha JKUAT/ITROMID. Kahi za mambo gahenzekanago kahi za shomo/taaluma ii ni kuhenda uyeyi. Uu ni mwiho wa kukuvoya ushiriki kahi za uyeyi uu. Fomu ii ni ya kuhakikisha kukala una habari zosini kuhusiana na uyeyi uu kabila ya kuhala hatua ya kushiriki. Tafadhali isome fomu ii kwa makini na uchikala na vahali usivoelewa ni tayari kulavya maelezo. Unaweza kuza maswali kuhusu athari au tahadhari, manufaa au hachi zo here adzitoleae au mambo manjine gogosi dzulu ya uyeyi uu.*

*Bada ya kuuelewa na kutosheka na majibu ga maswali go unaweza kuhenda wamuzi wa kushiriki au kusashiriki. Kumbukira kukala kushiriki ko ni kwa hiari. Habari undizozilavya zindaikwa faraghani na zindahumirwa kwa mambo ga uyeyi uu hacheye.*

**Chisi cha uyeyi**

*Shomo riri rinahusisha uyeyi dzulu ya vimera vya ndani na hatari wezekani za kufyoka milatsa kahiza wilaya za NairobiMagaribi na Chilifi. Madhumuni ga shomo riri ni kutambua huluka za kiepidemolojia, chikliniki na za chimaabara za kufyoka milatsa zizuruzo atu a Matauni na Midzmidzi kahi za tsi ya Kenya, ili kulavya mlangaza wa chisayansi wa chinga na kuweza manyonge ga kubwizshana ga ndani.*

***Voyi:***

*Iye muyeyi yunakuvoya ushiriki kahi za uyeyi uu. Kwa ahoho, avyazi au arezi ao andahewa maelezo kabila ya kulavya idhini.*

***Utaratibu wa Uyeyi***

*Shomo riri rinahusisha mahojiano ga matukio gosi na uzibiti kwa kuhumira hujaji.Undauzwa maswali ga chidemografia (umuri, jinsia, viwango vya elimu, hali za chijamii na chiutsumi, chitamaduni na manjinego). Pia undauzwa mambo gahusianago na ufahamu o kuhusu uzibiti wa manyonge ga kufyoka, madzi na usafi wa chibinafsi. Maswali gosi gandauzwa ili kulavya habari husika za chikliniki; dukizo muhimu here sindikizo ra milatsa, dzoho mwirini na kupiga kwa moyo vindapimwa.*

*Sampuli ya choo au madzimadzi galaago kahi za njira ya choo ya nyuma gandahalwa kula kwa atu osi ario na kufyoka milatsa na kuzibiti. Undahewa chia cha kubuma sampuliyo ya choo. Ichikala kaindawezekana kuhala sampuli ya choo basi madzimadzi ga njira ya choo ya nyuma gandahalwa.Hivyo basi undalala chitandani here apumzikaye kisha undabumwa chidude kahiza njira ya choo ya nyuma.Vivi vinaweza kukuhenda usikire kubujwa chidogo. Hicho choo au go madzimadzi ga njira ya choo ya nyuma vindavirikwa kahi za maabara na kupimwa ili vihenderwe uyeyi uu.*

*Sampuli ya madzi indahalwa kulaa kwa vyombo vya kuhifadhira madzi ga kunwa na kulaa kura uhekako madzi. Sampuli zosi mbiri zindavirikwa kwenye maabara kuhenderwa uchunguzi. Chombo cha chitaalamu chihiwacho Global Positioning System (GPS) chindahumirwa konyesa nyumbayo iriko kahiza ramani tambulizi.*

**HATARI/TAHADHARI**

*Kakuna hatari zozosi ambazo zinahusikana na uyeyi uu.Ichibidi ulaviwe madzimadzi kula njira ya choo ya nyuma inawezekana usikire usumbufu chasi chitite..*

***MANUFAA***

*Mahojiano gandasaidia chibinafsi kupima hali ya usafio na imanyikane chamba unahala hatua zihenzekanazo za kuzulia kubwirwa ni kufyoka. Habari undizozilavya zindahusaidia kuelewa hatari au uzuliaji wa kufyoka milatsa kurivyo.Habari zizi pia zindasaidia kahiza kutengeza njira za kusaidia kuzulia na kuzibiti manyonge ga kufyoka. Sampuli ya choo undiyo ilavya indahenderwa uchunguzi kahiza maabara bila marivo na majibu gandahumirwa ni dakitario kukulagula ichikala undapatikana na unyonge.*

***KUBANWA KWA UYEYI***

*Muyeyi yunalavya hakikisho kwamba habari zindizolaviwa zindaikwato bila kulaviwa kwa atu anjine oosi bali zindabaki kukala siri. Ni ahendaji kazi hachiayo andioruhusiwa kuzipata. Habari za chibinafsi kazidalaviwa kwa mtu yeyosi,bali zindahumirwa ni iye muyeyi hacheye. Hojaji zindafungirwa kahiza makasha. Sampuli nazo zindafungirwa kwenye makavazi.*

***HACHI ZO HERE MSHIRIKI***

*Kushirikiko kahiza uyeyi uu ni kwa hiari. Hali yo ya afya ya kawaida kaindazuriwa ichikala undamua kusashiriki kahiza uyeyi uu. Unaweza kukahala kujibu swali rorosi usirorihenza. Una uhuru wa kombola kahiza mahojiano wakati wowosi.*

***FIDIA***

*Baada ya kumarigiza uyeyi uu, ashirika osi andaambirwa majibu gao ga jimula na kila mshiriki yundaambirwa majibuge gahusianago na haliye ya chiafya. Kakundakala na marivo gogosi kwa kushiriki au fidia yoyosi.*

***HABARI YA MSHIRIKI***

*Nidziisoma fomu ii ya kulavya idhini. Mambo gosi garigo kahiza fomu ii dzigaelezwa. Hali zosi kuhusiana na shomo riri nidzizielezwa na zindahusisha mahojiano, uchunuguzi wa jamaa a nyumba yangu na kulaviwa sampuli ya choo changu au madzimadzi ga njira ya choo ya nyuma. Wakati uo uo sampuli ya choo changu au*

*madzimadzi ga njira yangu ya choo ya nyuma vindivohalwa,habari zizi zindabaki kukala siri ya iye muyeyi,kuno majibu muhimu gawezago kunizuru kahiza kuimudu hali yangu ya chiafya na ya chiganga nindaimanyishwa. Maswali gangu gosi gadzijibiwa na kwa hiari yangu nadzitolea kushiriki kahiza uyeyi uu. Ninaelewa kwamba nina hachi ya kukahala kujibu swali rorosi au kombola kahiza mahojiano wakati wowosi uratu na vivyo kavindazuru uganga wangu macheroni kwa njira yoyosi irahu. Ninaelewa hachi zangu na faragha yangu indaheshimiwa wakati wosi.*

*Nidzimanyishwa kuhusu uyeyi uu, manufaage,atharize na hachi zangu here mshiriki.Kwa hivyo basi, nalavya idhini ya kushiriki kahiza uyeyi uu.*

*Dina(Hiari)_____sahihi/Alama ya dzalagumba_____Tarehe_____*

***Muyeyi au Mwimirizi we***

*Sahihi yangu ni chizibiti kwamba nidzielezwa chisi na taratibu za uyeyi uu kwa mshiriki na nidzijibu maswali gosi ambago mshiriki yudzikala nago kuhusu uyeyi uu na mshiriki kwa hiariye yudzikubali kushiriki.*

***Dukizi***

*Ichikala una swali ama shaka kuhusu uyeyi uu tafathali wasiliana na muyeyi mwenye: Charles Njuguna, c/o ITROMID Director Telephone Number 067-52095; PO BOX 62000-00200 Nairobi. Cell phone 0722882214; Email:* njugunack@gmail.com *au Katibu* KEMRI National Ethics Review Committee PO BOX 54840, 00200 Nairobi; Telephone Number 020-2722541 Cell phone 0722205901 or 0733400003.

**Appendix 8: Assent Form (English version)**

**Identification No.** | | | | | **Interviewer:** _____

***Note: This form is to be used by children who are above 7 years and less than 18 years***

| |
|---|
| **Project Title:** Enteric Pathogens and Potential Risk Factors for Acute Bloody Diarrhoea in Nairobi West and Kilifi Districts. A case control study |
| **Name of Principal Investigator:** Charles K. Njuguna |
| **Institution:** Institute of Tropical Medicine and Infectious Diseases, Jomo Kenyatta University of Agriculture and Technology |

My name is Charles Njuguna. I am doing a PhD degree at the Jomo Kenyatta University of Agriculture and Technology. I am trying to learn more about why people are suffering from diarrhoea with blood. This may provide answers to what is causing the problem in towns and rural areas and as a result, we can be able to come up with a way of dealing with this problem. To do this, I am asking you and others to take part in my research study. A research study is a way to learn more about something. You are being asked to join this research study because you have diarrhoea with blood (case) or you are in the same age category (control) with another child who has diarrhoea with blood. This form explains the study.

If you decide you want to be in my study, I will ask you to do the following:

- Answer questions that i will ask you which are written in a paper. I will ask you questions about your age, sex, education, health, food, water, cleanness and how to prevent yourself from getting this condition.
- You will go to toilet for a long call and put some stool in a container which I will give to you. In case you will have a problem in passing your stool, a swab will be taken from your anus. In such a case, you will be made to lie down on a

coach, a swab will be put in your anus and this may cause some small discomfort. I will take your stool to laboratory for testing.

Being in this study may not have a direct benefit for you. There are no dangers when you are involved in the study. In case a swab will be taken from your anus, it may cause small discomfort. Your stool will be tested in laboratory free of charge, and the results will be used by your doctor in deciding which drug to give you should any problem be found. Your taking part in this study may help us to learn something that will help other children with this condition.

Other people will not know if you are in my study. The information I write down about you and other children will be kept safely locked up. When I tell other people or write an article about my research, I will not use your name. This way, no one will know that you took part. Your parents or guardian have said it is OK for you to be in the study. You are to choose if you want to do it or not. Before you decide, I will answer any questions you may have. You can also talk to your mom and dad or your guardian.

You do not have to be in this study. It is okay if you decide you do not want to be in the study or if you change your mind and wish to stop at any time. No one will be mad at you. You can say no even if your mom and dad (or guardian) say yes.

My telephone number is 0722882214. You can call me if you have questions about the study. If you decide to be in this study, please sign your name below. I will give you a copy of this form to keep.

**Agreement**

I have decided to be in the study even though I know that I do not have to. I understand that my parents/guardian have/has given permission for me to participate in this study. Charles Njuguna has answered all my questions.

_____ _____

Signature of Study Participant | Date

_____ | _____

Signature of Researcher | Date

**Appendix 9: Assent Form (Kiswahili Version)**

**Identification No.** | | | | | **Interviewer: _____**

***Dokezo: Fomu hii itatumiwa na watoto wa umri wa zaidi ya miaka 7 na chini ya miaka 18***

| |
|---|
| ***AnwaniyaUtafiti:****Vimelea vya matumbo na hatari wezekani za kuhara damu kunakokithiri katika wilaya za Nairobi West na Kilifi*. Somo la udhibiti. |
| ***Jina la Mtafiti Mkuu:****Charles K. Njuguna* |
| ***Chuo:*** *Chuo cha Utafiti wa Madawa na Magonjwa Ambukizi katika Chuo Kikuu cha Kilimo na Teknolojia cha Jomo Kenyatta.* |

*Jina langu ni Charles Njuguna. Ninasomea shahada ya PhD katika Chuo Kikuu cha Kilimo na Teknologia cha Jommo Kenyatta. Ninajaribu kutambua ni kwanini watu huugua magonjwa ya kuhara damu. Kwa kufanya hivi kuna uwezekano wa kutoa majibu ya kinachosababisha shida hii mijini na mashambani, na kwa kufanya hivi, huenda tukagundua njia za kukumbana na shida hii. Ili tuweze kufanya hivi ninakuomba wewe na wengineo kushiriki katika utafiti huu. Unaulizwa kushiriki kwa sababu unaugua kuhara damu ama uko katika umri mmoja na mtoto mwingine anayeugua*

*Ukifanya uamuzi wa kushiriki katika utafiti huu nitakuuliza kufanya mambo afuatayo:*

- *Ujibu maswali nitakayokuuliza ambayo yameandikwa kwa karatasi. Nitakuuliza maswali kuhusi umri wako, jinsia, elimu, afya, chakula, maji, usafi na jinsi unavyojizuia kupata hali hii (maambukizi)*

- *Utaenda msalani kwa haja kubwa na uweke kinyesi kwa chombo nitakachokupa. Endapo hutaweza kutoa kenyesi, usufi utachukuliwa kutoka njia ya kinyesi. Katika hali hiyo utaulizwa kulala kwenye kocha usufi utatiwa ndani ya njia ya kinyesi chako, hii inaweza kukufanya uhisi usumbufu mdogo.*

*Kuwa kati utafiti huu huenda haitakua na manufaa kwako moja kwa moja. Hamna hatari yeyote inayotokana na utafiti. Kama usufi utachukuliwa kutoka kwa njia ya kinyesi chako huenda uakahisi usumbufu mdogo. Kinyesi chako kitafanyiwa uchunguzi kwenye maabara bila malipo, na matokeo yake yatatumiwa na daktari wako kufanya uamuzi juu ya dawa utakazopewa endapo itapatikana kuwa ua shida.*
*Watu wengine hawtajua kama upo katika utafiti huu. Habari ninazoandika kukuhusu na kuhusu watoto wengine zitawekwa na kufungiwa salama. Nitakapowaambia watu wengine au kuandika kuhusu utafiti wangu, sitalitumia jina lako. Kwa kufanya hivi hakuna yeyote atajua ulishiriki.*

*Wazazi au walezi wako wamekubali wewe kuwa katika utafiti huu. Utafanya uamuzi kama ungependa kushiriki ama kutoshiriki. Kabla ya kuamua nitajibu maswali yoyote unaweza kuwa nayo. Unaweza pia kuongea na mama na baba ama mlezi wako.*

*Sio lazima ushiriki. Ni sawa ikiwa utaamua kutoshiriki ama ikiwa utabadilisha nia wakati wowote ule katika utafiti. Hakuna yeyote atakayekukasirikia. Unaza kukataa hata kama mama na baba (au mlezi) amekubali.*

*Nambari yangu ya simu ni 0722882214. Unaweza kunipigia simu kama una swali kuhusu utafiti huu. Ikiwa umeamua kushiriki, tafadhali weka sahihi chini ya fomu hii. Nitakupa nakala ya fomu hii uiweke.*

**Makubaliano**

*Nimeamua kuwa katika utafiti hata kama najua sio lazima niwepo. Nimeelewa kuwa wazazi/walezi wangu wamepeana kibali mimi nishiriki. Charles Njuguna ameyajibu maswali yangu yote.*

_____ _____

*Sahihi ya Mshiriki* *Tarehe*

_____ _____

*Sahihi ya Mtafiti* *Tarehe*

**Appendix 10: Assent Form (Giriama Version)**

**Identification No.** | | | | | **Interviewer: _____**

***Lola hi: Fomu ii ni ya kuhumirwa ni ahoho enye umuri wa zaidi ya miaka mihandahu(7) na tsini ya miaka kumi na minane (18).***

| |
|---|
| **Chitswa cha uyeyi:**Vimera vya ndani na hatari wezekani za chipanya cha kufyoka milatsa kahi za wilaya za Nairobi magaribi na Chilifi.Shomo ra uzibiti. |
| **Dzina ra muyeyi mbomu:**Charles K. Njuguna |
| **Chuo:**Idara ya Mihaso ya kanda ii na manyonge ga kubwizshana,Chuo chikulu cha Chirimo na Tekinolojia cha Jomo Chinyata. |

Dzina rangu ndimi Charles wa Njuguna.Ninashomera shahada ya PhD kahi za Chuo chikulu cha Chirimo na Tekinolojia cha Jomo Chinyata.Najeza kudzifundisha zaidi ni utu wani uhendao atu akale na chipanya cha Kufyoka milatsa.Uyeyi uu unaweza kulavya majibu ga hizo sababu zihendazo atu apatikane ni chipanya chichi kahi za matauni na midzimidzi,matokeo ambago gandahuwezesha kubuni njira za kushuhulikira tatizo ra chipanya chichi. Ili kusaidia kahi za kutatua shida ii,nakuvoya ushiriki kahi za uyeyi uu Kuhenda uyeyi ni njira mwenga yavo ya kudzifundisha zaidi kuhusu jambo fulani.Unasihiwa ushiriki kahi za uyeyi uu kwa sababu una chipanya cha kufyoka milatsa(kudi husika) au ukahi za chiwango cha umuri wa sawa na (kundi ra kuzibiti) muhoho munjine ambaye yunafyoka milatsa.Fomu ii inalavya maelezo ga uyeyi uu.

Uchihenda wamuzi wa kushiriki kahi za uyeyi uu,nindakusihi uhende mambo gaga gatuizirago:

- Ujibu maswali nindigokuza garigoandikwa kahi za karatasi.Nindakuza maswali kuhusu umurio,u mutu wa vi,elimu,afya chakurya,madzi, usafi na jinsi za kudzichinga na chipanya chichi.
- Undakwenda chooni ukalavye choo cha nyuma,uhale sampuli ya chicho choo uibume kahi za chombo ambacho nindakuva.Ichikala vigumu kwako kulavya choo kwa wakati uu,indabidi ulaviwe madzimadzi kulaira na njira yo ya choo ya

nyuma,ambavo undalala kwenye kochi alafuye undabumwa chidude maalumu ko nyuma kwa muda mufuhi ili kulavya go mazimadzi.Vivi vinaweza kukuhenda usikire usumbufu chidogo.Sampuliyo ya choo au go madzimadzi ga nyuma nindavihala ni vivirike  kahi za maabara ili vikachunguzwe

Kushiriki kahi za uyeyi uu kakundakala na manufaa ga mara mwenga kwako.Pia kakuna hatari yoyosi kwako kahi za kushiriki uyeyi uu.Ichibidi ulaviwe madzimadzi kulaira na njira ya choo ya nyuma inawezekana uhisi usumbufu chasi chitite.Sampuliyo ya choo au madzimadzi ga njira ya nyuma vindahenderwa uchunguzi kahi za maabara na majibu gandahumirwa ni dakitario kwamua ni dawa hizo zindizo kufaa,ichikala vanjine udzipatikana na  unyonge uu.Kushirki kwakwako kahi za uyeyi uu kunaweza kuhufundisha manji ambago gandasaidia sana ahoho anjine ario na chipanya chichi.

Atu anjine kandamanya kukala unashiriki kahi za uyeyi uu.Habari zosi nindizo  andika kula kwako na ahoho anjine zindafungirwa zikale siri.Nindivo kala nalavya habari za uyeyi uu kwa atu anjine kahiza taarifa ya uyeyi uu sindahadza dzinaro.Kwa vivyo kakuna andiye manya kukala washiriki kahiza uyeyi uu. Muvyazio/murezio yudzikubali kukala unaweza kushirki kahi za uyeyi uu.Una nafasi ya kuhenda wamuzi wa kushiriki au kusashiriki.Kabila ya kuhenda wamuzi uo nindajibu maswali gogosi undigo kala nago dzulu ya uyeyi uu.Pia unaweza kuzungumuza na mameyo,babayo hebu murezio.

Si vya sharuti kushiriki kahi za uyeyi uu.Uchamua kusashiriki au uchamua kombola kahi za uyeyi uu wakati wowosi dzisikire huru,vyovyosi ni sawa.Unaweza kukahala kushriki hata kala mameyo,babayo au murezio yudzikukubarira

Namba yangu ya simu ni 0722882214. Unaweza kunipigira simu ukanuza swali rorosi undirokalanaro dzulu ya uyeyi uu.Uchamua kushiriki kahi za uyeyi uu ika sahihio/buma chidole kahi za fomu vava tsini.Ndakuva nakala ya ii fomu wike

**Makubaliano**

Dzihenda wamuzi wa kushiriki kahi za uyeyi uu mbali na kukala naelewa si kwa sharuti.Naelewa kukala avyazi angu/murezi wangu yudzinipa ruhusa ili nishiriki kahi za uyeyi uu. Charles Njuguna yudzinijibu maswali gangu gosi.

_____ _____

Sahihi ya mshiriki Tarehe

_____ _____

Sahihi ya Muyeyi. Tarehe

**Appendix 11: Classification of Dehydration**

Dehydration will be classified according to the Ministry of Health Clinical guidelines for level 4-6 Hospitals (2009)

**(1) Children below 5 years**

| No dehydration | Some dehydration (2 signs) | Severe dehydration (2 or more signs) |
|---|---|---|
| Normal | • Restless and irritable<br>• Sunken eyes<br>• Dry mouth<br>• Thirsty, drinks eagerly<br>• Skin pinch goes back slowly (<2 sec)<br>• No tears | • lethargic, unconscious, floppy<br>• sunken eyes<br>• not able to drink or drinking poorly<br>• mouth very dry<br>• skin pinch goes back very slowly (>2 sec)<br>• no tears |

**(2) Older children and adults**

| **Clinical Future** | **Mild dehydration** | **Some dehydration (2 signs present)** | **Severe dehydration (2 or more signs present)** |
|---|---|---|---|
| **General appearance** | Thirsty, alert | Thirsty, Alert | Generally conscious, anxious, cold extremities, cyanosis, muscle cramps |
| **Pulse** | Normal | Rapid | Rapid, thready, sometimes absent |
| **Respiration** | Normal | Deep, sometimes rapid | Deep and rapid |
| **Systolic BP** | Normal | Normal | Low, sometimes un measurable |
| **Eyes** | Normal | Sunken eyes | severely sunken eyes |
| **Tears** | Present | Absent | Absent |
| **Mucous** | Moist | Dry | Very dry |

| | | | |
|---|---|---|---|
| **membranes** (test mouth with a clean figure) | | | |
| **Urine output** | Normal | Reduced, urine dark | Anuria,, empty bladder |
| **% weight loss** | 1-5% | 6-9% | 10% or plus |

**Appendix 12: Glasgow Coma Scale**

The Glasgow Coma scale comprises three tests: eye, verbal and motor responses. The Scale provides a score in the range 3-15; **patients with scores of 3-8 are usually said to be in a coma**. The total score is the sum of the scores in three categories. For adults the scores are as follows:

| **TEST** | **GRADE/ SCORE** | **RESPONSE** |
|---|---|---|
| **Best eye response (E)** | 1 | No eye opening |
| | 2 | Eye opening in response to pain. (Patient responds to pressure on the patient's fingernail bed; if this does not elicit a response, supraorbital and sternal pressure or rub may be used.) |
| | 3 | Eye opening to verbal command, speech, or shout. (Not to be confused with an awaking of a sleeping person; such patients receive a score of 4, not 3.) |
| | 4 | Eyes opening spontaneously |
| **Best verbal response (V)** | 1 | No verbal response |
| | 2 | Incomprehensible sounds. (Moaning but no words.) |
| | 3 | Inappropriate words. (Random or exclamatory articulated speech, but no conversational exchange) |
| | 4 | Confused. (The patient responds to questions coherently but there is some disorientation and confusion.) |
| | 5 | Oriented. (Patient responds coherently and appropriately to questions such as the patient's name and age, where they are and why, the year, month, etc.) |
| **Best motor** | 1 | No motor response |
| | 2 | Extension to pain (abduction of arm, external rotation of shoulder, supination of forearm, extension of wrist, decerebrate response |

| | | |
|---|---|---|
| **response (M)** | 3 | Abnormal flexion to pain (adduction of arm, internal rotation of shoulder, pronation of forearm, flexion of wrist, decorticate response) |
| | 4 | Flexion/Withdrawal to pain (flexion of elbow, supination of forearm, flexion of wrist when supra-orbital pressure applied ; pulls part of body away when nailbed pinched) |
| | 5 | Localizes to pain. (Purposeful movements towards painful stimuli; e.g., hand crosses mid-line and gets above clavicle when supra-orbital pressure applied.) |
| | 6 | Obeys commands. (The patient does simple things as asked.) |

For children under 5, the verbal response criteria are adjusted as follow;

| **SCORE** | **0 to 23 Months.** | **2 to 5 YRS** |
|---|---|---|
| 1 | No response | No response |
| 2 | Grunts or is agitated or restless | Grunts |
| 3 | Persistent inappropriate crying &/or screaming | Persistent cries and/or screams |
| 4 | Cries and consolable | Inappropriate words |
| 5 | Smiles or coos appropriately | Appropriate words or phrases |

**Appendix 13: Water Supply Minimum Standards**

Water quantity 15 liters/person/day

Max. Distance from any household to nearest water source -500m

Max. Queuing time at a water source -15 minutes

Max. Time to fill a 20-litre container of water -3 minutes

Water quality -No faecal coliforms per 100ml at delivery point

Max. Number of people per toilet -20 (up to 50 in emergencies). Sanitation < 1 toilet per 20 people

Max. Distance of toilets from dwellings - 50 m

Max. Distance from any household to refuse pits -100 m

**Guidelines on free chlorine levels in Water distribution system**

Recommended free chlorine levels in water distribution systems in areas affected by epidemic dysentery

The minimum levels of free residual chlorine necessary for safe water are:

- at all points in a piped water system 0.2 - 0.5 mg/litre
- at stand-posts and wells 1.0 mg/litre
- in tanker trucks, at filling 1.5 mg/litre

Regular monitoring is required to ensure that these minimum levels of chlorine are maintained.

**Guidelines on Bacteriological Analysis (Kenya Government Chemist)**

| **Examination of Water** | **CHLORINATED SUPPLY** | **WELL WATER** |
|---|---|---|
| Total Coliforms Count (MPN/100mls | Max Limit= Nil | Max Limit= 10 |
| Faecal Coliforms (E.Coli) Count (MPN/100mls | Max Limit= Nil | Max Limit= Nil |
| Total Plate Count (37$^{o}$c, 48hrs) | Max Limit= $10^{3}$/ml | Max Limit= $10^{3}$/ml |
| Strept. Faecalis | Max Limit= Nil | Max Limit= Nil |
| Strept. aureus | Max Limit= Nil | Max Limit= Nil |

## Appendix 14: Guidelines on Standard Operating Procedures for Microbiology

### Parasitological Examination of Faeces

The examination of faeces for parasitological diagnosis is done to detect:

- Adult worms
- Segments of tapeworms
- Ova and cysts
- Larvae
- Trophozoites
- Cellular exudates such as WBCs, RBCs, macrophages and Charcot-Leyden (CL) crystals

For this, the sample should be properly collected and preserved.

### Collection of faecal sample

- Ask the patient to pass the stool sample directly into a waxed cardboard or a plastic cup with a tight fitting lid. Collection of sample in a match box or on plant leaves is not a satisfactory method.

- About 20-40 grams of well-formed stool or 5-6 table spoonfuls of watery stool will suffice for a routine examination.

- Ingestion of some medicines prior to collection of faecal sample may interfere with the detection of parasites. These include tetracyclines, sulfonamides, antiprotozoal agents, laxatives, antacids, castor oil, magnesium hydroxide, barium sulphate, bismuth kaolin compounds and hypertonic salts etc. These should not be taken 1-2 weeks before the examination of stool sample.

- All specimens must be properly labelled with patient's name, age, sex, and date of collection.

- The specimen must reach the laboratory within 30 minutes of passing of the stool, since amoebic trophozoites die and become unrecognizable after that.

**Note**

- Do not keep the specimen at warm temperatures. Try to keep it in cool, shady places.

- Prevent the drying of the specimen.

- Prevent contamination with urine or dirt particles.

- Multiple stool examinations are required before the presence of parasitic infections is ruled out.

- Stool should not be collected from bed-pans containing disinfectants.

**Transportation of samples**

If looking for trophozoites, stool specimen must be transported very rapidly to the laboratory to avoid disintegration of trophozoites. Stool samples should be examined within 30 minutes of collection of the specimen and not receipt of the specimen in the laboratory. Stool specimens should never be frozen and thawed or placed in an incubator because parasitic forms deteriorate very rapidly.

For permanent fixation of the stool specimen, 10% formol-saline (prepared by adding 100 ml formaldehyde to 900 ml of 0.85% sodium chloride) is a well-known preservative. Polyvinyl alcohol (PVA) is a widely used preservative because the performance of concentration procedures and preparation of permanent stained smears are both possible with this.

**Macroscopic examination**

Various points to be noted are:

- Consistency: The consistency of the stool could be formed, soft, loose or watery. The cysts are found maximum in the formed stool while trophozoites are most abundant in watery stool.

- Presence of blood and mucus.

- Presence of round worms, thread worms or tapeworm proglottids.

- Colour and smell of the stool.

**Microscopic examination (temporary wet mounts)**

It is the simplest and easiest technique. A wet mount can be prepared directly from faecal material or from the concentrated specimens. The basic types of wet mounts that should be made from each sample include:

a) **Saline wet mount:** It is used to detect worm eggs or larvae, protozoan trophozoites and cysts. In addition it can reveal the presence of RBCs and WBCs.

b) **Iodine wet mount:** It is used to stain glycogen and nuclei of the cysts.

**Procedure**

- Place a drop of saline on left half of the slide and one drop of iodine on the right half.

- With an applicator stick pickup a small portion of the specimen (equivalent to the size of a match head) and mix with saline drop.

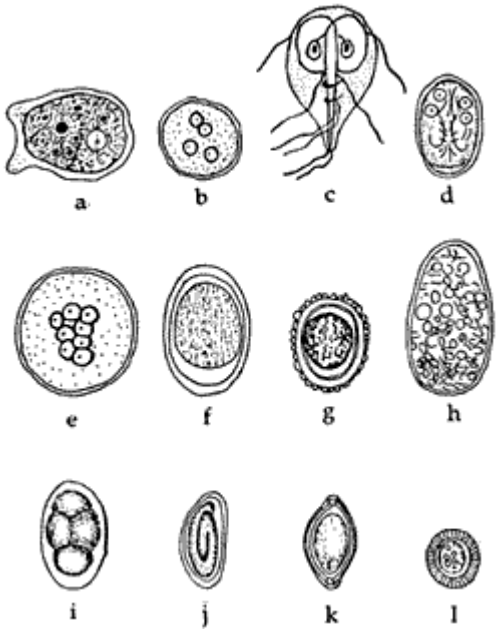
- Similarly pickup similar amount and mix with a drop of iodine.

- Put the cover slip separately on both and examine under the microscope.

- Ova, cysts, trophozoites and adult worms can be identified as per their characteristic features (Fig 1 and Table 1).

- Iodine wet mount is examined for amoebic and flagellar cysts.

**Morphological features of common parasites/eggs/ova/cysts**




**Concentration techniques**

If the number of parasites in the stool specimens is low, examination of a direct wet mount may not detect them, hence the stool should be concentrated. Eggs, cysts and larvae are recovered after concentration procedures whereas trophozoites get destroyed during the procedure. This makes direct wet mount examination obligatory as the initial phase of microscopic examination.

The concentration procedures can be grouped under 2 categories:

a) Sedimentation procedures: In which the eggs and cysts settle down at the bottom.

b) Flotation procedures: In which the eggs and cysts float at the surface due to specific gravity gradient.

The basic disadvantage of sedimentation technique is that examination of the sediment is often difficult due to the presence of excessive faecal debris that may mask the presence of the parasites. The basic disadvantage of flotation technique is that not all eggs and cysts float in the flotation procedures.

Two commonly used concentration techniques are formalin-ether and saturated salt solution technique.

**Formal ether sedimentation technique**

- **Procedure**

- Transfer half teaspoonful of faeces in 10 ml of water in a glass container and mix thoroughly.

- Place 2 layers of gauze in a funnel and strain the contents into a 15 ml centrifuge tube.

- Centrifuge for 2 minutes at about 500 g.

- Discard the supernatant and resuspend the sediment in 10 ml of physiological saline. Centrifuge at 500 g and discard the supernatant.

- Resuspend the sediment in 7 ml of 10% formaldehyde (1 part of 40% formalin in 3 parts of saline).

- Add 3 ml of ether (or ethyl acetate).

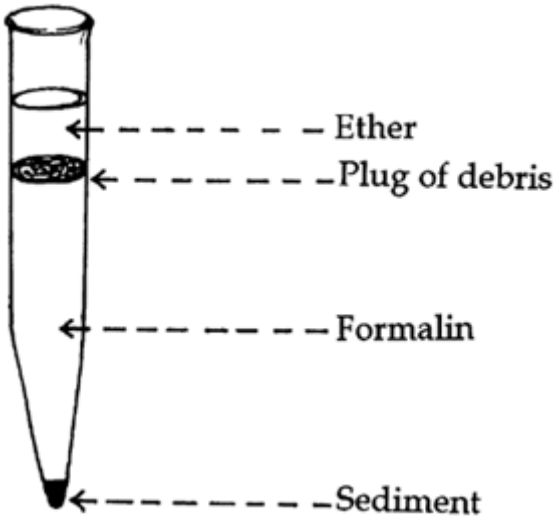
- Close the tube with a stopper and shake vigorously to mix. Remove the stopper and centrifuge at 500g for 2 minutes.

- Rest the tube in a stand. Four layers now become visible the top layer consists of ether, second is a plug of debris, third is a clear layer of formalin and the fourth is the sediment (Fig 2).

- Detach the plug of debris from the side of the tube with the aid of a glass rod and pour off the liquid leaving a small amount of formalin for suspension of the sediment.

- With a pipette, remove the sediment and mix it with a drop of iodine. Examine under the microscope.

**Formal ether sedimentation technique**




**Advantages**

- Faecal odour is removed.

- The sensitivity of detecting the ova or cysts increases by 8-10 folds.

- The examination is easier than examining a direct wet smear.

- The size and shape of the parasitic structures is maintained.

- It is inexpensive, easy to perform and can be done at any level of health infrastructure.

**Disadvantages**

- Faecal debris may mask the parasitic structure.
- Trophozoite forms are not detected in this method.

**Saturated salt flotation technique**

- Place about one millilitre of faeces in a container which is flat bottomed and has a diameter of less than 1½ inches and capacity of about 15-20 ml (Fig 3).
- Add a few drops of saturated salt solution (specific gravity 1.200) and stir it to make a fine emulsion.
- Add more salt solution so that the container is nearly full, stirring the solution throughout.
- Remove any coarse matter which floats up.
- Place the container on a level surface. Do the final filling by a dropper until a convex meniscus is formed.
- A glass slide 3"x 2" is carefully laid on the top of the container so that the centre is in contact with the fluid.
- Preparation is allowed to stand for 20 minutes after which the glass slide is quickly lifted, turned over smoothly as to avoid spilling of the fluid and examined under the microscope after putting a coverslip.

**Flotation technique**

**Disadvantages of flotation techniques**

- Unfertilised eggs of Ascaris lumbricoides, eggs of Taenia solium and Taenia saginata, all trematodal eggs and larvae of Strongyloides do not float in the salt solution.

- Due to high specific gravity of the solution, protozoan cysts and thin walled nematode eggs will collapse and become distorted in appearance, if left for more than 20 minutes.

**Biosafety**

- Follow general laboratory principles for biosafety such as washing of hands, wearing of gloves, disinfecting work place and practising good personal hygiene measures.

- Handle the chemicals with care. Special precautions should be taken to store explosive chemicals (picric acid and phenol crystals) and flammable solvents such as acetone, ether, benzene, xylene etc.

- Equipment and glassware should be handled carefully to minimize risk of injury and aerosol production.

- Dispose off infectious material appropriately.

**Disposal of morbid material**

- After examination the stool specimen should either be incinerated or soaked in disinfectant solution and then buried in disposable specimen container.

- Used glass slides should be discarded in a pot containing 1% hypochlorite solution and cleaned if to be reused or buried if not to be used again.

**Quality assurance**

Attention be given to all pre-analytical, analytical and post-analytical factors affecting quality (Chapter 9).

Laboratories should volunteer to participate in external quality assessment schemes.

**Reporting of results**

The report should include positive/negative comments on the following:

- Adult worms/segments of worms/larvae.
- Cellular exudate such as RBCs, WBCs, Macrophages and CL crystals.
- Trophozoites (only if the specimen was fresh otherwise comment that specimen was not fit to comment on this).
- Ova and cysts.
- Any advise for further examination.

**Referral**

- As a part of any quality assurance programme.
- In case of unusual findings or an outbreak situation.

**Table: Salient features of common trophozoites,cysts and eggs of parasites**

| Cyst/egg/trophozoite | Features |
|---|---|
| *Entamoeba histolytica* trophozoite | 12-60 µ, asymmetric, purposeful directional motility, single spherical nucleus, single central karyosome, delicate and evenly distributed chromatin. |
| *Entamoeba histolytica* cyst | Spherical, 10-20 µ mature cyst has four nuclei with compact centrally located karyosome; chromatin is delicate. Some cysts may have chromatoid bars. |
| *Giardia lamblia* trophozoite | 9-21x5-15 µ, pear shaped with tapering ends, actively motile like falling leaf, 2 centrally placed nuclei, uniform granular cytoplasm. |
| *Giardia lamblia* cyst | Oval, 8-12 µ long and 7-10 µ wide, nucleus has 4 karyosomes, tend to be eccenterically placed; clear space between cell wall and cytoplasm. Four median bodies are present. |
| *Entamoeba coli* cyst | 10-35 µ, usually spherical, mature cyst may contain 8 or rarely 16 nuclei. Peripheral chromatin is coarse and granular; unevenly distributed in clumps; karyosome is usually eccentric. Chromatid bars not frequently seen. |
| Fertile egg of roundworms | 60x45 µ, round or ovoid with thick shell; covered by a thick albuminous coat, inner cell in various stages of cleavage, brown in colour. |
| Decorticated egg of roundworm | Albuminous coat is lost. All other features are same as in fertile egg. |
| Infertile egg of roundworm | 90x40 µ, elongated, shell is often thin, internal material is a mass of globules. |
| Hookworm egg | Oval, ellipsoid, 60x40 µ. Shell is thin walled, smooth and |

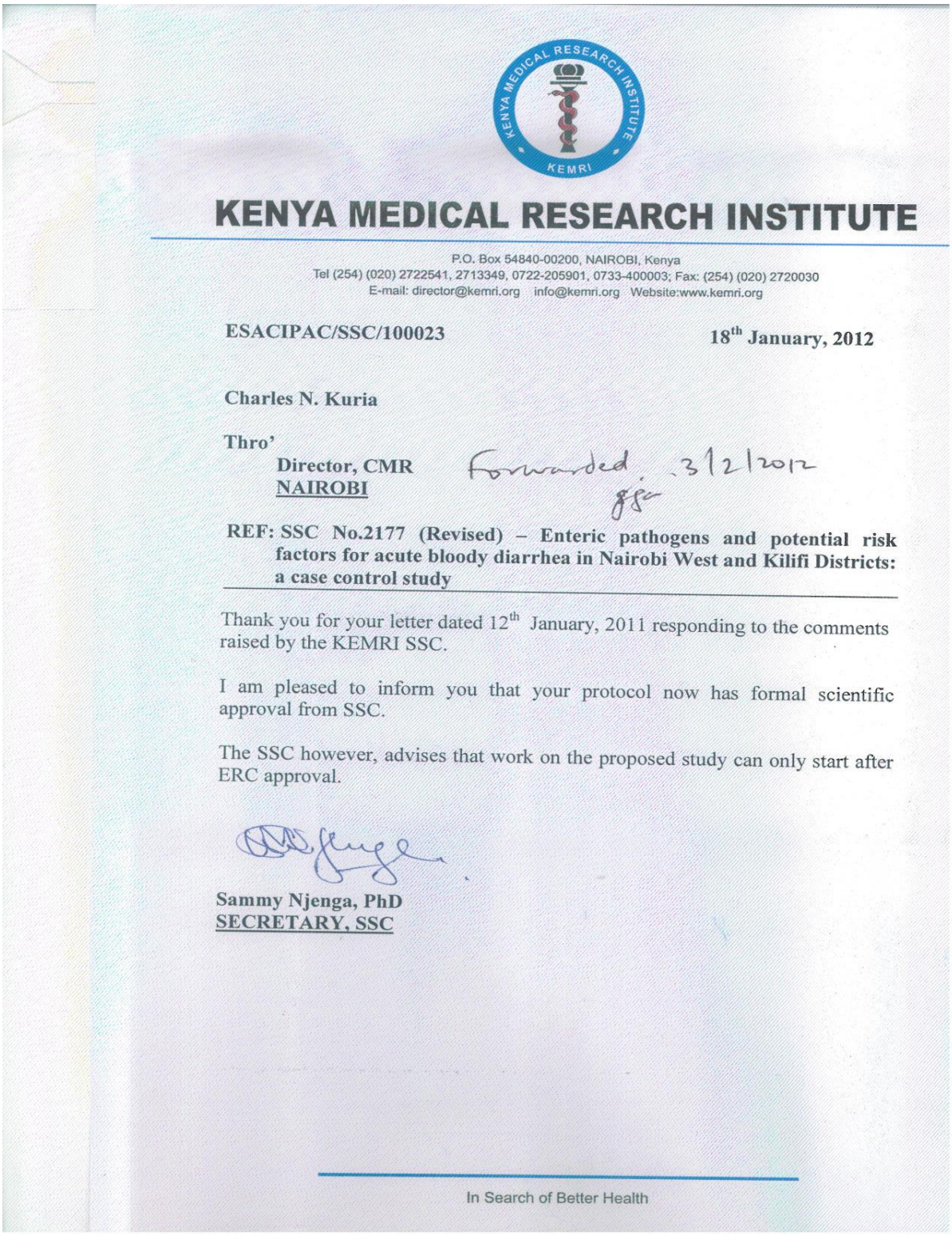
| | |
|---|---|
| | colourless. Internal cleavage is well developed at 4-8 cell stage which pulls away from the shell leaving an emplty space. |
| Threadworm egg | Planoconvex, elongate, asymmetric eggs, 55x26 µ, shell is thin and smooth. Fully developed larvae are seen in the eggs. |
| Whipworm egg | Elongate, barrel shaped with polar hyaline plug, 54-22 µ. Shell is yellow to brownish, plugs are colourless |
| Tapeworm egg | Spherical, 31-43 µ with thick shell with prominent radial striations. Embryonated oncosphere possessing 3 pairs of hooklets within the shell is diagnostic of the genus. Species identification on the basis of morphology is not possible. |

**Appendix 15: Top Ten Diseases in Kilifi District, 2009**

| TOP 10 DISEASE IN KILIFI DISTRICT, 2009 | | | | |
|---|---|---|---|---|
| | <5YEARS | | > 5years | |
| | **Condition** | **Cases** | **Condition** | **Cases** |
| 1 | Other Dis. Of Respiratory System | 106578 | Other Dis. Of Respiratory System | 130361 |
| 2 | Clinical Malaria | 75169 | Clinical Malaria | 82710 |
| 3 | Diarrhoea | 31364 | Dis. Of the skin (incl. wounds) | 39982 |
| 4 | Dis. Of the skin (incl. wounds) | 24203 | Confirmed Malaria | 21593 |
| 5 | Pneumonia | 19413 | Diarrhoea | 16832 |
| 6 | Intestinal worms | 11787 | Urinary Tract Infection | 14072 |
| 7 | Confirmed Malaria | 9466 | Rheumatism, Joint pains etc. | 12616 |
| 8 | Ear Infections | 6105 | Accidents – Fractures, injuries,etc. | 10248 |
| 9 | Eye Infections | 5084 | Sexually Transmitted Infections | 7874 |
| 10 | Dysentery | 2883 | Aneamia | 7382 |

Source: Ministry of Public Health and sanitation, Kilifi district

**Appendix 16: KEMRI SCC Clearance Letter**



KENYA MEDICAL RESEARCH INSTITUTE

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**ESACIPAC/SSC/100023** **18th January, 2012**

**Charles N. Kuria**

**Thro'**
**Director, CMR**
**NAIROBI**

Forwarded 3/2/2012

**REF: SSC No.2177 (Revised) – Enteric pathogens and potential risk factors for acute bloody diarrhea in Nairobi West and Kilifi Districts: a case control study**

Thank you for your letter dated 12th January, 2011 responding to the comments raised by the KEMRI SSC.

I am pleased to inform you that your protocol now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval.

**Sammy Njenga, PhD**
**SECRETARY, SSC**

## Appendix 17: KEMRI ERC Clearance Letter

# KENYA MEDICAL RESEARCH INSTITUTE

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**KEMRI/RES/7/3/1** **April 27, 2012**

**TO:** **MR. CHARLES KURIA NJUGUNA (PRINCIPAL INVESTIGATOR)**

**THROUGH:** **DR. SAMUEL KARIUKI, THE DIRECTOR, CMR, NAIROBI**

Forwarded on 02/05/2012

Dear Sir,

**RE:** **SSC PROTOCOL No. 2177 (*RE-SUBMISSION*): ENTERIC PATHOGENS AND POTENTIAL RISK FACTORS FOR ACUTE BLOODY DIARRHOEA IN NAIROBI WEST AND KILIFI DISTRICTS: A CASE -CONTROL STUDY (*VERSION DATED 24TH APRIL 2012*)**

The ERC Secretariat acknowledges receipt of the revised proposal on 26th April 2012.

This is to inform you that the Committee determines that the issues raised at the 198th ERC meeting of 21st February 2012 are adequately addressed. Consequently, the study is granted approval for implementation effective this **27th day of April 2012** for a period of one year. Please note that authorization to conduct this study will automatically expire on **April 26, 2013**.

If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to the ERC Secretariat by **March 15, 2013**. The regulations require continuing review even though the research activity may not have begun until sometime after the ERC approval.

Note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of the ERC. You are also required to submit any proposed changes to this study to the SSC and ERC for review and approval prior to initiation and advise the ERC when the study is completed or discontinued.

Work on this project may begin.

Sincerely,

**CHRISTINE WASUNNA,**
**Ag. SECRETARY,**
**KEMRI ETHICS REVIEW COMMITTEE**

**Appendix 18: Factors Associated with Acute Bloody Diarrhoea in Rural and Urban Settings**

| Variables | Rural (Kilifi) | | | | | | | Urban (Nairobi) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cases | | Controls | | | | | Cases | | Controls | | | | |
| | Yes n(%) | No n(%) | Yes n(%) | No n(%) | Odds Ratio | 95% CI | *P* value | Yes n(%) | No n(%) | Yes n(%) | No n(%) | Odds Ratio | 95% CI | *P* value |
| Other diarrhoea cases in household in previous 2 weeks | 60 (37) | 103 (63) | 91 (28) | 233 (72) | 1.49 | 1.00-2.225 | 0.050 | 13 (16) | 67 (84) | 9 | 165 | 3.56 | 1.452-8.715 | **0.0036*** |
| Main water source treated | 49 (35) | 91 (65) | 111 (40) | 167 (60) | 0.81 | 0.53-1.24 | 0.328 | 30 (65) | 16 (35) | 72 | 33 | 0.86 | 0.41-1.79 | 0.710 |
| Chlorine in the house | 16 (10) | 145 (90) | 39 (12) | 283 (88) | 0.80 | 0.43-1.48 | 0.540 | 20 (23) | 68 (77) | 34 | 145 | 1.25 | 0.67-2.34 | 0.520 |
| Drinking water stored in superdrum | 17 (10) | 157 (90) | 36 (11) | 293 (89) | 0.88 | 0.48-1.62 | 0.680 | 12 (11) | 98 (89) | 54 | 138 | 0.31 | 0.16-0.62 | **0.001*** |
| Drinking water stored in bucket | 35 (20) | 139 (80) | 91 (28) | 238 (72) | 0.66 | 0.42-1.03 | 0.060 | 36 (32) | 75 (68) | 47 | 144 | 1.47 | 0.88-2.46 | 0.140 |
| Drinkimg water stored in tank | 10 (6) | 164 (94) | 16 (5) | 313 (95) | 1.19 | 0.53-2.69 | 0.680 | 1 (1) | 110 (99) | 1 | 190 | 1.73 | 0.11-27.89 | 1.000 |
| Wide mouthed water storage container | 108 (68) | 51 (32) | 209 (64) | 116 (36) | 1.18 | 0.79-176 | 0.480 | 64 (74) | 23 (26) | 104 | 68 | 1.82 | 1.033-3.20 | **0.037*** |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Drinkimg water stored in Jerrican | 130 (75) | 44 (25) | 255 (78) | 74 (22) | 0.86 | 0.56-1.32 | 0.510 | 44 (40) | 67 (60) | 78 | 113 | 0.95 | 0.59-1.53 | 0.900 |
| Specific container for drawing water from storage container | 42 (24) | 132 (76) | 88 (27) | 241(73) | 0.87 | 0.57-1.33 | 0.525 | 28 (48) | 30 (52) | 53 | 79 | 1.39 | 0.75-2.59 | 0.340 |
| Washing storage container at least every month | 103 (80) | 26 (20) | 199 (82) | 43 (18) | 0.86 | 0.51-1.47 | 0.580 | 49 (60) | 33 (40) | 107 | 62 | 0.86 | 0.50-1.48 | 0.580 |
| Drinking water covered | 140 (89) | 18 (11) | 300 (93) | 24 (7) | 0.62 | 0.33-1.18 | 0.170 | 82 (94) | 5 (6) | 170 | 8 | 0.77 | 0.24-2.43 | 0.760 |
| Drinking water stored separately | 66 (41) | 94 (59) | 166 (51) | 157 (49) | 0.66 | 0.45-0.97 | **0.036*** | 58 (64) | 31 (35) | 124 | 55 | 0.83 | 0.48-1.42 | 0.580 |
| drunk water outside home last 1 week | 64 (41) | 91 (59) | 129 (40) | 190 (60) | 1.04 | 0.70-1.53 | 0.920 | 45 (52) | 42 (48) | 72 | 107 | 1.59 | 0.95-2.67 | 0.077 |
| Presence of coliforms in household water | 35 (39) | 54 (61) | 35 (43) | 47 (57) | 0.87 | 0.47-1.60 | 0.760 | 9 (15) | 52 (85) | 5 | 65 | 2.25 | 0.71-7.12 | 0.260 |
| Presence of coliforms in main water source | 36 (44) | 46 (56) | 23 (32) | 48 (68) | 1.63 | 0.84-3.16 | 0.180 | 7 (13) | 49 (88) | 3 | 63 | 3.00 | 0.74-12.20 | 0.180 |
| Presence of fecal coliform in household water | 13 (19) | 56 (81) | 16 (24) | 50 (76) | 0.73 | 0.32-1.66 | 0.530 | 4 (7) | 56 (93) | 4 | 66 | 1.18 | 0.28-4.93 | 1.000 |
| Presence of fecal coliform in main water source | 8 (13) | 53 (87) | 6 (11) | 51 (89) | 1.28 | 0.42-3.96 | 0.665 | 6 (11) | 49 (89) | 2 | 65 | 3.98 | 0.77-20.57 | 0.079 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Household water supply chlorinated | 38 (32) | 82 (68) | 36 (32) | 76 (68) | 0.98 | 0.56-1.69 | 1.000 | 17 (28) | 44 (72) | 22 | 56 | 0.98 | 0.47-2.07 | 1.000 |
| Main water supply chlorinated | 34 (31) | 74 (69) | 31 (35) | 58 (65) | 0.86 | 0.47-1.56 | 0.650 | 17 (30) | 39 (70) | 20 | 48 | 1.05 | 0.48-2.26 | 1.000 |
| Main water source protected | 68 (67) | 33 (33) | 63 (79) | 17 (21) | 0.56 | 0.28-1.09 | 0.097 | 64 (94) | 4 (6) | 71 | 4 | 0.90 | 0.22-3.75 | 0.886 |
| Eating raw foods/vegetables | 144 (89) | 18 (11) | 301 (93) | 23 (7) | 0.61 | 0.32-1.17 | 0.165 | 86 (97) | 3 (3) | 174 | 6 | 0.98 | 0.24-4.05 | 0.480 |
| Washing fruits and vegetables | 53 (38) | 88 (62) | 119 (40) | 177 (60) | 0.89 | 0.59-1.35 | 0.300 | 41 (51) | 40 (49) | 106 | 64 | 0.62 | 0.36-1.06 | 0.078 |
| Always reheat food before eating | 79 (55) | 64 (45) | 188 (62) | 115 (38) | 0.76 | 0.50-1.13 | 0.090 | 48 (56) | 38 (44) | 98 | 80 | 1.03 | 0.61-1.73 | 0.450 |
| Eating food cooked previous day | 55 (36) | 99 (64) | 143 (46) | 169 (54) | 0.66 | 0.44-0.98 | **0.038*** | 64 (72) | 25 (28) | 127 | 50 | 1.01 | 0.57-1.78 | 0.490 |
| Separating cooked food from uncooked | 153 (99) | 2 (1) | 306 (97) | 11 (3) | 2.75 | 0.60-12.56 | 0.240 | 81 (92) | 7 (8) | 169 | 9 | 0.62 | 0.22-1.71 | 0.180 |
| Protecting food from flies by fly screen | 77 (61) | 49 (39) | 153 (59) | 105 (41) | 1.08 | 0.70-1.67 | 0.370 | 66 (77) | 20 (23) | 137 | 34 | 0.82 | 0.44-1.53 | 0.270 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Eat from outside home in the last 2 weeks | 61 (38) | 98 (62) | 103 (32) | 215 (68) | 1.30 | 0.87-1.93 | 0.099 | 59 (67) | 29 (33) | 105 | 75 | 1.45 | 0.85-2.48 | 0.170 |
| Eating in social gathering | 26 (16) | 132 (84) | 66 (21) | 245 (79) | 0.73 | 0.44-121 | 0.110 | 17 (20) | 70 (80) | 30 | 145 | 1.17 | 0.61-2.27 | 0.320 |
| Toilet present in the compound | 89 (55) | 74 (45) | 186 (58) | 132 (42) | 0.85 | 0.58-1.25 | 0.210 | 85 (96) | 4 (4) | 172 | 8 | 0.99 | 0.29-3.37 | 0.600 |
| Toilet use | 72 (71) | 29 (29) | 169 (81) | 40 (19) | 0.59 | 0.34-1.02 | 0.057 | 80 (92) | 7 (8) | 163 | 15 | 1.05 | 0.41-2.68 | 0.470 |
| Sharing toilets with neighbors | 47 (47) | 54 (53) | 77 (42) | 105 (58) | 1.19 | 0.73-1.94 | 0.250 | 42 (58) | 31 (42) | 108 | 66 | 0.83 | 0.47-1.44 | 0.250 |
| Condition of toilet is clean | 155 (89) | 19 (11) | 310 (94) | 19 (6) | 0.50 | 0.26-0.97 | **0.038*** | 68 (62) | 42 (38) | 99 | 93 | 1.52 | 0.94-2.45 | 0.085 |
| Always hand-wash after defecating | 74 (47) | 85 (53) | 193 (61) | 124 (39) | 0.56 | 0.38-0.82 | **0.003*** | 59 (66) | 30 (34) | 131 | 49 | 0.74 | 0.43-1.27 | 0.140 |
| Hand washing with water and soap | 23 (24) | 71 (76) | 63 (30) | 149 (70) | 0.77 | 0.44-1.33 | 0.180 | 49 (75) | 16 (25) | 92 | 41 | 1.36 | 0.68-2.68 | 0.190 |
| Washed hands after last defecation | 103 (69) | 46 (31) | 243 (82) | 53 (18) | 0.49 | 0.31-0.77 | **0.002*** | 71 (85) | 13 (15) | 160 | 11 | 0.38 | 0.16-0.88 | **0.02*** |
| Always wash hands after disposing child's stool | 56 (50) | 55 (50) | 146 (63) | 87 (37) | 0.61 | 0.38-0.96 | **0.032*** | 49 (66) | 25 (34) | 107 | 47 | 0.86 | 0.48-1.56 | 0.310 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Always washing hands before food preparation | 65 (50) | 66 (50) | 151 (59) | 107 (41) | 0.70 | 0.46-1.07 | 0.095 | 54 (61) | 35 (39) | 105 | 69 | 1.01 | 0.60-1.71 | 0.480 |
| Always washing hands before eating | 158 (98) | 4 (2) | 307 (97) | 10 (3) | 1.29 | 0.40-4.17 | 0.460 | 61 (69) | 28 (31) | 134 | 44 | 0.72 | 0.41-1.26 | 0.120 |
| Washing hands with soap before eating | 6 (4) | 137 (96) | 8 (3) | 270 (97) | 1.48 | 0.50-4.35 | 0.240 | 44 (59) | 31 (41) | 86 | 61 | 1.01 | 0.57-1.77 | 0.490 |
| Using Running water for handwashing | 23 (14) | 140 (86) | 38 (12) | 277 (88) | 1.20 | 0.69-2.1 | 0.560 | 10 (12) | 76 (88) | 23 | 149 | 0.85 | 0.39-1.89 | 0.840 |
| Washing utensils after use | 130 (85) | 23 (15) | 264 (85) | 45 (15) | 0.96 | 0.56-1.66 | 0.890 | 51 (57) | 38 (43) | 106 | 70 | 0.88 | 0.53-1.49 | 0.690 |
| Drying cooking utensils after washing | 57 (47) | 64 (53) | 117 (48) | 126 (52) | 0.96 | 0.62-1.48 | 0.430 | 39 (44) | 50 (56) | 88 | 89 | 0.79 | 0.47-1.32 | 0.180 |
| Keeping clean utensils separate from unwashed | 154 (99) | 2 (1) | 304 (98) | 6 (2) | 1.52 | 0.30-7.62 | 0.460 | 85 (97) | 3 (3) | 171 | 6 | 0.99 | 0.24-4.07 | 0.620 |
| Observed faeces in the compound | 21 (13) | 138 (87) | 44 (14) | 272 (86) | 0.94 | 0.54-1.64 | 0.889 | 17 (19) | 71 (81) | 39 | 139 | 0.85 | 0.45-1.61 | 0.320 |
| Presence of refuse pit in the compound | 46 (29) | 112 (71) | 79 (25) | 237 (75) | 1.23 | 0.80- 1.89 | 0.170 | 18 (20) | 70 (80) | 37 | 140 | 0.97 | 0.52-1.83 | 0.470 |
| Presence of rack for drying utencils | 24 (16) | 125 (84) | 43 (15) | 248 (85) | 1.12 | 0.64-1.91 | 0.350 | 12 (14) | 74 (86) | 28 | 138 | 0.79 | 0.38-1.66 | 0.280 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Presence of sheep in compound | 15 (10) | 131 (90) | 22 (7) | 299 (93) | 1.56 | 0.78-3.09 | 0.110 | 1 (1) | 88 (99) | 1 | 180 | 2.05 | 0.13-33.09 | 0.550 |
| Presence of goats in compound | 77 (50) | 78 (50) | 148 (46) | 173 (54) | 1.15 | 0.79-1.69 | 0.230 | 4 (5) | 84 (95) | 5 | 177 | 1.69 | 0.44-6.44 | 0.330 |
| Presence of chicken in compound | 106 (67) | 53 (33) | 212 (66) | 109 (34) | 1.03 | 0.687-1.538 | 0.892 | 15 (17) | 73 (83) | 29 | 152 | 1.08 | 0.54-2.13 | 0.410 |
| Walked in water previous 2 weeks | 22 (13) | 141 (87) | 42 (13) | 276 (87) | 1.03 | 0.59-1.78 | 0.460 | 6 (7) | 82 (93) | 12 | 170 | 1.04 | 0.38-2.86 | 0.460 |
| Swimming in the last 2 weeks | 2 (1) | 159 (99) | 5 (2) | 312 (98) | 0.78 | 0.15-4.09 | 0.560 | 1 (1) | 87 (99) | 4 | 174 | 0.50 | 0.06-4.54 | 0.465 |
| Equipped with diarrhoea knowledge in the 1 year | 21 (13) | 142 (87) | 60 (19) | 257 (81) | 0.63 | 0.37-1.08 | 0.094 | 12 (13) | 77 (87) | 23 | 159 | 1.08 | 0.51-2.29 | 0.420 |
| Poor compound cleanliness | 27 (17) | 136 (83) | 19 (6) | 295 (94) | 3.08 | 1.66-5.74 | **0.00023*** | 21 (24) | 66 (76) | 40 | 135 | 1.07 | 0.59-1.97 | 0.410 |
| Contact with livestock drinking water in the past 2 weeks | 20 (16) | 107 (84) | 31 (11) | 244 (89) | 1.47 | 0.80-2.69 | 0.120 | 1 (1) | 82 (99) | 2 | 169 | 1.03 | 0.09-11.53 | 0.470 |