ENTEROHAEMORRHAGIC ESCHERICHIA COLI INFECTIONS AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS AMONG CHILDREN ATTENDING OUT-PATIENT CLINIC AT EMBU PROVINCIAL GENERAL HOSPITAL-KENYA.

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JANUARY 2013

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DEDICATION

This research work is dedicated to my parents who though unschooled, taught and encouraged me to sip from the font of knowledge.
ACKNOWLEDGMENTS

For their professional role in making this research project such a success, I wish to register my gratitude with my supervisors: The late Dr. John Mbithi: Department of medical laboratory sciences-Kenyatta University, Dr. Augustine Afullo: Department of Health Systems Management-Kenya Methodist University and Dr. Ochieng George Otieno: School of public health-Kenyatta University. I am also greatly indebted to the following people for their support during the entire project implementation stage at Embu provincial General hospital: Dr C.M Muli, Dr. Ngwiri, and Dr. Maina, clinicians: Kaimu, Faith Njogu and Khaltumah Tisho, laboratory technicians: Peter Njuki and Ruth, research assistant Eunice Ndwiga.

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Of course, all this was through the grace of God. To Him be praise and honor.
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DEFINATION OF TERMS

Antibiotic susceptibility test: Measure of a given antimicrobial agent to inhibit bacterial growth in vitro

Diarrhea: Having three or more loose/watery bowel movements in a day

*Escherichia coli* 0157:H7: A pathotype of pathogenic *E. coli* belonging to Enterohaemorrhagic *E. coli* Strain which induces Hemolytic Uremic Syndrome in humans particularly children below five years.

Multi-drug resistance: resistance to more than three different drug combinations at recommended *Mic* concentrations

Prevalence: Number of candidates with positive EHEC 0157:H7 isolates among children below five years attending out-patient clinic at Embu provincial general hospital over the total number for diarrheal cases in under-fives screened for the bacteria during the study period

Resistant: Organism under investigation not expected to respond to the antimicrobial agent in question at the recommended *Mic* and given location of infection.

Susceptible: Organism under investigation responds to the drug in question at the recommended *Mic* and given location of infection.

Intermediate: Moderately susceptible, buffer zone between resistant and susceptible.
ABBREVIATIONS AND ACRONYMS

A: Ampicillin
C: Chloramphenicol
CDC: Centre for diseases control.
COT: Cotrimoxazole
EHEC: Enterohaemorrhagic Escherichia coli.
EPGH: Embu Provincial General Hospital
FAO: Food and Agriculture Organization.
GEN: Gentamicin
HC: Haemorrhagic colitis
HUS: Hemolytic uremic syndrome.
K: Kanamycin
KEMRI: Kenya Medical Research Institute.
LCDC: Laboratory centres for disease control
MHA: Mueller Hinton Agar.
Mini-API: Mini-Analytical Profile Indexing.ID 32
MIC: Minimum Inhibitory Concentrations.
MKU: Mount Kenya University.
MOH: Ministry of Health-Kenya.
NUIITIM: Nagasaki University Institute of Tropical Medicine.
PCR: Polymerase chain reaction
PHLS: Public Health Laboratory Surveillance
PiP: Piperacillin
S: Streptomycin
SMAC: Sorbitol MacConkey Agar
TE: Tetracycline
VTEC: Verotoxigenic Escherichia coli.
Vtx: Verotoxin/shiga toxin
WHO: World Health Organization
XLD: Xylose Lysine Desoxycholate agar
ABSTRACT

Diarrhea is the second leading cause of death in under-fives in the world. EHEC 0157:H7 is an important etiologic agent of diarrhea with public health significance since exposure to low doses can lead to an infection and could cause an epidemic. EHEC 0157:H7 induces HUS, the leading cause of acute renal failure in children below five years. The global prevalence of EHEC 0157:H7 is about 2% while most isolates of this bacterium exhibit multi-drug resistance to most antibiotics in use. To clarify the role of 0157:H7 as an important etiologic agent of acute gastroenteritis in children below five years in Kenya, a descriptive cross-sectional study was conducted to establish the prevalence and antibiotic susceptibility patterns associated with EHEC 0157:H7 isolates from human sources. Diarrhea accounted for 7% of all illnesses causing out-patient morbidity and mortality in children below five years in the study area during the study period. Stool samples were obtained from 302 consenting children below five years in Embu District who were systematically selected between November 2009 and June 2010 and characterized as EHEC 0157:H7 using Cowan method as well as sero-typing. Confirmation was through analytical profile indexing as well as CerTest. Antibiotic susceptibility testing of the isolates was through Kirby Bauer disc diffusion method. 201 (66.7%) samples out of 302, had E. coli, 84 (27.8%) cases had parasites while 17 (5.63%) cases did not have any growth. Out of 201 stool samples with E. coli, 32 (10.6%) isolates were found to be positive for EHEC 0157:H7 on Sorbitol MacConkey agar, 2 (0.66 %) cases were confirmed to be positive for EHEC 0157:H7 by CerTest method, while 12 (4%) cases were confirmed positive EHEC 0157:H7 by slide agglutination. Based on sero-typing test results, the area under study was found to be having a prevalence of about 4% which is above the global prevalence. 58% of the 12 confirmed cases of E. coli were found to be resistant to Trimethoprim/sulfamethoxazole, 50% were resistant to Ampicillin while 33% of EHEC 0157:H7 isolates were resistant to Chloramphenicol, Tetracycline and cotrimoxazole. All EHEC 0157:H7 isolates were susceptible to Streptomycin, Kanamicin and Gentamicin. Z-test statistic was used to test for significance while a two-tailed chi-square test ($\chi^2$) was used to test for associations between various demographic factors. The isolation of EHEC 0157:H7 in stool samples from children confirms the circulation of this bacterium in the immediate environment while the detection of multidrug resistance is a course for concern. The research findings shall be used to inform policy on; the need for improvement on provision of clean, safe drinking water and general hygiene for the general public, the importance of making laboratory confirmations a routine undertaking in hospitals to ascertain the actual causes of diarrhea particularly in under-fives and state mandating of cases reporting. Introduction of faster presumptive diagnostic tools such as Rapid-Antigen testing is highly recommended.
CHAPTER ONE

1.0 INTRODUCTION

Diarrhea remains the second leading cause of death among children under five years globally with nearly one in every five child deaths (about 2.2 million deaths each year) being due to diarrhea (Sang 'et al'. 2011). It kills more children than AIDS, malaria and measles combined (WHO/UNICEF, 2009).

Diarrhea is one of the main causes of morbidity and mortality in children under five years in developing countries where the average number of diarrhea episodes per child per year within this age group is 3.2 (Bryce 'et al'. 2005) In Sub-Saharan Africa, mortality caused by acute diarrhea varies from 1.9% of all child deaths in the Gambia to 37% in Nigeria with most of the deaths occurring during the first year of life. Even though morbidity caused by diarrhea is still high, mortality has been decreasing worldwide mainly due to improved management. (Kosek 'et al'. 2003). Appropriate antimicrobial therapy can shorten both the bacterial excretion and clinical periods. However, the incidences of multidrug-resistance are increasing (Tjaniadi 'et al'. 2003) thus, adequate fluid and electrolyte replacement and maintenance remains the central key to managing diarrheal illness (Casburn 'et al'. 2004) use of antibiotics is recommended only in acute diarrhea or chronic diarrhea.

In Kenya, the number of diarrheal cases may be decreasing due to improved general hygiene, advances in healthcare provision and proper sanitation (Sang 'et al'. 2011), however, despite these declines, few studies have been conducted to either clarify the risks of diarrhea or account for the etiological agents of diarrhea in urban/suburban areas as well as in remote areas (Sang 'et al'. 2011)

About 30% of all cases of infantile diarrhea in Kenya are caused by bacterial diarrhea [Sang 'et al'.2011]. Diarrheagenic Escherichia coli is among enteric pathogens that cause intestinal infections in both the temperate and the tropical areas of the world. Most of the pathogenic E. coli isolates exhibit multidrug resistance to common antibiotics available in Kenya through prescriptions to the general public (Oundo 'et al'.2008).

Escherichia coli (E. coli) is a bacterium that commonly lives in the intestines of people and animals (Ostroff 'et al'. 1990). There are many strains of E. coli and most of them are normal inhabitants of the small intestines and colon and are non-pathogenic. Nevertheless, these non-pathogenic E. coli can cause disease if they spread outside the intestines, for example, into the urinary tract where they cause bladder or kidney infections or sepsis (Carter 'et al'. 1987). Enteric E. coli are divided on the basis of virulence properties into enterotoxigenic E. coli (ETEC) (causative agent of diarrhea in humans, pigs, sheep, goats, cattle, dogs and horses), enteropathogenic E. coli (EPEC) which causes diarrhea in humans, rabbits, dogs,
cats and horses, enteroinvasive *E. coli* (EIEC), found only in humans, verotoxigenic *E. coli* (VTEC) found in pigs, cattle and dogs. Enterohaemorrhagic *E. coli* (EHEC) found in humans, cattle and goats and attacking porcine strains that colonize the gut in a manner similar to human EPEC strains and enteroaggregative *E. coli* (EAggEC), found only in humans. (Feng 'et al'. 2002).

Some strains of *E. coli* are pathogenic and cause disease in the small intestines and colon. Enterotoxigenic *E. coli* (ETEC) may cause diarrhea by producing toxins that cause the intestine to secrete fluid while enteropathogenic *E. coli* cause diarrhea by invading and inflaming the lining of the small intestine and the colon. A third strain of *E. coli* has a tendency of causing inflammation of the colon and bloody diarrhoea, these are enterohamorrhagic *E. coli* (EHEC) (Feng 'et al'. 2002). *E. coli* 0157:H7 is a strain of EHEC that causes colitis and bloody diarrhea by producing shiga-like toxins which damages the intestines and is a major health problem.

This diarrheal illness was first recognized when the centre for diseases control (CDC) isolated *E. coli* 0157:H7 from patients in two separate outbreaks in Oregon and Michigan states with the illness being associated with eating hamburgers at the restaurant of one national chain (CDC, 1991).

EHEC 0157:H7 is responsible for an array of several clinical syndromes including:-

Hemorrhagic diarrhea(hemorrhagic enterocolitis)-the incubation period between exposure to EHEC bacteria and the onset of symptoms is usually three to four days with the symptoms including severe abdominal pain and abdominal tenderness which often is associated with bloody diarrhea which typically lasts for six to eight days.

Thrombotic thrombocytopenic purpura, Persons infected with *E. coli* 0157:H7 particularly the elderly can develop a syndrome similar to hemolytic-uremic syndrome called thrombotic thrombocytopenic purpura (TTP) with clotting of blood within small blood vessels, anaemia due to fragmentation of red blood cells and shortage of platelets (thrombocytopenia) that results in easy bruising, neurologic abnormalities, impaired kidney function and fever. Thrombotic thrombocytopenic purpura, once almost always fatal, is still a serious consequence of *E. coli* 0157:H7.

Hemolytic- Uremic Syndrome (HUS).This is the most worrisome complication of EHEC 0157:H7 infection because, it is a serious and potentially fatal illness. "hemolytic" refers to the breakup of red blood cells leading to anaemia. There is also destruction of platelets which leads to low blood levels of platelets (thrombocytopenia) which in turn promotes abnormal bleeding. "uremic" refers to failure of the kidneys. In addition, problems in the brain with seizures and
coma may occur. HUS most commonly affects children below five years and is the most common cause of acute renal failure in infants and young children. It occurs in about 9% of hemorrhagic colitis caused by \textit{E. coli} 0157:H7 and usually occurs approximately 7 to 10 days after the onset of diarrhea.

Although outbreaks of \textit{E. coli} O157:H7 infections are frequently associated with food or milk derived from cattle, other sources, including fresh fruits, vegetables and water, have been implicated (Mead & Griffin, 1998). Varied prevalence rates have been reported in Kenya including: 0.2% among food handlers in Nairobi (Onyango ‘et al’. 2009) 0.8% in unpasteurized milk (Arimi ‘et al’. 2005), 5.2% in cattle feaces (Kangethe ‘et al’. 2007) and 24.1% among adults in Maasailand Kenya (Sang ‘et al’.2012), However, the epidemiology and pathogenicity of EHEC 0157:H7 among children below five years in Kenya and more so in Embu District has not been evaluated.

\textbf{1.2 RESEARCH QUESTIONS}

The study intended to make enquiries into the following areas:-

1. What is the prevalence of diarrhea among children below five years in Embu district?

2. What is the proportion of diarrhea due to Enterohemorrhagic \textit{Escherichia coli} in children below five years in the same region and the associated risk factors?

3. What is the level of antibiotic resistance associated with EHEC 0157:H7?

\textbf{1.3 PROBLEM STATEMENT}

No systematic study has ever been carried out to establish the national situation with regard to EHEC 0157:H7 infections among children below five years despite the fact that this bacterium is a known local environmental pollutant. This situation is further worsened by the fact that bacterial culture is not a routine laboratory practice in Kenyan hospitals for ascertaining the exact causes of persistent diarrhea particularly among children below five years. Kenya occupies the 10th position among the 15 countries in the world accounting for 80% of all under-five child mortality due to diarrhea with 27,400 under-five’s deaths being reported annually (WHO, 2007).

Embu District had the highest number of diarrheal cases in under-fives in 2008 Ministry of Health (MOH, 2009).
Multi-drug resistance associated with EHEC 0157:H7 has also been reported in various parts of the world and this is largely attributable to horizontal gene transfer as well dispensing of antibiotics over the counter without proper medical supervision, a problem that is quite rampant in Kenya.

"Kenya may not achieve the 4th and 6th millennium development goal" Minister for Public Health and Sanitation-Kenya October 2011.

1.4 JUSTIFICATION

Diarrhea is one of the major causes of death in children below five years in Kenya, (MOH, 2007). For every nine children born in Kenya, one child dies before reaching age five from preventable diseases such as diarrhoea. In the first half of year 2008, Eastern province accounted for 30% of the total number of diarrheal cases in under-fives recorded in Kenya with 251,738 cases while Rift valley had total of 189,332 cases, Nyanza had 106,658 cases, North eastern 2,552, Coast 77,217, Nairobi 23,212 while Western had a total of 87,811 cases, thus, the total number of cases of diarrhea recorded in the country was 858,292. (MOH, 2008).

At Embu general hospital, for every five children below five years seen at the pediatric out-patient clinic, three of the children present with diarrhoea (MOH, 2007). Most of these cases are treated symptomatically with antibiotics without confirmation of the exact cause of diarrhea through laboratory tests. 60% of all diarrheal cases in children below five years at this health facility are caused by E. coli (MOH, 2008) but the level of EHEC 0157:H7 is unknown. This therefore creates an urgent need to carry out a survey to determine the levels of this bacterium in the region and account for the clinical consequences.

The study findings shall be used as a guide for policy development on the need to equip health facilities with diagnostic equipments and personnel and make screening for this bacterium a routine undertaking in order to arrest development of EHEC complications particularly in children below five years which is the group most affected and to make cases reporting mandatory besides highlighting the importance of improving on general hygiene and provision of safe drinking water. The findings shall also contribute towards the achievement of the 4th millennium development goal which is reduction by two-thirds, the under-five year old mortality by 2015.

1.5 HYPOTHESIS STATEMENT

Enterohaemorrhagic Escherichia coli 0157:H7 infection is not a major cause of diarrhoea in children below five years in Embu District.
1.6 OBJECTIVES

1.6.1 BROAD OBJECTIVE
The broad objective was to determine the prevalence of Enterohaemorrhagic Escherichia coli infections as well as antibiotic resistance patterns associated with EHEC 0157:H7 among children below five years in Embu district.

1.6.2 SPECIFIC OBJECTIVES
The specific objectives were:

1. To determine the prevalence of E. coli induced diarrhea among children below five years in Embu district.

2. To determine what proportion of diarrhea in children below five years in Embu district is caused by EHEC 0157:H7 and the associated risk factors for its infection.

3. To profile the antibiotic susceptibility patterns of the EHEC 0157:H7 isolates.

1.7 SIGNIFICANCE AND OUTPUT OF THE STUDY
The study’s aim was to establish the prevalence of diarrhea in children below five years in Embu district, account for the proportion of diarrhea caused by EHEC 0157:H7 in the same defined population and as such, establish the prevalence of EHEC 0157:H7 in this population. The second objective was to profile antimicrobial susceptibility patterns associated with the same strain of E. coli. The findings shall find use in informing policy with regard to the need for the healthcare facilities to be equipped with the necessary wherewithal to carry out routine laboratory tests with a view of ascertaining the exact causes of diarrhea in children below five years besides highlighting the need for provision of safe drinking water, design and implementation of public health measures aimed at educating parents/caretakers on the dangers of consuming under-cooked beef, raw vegetables as well as observance of proper personal hygiene particularly when it comes to children below five years.

1.8 LIMITATIONS & DELIMITATIONS OF THE STUDY
Due to resource constraints, the study could not adequately address the issue of co-infections while taking cognizance of the fact that there could be other causes of diarrhea in under-fives in the study area including viruses (Rota virus, Noro virus), other diarrhegenic bacteria such as Salmonella and Shigella spp and other strains of pathogenic E. coli.
Figure: 1.1 Logical framework
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Etiology of diarrhea and modes of transmission

Diarrhea is one of the leading causes of death among children below five years globally. More than one in every ten child deaths each year is due to diarrhea (WHO/UNICEF, 2009). Today, only 44% of children with diarrhea in low income countries receive the recommended treatment and limited trend data suggest that there has been little progress since year 2000 (WHO/UNICEF, 2009).

The commonest causes of infectious diarrhea are as shown in Table 2.1. The incidence of these pathogens varies between developed and developing country settings. In developed countries, about 70% of diarrhea cases are of viral (40% rotavirus) nature, 10–20% of bacterial and < 10% of protozoal origin (Cheng et al., 2005). In developing countries, 50–60% of cases are of bacterial (Enteropathogenic E. Coli 25%, Campylobacter jejuni 10–18%, Shigella spp and Salmonella spp 5% each), 35% of viral (15–25% rotavirus) origin, and in many of such countries, the cause is unidentified or mixed (Cheng et al., 2005; Eliot, 2007; Naghipour et al., 2008). In developing countries the prevalence of diarrhea also varies widely by country. For instance, there are many more cases of cholera in India and South East Asia, whilst in Africa rotavirus has been shown to be the causative agent in 28–49% of cases in Ethiopia but only 14% of cases in Tanzania (Naghipour et al., 2008). The incidence of rotavirus diarrhea varies widely even within each country with studies from South Africa indicating a range of 14–34% of cases in Johannesburg, 20–55% in Durban and 18% in Cape Town (Naghipour et al., 2008). In Kenya, bacterial diarrhea has been reported to account for up to 30% of all cases of infantile diarrhea (Sang et al., 2011) and is the most common cause of travelers’ diarrhea (Jiang et al., 2002).

Table 2.1: Common pathogens causing childhood diarrhea (Modified from Cooke ML, 2010)

<table>
<thead>
<tr>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>— Campylobacter jejuni</td>
</tr>
<tr>
<td>— Non-typhoid Salmonella sp</td>
</tr>
<tr>
<td>— Enteropathogenic E. Coli</td>
</tr>
<tr>
<td>— Shigella spp</td>
</tr>
<tr>
<td>— Salmonella typhi</td>
</tr>
<tr>
<td>— Shiga-toxin producing E. Coli (ETEC)</td>
</tr>
<tr>
<td>— Vibrio cholera</td>
</tr>
<tr>
<td>Viruses</td>
</tr>
<tr>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>— Rotavirus</td>
</tr>
<tr>
<td>— Norovirus</td>
</tr>
<tr>
<td>— Enteric adenovirus</td>
</tr>
<tr>
<td>— Other: caliciviruses, astroviruses, enteroviruses</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>— Cryptosporidium parvum</td>
</tr>
<tr>
<td>— <em>Giardia lamblia</em></td>
</tr>
<tr>
<td>— Entamoeba histolytica</td>
</tr>
</tbody>
</table>

Escherichia coli are a more common bacterial cause of diarrhea in developing than in developed countries. It is the most common cause of infectious diarrhea leading to hospitalizations in developing countries (Cooke, 2010). It usually occurs in epidemics in the summer season. Sources of infection include: beef, pork, fast food restaurants (undercooked hamburger), apple cider, leaf lettuce, milk, cheese, spinach, and sprouts.

All diarrheal diseases stated above are transmitted directly or indirectly by faecal-oral routes, as shown in Figure 2.1.

Figure 2.1: The main modes of transmission for most diarrhoeal diseases are by ingestion of contaminated food and water. (Source: adapted from AMREF, 2007)
2.2 Diarrheagenic *Escherichia coli* among children aged below 5 years

*Escherichia coli* is a bacterium that commonly lives in the intestines of people and animals (Ostroff 'et al', 1990) there are many strains of *E. coli* and most of them are normal inhabitants of the small intestines and colon and are non-pathogenic. Nevertheless, this non-pathogenic *E. coli* can cause disease if they spread outside the intestines, for example, into the urinary tract where they cause bladder or kidney infections or sepsis (Carter 'et al', 1987).

The enteric *E. coli* are divided on the basis of virulence properties into enterotoxigenic *E. coli* [ETEC] (causative agent of diarrhea in humans, pigs, sheep, goats, cattle, dogs and horses), enteropathogenic *E. coli* [EPEC] which causes diarrhea in humans, rabbits, dogs, cats and horses, enteroinvasive *E. coli* [EIEC], found only in humans, verotoxigenic *E. coli* [VTEC] found in pigs, cattle and dogs. Enterohaemorrhagic *E. coli* [EHEC] found in humans, cattle and goats and attacking porcine strains that colonize the gut in a manner similar to human EPEC strains and enteroaggregative *E. coli* [EAggEC], found only in humans (Feng 'et al', 2002).

Some strains of *E. coli* are pathogenic and cause disease in the small intestines and colon. Enterotoxigenic *E. coli* (ETEC) may cause diarrhea by producing toxins that cause the intestine to secrete fluid while enteropathogenic *E. coli* cause diarrhea by invading and inflaming the lining of the small intestine and the colon. A third strain of *E. coli* has a tendency of causing inflammation of the colon and bloody diarrhoea, these are enterohaemorrhagic *E. coli* (EHEC) (Feng 'et al'. 2002). Enterohaemorrhagic *E. coli* 0157:H7, is one of the strains of EIEC responsible for an array of symptoms including: Hemorrhagic colitis, Hemolyticuremic syndrome and Thrombotic thrombocytopenic purpura.

Diarrheagenic *E. coli* produce toxins or possess certain virulence traits. Enterotoxigenic *E. coli* and EHEC produce toxins, i.e., heat-labile (LT) and/or heat-stable toxin (ST) and shiga-like toxins I and II (SLT I/II), respectively. Enteroinvasive *E. coli* typically invade and destroy the bowel mucosa. Enteropathogenic *E. coli* damage the bowel mucosa with characteristic attaching and effacing lesions mediated by a protein encoded by a gene called the attaching and effacing locus (*eal*). The epidemiology and pathogenicity of EAEC are less clear, but are associated with the presence of a large 60-kD plasmid encoding different virulence factors and toxins. Enteroaggregative *E. coli* are distinct in their adherence pattern with a so-called stack-brick adherence to Hep-2 cells (Nataro and Kaper, 1998). In addition, EAEC may harbor additional virulence factors including fimbriae I and II (AAFI and AAFII), an ST-like enterotoxin (EAST), and a plasmid-encoded heat-labile toxin (PET) that are involved in aggregation and pathogenesis of associated diarrhea (Agin 'et al'. 1997).
2.3 Escherichia coli O157:H7 diarrhea among children aged below 5 years

*Escherichia coli* O157:H7 was first recognized as a cause of human illness in two separate outbreaks of hemorrhagic colitis in Michigan and Oregon in 1982 (Riley *et al.*, 1983). The organisms were transmitted by the same source of undercooked beef, and Shiga-like toxin-producing strains of *E. coli* O157:H7 were isolated from the stools of the affected persons and from a sample of the implicated beef burgers but from no healthy controls. Increasing numbers of diseases related to *E. coli* O157:H7 have been reported since 1982; most have been sporadic (PHLS, 1987), but many institutional and community-wide outbreaks have occurred in nursing homes (LCDC, 1983), schools (LCDC, 1987), and day care centers (Spika *et al.*, 1986) or have been related to eating at fast food restaurants (Bell *et al.*, 1994), drinking untreated municipal water or fresh-pressed apple cider (Besser *et al.*, 1993), or swimming in lake water (Keene *et al.*, 1994).

It is estimated that 0.6% to 2.4% of all cases of diarrhea (Cahoon *et al.*, 1987) and 15% to 36% of all cases of bloody diarrhea or hemorrhagic colitis (PHLS, 1987) are associated with *E. coli* O157:H7. In a 1-year (1985, 1986), population-based study in the Puget Sound area of Washington State, *E. coli* O157:H7 was the third most common cause of bacterial diarrhea. Among the 4539 patients who submitted stool specimens, *E. coli* O157:H7 was isolated in 25 cases (0.6%) and followed *Campylobacter* (165 cases, 3.6%) and *Salmonella* organisms (70 cases, 1.5%) in frequency. *Shigella* organisms were isolated slightly less frequently than *E. coli* O157:H7 (23 cases, 0.5%). The population-based incidence rates in the same study (Martin *et al.*, 1990) were 8 cases per 100 000 person-years for *E. coli* O157:H7; 50 cases per 100 000 person years for *Campylobacter* organisms; 21 cases per 100 000 person-years for *Salmonella* organisms; and 7 per 100 000 person-years for *Shigella* organisms.

Other prospective studies (Gransden *et al.*, 1986) have found *E. coli* O157:H7 to be second to *Salmonella* organisms in areas of Canada (2.4%) and to *Campylobacter* organisms in Great Britain (1.9%) as the most common cause of bacterial diarrhea. In both studies (Gransden *et al.*, 1986), *E. coli* O157:H7 was isolated more often than *Shigella*, *Yersinia*, or *Aeromonas* organisms. In a prospective study limited to persons with grossly bloody diarrhea in Calgary, Canada, *E. coli* O157:H7 was isolated from 15% of patients (19 of 125). A 21-month surveillance study in the United States, established after initial outbreaks, identified 103 cases of hemorrhagic colitis (Remis *et al.*, 1984), 28 of which (27%) were associated with *E. coli* O157:H7. *Escherichia coli* O157:H7 was also found in 36% of sporadic cases (30 of 83) of hemorrhagic colitis in a British surveillance study (PHLS, 1987). Thus, the frequency of *E. coli* O157:H7 in infectious diarrhea rivals that of other major bacterial organisms, and *E. coli* O157:H7 is an important cause of bloody diarrhea and hemorrhagic colitis.
The estimated incidences cited in the above studies, however, are problematic and probably underestimate the true incidence of *E. coli* O157:H7 infection. Certainly, the best way to examine the incidence of an organism is to do prospective, laboratory-based studies within defined populations, and such studies have been the major sources of data on the reported incidences of *E. coli* O157:H7. However, case reporting through a surveillance system is affected by many factors: the variety and severity of clinical manifestations; the number of infected persons seeking medical attention; whether a stool culture is ordered and its timing in relation to the onset of illness and possible use of antibiotics; whether the laboratory tests correctly identify the organism; and whether the results are reported to public health officials. Clinical laboratories are becoming increasingly familiar with the varied spectrum of illness produced by *E. coli* O157:H7 and the ability to screen for this organism is becoming more widespread.

The minimum estimated attack rates of *E. coli* O157:H7 among persons who consumed the suspected food product were 3.5% in a community outbreak (CDC, 1991) and 8% in a junior high school outbreak (Belongia *et al*. 1991). These estimated attack rates include only cases in which patients had bloody diarrhea or a positive stool culture; thus, "possible" cases or those with milder symptoms were excluded. In a nursing home outbreak, the estimated attack rates from both food-borne and person-to-person transmission were 33% among the nursing home residents and 13% among the staff (Carter *et al*. 1987). The attack rate was reported to be as high as 67% (42 of 63 persons) in a kindergarten outbreak involving unpasteurized milk (PHLS, 1987).

Most reported cases of *E. coli* O157:H7 infection have occurred in the United States, Canada, and Great Britain, but cases have also been documented in Japan (Hamano *et al*. 1993), Australia (Albert *et al*. 1989), South Africa (Browning *et al*. 1990), Europe (Caprioli *et al*. 1992), Argentina (Lopez *et al*. 1989), and Chile (Cordovez *et al*. 1992). *Escherichia coli* O157:H7 has been detected in most areas of the United States; the largest numbers of isolations have been found in Washington State, Oregon, Minnesota, and Massachusetts (Ostroff *et al*. 1990). The geographical position of these states and of the two countries other than the United States (Canada and Great Britain) in which most reported cases have occurred suggests a predominance of infections in northern latitudes (Ostroff *et al*. 1990). However, the high number of reported cases in these regions may also reflect increased awareness among physicians in those areas and the fact that case reporting is required in some states.

*Escherichia coli* O157:H7 infections occur in all age groups, and the young are most often affected. In one study (Ostroff *et al*. 1989), the age-specific annual incidence rate was highest for children younger than 5 years of age (6.1 cases per 100 000 persons compared with an overall incidence rate of 2.1 cases
per 100,000 persons). The lowest rate was for adults 50 to 59 years of age, who had an annual incidence rate of 0.5 per 100,000 persons (Ostroff et al., 1989).

The trends in age-specific incidence of the hemolytic-uremic syndrome in the pediatric population parallel those in the incidence of *E. coli* O157:H7 infection. A 10-year retrospective, population-based study of the hemolytic-uremic syndrome in Minnesota reported a substantial increase in the incidence of the hemolytic-uremic syndrome during the study period, and a disproportionate number of cases occurred in children younger than 5 years of age (Martin et al., 1990). Although *E. coli* O157:H7 infections occur most often in young children and elderly persons, the elderly especially those in institutional settings have the highest morbidity and mortality rates (Pavia et al., 1990). *Escherichia coli* O157:H7 generally affects both sexes equally, and no data are available on the ethnicity-specific incidence rate of infection; most outbreaks seem to have affected patients with an ethnic distribution similar to that of the general population.

The rate of *E. coli* O157:H7 infection follows a seasonal pattern, with a peak incidence from June through September (LCDC, 1987). Sixty percent of *E. coli* O157:H7 infections and 73% of cases of the hemolytic-uremic syndrome and thrombotic thrombocytopenic purpura presented with bloody diarrhea between June and September, and patients affected during the summer months were younger than those seen during the rest of the year (Ostroff et al., 1989). In contrast to the pattern seen with *Salmonella* infection, the number of cases of *E. coli* infection does not increase after the December holiday period (LCDC, 1987).

Food-borne transmission of *E. coli* O157:H7 is the most important means of infection. Transmission has primarily been linked to undercooked meat, and, during the 1982 outbreaks, the organism was cultured from a suspected lot of hamburger patties (Riley et al., 1983). Sources other than undercooked hamburger meat (LCDC, 1987) that have been implicated in transmission of *E. coli* O157:H7 include heat-processed meat patties, which should be pathogen-free (Belongia et al., 1991); roast beef (CDC, 1991); ham, turkey, and cheese sandwiches (Carter et al., 1987); and potatoes (PHLS, 1987). Unpasteurized milk has been implicated as the vehicle for two cases of the hemolytic-uremic syndrome (Martin et al., 1990) and for a kindergarten outbreak of *E. coli* O157:H7 infection (LCDC, 1987).

*E. coli* O157:H7 was isolated from the feces of healthy cows who had supplied raw milk consumed by the patients affected in the outbreak. Even fresh-pressed, unpreserved apple cider, a seemingly unlikely vehicle, was implicated in one outbreak (Besser et al., 1993). The transmission probably occurred through the pressing of apples contaminated on the ground or during the production process. The isolation of *E. coli* O157:H7 from milk and from the feces of healthy cattle (Martin et al., 1990) and the fact that
hamburger is a major vehicle associated with food-borne outbreaks of *E. coli* O157:H7 infection (LCDC, 1987) suggest that cattle are an important reservoir for the pathogen. *Escherichia coli* O157:H7 has been isolated more often from dairy than from beef cattle (Martin et al., 1990), but both beef and dairy cattle are thought to be principal domestic reservoirs for the organism. In one study (Doyle et al., 1987) a particularly high rate of isolation of the organism from beef (31%) was found to correlate with the increasing incidence of human infection in the region studied. Because cattle are an important reservoir for *E. coli* O157:H7.

The apparent increase in *E. coli* O157:H7 infections during the past several years seem to suggest that an epizootic infection may be occurring in the animal reservoir (Martin et al., 1990). *Escherichia coli* O157:H7 has also been isolated from 1.5% to 3.7% of retail samples of beef, pork, poultry, and lamb from grocery stores in Canada and the United States (Doyle et al., 1987). Because of the wide array of contaminated food products, the precise sources of organisms are often difficult to trace and thus remain unknown in most cases.

Non-food-borne vehicles have also been implicated in the spread of *E. coli* O157:H7. Water-borne transmission has been implicated in two outbreaks (Dev et al., 1991), and transmission by person-to-person contact or by formites has been suggested in sporadic cases (Pai et al., 1988) and outbreaks (Duncan et al., 1986). Secondary person-to-person contact can be an important method of spread in institutional settings, especially day care centers (Belongia et al., 1993) and nursing homes (Carter et al., 1987), but it is less common in community-wide outbreaks. Nosocomial *E. coli* O157:H7 infections have also been reported (Yannelli et al., 1990).

### 2.4 Pathogenesis

*Escherichia coli* O157:H7 infection produces its most severe abnormalities in the ascending and transverse colon (Griffin et al., 1990); this is consistent with endoscopic and radiologic findings showing right-sided predominance (Remis et al., 1984). Colonic tissues show a spectrum of appearances ranging from normal to gross dilation with hyperemia of the involved segments (Pavia et al., 1990). In one study (Kelly et al., 1990), all specimens showed patchy, shallow mucosal ulcers with partial loss of normal mucosal folds, and many ulcers were covered by a thin layer of yellow or green exudates. Extreme submucosal edema, hemorrhage, and thickening of the bowel wall were present and, in one case, were so severe that the lumen of the ascending colon was almost obliterated.

Microscopically, no single histologic feature is diagnostic of *E. coli* O157:H7 infection, but the colonic pathology in colitis associated with *E. coli* O157:H7 often resembles a combination of ischemic colitis and infectious injury similar to that seen in toxin-mediated *Clostridium difficile*-associated colitis (Griffin
Submucosal hemorrhage, edema, and fibrin exudation are the most prominent features; ulceration, hemorrhage, capillary thrombi, and mild neutrophil infiltration in the mucosa are less common (Griffin 'et al'. 1990). Immunocytochemical examination showed that the submucosal plasma cells were primarily IgG, IgA, and IgM cells (Kelly 'et al'. 1990).

In one study of 11 patients, all 20 colonic specimens showed variable hemorrhage and edema in the lamina propria. Nine patients had colonic pathology similar to the pattern of injury associated with acute ischemic colitis (Griffin 'et al'. 1990): focal coagulative necrosis, hemorrhage, and acute inflammation in the superficial mucosa and preservation of the deep colonic crypts. Five patients showed both neutrophilic infiltration of the lamina propria and crypts and formation of crypt abscesses, resembling the pattern of injury seen in infectious colitis (Griffin 'et al'. 1990). Pseudomembranous lesions similar to those in C. difficile-associated pseudomembranous colitis may also be present (Richardson 'et al'. 1988). The ischemic and infectious patterns of injury can be seen alone or in combination (Griffin 'et al'. 1990); occasionally, normal specimens have also been described (Richardson 'et al'. 1988). In one case of nonbloody diarrhea, the ascending colon showed only patchy eosinophilic infiltrates (Pavia 'et al'. 1990). No single histologic feature is diagnostic of colitis associated with E. coli O157:H7, but the combination of infectious and ischemic patterns of injury, especially in association with capillary micro thrombi and a compatible clinical picture, should suggest the diagnosis (Griffin 'et al'. 1990). Obtaining more than one biopsy specimen from any patient increases the likelihood of identifying an abnormality, because abnormalities are often patchy (Griffin 'et al'. 1990).

Light microscopy showed no evidence of bacterial adherence or invasion in either the diseased areas or normal mucosa (Pavia 'et al'. 1990). To date, immunocytochemical (Kelly 'et al'.1990) or immunofluorescent (Pavia 'et al'. 1990) studies for E. coli O157 and H7 antigens have also failed to detect the organism in tissues. In a recent pilot study, immunohistochemical staining with peroxidase-labeled antibody to E. coli O157:H7 successfully detected the organism in biopsy or surgical specimens from four patients known to have colitis associated with E. coli O157:H7 and from six patients with ischemic colitis (Brandt 'et al'. 1995).

### 2.5 Isolation and Characterization of EHEC O157:H7

Although assays to identify all categories of diarrheagenic E. coli are available, in many situations it is not necessary to implicate a specific E. coli pathogen in a particular patient. Escherichia. coli is the sub-species of the genus Escherichia, which contains mostly motile gram-negative bacilli within the family Enterobacteriaceae and the tribe Escherichia (Bettelheim, 1994) (Edwards 'et al'. 1972).
*E. coli* can be recovered easily from clinical specimens on general or selective media at 37°C under aerobic conditions. *E. coli* in stool are most often recovered on MacConkey or eosin methylene-blue agar, which selectively grow members of the *Enterobacteriaceae* and permit differentiation of enteric organisms on the basis of morphology (Balows et al. 1991).

*Enterobacteriaceae* are usually identified via biochemical reactions. These tests can be performed in individual culture tubes or by using test “strips” which are commercially available with either method producing satisfactory results.

For epidemiologic or clinical purposes, *E. coli* strains are often selected from agar plates after presumptive visual identification. However, this method should be used with caution, because only about 90% of *E. coli* strains are lactose positive; some diarrheagenic *E. coli* strains, including many of the EIEC strains, are typically lactose negative. The indole test, positive in 99% of *E. coli* strains, is the single best test for differentiation from other members of the *Enterobacteriaceae*.

**Serotyping**

Serotyping of *E. coli* occupies a central place in the history of these pathogens (Lior, 1996). Prior to the identification of specific virulence factors in diarrheagenic *E. coli* strains, serotypic analysis was the predominant means by which pathogenic strains were differentiated. In 1933, Adam showed by serologic typing that some strains of “dyspepsiekoli” could be implicated in outbreaks of pediatric diarrhea. In 1944, Kauffman proposed a scheme for the serologic classification of *E. coli* which is still used in modified forms today (Lior, 1996).

According to the modified Kauffman scheme, *E. coli* are serotyped on the basis of their O (somatic), H (flagellar), and K (capsular) surface antigen profiles (Edwards et al. 1972) (Lior, 1996). A total of 170 different O antigens, each defining a serogroup, are recognized currently. The presence of K antigens was determined originally by means of bacterial agglutination tests: an *E. coli* strain that was inagglutinable by O antiserum but became agglutinable when the culture was heated was considered to have a K antigen. The discovery that several different molecular structures, including fimbriae, conferred the K phenotype led experts to suggest restructuring the K antigen designation to include only acidic polysaccharides (Lior, 1996). Proteinaceous fimbrial antigens have therefore been removed from the K series and have been given F designations (Orskov et al. 1982).

A specific combination of O and H antigens defines the “serotype” of an isolate. *E. coli* of specific serogroups can be associated reproducibly with certain clinical syndromes (appendix 5), but in general, it
is not the serologic antigens themselves that confer virulence but rather, the serotypes and serogroups serve as readily identifiable chromosomal markers that correlate with specific virulent clones (Whittam et al. 1993).

2.6 DIAGNOSIS
Most patients with E. coli O157:H7 infection that occurs in epidemics should be suspected of having infectious diarrhea. More laboratories are now screening for E. coli O157:H7, but infection with this organism is often unrecognized because most clinical laboratories still do not routinely test stool samples for this organism. Other differential diagnoses that have often been considered include inflammatory bowel disease, ischemic colitis, antibiotic-associated pseudomembranous colitis, intussusception, or various causes of an acute abdomen (Ryan et al. 1986).

The strongest evidence for E. coli O157:H7 infection is the presence of organisms in stool culture, but diagnosis can also be supported by the presence of Shiga-like toxin, an increase in serum Shiga-like toxin antibody titers, or a host of new genotypic and phenotypic assays. Stool culture for this organism requires a special growth medium since, E. coli O157:H7 ferments lactose rapidly and thus cannot be picked out from normal, fecal flora when grown on a lactose-containing medium for routine stool cultures. However, serotype O157:H7 can be distinguished from most other strains of E. coli by its slow fermentation of sorbitol. When plated on MacConkey agar (indicator medium) and sorbitol agar (selective medium), E. coli O157:H7 appears sorbitol negative at 24 hours (Farmer et al. 1985).
This MacConkey-sorbitol agar medium is 100% sensitive, 85% specific, and 86% accurate for detecting E. coli O157:H7 (March et al., 1986). Sorbitol-negative colonies can be picked and further tested by characterizing responses to other biochemical parameters (Doyle et al. 1984), serotyping using antisera to H7 and O157 antigens, or determining the presence of Shiga-like toxins. One limitation to this approach is that the rate of isolation decreases with delay in collection of stool samples; cultures collected more than 6 days after the onset of illness or after the administration of antibiotics often produce negative results (Wells et al. 1983).

Escherichia coli O157:H7 was isolated from 75% to 100% of the stool samples obtained within 7 days of the onset of illness, but the recovery rates from samples collected after day 7 ranged from 0% to 33% (Wells et al. 1983). In one study (Tarr et al. 1990), the rate of positive stool culture decreased from 100% for samples collected within 2 days of the onset of diarrhea to 92% for samples collected on days 3 through 6 and to 33% for samples collected after day 7. The duration of carriage seems to be longer in children than in adults (Pai et al. 1986). Finally, there are sorbitol-fermenting E. coli O157
strains that have been reported to cause human disease (Gunzer 'et al'. 1992) but their prevalence and significance are still unclear.

Screening specimens on sorbitol-containing MacConkey culture medium and then testing the non-sorbitol-fermenting colonies for *E. coli O157:H7* by using biochemical parameters and by serotyping with O157 and H7 antisera (Krishnan 'et al'. 1987) can be laborious and time-consuming. Antisera to both H7 and O157 are now commercially available, so that after screening with Sorbitol-MacConkey medium, the sorbitol-negative colonies can be rapidly confirmed with O serum and H serum in the slide agglutination test (Chapman 'et al'. 1989). Investigators have shown that the commercially available latex slide agglutination tests for O157 serum are an efficient and reliable alternative to conventional serotyping with the standard-tube agglutination test, making rapid presumptive detection of *E. coli O157:H7* possible (Chapman 'et al'. 1989). However, colonies that agglutinate should be confirmed serologically, using agglutination or direct immunofluorescent antibody tests (March 'et al'. 1989). An alternative screening method was reported by Farmer and Davis (Farmer 'et al'. 1985), who devised an H7 antiserum-sorbitol fermentation medium as a single-tube screening medium; strains that were presumptive positives (negative for sorbitol fermentation and positive for H7 reaction) were then tested by slide or tube agglutination with *E. coli O157* serum (Farmer 'et al'. 1985).

Another sensitive method of diagnosing *E. coli O157:H7* infection is to look for Shiga-like toxins. These toxins have been detected in *E. coli* culture broth filtrate and in stool extracts (O'Brien 'et al'. 1983). Demonstration of free fecal Shiga-like toxins can be made by tissue culture assays with neutralization by appropriate antisera (Downes 'et al'. 1988). The disadvantage of this approach is that classic tissue culture assays using HeLa or Vero cell culture cytotoxicity (Konowalchuk 'et al'. 1977) require appropriate facilities and are slow and cumbersome. On the other hand, testing for Shiga-like toxin allows the detection not only of *E. coli O157:H7* but of Shiga-like toxin-producing serotypes other than O157:H7, which may be increasing in importance as causes of human illness. Moreover, Shiga-like toxins have been found in fecal filtrates long after *E. coli* cannot be cultured from stools (Karmali 'et al'. 1985): More than 4 to 9 for as long as 4 to 6 weeks.

Free fecal Shiga-like toxin assay has been reported to be more sensitive than stool days after an *E. coli O157:H7* infection, the excretion of organisms into stools usually decreases to an undetectable amount, but free fecal Shiga-like toxin may remain measurable culture for the organism (Carter 'et al'. 1987). In a nursing home outbreak, the rate of isolation from stool samples was 34% and the detection rate for free fecal toxin was 50% (Carter 'et al'. 1987). Although the organism has never been isolated without fecal Shiga-like toxin, the latter was often present even when stool culture was negative (Karmali 'et al'. 1985).
Other methods for detecting toxins include genetic probes and immuno specific assays, which are simpler and more sensitive than culture techniques, although some may be less practical for use in clinical laboratories. Deoxyribonucleic acid hybridization assays using synthetic nucleotides or fragments of structural genes specific for the toxins can also be used to detect Shiga-like toxin-producing \textit{E. coli} (Gunzer et al. 1992).

Gene probes are sensitive and specific (Brown et al. 1989). Using colony blot hybridization, only 2 of 102 strains were toxin-probe positive when toxin was not present (Newland et al. 1988), suggesting that the use of DNA probes to detect Shiga-like toxin production is as accurate as the use of toxin-specific antibodies. These specific DNA probes were able to detect colonies of Shiga-like toxin-producing \textit{E. coli} present in numbers as small as 1 in 1200 colonies (Scotland et al. 1988). In one study (Brown et al. 1989) that used synthetic oligonucleotides from selected sequences of genes encoding A-subunit of Shiga-like toxin I and B-subunit of Shiga-like toxin II at different degrees of stringency, the A-I probe had 92% sensitivity and 91% specificity for identifying Shiga-like toxin I-producing \textit{E. coli}, and the B-II probe had 100% sensitivity and 97% specificity for identifying Shiga-like toxin II-producing \textit{E. coli} (Brown et al. 1989). Both probes were able to identify strains that produce variants of Shiga-like toxins. Gene probes are diagnostically useful, but the cost and concern associated with radioactive safety have limited their widespread applicability.

Various enzyme-linked immunosorbent assays using polyclonal and monoclonal antibodies against Shiga-like toxins I and II to detect the presence of toxins in culture or fecal extract have also been developed (Perea et al. 1988). On the basis of its specific binding to the globotriaosylceramide natural receptor, a modified enzyme-linked immunosorbent assay for the rapid detection of Shiga-like toxin I has been reported (Ashkenazi et al. 1989), in which toxin bound to the globotriaosylceramide receptor was detected by enzyme-linked immunosorbent assay with monoclonal antibodies against Shiga-like toxin I. Both techniques are highly sensitive and specific in detecting toxin production, and they promise to shorten the time to diagnosis of \textit{E. coli} O157:H7 infection.

Another genetic technique involves polymerase chain reaction (PCR) amplification to test for the presence of Shiga-like toxin genes (Paton et al. 1993). Because PCR should detect organisms in low numbers, it can detect Shiga-like toxin production when culture fails (Brian et al. 1992). In addition, like other methods for detecting the presence of Shiga-like toxin, PCR can identify Shiga-like toxin-producing \textit{E. coli} other than O157:H7. Techniques for the direct detection of Shiga-like toxin sequences in stool specimens have also been reported (Brian et al. 1992), overcoming the difficulty of high-frequency loss of toxin genes with repeated cultures (Garch et al. 1992).
Additional phenotypic and genotypic assays have been developed to assist in epidemiologic studies, allowing investigators to determine the extent of outbreaks, trace human outbreaks to animal sources, and differentiate and analyze linkage between strains of *E. coli* O157:H7. These schemes include Shiga-like toxin genotyping (Tarr et al. 1989), plasmid DNA profiling (Paros et al. 1993), bacteriophage typing (Tarr et al. 1989), restriction digests of plasmid (Wells et al. 1983), restriction fragment length polymorphism with a bacteriophage probe (Paros et al. 1993), electrophoresis of plasmids and multilocus enzyme electrophoretic typing (Whittam et al. 1988), pulsed-field gel electrophoresis of restriction fragment length polymorphism (Bohm et al. 1992), and patterns of antibiotic susceptibilities (Swerdlow et al. 1992).

Another useful diagnostic tool is serologic testing to detect antibodies to Shiga-like toxin or O157 lipopolysaccharides. Increases in serum Shiga-like toxin-neutralizing antibody titers during *E. coli* O157:H7 infections have been used to detect or support the diagnosis of infections (Karmali et al. 1983). The antibody titers ranged from 4 to 80 in acute serum specimens collected between days 4 and 18 after the onset of illness; they ranged from 32 to 1280 in convalescent serum specimens collected between days 13 and 43 (Karmali et al. 1985). In one case, the acute and convalescent serum specimens yielded titers of 4096 and 32,000, respectively. In the same study, a fourfold or greater increase in Shiga-like toxin-neutralizing antibody titer was used to diagnose infection (Karmali et al. 1985). Fifty-nine percent of patients (16 of 27) met the requirement, and this criterion was the only evidence of infection in 15% of those tested (Karmali et al. 1985). This serologic test may be an alternative way to diagnose *E. coli* O157:H7 infection, especially during epidemics of this infection or when other methods fail to detect *E. coli* O157:H7.

Similarly, serologic response to O157 lipopolysaccharides of *E. coli* O157:H7 has also been reported (Chart et al. 1991) and can be a useful adjunct for diagnosing *E. coli* O157:H7 infection. In one study (Chart et al. 1991), this serologic test detected evidence of *E. coli* O157:H7 infection in 73% of children with the hemolytic-uremic syndrome and was more sensitive than either isolation of the organism or the detection of fecal Shiga-like toxin. In studies involving patients with the hemolytic-uremic syndrome, the presence of antibodies to O157 lipopolysaccharide was able to provide evidence of *E. coli* O157:H7 infection when fecal bacteria or Shiga-like toxin activity could no longer be detected (Chart et al. 1991). Most IgM antibodies became undetectable 2 to 3 months after the acute phase of the hemolytic-uremic syndrome (Bitzan et al. 1991). However, the interpretation of the serologic study for O157 lipopolysaccharide may be affected by possible cross-reactivity with other organisms and detection of nontoxigenic or non-H7 strains of *E. coli* O157.
In summary, the most common algorithm for diagnosing *E. coli* O157:H7 infection in current clinical practice is to cultures stool specimens for the organisms using Sorbitol-MacConkey agar; this can be done at local hospital laboratories. The sorbitol-negative colonies can be serotyped using commercially available anti-sera to O157 while the sample is sent to a reference laboratory. Presumptive diagnosis can also be made by biochemical testing. In either case, diagnosis is confirmed by the reference laboratory, where the O157 latex test or O157 direct fluorescent antibodies and H7 antisera are used to test for O157:H7. In addition, DNA probes are used to detect Shiga-like toxin in stools at some reference laboratories. If the initial culture is negative but clinical suspicion is still high, stool samples can be sent to a reference laboratory, where more sophisticated techniques, such as PCR for toxin genes, can be used. In practice, serologic determination of Shiga-like toxin titers is used primarily as a diagnostic aid and is not done routinely. In areas where infection with Shiga-like toxin-producing *E. coli* is common, Shiga-like toxin titers on one serum specimen may be difficult to interpret.

2.7 EHEC O157:H7 OUTBREAK RECOGNITION

Symptoms of the diseases caused by EHEC include abdominal cramps and diarrhoea that may in some cases progress to bloody diarrhoea (haemorrhagic colitis), fever and vomiting may also occur. The incubation period can range from three to eight days, with a median of three to four days. Most patients recover within 10 days, but in a small proportion of patients (particularly young children and the elderly), the infection may lead to a life-threatening disease, such as haemolytic uraemic syndrome (HUS). HUS is characterized by acute renal failure, haemolytic anaemia and thrombocytopenia. It is estimated that up to 10% of patients with EHEC infection may develop HUS, with a case-fatality rate ranging from 3 to 5%. Overall, HUS is the most common cause of acute renal failure in young children. It can cause neurological complications (such as seizure, stroke and coma) in 25% of HUS patients and chronic renal sequelae, usually mild, in around 50% of survivors (WHO, 2011).

Persons who experience bloody diarrhoea or severe abdominal cramps should seek medical care.

*E. coli* O157:H7 is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products and raw milk. Faecal contamination of water and other foods, as well as cross-contamination during food preparation (with beef and other meat products, contaminated surfaces and kitchen utensils), will also lead to infection. Examples of foods implicated in outbreaks of *E. coli* O157:H7 include undercooked hamburgers, dried cured salami, unpasteurized fresh-pressed apple cider, yoghurt and cheese made from raw milk (WHO, 2011).
An increasing number of outbreaks are associated with the consumption of fruits and vegetables (sprouts, spinach, lettuce, coleslaw, salad) whereby contamination may be due to contact with faeces from domestic or wild animals at some stage during cultivation or handling. EHEC has also been isolated from bodies of water (ponds, streams), wells and water troughs, and has been found to survive for months in manure and water-trough sediments. Waterborne transmission has been reported, both from contaminated drinking-water and from recreational waters (WHO, 2011).

Person-to-person contact is an important mode of transmission through the oral-faecal route. An asymptomatic carrier state has been reported, where individuals show no clinical signs of disease but are capable of infecting others. The duration of excretion of EHEC is about one week or less in adults, but can be longer in children. Visiting farms and other venues where the general public might come into direct contact with farm animals has also been identified as an important risk factor for EHEC infection (WHO, 2011).

2.8 ANTIMICROBIAL SUSCEPTIBILITY

Most diarrheas are self-limiting and the principal treatment is rehydration and replacement of electrolytes until the patient’s immune system clears the infection (Okeke IN, 2009). In some diarrheas particularly those caused by shiga-like toxin producing \( E. \text{coli} \), antimicrobials may be contra-indicated as they cause release of more toxins from lysed or SOS-induced bacteria (Prester 'et al'. 2003). When pathogens are invasive, antimicrobials may be essential to clear them and prevent long-term sequelae whereas, for persistent infections, antimicrobial agents may be the only recourse, diarrheagenic \( E. \text{coli} \) span the spectrum of these examples which means treatment protocols may not be optimal for example, bloody diarrhea is presumed to be due to \( Shigella \) or \( Entamoeba \) infections in most parts of Africa and is therefore treated with antimicrobials which are contra-indicated in shiga-like toxin producing \( E. \text{coli} \). (Okeke IN, 2009).

In the majority of diarrheas for which antimicrobials are not indicated, they are more often than not prescribed because of the difficulty in distinguishing between self-resolving infections and drug induced ones. Prescribers and self-medicators would use antimicrobials less this way if there was a way to determine which infections might be life threatening (Chunge 'et al'.1992). When antimicrobials are required, more recent studies have shown that common \( E. \text{coli} \) pathotypes including \( \text{EPEC, ETEC, EIEC, EHEC and EAEC} \) are resistant to almost all drugs available and affordable to patients particularly in developing countries such that optimal treatments do not exist (Biì 'et al'. 2005). Results from a Tanzanian study showed that 25-90% of ETEC, EAEC and EPEC isolates were resistant to Ampicillin,
Trimethoprim/sulphamethoxazole, Tetracycline and Chloramphenicol. Resistance to quinolones was also detected (Vila 'et al'. 1999). Comparable results have been seen in bacterial isolates from other studies including from HIV-positive patients with diarrhea in Senegal and from EAEC isolates from Nigerian children where strains from children with diarrhea were more likely to show resistance to commonly used antibiotics than strains from healthy children (Mandomando 'et al'. 2007).

Antimicrobial resistance rates in most African countries although very high, have been lower than observed in other parts of the developing world. In one comparative study of travelers' diarrhea bacterial isolates, strains from Mombasa Kenya were significantly less likely to be resistant to three or more agents than isolates from Goa, India and Montego Bay in Jamaica (Jiang 'et al'. 2002).

Case fatality rates in outbreaks caused by antimicrobial resistant Shigella and other diarrheal agents has been raised by antibiotic resistance, in these situations, susceptibility testing performed early in the epidemic could reduce mortality (Cunin 'et al'. 1999). Due to development of resistance, quinolones have replaced drugs like Doxycycline and Trimethoprim/Sulphamethoxazole as drugs of choice for travelers' diarrhea but resistance to quinolones has been noted and it's on the rise (Okeke IN, 2009).

Over the last 20 years, antibiotic resistance has been reported for all classes of diarrheagenic E. coli and more so from African isolates (Vila 'et al'. 1999). African countries must therefore prioritise resistance containment if efficacy of affordable drugs is to be maintained. Non-antibiotic strategies for diarrheal disease management would save antimicrobials for those cases where there are no alternatives and would reduce the overall selective pressure on these drugs (Okeke IN, 2009). Nutritional supplementation, in particular Zinc, has been proposed as a means for promoting small-bowel movement repair after infection leading to shorter episodes of persistent diarrhea and resistance to infection (Okeke IN, 2009). Such strategies could reduce the burden of disease from diarrheagenic E. coli.

2.9 PREVENTION AND CONTROL OF EHEC O157:H7 INFECTIONS

The prevention of infection requires control measures at all stages of the food chain, from agricultural production on the farm to processing, manufacturing and preparation of foods in both commercial establishments and household kitchens (WHO, 2011).

Industry

The number of cases of disease might be reduced by various mitigation strategies for ground beef (for example, screening the animals pre-slaughter to reduce the introduction of large numbers of pathogens in
the slaughtering environment). Good hygienic slaughtering practices reduce contamination of carcasses by faeces, but do not guarantee the absence of EHEC from products. Education in hygienic handling of foods for workers at farms (FAO/WHO, 2008), abattoirs and those involved in the food production is essential to keep microbiological contamination to a minimum (FAO and WHO, 2009). The only effective method of eliminating EHEC from foods is to introduce a bactericidal treatment, such as heating (e.g. cooking, pasteurization) or irradiation (FAO and WHO, 2009).

Household

Preventive measures for *E. coli* O157:H7 infections are similar to those recommended for other foodborne diseases. Basic good food hygiene practice, as described in the WHO *Five keys to safer food*, (WHO, 2011) can prevent the transmission of pathogens responsible for many foodborne diseases, and also protect against foodborne diseases caused by EHEC. Such recommendations should in all cases be implemented, especially "Cook thoroughly" so that the centre of the food reaches at least 70°C. Make sure to wash fruits and vegetables carefully, especially if they are eaten raw. If possible, vegetables and fruits should be peeled. Vulnerable populations (e.g. small children, the elderly) should avoid the consumption of raw or undercooked meat products, raw milk and products made from raw milk.

Regular hand washing, particularly before food preparation or consumption and after toilet contact, is highly recommended, especially for people who take care of small children, the elderly or immunocompromised individuals, as the bacterium can be passed from person-to-person, as well as through food, water and direct contact with animals.

A number of EHEC infections have been caused by contact with recreational water. Therefore, it is also important to protect such water areas, as well as drinking-water sources, from animal waste (WHO, 2011).
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY DESIGN
The study employed a descriptive cross-sectional study design between November 2009 and June 2010.

3.2 STUDY AREA
Embu District within Eastern province (Figure 3.1) of Kenya is an agricultural area with the main economic activities being tea and coffee growing coupled with daily cattle farming. The district has a population of approximately 306,224 persons (Kenya Bureau of statistics, 2009). It is served by one provincial general hospital, four sub district hospitals, one mission hospital, three health centers and twenty three dispensaries; the district also has eighty four private clinics (Embu District Strategic Plan 2005-2010). Diarrhea among children below five years attending hospitals in Embu is mainly caused by Rota virus, *E. coli* but parasites may also play a significant role.

Diarrhea is endemic in this area probably due to inadequate treatment of raw municipal water or contamination of piped water for domestic use, consumption of raw milk or improperly cooked meat products, close contacts between animals and humans, failure to take necessary measures to de-contaminate water before use and/or lack of proper general hygiene. It was therefore imperative to carry out a survey to establish the prevalence of diarrhea among children below five years who are the most venerable furthermore, epidemiological studies have revealed varied prevalence rates of EHEC 0157:H7 among adults in Kenya but the epidemiology and pathogenicity of EHEC 0157:H7 among children in Embu District has not been characterized. EPGH was selected because it serves as a referral hospital for the entire Eastern province which had the highest number of diarrheal cases in the first half of the year 2008 at 251,738 cases which accounted for 30% of all cases countrywide.
EMBU DISTRICT MAP

Figure 3.1 map of study area
3.30 STUDY POPULATION
The study population was children below five years attending out-patient clinic at Embu provincial General Hospital during the study period.

3.3.1 TARGET POPULATION
The target population was children below five years, presenting with diarrhea at Embu PGH out-patient clinic, not on any antibiotics for at least seven days and who must have been residents of Embu district for at least three months prior to the commencement of the study.

3.3.2 SAMPLE POPULATION
The sample population consisted of 302 children below five years who had been randomly selected as follows: Every second child, presenting with diarrhea at the outpatient clinic, not on any antibiotics for at least 7 days before specimen collection and a resident of Embu district for at least three months prior to the commencement of the study. The sample comprised of 42% female candidates (126 of 302) and 58% male candidates (176 of 302).

3.4 INCLUSION AND EXCLUSION CRITERIA

3.4.1 INCLUSION CRITERIA
Those who were to be included in the study were children below five years presenting with diarrhea at the out-patient division of the hospital, not on any antibiotics and who were residents of the District for at least three months prior to the commencement of the study and whose parent/guardian had consented to the study.

3.4.2 EXCLUSION CRITERIA
Those children not conforming to the above criterion were excluded from the study.

3.5 SAMPLING TECHNIQUE
The technique that was employed to come up with an optimal sample size is systematic random sampling whereby, every second child below five years who presented with diarrhea at Embu general hospital out-patient clinic and who must have been a resident here for at least three months and from whom consent had been obtained, was considered a case until the optimal sample size was attained.

3.6 SAMPLE SIZE ESTIMATION
The study’s aim was to estimate a population proportion, as such, the formula employed in the determination of a suitable sample size is,
However, use is made of the fact that one half of the desired interval (d) is set equal to the product of the reliability coefficient and the standard error. Since sampling was random and assumptions were made that conditions allow for approximate normality of the distribution of \( p \), which then leads to the following formula:

\[
N = \frac{z^2 \sigma^2 + d^2}{d^2}
\]

where, \( q = 1 - p \).

Using this formula, \( N = \frac{z^2 p(1-p)}{d^2} \) (Fischer et al., 2004)

Where,

- \( N \) is optimal sample size,
- \( z \) is the standard normal deviate (95\% for this case)
- \( p \) is the hypothesized proportion of children under five years who present with diarrhea in a month (0.542 for this study)
- \( d \) is the maximum allowable error (0.06 or 6\%)

On ignoring the finite population correlation, the estimated sample size is,

\[
1.96^2 \times 0.542 \times 0.458 \div 0.06^2 = 265 \approx 302 \text{ children,}
\]

From the hospital records at Embu provincial general hospital, on average, 271 children below five years presented with diarrhea every month at the hospital (MOH, 2008) out of an average 500 children below five years who were attended to at the out-patient clinic every month in 2008 therefore, \( 271 \div 500 \) gives 0.542 (p) the proportion of children \( \leq 5 \) who present with diarrhea. A systematic random sample of every second child below five years presenting with diarrhea was recruited into the study until the sample size of 302 was achieved. The optimum sample size (\( N \)) was conveniently extrapolated to 302 to improve the power of the study. Therefore, 302 diarrheagenic stool samples were collected and analyzed for the presence of EHEC 0157:H7.
3.7 ETHICAL CONSIDERATIONS
Before commencement of the study, relevant authorization, permission and consent was obtained from the relevant authorities i.e., Kenyatta University scientific ethics committee, National Council for Science and Technology, as well as the Ministry of Medical Services (Appendix 8). Written consent from the subject was obtained of which the parent/guardian gave consent on behalf of the child (Appendix 7).

3.8 DEPENDENT AND INDEPENDENT VARIABLES

**Independent Variables**
- Demographic, socio-economic, cultural and environmental factors
- Child age
- Gender
- Child guardianship
- Child schooling
- Parents educational level
- Abode
- Water source
- Animal husbandry

**Intermediate/proximate**
- Water treatment knowledge
- Vegetable/fruit washing practices
- Milk preservation and treatment practices

**Outcome/ Dependent Variables**
- EHEC Negative
- EHEC Positive

Figure 3.2 dependent & independent variables.

3.9 VALIDITY AND RELIABILITY OF RESEARCH TOOLS
The methods involved in this research study are methods and techniques that have been used variously worldwide and have been approved for use in clinical research work for instance:

MacConkey-sorbitol agar medium is 100% sensitive, 85% specific, and 86% accurate for detecting *E. coli* 0157:H7 (March 'et al'. 1986), similarly, Certest method for confirmation of EHEC 0157:H7 strains has
been tested and found to have a 100 % sensitivity, 85% specificity and 94 % accuracy for EHEC 0157:H7 (Thompson. J, Hodge. D, and Borczyk, 1990) while slide agglutination test has been proved to have a100% sensitivity, 85% specificity and 86% accuracy for EHEC 0157:H7, however, use of controls was employed for validation of results e.g., *E. coli* ATCC 25922 was used as a negative control in all stages as well as for bacterial growth and anti-biotic susceptibility testing, CerTest kit is supplied with a positive control while a positive control for EHEC 0157:H7 was borrowed from the Nagasaki University Institute of Tropical Medicine-Nairobi Station at the sero-typing stage.

The questionnaire was pretested at Dallas Dispensary which is a public facility where 10 interviews took place to ensure validity, accuracy and completeness of information gathered.
3.10 ISOLATION AND CHARACTERIZATION OF SPECIMEN

3.10.1 ISOLATION

3.10.1.A: SAMPLE COLLECTION

Stool specimens (loose, watery, bloody/diarrheagenic stool) were collected from potentially infected cases using standard procedure as outlined by Monicah cheeseborough (Monicah cheeseborough, 2006). After randomization and after giving a written consent, the parent/guardian accompanying the child selected was taken through the structured, open ended questionnaire in order to capture socio-demographic data such as age, school attendance, sources of drinking water at home, fruits/vegetable handling practices as well as possible use of antibiotics. The interviewee was then issued with a standard sterile poly pot for stool collection and requested to pass the stool sample into the poly pot-about 40gm of formed stool or 6 table spoonfuls of watery/bloody stool. The poly pots were tightly cupped and properly labeled with the patient’s outpatient number and date of collection. The stool specimens were then taken to the laboratory within 15 minutes after collection. Microscopic examination for parasites was done within 30 minutes of stool collection.

3.10.1.B: MICROSCOPY

The proportion of cases of diarrhea caused by parasites was determined using basic microscopy as outlined in the Cowan & Steel’s manual (Microscopic examination was for purposes of ruling-out the possibility of co-infections by accounting for the proportion of diarrhea caused by parasites). 1ml of diarrheal stool was aliquotted into a separate poly pot. 2ml of normal saline was added into the aliquoted sample and the preparation mixed well. Two slides were prepared, one with a drop of diluted stool and cover slipped and the other with a drop of the diluted stool plus a drop of Lugol’s iodine, stirred and cover slipped. The slide preparation without iodine was examined for trophozoites and cysts while the slide to which Lugol’s iodine was added, was examined for further identification and confirmation of cysts. The slide preparations were examined under x10 objective magnification for screening and under x40 objective magnification for identification and confirmation. The data obtained was recorded into a table immediately upon completion of identification.

3.10.2 MICROBIAL CULTURE AND IDENTIFICATION

DIRECT INOCULATION OF SPECIMEN

Cultivation of E. coli isolates on indicator as well as selective media was done by Cowan method; diarrheagenic stool samples were inoculated onto Xylose Lysine Dextrose (XLD) agar plates by streaking using sterile wire loops. After incubation for 24 hours, the plates were observed for presence of large yellow colonies. For the plates that had large yellow colonies, single colonies were picked using
sterile straight wire and sub-cultured on MacConkey agar and incubated at 37°C for 24 hours upon which the plates were observed for the presence of medium sized (1-2mm), pink, dry, flat colonies with sunken centers. Single colonies from MacConkey sub-culture plates containing colonies with the four distinct characteristics were again inoculated onto MacConkey agar plates using sterile straight wires and incubated at 37°C for 24 hours until pure colonies were obtained.

EHEC 0157:H7 is a non-sorbitol fermenting bacteria and therefore produces colourless colonies within 24 hours upon cultivation on Sorbitol MacConkey (SMAC) agar (March 'et al'. 1986). The medium contains sorbitol instead of lactose thus is recommended for the detection of enterohaemorrhagic strains of E. coli 0157:H7 which ferments lactose but does not ferment sorbitol producing colorless colonies whereas sorbitol fermenting strains produce pink/red colonies. The red color is due to production of acid from sorbitol, absorption of neutral red and a subsequent color change of the dye when the pH of the medium falls below 6.8. Biochemical (B.C.) indicator is added to detect the presence of an enzyme β-glucuronidase which is specific for E. coli. Strains of E. coli possessing β-glucuronidase appear as blue colonies on the medium. E. coli 0157:H7 do not possess beta-glucuronidase activity and thus produce colorless colonies. Since E. coli 0157:H7 does not ferment sorbitol, it uses peptone to grow. This raises the pH of the medium allowing the 0157:H7 strain to be differentiated from other E. coli strains. (Farmer 'et al'. 1985)

The remaining stool specimen for each case was preserved at -20 degrees celcius awaiting confirmation through Rapid-Antigen test.

Suspected E. coli isolates from pure cultures on MacConkey agar plates were sub-cultured aseptically on peptone broth & SMAC agar upon which, observation of five characteristic colonies was done and the colonies inoculated into peptone broth tubes and SMAC agar plates respectively. Labeling was done accordingly. The cultures were incubated aerobically at 37°C for 24 hours after which, observation for colourless colonies as illustrated in figure 3.3 was done and biochemical tests carried out.

3.11 CONFIRMATORY TESTING

3.11.A: INDOLE TEST

After incubation for 24 hours, the plate containing peptone broth media was removed and three drops of Kovac’s reagent added to it, colour change of the media to red or pink, the conclusion was indole positive and the corresponding plate containing SMAC agar was observed for colourless or pink/red colonies and if it turned green, indole negative. Observation of red colonies in the plate containing SMAC agar, the results were ignored i.e. no EHEC present. However, on observation of colourless colonies in the SMAC
plate, Certest was performed on the frozen stool specimen for confirmation of presence of EHEC 0157:H7 antigens.

![Colourless colonies on SMAC media.](image)

Figure 3.3: Scanned photo; Colourless colonies on SMAC media.

Pure colonies of suspected EHEC 0157:H7 were then sub-cultured on Mueller hintoin agar and stocked in tryptone soy broth with 15% glycerin at -80°C awaiting confirmation through sero-typing.

### 3.11. B: CERTEST CONFIRMATION

The CerTest *E. coli* 0157:H7 card is a one step coloured chromatographic immunoassay for the rapid, qualitative detection of Escherichia coli 0157:H7 in stool samples.

The membrane is pre-coated with mouse polyclonal antibodies on the test band region, during testing, the sample is allowed to react with the coloured conjugate (anti-*E. coli* 0157:H7 antibodies-red microspheres) which was pre-dried on the test. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the coloured particles migrate. In the case of a positive result, the specific antibodies present on the membrane will capture the coloured conjugate (result region). The mixture continues to move across the membrane to the immobilized antibody placed in the control band region, a green coloured band always appears. The presence of this GREEN band serves to:

1. Verify that sufficient volume is added
2. Verify that proper flow is obtained
3. Serves as an internal control of the reagent
The tests, stool specimens and controls were allowed to come to room temperature (25°C). The stool collection tube containing 1ml of liquid stool plus diluent was shaken to ensure good sample dispersion, using separate stool collection tube and device for each sample or control, 5 drops of stool specimen were dispensed into the circular window on the *E. coli* 0157:H7 device. Observation of coloured bands was done after ten minutes.

The results were interpreted as follows:

**NEGATIVE**- Only one GREEN band appeared across the central window in the site marked with the letter C (control line)

**POSITIVE**- In addition to the green control band, a distinguishable RED band also appeared in the site marked with letter T (Result line)

**INVALID**- A total absence of the control coloured band (GREEN) in the control line regardless of the appearance/or lack of the RED band in the result line. This can be brought about by insufficient specimen volume or incorrect procedural technique.

Quality control: A green line appearing in the control line is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique.

**LIMITATIONS OF CerTest**

The test must be carried out within two hours of opening the sealed bag.

An excess of sample could cause wrong results (brown bands appearing) this is corrected by diluting the sample with a buffer and repeating the test.

A negative result is not meaningful because it is possible the *E. coli* content in the stool sample is too small. Enrichment of the culture could solve this problem.

This test provides a presumptive diagnosis of infection with *E. coli* 0157:H7. Confirmation should be with further clinical observations.

CerTest is 100% sensitive for *E. coli* 0157:H7, 85% specific for *E. coli* 0157:H7 and 94% accurate for *E. coli* 0157:H7.

3.11. **C: SERO TYPING**

Pure isolates from SMAC (sorbitol MacConkey agar) were sub-cultured on Mueller Hintoin agar and then serotyped with *E. coli* 0157 and H7 antisera. A drop of 0157 anti-sera was placed on a sterile slide. A colony of the suspected *E. coli* 0157:H7 was then added onto the slide using an applicator stick, mixed
with the anti-serum and observed for agglutination. Observation for agglutination was done within the first 60 seconds of the reaction to avoid false positives. This was done by rocking the slides 3-5 times while observing under the light. The process was repeated after 24 hours with H7 anti-sera.

Figure 3.4: Scanned photo; Agglutination. Legend to figure 3.4: 24 & 25- positive isolates for EHEC 0157, 26- Control

On sero-typing with 0157 and H7 anti-sera, EHEC 0157:H7 positive isolates produce distinct agglutination as illustrated in figure 3.4. For the isolates that produced distinct agglutination on both 0157 and H7 anti-sera, they were considered positive for EHEC 0157:H7.

3.11. D: ANALYTICAL PROFILE INDEXING

The stocked isolates that produced agglutination on the slide agglutination test were revived on MacConkey agar to confirm absence of contamination. A sterile wire loop was dipped into TBS vials and an inoculum made on the MacConkey plate, the inoculum was streaked and incubated at 37°C for 24 hours. Further analysis was done using Mini-API ID 32 biochemical test as outlined in Color, Atlas of medical bacteriology (Luis ‘et al’.2004). ID 32 is a standardized system for the identification of Enterobactericeae and other non-fastidious gram-negative rods which uses 32 miniaturized biochemical tests as well as a specific database (Api Web). Each ID 32 strip consists of 32 test cupules which contain dehydrated test substrates. After an incubation period of 24 hours, the reactions are read using either the ATB™ Expression or Mini-Api instruments or visually. Identification is obtained using the identification software.

For each of the 12 isolates, after incubation for 24 hours, pink, medium sized dry colonies with sunken centers were then aseptically picked and inoculated onto Mueller Hintoin agar. Incubation was again allowed for 24 hours at 37°C. 0.5% McFarland standards of the organisms on Mueller Hintoin agar plates
were prepared using a turbidometer. 55μl of each isolate were transferred aseptically into each vial of the ID 32 E strip. One drop of mineral oil was added to each underlined test on the strip. The preparation was then placed in a moist anaeropack and incubated for 24 hours at 37°C. The colour changes were read and interpreted using ID 32 E manual. Confirmation of the organism was done using API web.

3.12 ANTIBIOTIC SUSCEPTIBILITY TESTING

The 12 isolates that tested positive for EHEC 0157:H7 on slide agglutination test were tested for antibiotic susceptibility profile. 0.5 McFarland standards of pure *E. coli* colonies of these isolates grown on Sorbitol MacConkey agar and sub-cultured on nutrient agar were made using sterile normal saline. Sterile cotton swabs were dipped into the suspensions containing pure colonies and allowed to soak adequately; excess fluid was removed by squeezing on the sides of the tube. The swabs were then removed and then seeded onto the Muller Hinton agar plates through repeated streaks. The swabs were then discarded in 10% bleach.

The drug discs of eight different antibiotics used in management of diarrheal illnesses in the study area (Trimethoprim/sulphamethoxazole, Chloramphenicol, Tetracycline, Ampicillin, Gentamicin, Streptomycin and Kanamicin) were then placed onto the plates within 15 minutes after inoculation through Kirby-Bauer Disc Diffusion method (Kirby Bauer 'et al'.1966). Tapping was done to ensure the discs were firm. The plates were then incubated aerobically at 37°C within 15 minutes for 20 hours after which the plates were removed and measurements of the zones of inhibition taken in mm using a ruler. The zones of inhibition were then interpreted as being either R (resistant), I (intermediate) or S (sensitive) in accordance with the MIC interpretation scheme provided by the manufacturer and performance standards for antimicrobial susceptibility testing (CLSI, 2010) as illustrated in table 3.1.
Table 3.1: Zones of inhibition Chart-1

<table>
<thead>
<tr>
<th>DRUG/CON.(ug/ml)/CODE</th>
<th>RESISTANT(R)</th>
<th>INTERMEDIATE (I)</th>
<th>SENSITIVE (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim/Sulfamethoxazole 200ug/ml SXT</td>
<td>≤10mm</td>
<td>11-15mm</td>
<td>≥16mm</td>
</tr>
<tr>
<td>Chloramphenicol 30μg/ml C</td>
<td>≤12mm</td>
<td>13-17mm</td>
<td>≥18mm</td>
</tr>
<tr>
<td>Ampicillin 25μg/ml AMP</td>
<td>≤13mm</td>
<td>14-16mm</td>
<td>≥17mm</td>
</tr>
<tr>
<td>Tetracyclin 25μg/ml TE</td>
<td>≤14mm</td>
<td>15-18mm</td>
<td>≥19mm</td>
</tr>
<tr>
<td>Streptomycin 10μg/ml S</td>
<td>≤6mm</td>
<td>7-9mm</td>
<td>≥10mm</td>
</tr>
<tr>
<td>Kanamycin 30μg/ml K</td>
<td>≤13mm</td>
<td>14-17mm</td>
<td>≥18mm</td>
</tr>
<tr>
<td>Gentamicin 10μg/ml GEN</td>
<td>≤12mm</td>
<td>13-14mm</td>
<td>≥15mm</td>
</tr>
</tbody>
</table>

Source: CLSI, 2010

3.13 DATA PROCESSING AND ANALYSIS

Data analysis is descriptive and the computer software SPSS Version 17.0 was used in Data analysis. Data obtained was sorted, coded and entered into the computer. The effect of various demographic characteristics of study participants such as age, school attendance, parent/caretaker education level, sources of drinking water at home, water, milk and vegetable handling practices as well as type of animal reared at home and infection with EHEC 0157:H7 were tested at 5% significance level using Chi-square (χ²) test of homogeneity while Z- test statistic was used to test for statistical significance. Descriptive statistics such as mean, frequencies and percentages were used in summarizing and describing data. Data presentation is through pie charts, bar graphs and frequency tables.
CHAPTER FOUR

4.0 RESULTS, ANALYSIS AND DISCUSSION

4.1 RESULTS

4.1.1 PREVALENCE OF DIARRHEA IN UNDER-FIVES IN EMBU DISTRICT

Of all the cases screened for presence of EHEC 0157:H7, 41.7% (126 of 302) were females while 58.3% (176 of 302) were males.

Figure 4.1: Frequency: Diarrhea cases in under-fives at EPGH by a gender (N=302)
Source: Primary data

Figure 4.2: Frequency: Diarrhea cases by age (N=302), Source: Primary data
The highest number of diarrheal cases was recorded in the very young ages with 75% of all diarrheal cases screened for EHEC 0157:H7 being accounted for by ages below two years while the ages between 2 and 5 years accounted for only 25% of all the cases of diarrhea recorded (figure 4.2).

From the figures in Figure 4.3, diarrhea among children below five years accounted for approximately 7% (582 of 8174) of all the causes of outpatient morbidity at Embu PGH during the period of the study and was the 4th leading cause of out-patient morbidity in under five’s at the PGH with the first position being occupied by diseases of the respiratory system at 51.0% (4166 of 8174) followed by clinical malaria at 23.8% (1946 of 8174). The schedule of the first seven leading causes of outpatient morbidity in under-five year old children at Embu PGH in the period between November 2009 and June 2010 is as illustrated in figure 4.3.

![Figure 4.3: Frequency: Leading causes of morbidity in under-fives at EPGH. Nov. 2009-May 2010 (N=7362), Source: EPGH clinical records.](image)

![Figure 4.4: Frequency: Diarrhea cases in under-fives at EPGH by months. (N=302), Source: Primary data.](image)
There was no definite seasonality pattern of diarrheal cases during the study period; however, the highest number of diarrhea cases was recorded during the months of April and May 2010 (figure 4.4)

4.1.2 PREVALENCE OF PATHOGENIC E. COLI IN UNDER-FIVES IN EMBU DISTRICT.

Out of the 302 paediatric stool samples analysed for causative agents of diarrhea, 84 cases (27.8%) were found to contain parasites, 32 (10.3%) cases were of highly suspected cases of EHEC 0157:H7 through culture while 186 cases (61.9%) could be accounted for by Viruses (Rota virus, noro virus) which are known to cause diarrhea in under five’s in this region, other bacterial causes of diarrhea including Salmonella, Shigella and other strains of pathogenic E. coli that could not be accounted for due to resources constraints. 17 (5.63%) of all cases screened for EHEC did not have any growth which could mean the patient was already using antimicrobial agents by the time stool was collected for analysis (figure 4.5)

4.1.3. PARASITOLOGICAL TEST RESULTS

Parasites were isolated in 84 (27.8%) cases out of 302 cases of diarrhea screened for infection with EHEC 0157:H7 with two main parasites being prominently identified, namely: Entamoeba histolytica accounting for 68 (81%) out of 84 and Giardia lamblia accounting for 16 (19%) cases out of 84. The distribution is as illustrated in (figure 4.6).
40

• Entamoeba histolytica Cysts
• Gianlia lamblia Cysts
• Giardia lamblia trophozoite
• Entamoeba histolytica trophozoites

Figure 4.6: Frequency: Diarrhea in under-fives at EPGH caused by parasites. (N=84), Source: Primary data

4.1.4 MICROBIAL CULTURE TEST RESULTS
Out of the 285 cases of *E. coli* isolated upon sub-culturing on XLD agar, MacConkey agar and subsequently on peptone broth and SMAC media. 32 (10.6%) plates out of 285 plates (10.6%) were found to contain non sorbitol-fermenting isolates with colorless colonies. The 32 isolates were however, not among the 84 cases that had parasites.

Figure 4.7: Frequency: Etiologic agents of diarrhea in under-fives at EPGH on SMAC agar (N=302), Source: Primary data
4.1.5 CerTEST RESULTS
CerTest was performed on the previously preserved stool for the 32 isolates that turned positive for EHEC 0157:H7 upon sub-cultivation on selective medium. Only 2 (0.7%) cases out of the 302 turned positive for EHEC 0157:H7 on the rapid-antigen test (figure 4.8).

![Graph showing 1% positive and 99% negative for CerTest]

Figure 4.8: Frequency: Etiologic agents of diarrheal agents through rapid-antigen test (N=302), Source: Primary data

4.1.6 SERO-TYPING TEST RESULTS
Sero-typing was done on the 32 isolates that were found to be non-sorbitol fermenting on SMAC media. 12 isolates out of 32 or 4% of the total sample population were found to be positive for EHEC 0157:H7 in that they all produced distinct agglutination on slide agglutination test. The cases were distributed equally between the two genders i.e 6 male and 6 females (figure 4.9).

![Graph showing 4% positive and 96% negative for Sero-typing]

Figure 4.9: Frequency: EHEC 0157:H7 through Sero-typing (N=32), Source: Primary data
4.1.7 ANALYTICAL PROFILE INDEXING RESULTS
The 12 isolates that were found to be positive for EHEC 0157:H7 through sero-typing were further confirmed to be true E. coli isolates through Mini-API ID 32 E. Through this test, 75% (9) isolates were confirmed to be over 95% similar to E. coli, 8.3% (1) was confirmed to be over 99.9% similar to Escherichia fergusonii while 16.7 (2) were confirmed to be 25% similar to Shigella sonnei or 75% similar to E. coli.

4.1.8.0 DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS
The purpose of collecting this data was to aid in determining the possible sources of exposure/contamination with EHEC in under-five year old children in Embu District. 302 children below five years were enrolled into the study, out of 302 parents interviewed, 43.4% (131) of them had primary level education while 42.7% (129), had secondary level education with 13.9% (42) having tertiary level education.

89.7% (271) of the children were staying with the parent most of the time, 8% (24) were staying with house help for most of the time while only 2.3% (7) of the children were staying with other people including grandparents, aunt, sister e.t.c It is worthwhile noting however that of all the children born of parents with tertiary level education, 81% (34) of 42, were staying with either house help or other people other than the parent. Of all the children screened for infection with E. coli 0157:H7, 17.9% (54) of 302 of them were attending school while 82.1% (248) were not attending school.

The main source of drinking water in Embu district is piped water, of the 302 respondents interviewed, 96% (290) out of 302 reported having piped water as the main source of drinking water at home. Only 1.7% (5) and 1.35 (4) had bore hole and river/stream as the source of drinking water at home respectively.
while 1% (3) out of 302 had rain water as the main source of drinking water at home. 74.2% (224) of all the respondents reported boiling drinking water before use as a routine while 25.8% (78) did not routinely boil water before drinking. 19.2% (58) of the respondents routinely chemically treated water for domestic use at home while 80.8 (244) did not routinely chemically treat water for domestic use. Of all those who treated water at home, 95% (55) used waterguard as the preferred method of treating water for domestic use while only 5% (3) used aqua tab for treating water. These are the only reported two ways of chemically treating water at home in this region.

37% (111) of 302 of all those interviewed had domestic animals at home while 63% (191) of 302 did not have any domestic animals at home. Of the 37% who had domestic animals 49.4% (55) out of 111 had cattle, 41.3% (46) out of 111 had sheep while only 9.3% (10) had goats at home.

2.7% (8) out of 111 of those who had domestic animals at home had a habit of sharing the house with domestic animals while 97.3% (103) did not share houses with domestic animals. Of all the interviewees, 95% (287) were in the habit of washing fruits and vegetables before eating while 5% (15) did not routinely clean fruits and vegetables before eating.

94% (284) respondents did boil fresh milk before drinking while 6% (18) out of 302 did not routinely boil fresh milk before drinking. Of the 302 respondents, all reported having sanitary facilities (toilet) at home i.e. 100% availability of toilet facilities at home.

4.1.9 DEMOGRAPHIC CHARACTERISTICS ASSOCIATED WITH EHEC O157:H7 INFECTION.

Of the 32 isolates that turned positive for EHEC 0157:H7 upon cultivation on Sorbital MacConkey agar, all were analysed for demographic factors that could have predisposed the participant to infection with EHEC strains.

A significant association exists between the age of the child \((\chi^2 = 19.88, p\text{-value}=0.0005)\) and infection with EHEC 0157:H7 as can be deduced from table 4.1, out of 302 cases of diarrhea in under-five’s, 135 (45%) cases were recorded in children below 1 year and 15 (11%) suspected EHEC 0157:H7 isolates were isolated from children below one year. As the child advances in age, the number of cases of diarrhea and suspected EHEC 0157:H7 isolates appear to decrease, 9.8% of positive isolates were isolated from children between 1 and 2 years, 9.3% in those between 2 and 3 years, and then a sharp increase in the number of EHEC 0157:H7 isolates in those aged between 3 and 4 years was noted, i.e. 15.8%, this may coincide with the entry of the child into school besides, at this age, the child is likely to be mobile and active such that without adequate parental care, the child can come into contact with contaminated water,
vegetables, raw milk etc. Only 7.7% of the isolates were obtained from children aged between 4 and 5 years. These findings are consistent with findings in other studies conducted elsewhere in the world by World Health Organization (WHO, 2007).

**TABLE 4.1: DISTRIBUTION OF EHEC 0157:H7 ISOLATES AT EPGH ON AGE**

<table>
<thead>
<tr>
<th>AGE CATEGORY</th>
<th>EHEC +VE</th>
<th>EHEC -VE</th>
<th>TOTALS</th>
<th>(%) OF EHEC 0157:H7 POSITIVES</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-12 MONTHS</td>
<td>15</td>
<td>120</td>
<td>135</td>
<td>11.1%</td>
</tr>
<tr>
<td>13-24 MONTHS</td>
<td>9</td>
<td>83</td>
<td>92</td>
<td>9.78%</td>
</tr>
<tr>
<td>25-36 MONTHS</td>
<td>4</td>
<td>39</td>
<td>43</td>
<td>9.3%</td>
</tr>
<tr>
<td>37-48 MONTHS</td>
<td>3</td>
<td>16</td>
<td>19</td>
<td>15.8%</td>
</tr>
<tr>
<td>49-60 MONTHS</td>
<td>1</td>
<td>12</td>
<td>13</td>
<td>7.69%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>32</td>
<td>270</td>
<td>302</td>
<td></td>
</tr>
</tbody>
</table>

\[ N (302) \chi^2 = 19.88, \text{df}=4, \text{p-value}=0.0005 \]

With regard to gender, statistically, no association was established between gender and infection with EHEC 0157:H7 (\( \chi^2 \text{(1)} = 1.23, \text{p-value}=0.2888 \)) though from the figures in table 4.2, the percentage (15%) of females infected with EHEC 0157:H7 is higher than that of males (7.4%) even though males accounted for the higher percentage (58%) of diarrheal cases recorded.

**TABLE 4.2: DISTRIBUTION OF EHEC 0157:H7 ISOLATES AT EPGH ON GENDER**

<table>
<thead>
<tr>
<th>GENDER</th>
<th>EHEC POSITIVE</th>
<th>EHEC NEGATIVE</th>
<th>TOTALS</th>
<th>%EHEC 0157:H7 POSITIVE ISOLATES</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALES</td>
<td>13</td>
<td>163</td>
<td>176</td>
<td>7.39%</td>
</tr>
<tr>
<td>FEMALES</td>
<td>19</td>
<td>107</td>
<td>126</td>
<td>15.08%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>32</td>
<td>270</td>
<td>302</td>
<td></td>
</tr>
</tbody>
</table>

\[ N (302) \chi^2 = 1.125, \text{df}=1, \text{p-value}=0.2888 \]

An association was also established between educational level of the parent/caretaker and infection with EHEC 0157:H7 (\( \chi^2 \text{(3)} = 22.75, \text{p-value}=0.0001 \)). As can be deduced from table 4.3, the level of education seems to have an inverse relationship with recorded number of EHEC 0157:H7 isolates obtained. 13% of the isolates were obtained from children whose parent or caretaker had primary level education while 9.6% of the isolates came from children whose parent/caretaker had secondary level education. Only 2.3% of the isolates were obtained from children with parent/caretaker having tertiary level of education.
As the level of education rises from primary to secondary and then tertiary, the number of isolates decrease possibly due to increased levels of awareness and higher standards of hygiene generally associated with higher levels of education.

**TABLE 4.3: DISTRIBUTION OF EHEC 0157:H7 ISOLATES ON EDUCATION LEVEL OF PARENT/CARETAKER**

<table>
<thead>
<tr>
<th>EDUCATION LEVEL</th>
<th>EHEC +VE</th>
<th>EHEC -VE</th>
<th>TOTALS</th>
<th>% EHEC 0157:H7 POSITIVES</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO EDUCATION</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>50%</td>
</tr>
<tr>
<td>PRIMARY</td>
<td>17</td>
<td>114</td>
<td>131</td>
<td>12.98%</td>
</tr>
<tr>
<td>SECONDARY</td>
<td>12</td>
<td>113</td>
<td>125</td>
<td>9.6%</td>
</tr>
<tr>
<td>TERTIARY</td>
<td>1</td>
<td>41</td>
<td>42</td>
<td>2.3%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>32</td>
<td>270</td>
<td>302</td>
<td></td>
</tr>
</tbody>
</table>

N (302) $\chi^2$ = 22.75, df = 3, p-value = 0.0001

An association also exists between source of drinking water at home and infection with EHEC 0157:H7 ($\chi^2$ = 21.44, p-value = 0.0001). Even though 95% of the study participants reported having piped water at home, 72% of the EHEC0157:H7 positive isolates came from stool samples obtained from children with piped water as the main source of drinking water at home (table-4.4). It is worthwhile noting however that, of all the respondents who reported using borehole or river/stream as the main source of drinking water at home, positive isolates of EHEC 0157:H7 were obtained from each of them i.e.,100% prevalence.

**TABLE 4.4: DISTRIBUTION OF EHEC 0157:H7 AT EPGH ON SOURCE OF DRINKING WATER**

<table>
<thead>
<tr>
<th>SOURCE OF WATER</th>
<th>EHEC +VE</th>
<th>EHEC -VE</th>
<th>TOTALS</th>
<th>% EHEC 0157:H7 POSITIVES</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIPED WATER</td>
<td>23</td>
<td>267</td>
<td>290</td>
<td>7.9%</td>
</tr>
<tr>
<td>BOREHOLE</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>100%</td>
</tr>
<tr>
<td>RIVER/STREAM</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td>RAIN WATER</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>0%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>32</td>
<td>270</td>
<td>302</td>
<td></td>
</tr>
</tbody>
</table>

N (302) $\chi^2$ = 21.44, df = 2, p-value = 0.0001

The overall analysis of all demographic characteristic associated with the study participants is as reflected in table 4.5.
Table 4.5: Characteristic-specific prevalence of EHEC O157:H7 infection and the corresponding Bivariate P-Value (s) at 95% CIs:

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Sample size</th>
<th>Children infected with Escherichia coli O157:H7</th>
<th>P Value</th>
<th>Bivariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>PR (95%CI)</td>
<td>PR (95%CI)</td>
<td>PR (95%CI)</td>
</tr>
<tr>
<td><strong>Age Group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to 12 months</td>
<td>132</td>
<td>13</td>
<td>0.85</td>
<td>0.61 (0.14 - 2.42)</td>
<td>0.339</td>
</tr>
<tr>
<td>13 to 24 months</td>
<td>95</td>
<td>11</td>
<td>0.58</td>
<td>0.71 (0.17 - 2.91)</td>
<td>0.48</td>
</tr>
<tr>
<td>25 to 36 months</td>
<td>42</td>
<td>4</td>
<td>0.92</td>
<td>0.57 (0.11 - 2.94)</td>
<td>0.498</td>
</tr>
<tr>
<td>37 to 48 months</td>
<td>22</td>
<td>2</td>
<td>0.09</td>
<td>0.55 (0.08 - 3.68)</td>
<td>0.647</td>
</tr>
<tr>
<td>49 to 60 months</td>
<td>13</td>
<td>2</td>
<td>0.38</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>129</td>
<td>13</td>
<td>10.08</td>
<td>0.91 (0.45 - 1.85)</td>
<td>0.697</td>
</tr>
<tr>
<td>Male</td>
<td>173</td>
<td>19</td>
<td>10.98</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td><strong>Care Taker</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parents</td>
<td>223</td>
<td>23</td>
<td>10.31</td>
<td>0.488</td>
<td>0.65 (0.19 - 2.17)</td>
</tr>
<tr>
<td>House help</td>
<td>60</td>
<td>6</td>
<td>0.518</td>
<td>0.63 (0.16 - 2.53)</td>
<td>0.231</td>
</tr>
<tr>
<td>Other guardian</td>
<td>19</td>
<td>3</td>
<td>15.79</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td><strong>Guardian Education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>182</td>
<td>23</td>
<td>12.64</td>
<td>2.14 (0.51 - 9.11)</td>
<td>0.176</td>
</tr>
<tr>
<td>Secondary</td>
<td>86</td>
<td>7</td>
<td>8.14</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Tertiary</td>
<td>32</td>
<td>2</td>
<td>6.25</td>
<td>1.38 (0.28 - 6.66)</td>
<td>0.445</td>
</tr>
<tr>
<td>None</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td><strong>School attendance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>56</td>
<td>7</td>
<td>12.5</td>
<td>1.23 (0.53 - 2.84)</td>
<td>0.655</td>
</tr>
<tr>
<td>No</td>
<td>246</td>
<td>25</td>
<td>10.16</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td><strong>Water source</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piped water</td>
<td>290</td>
<td>23</td>
<td>10.81</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Bore hole</td>
<td>5</td>
<td>5</td>
<td>100.00</td>
<td>0.99</td>
<td>Referent</td>
</tr>
<tr>
<td>Stream</td>
<td>4</td>
<td>4</td>
<td>100.00</td>
<td>0.001</td>
<td>Referent</td>
</tr>
<tr>
<td>Rain water</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>Referent</td>
</tr>
<tr>
<td><strong>Water treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiling</td>
<td>188</td>
<td>22</td>
<td>11.7</td>
<td>0.61</td>
<td>1.28 (0.48 - 3.39)</td>
</tr>
<tr>
<td>Chemical</td>
<td>59</td>
<td>5</td>
<td>8.47</td>
<td>0.912</td>
<td>0.93 (0.26 - 3.22)</td>
</tr>
<tr>
<td>No treatment</td>
<td>55</td>
<td>5</td>
<td>9.09</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td><strong>Animals reared</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>64</td>
<td>5</td>
<td>7.81</td>
<td>0.757</td>
<td>0.85 (0.31 - 2.31)</td>
</tr>
<tr>
<td>Goat</td>
<td>6</td>
<td>1</td>
<td>16.67</td>
<td>0.559</td>
<td>1.82 (0.24 - 13.6)</td>
</tr>
<tr>
<td>Sheep</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cattle and Goat</td>
<td>27</td>
<td>5</td>
<td>18.52</td>
<td>0.162</td>
<td>2.03 (0.75 - 5.45)</td>
</tr>
<tr>
<td>Cattle and Sheep</td>
<td>8</td>
<td>3</td>
<td>37.5</td>
<td>0.024</td>
<td>4.11 (1.21 - 13.9)</td>
</tr>
<tr>
<td>Cattle, Goat &amp; Sheep</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>None</td>
<td>188</td>
<td>18</td>
<td>9.47</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td><strong>Vegetable washing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Running water</td>
<td>105</td>
<td>12</td>
<td>11.43</td>
<td>0.384</td>
<td>0.51 (0.11 - 2.29)</td>
</tr>
<tr>
<td>Washing basin</td>
<td>188</td>
<td>18</td>
<td>9.57</td>
<td>0.259</td>
<td>0.43 (0.99 - 1.85)</td>
</tr>
<tr>
<td>No washing</td>
<td>9</td>
<td>2</td>
<td>22.22</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td><strong>Region of origin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Runyenjes</td>
<td>54</td>
<td>8</td>
<td>14.81</td>
<td>0.996</td>
<td>0.997</td>
</tr>
<tr>
<td>Municipality</td>
<td>136</td>
<td>16</td>
<td>11.76</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Manyatta Division</td>
<td>78</td>
<td>6</td>
<td>7.69</td>
<td>0.997</td>
<td>NS</td>
</tr>
<tr>
<td>Kyeni Division</td>
<td>33</td>
<td>2</td>
<td>6.06</td>
<td>0.997</td>
<td>0.997</td>
</tr>
<tr>
<td>Nembure Division</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Milk Boiling</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>288</td>
<td>28</td>
<td>9.72</td>
<td>2.63 (0.33 - 20.8)</td>
<td>NS</td>
</tr>
<tr>
<td>No</td>
<td>14</td>
<td>4</td>
<td>28.57</td>
<td>1.86 (0.16 - 20.6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS-Not significant; ND-Not done; PR-Prevalence Ratio; CI-Confidence intervals; No.-Number; %-Percentage
4.1.10 EHEC 0157:H7 ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

Antimicrobial susceptibility tests results on the 12 E. coli isolates that were positive for EHEC 0157:H7 by serological tests revealed that, 60% of EHEC 0157:H7 were resistant to Trimethoprim/sulfamethoxazole at 200μg/ml, while 50% were resistant to Ampicillin, 33% were found to be resistant to Chloramphenical, Cotrimoxazole(25μg/ml) and Tetracycline. All the isolates were found to be susceptible to Gentamycin, Kanamycin and Streptomycin with 0% resistance. Multi-drug resistance was detected in 75% (9 out of 12) of the EHEC 0157:H7 isolates that were found to be resistant to two drugs, 25% (3 out of 12) that were found to be resistant to three drugs and in 17% (2 out of 12) EHEC 0157:H7 Isolates that were found to be resistant to more than three drugs. (Figure 4.11)

<table>
<thead>
<tr>
<th>Drug Concentration</th>
<th>SXT 200 μg/ml</th>
<th>C 30μg/ml</th>
<th>AMP 25μg/ml</th>
<th>TE 25μg/ml</th>
<th>COT 25μg/ml</th>
<th>S 10μg/ml</th>
<th>K 30μg/ml</th>
<th>GEN 10μg/ml</th>
<th>ANTIMICROBIAL PATTERNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td></td>
<td></td>
<td></td>
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<td>6</td>
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<tr>
<td>% Resistant</td>
<td>58%</td>
<td>33%</td>
<td>50%</td>
<td>33%</td>
<td>33%</td>
<td>0%</td>
<td>0%</td>
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</tr>
</tbody>
</table>

Figure 4.11: Antibiotic resistance patterns. (N=12)

Legend to figure 4.11

Antibiotic susceptibility profile of suspected EHEC 0157:H7 isolates:
SXT=Trimethoprim/sulfamethoxazole, C= Chloramphenicol, AMP= Ampicillin, TE= Tetracycline, Cot=Cotrimoxazole, S=Streptomycin, K=Kanamycin, GEN=Gentamicin.
Source: Primary data
Out of the 32 diarrheagenic stool samples that were confirmed as pathogenic EHEC isolates on sub-cultivation on Sorbital MacConkey agar, 12 of them produced agglutination upon typing with 0157 and H7 anti sera i.e. on serology 4% of the 302 cases were confirmed to have been caused by EHEC 0157:H7. While 20 out of 32 or 66% of all the 302 cases of EHEC isolates could be accounted for by other sero types of EHEC such as EHEC 0104:H4, 0145 etc that are usually non-typable with the available 0157 and H7 anti-sera.

4.1.1 HYPOTHESIS TESTING

The obtained prevalence of EHEC 0157:H7 among children below five years in Embu district was found to be 4% based on sero-typing test results as well as Analytical profile Indexing.

Taking the global prevalence to be 2% and using the formula

\[ Z = \frac{p_s - p_0}{\sqrt{p_0(1-p_0)/n}} \]  

(Wane, 2005)

where, \( p_s \) = Sample population prevalence, \( p_0 \) = Total population prevalence, \( q=1-p \) and \( N=\text{total sample size} \), then 

\[ z = \frac{.04-.02}{0.080560947301} = 2.4826 \]  

With \( Z \)-calculated being 2.4826, the significance level at \( Z_{n.1} = 2.4826 \) with confidence level of 95% then, 

\[ p \text{ value observed}=0.01304 \]

\( p \)-value (0.01304) observed is LESS than 0.05 or 95% confidence level. Therefore,

There is a statistical difference between the global prevalence of EHEC 0157:H7 and observed sample population prevalence. i.e, significance levels fall within the acceptance region and as such the null hypothesis is rejected. Therefore, Enterohaemorrhagic Escherichia coli infection is a major cause of diarrhea in young children below five years in Embu District.
4.2 DISCUSSION

Diarrhea poses a serious public health problem particularly in developing countries and it is among the leading cause of morbidity and mortality among children below five years. Globally, diarrheal diseases account for an estimated 2.2 million deaths of children below five years each year (Sang 'et al'. 2011).

In developed and a few developing countries, recent improvement in biological techniques have drastically increased the rate of diagnosis/isolation of bacterial pathogens and consequently reduced the global death rate due to bacterial diarrhea diseases (Sarantuya 'et al'. 2004), however, the situation in Kenya still calls for more intervention because the incidences of diarrhea are still high.

Aetiological agents of diarrhea are many and varied. EHEC 0157:H7 has been implicated in various studies as an important diarrheagenic agent (EFSA, 2011). However, in Kenya, the relative occurrence of this pathogenic bacterium in the defined age group most vulnerable to enterohaemorrhagic Escherichia coli infection has not been well documented. Furthermore, antimicrobials are generally rarely used in the treatment of diarrheal illnesses but widespread use of antibiotics diminishes the efficacy of affordable and available drugs which can lead to antimicrobial resistance (Sang 'et al'.2012).

4.2.1 PREVALENCE OF DIARRHEA IN UNDER-FIVES IN EMBU DISTRICT

The study had sort to establish the prevalence of diarrhea in children below five years in Embu District, account for the portion of diarrhea caused by EHEC 0157:H7 through isolation and characterization of this strain of E. coli and establish antimicrobial susceptibility patterns associated with EHEC 0157:H7 isolates

In this study, which is the first study focusing specifically on EHEC O157:H7 infections in children below five years in Kenya, diarrhea accounted for 7% of all the diseases affecting children below five years attending out-patient clinic at EPGH in Embu district between November 2009 and June 2010. It was the 4th leading cause of outpatient morbidity at EPGH during that period with the first position being occupied by diseases of the respiratory system (51%) and the second position was taken up by clinical malaria (23.8%). The highest percentage of diarrheal cases was in children below two years (75.2%) while ages between 2-5 years accounted for only 24.8% of the cases. Males were more than females at 58.3% and 41.7% respectively.

These results concur with findings in another study conducted in Denmark where three quarters of diarrheal cases in children below five years was recorded in those below three years (Bente 'et al'. 2005) and in yet another study conducted in Nigeria (Nweze 'et al'. 2010) this scenario could be explained by the fact that at very young age, the human immunity in not well developed but the incidences of diarrhea
decrease as one gets older corresponding to development of specific immunity. This therefore calls for more public health measures focusing on mothers with infants and very young children to reduce incidences and burden of diarrhea.

The highest incidences of diarrhea among under-five’s in the study area during the study period were recorded during the month of May 2010 followed by the month of April and then January 2010. It is worthwhile noting that during the months of December 2009 as well as April 2010, there was some rainfall and this might account for the higher number of cases of diarrhea recorded in the stated months (Figure 4.4). Of the 302 cases of diarrhea screened for \textit{E. coli}, 285 (94.2%) of 302 cases had \textit{E. coli}. 84 (27.8%) cases out of 302 had parasites whereas 17 (5.63%) cases had no growth implying the children could have been on antibiotics by the time the stool was collected for analysis (figure 4.5). These findings are consistent with findings in other studies conducted in Lagos-Nigeria (Smith et al. 2009) (Nweze et al. 2010) where \textit{E. coli} accounted for the highest percentage of diarrhoeagenic bacteria in under-fives’s.

Of the 84 cases of diarrhea caused by parasites, only two parasites were identified \textit{Entamoeba histolytica} which accounted for 81% of all the cases caused by parasites and \textit{Giardia lamblia} which accounted for only 19% of the cases caused by parasites (figure 4.6). These figures differ with findings contained in the study conducted in Denmark by Bente (Bente et al. 2005) where parasites accounted for only 4 % of all causes of diarrhea in under-fives. This implies that parasites may play a significant role as aetiologic agents of diarrhea in children in the study area which needs to be investigated. Considering that Denmark is a developed country where as Kenya is a developing country, the difference could be explained by the differences in levels of hygiene both at personal as well as community levels, provision of better health care and proper nutrition.

**4.2.2 PREVALENCE OF PATHOGENIC \textit{E.COLI} IN UNDER-FIVE YEARS OLD IN EMBU DISTRICT.**

\textit{Escherichia coli} was isolated from most of the diarrheal samples, 285 (94%) of the 302 isolates were confirmed as \textit{E. coli}. This bacterium is part of the normal flora in the human gut and as such it was necessary to carry-out further tests aimed at verifying the proportion that was caused by pathogenic \textit{E. coli} including Enterohaemorrhagic \textit{E. coli}. Upon sub-cultivation of the isolates onto Sorbitol MacConkey agar which is selective for \textit{EHEC 0157:H7}, 32 (10.6%) isolates out of 302 diarrheal samples were found to be non-sorbitol fermentors (figure 4.7). The culture had been enriched with cefixime & tellulite (Zheijing-China) to inhibit growth of other non-sorbitol fermenting bacteria and increase specificity of SMAC media. This is a presumptive test since other bacteria share this phenotype and because there are strains of \textit{0157:H7} that can ferment sorbitol (Thomas et al. 1994) it is on the bases of this that
confirmation through rapid antigen test was done on the frozen stool of the 32 suspected cases out of which, 2 cases i.e. 0.7% were found to be positive for EHEC 0157:H7 (figure 4.8), this method has several advantages, it is faster and convenient in that results can be obtained within 15 minutes, it does not require elaborate laboratory infrastructure and therefore can be used in the remotest parts of Kenya and other developing parts of the world, CerTest has a sensitivity of 100% for EHEC 0157:H7 i.e., the chances of getting a false positive are almost zero however, the recovery of antigen from the stool depends on the amount in the stool as such, low levels cannot be detected through this method which necessitated further confirmation through sero-typing and analytical profile indexing.

The 32 cases positive for EHEC 0157:H7 on phenol-typing were further subjected to serological tests i.e. typing with 0157 and H7 anti sera, of which, 12 cases out of 32 i.e., 4% of the total sample size, (Figure 4.9) produced distinct agglutination which is the confirmatory observation expected for any isolate suspected to be EHEC 0157:H7. The cases were equally distributed between the two genders i.e, 6 cases or 50% were male and 6 cases (50%) were females. These findings are consistent with findings in the study done in Denmark (Bente `et al`.2005) as well as the study done in Nigeria (Nweze `et al`.2010) in which the distribution among genders was 50:50. The findings however differ in terms of the prevalence levels since the study in Denmark found a prevalence level of 0.5% of EHEC 0157:H7 among children with diarrhea and 0.2% among the control group (Bente `et al`.2005). The two cases identified as positive for EHEC 0157:H7 by the CerTest also produced distinct agglutination on serological test. Therefore, the prevalence level of EHEC 0157:H7 among children below five years in Embu District is 4% which indicates that this bacterium could be a major cause of sporadic gastroenteritis in children in the study area.

The 12 cases that produced agglutination on serology were confirmed further using Analytical Profile Indexing (Mini-API ID 32E) upon which 9 out of the 12 cases i.e. 75%, were found to be over 95% similar to E. coli, 1 case was confirmed to be 99.9% similar to Escherichia fergusonii (this is also an important emerging diarrheagenic agent in the world) While 2 cases out of 12 were found to be 25% similar to Shigella sonnei or 75% similar to E. coli (figure 4.10). In kenya, epidemiological studies have found varied prevalence rates of EHEC 0157:H7 including 0.2 % among food handlers in Nairobi (Onyango `et al`.2009) 0.8 % in unpasteurized milk (Arimi `et al`.2005) 5.2 % in cattle feaces (Kangethe `et al`.2007) and 24.1 % among adults in masailand (Sang `et al`.2011) while a similar study in Nigeria found a 32% prevalence though the sample population consisted of children, adults and elderly patients with diarrhea, studies in South Africa and Tanzania have revealed a 9% and 10% prevalence of EHEC 0157:H7 in humans respectively (Ateba `et al`.2008),( Raji `et al`.2008) but all these studies have
focused more on the adult population and cattle as the domestic reservoir unlike the current study which focused on children below five years.

4.2.3 DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS ASSOCIATED WITH INFECTION WITH EHEC 0157.

Due to the versatile nature of the strain of bacteria under investigation including the fact that other bacteria share same phenotype and some strains of 0157:H7 can ferment sorbitol, this coupled with the fact that other pathogenic *E. coli* strains such as 0108:H4, 026 have acquired the plasmid that enables them to synthesize shiga-like toxins responsible for the pathogenesis associated with 0157:H7 infection, necessitated analysis of the demographic characteristics of the study participants that could have predisposed the candidate to infection with EHEC 0157:H7. The analysis using a two-tailed Chi-square ($\chi^2$) test was therefore based on pheno-typing test results i.e., 32 isolates positive for *E. coli* 0157:H7 on Sorbitol MacConkey agar culture.

Significant associations were established between infections with suspected *EHEC 0157:H7* bacterium and age, educational level of the parent/caretaker and sources of water for drinking at home. With regard to age ($\chi^2 = 19.88, \text{df}=4, \text{p-value}=0.0005$), out of 32 isolates that tested positive for *EHEC 0157:H7* upon cultivation on Sorbitol MacConkey agar, 75% of the cases were detected from children below two years (table 4.1). Interestingly, between the age of 3 to 4 years, a sharp rise in infection rate was noted which may correspond with entry into school of the child. This is consistent with findings in the study done in Denmark where all the EHEC 0157:H7 isolates were obtained from children below two years (Bente et al., 2005). This may be explained by the fact that, *E. coli* is known to be among the first microorganisms to colonize a human gut besides which, the human immunity is normally not well developed at such tender age. This scenario points towards person to person mode of transmission from the parent/caretaker to the child as the most possible mode of transmission which calls for public health measures aimed at preventing infection with EHEC 0157:H7 focusing on parents/caretaker with children below two years and also those running baby schools in the study area.

With regard to educational levels of the parent/caretaker ($\chi^2 = 22.75, \text{df}=3, \text{p-value}=0.0001$), 50% of all EHEC 0157:H7 isolates were obtained from children whose parent/caretaker had no education, 12.3% from children whose parent/caretaker had primary level of education, 9.6% of the cases were detected in children whose parent/caretaker had secondary level of education while only 1 case (3%) was detected in a child whose parent had tertiary level education. (Table 4.3), i.e., there is an inverse relationship between level of education and infection with EHEC 0157:H7 in the study area which could be due to the fact that the more one is educated, the more one is aware of health risks in the immediate environment e.g.
consumption of undercooked meat, raw vegetables and unpasteurized milk e.t.c and how to mitigate those risks. Healthcare providers therefore need to take more time with parents/caretakers with infants presenting with diarrhea and with no or low levels of education to educate them on general hygiene as well as elucidating the dangers associated with consumption of raw milk, vegetables, raw untreated municipal water and under-cooked beef.

With regard to sources of water ($\chi^2=21.44, \text{df}=2, p\text{-value}=0.0001$), for domestic use, the majority of the homesteads (96%) reported having piped water at home while only 1.7% and 1.3% reported having either borehole, river/stream as the main source of drinking water at home respectively, (Table 4.4). 72% of all the positive isolates were from children having piped water as the main source of drinking water at home which suggests possible contamination of water in the storage facilities at home or in the pipes with sewage which needs to be investigated. Of much interest however, is the observation that, of all the 5 out of 302 respondents who reported having bore hole as the main source of drinking water at home, suspected EHEC 0157:H7 was isolated from all of them similarly, out of 4 respondents out 302 who reported having river/stream as the main source of drinking water at home, suspected EHEC 0157:H7 was isolated from all. This data points to water a possible source of contamination with suspected cases of EHEC 0157:H7 in the study area which a more elaborate study could explain. Gender had no role on infection with EHEC 0157:H7 (Table 4.2) which is consistent with findings in the study conducted in Nigeria (Nzewe ‘et al’.2010)

Globally, many institutional and community-wide outbreaks of E. coli 0157:H7 have occurred in nursing homes (LCDC, 1983), Schools (LCDC, 1987) and day care centres (Spika ‘et al’.1986) or have been related to eating at fast-food restaurants (Bell ‘et al’.1994), drinking untreated municipal water or fresh-pressed apple cider (Besser ‘et al’.1993) or swimming in lake water (Keene ‘et al’.1994) person to person transmission has also been reported. Other sources of Shiga toxin Escherichia coli are contaminated fruits and water (Smith ‘et al’.2009). EHEC has been reported to persist and remain infectious for several weeks in sewerage sludge, pasture land, farmyard manure and slurries (Maule, 2000).

In summary, there are four routes through which human beings can become exposed and potentially infected with EHEC 0157:H7, these are: Food-borne-including contaminated products of animal origin, contaminated fruits or vegetables. Water-borne:-contaminated water supply. Animal contact: direct or indirect contact with animals and their faeces. Person-to-Person: faecal-oral route.(EFSA , 2011) In the study area, although 93.6 % of the respondents reported having piped water as the main source of drinking water, only 19.2% used chemicals to treat water for drinking while 26% of them did not routinely boil water for drinking. Several cases of suspected EHEC 0157:H7, 22% (7 out of 32), were
reported from Stadium village which is located next to the municipal sewerage system and where there have been reported cases of contamination of drinking water with sewage which could point to the main source of contamination of water with suspected EHEC 0157:H7 in the study area. Taking all this into consideration, a more elaborate study could possibly establish the source of infection with EHEC 0157:H7 in the study area.

It is estimated that 0.6% to 2.4% of all cases of diarrhea worldwide (Cahoon 'et al'.1987) and 15% to 36% of all cases of bloody diarrhea or haemorrhagic colitis (PHLS, 1987) are associated with \textit{E. coli} 0157:H7. In a study done in Australia on aetiology of diarrhea (Sinclar, 2005), the most common pathogens isolated were pathogenic \textit{E. coli} 0157:H7 (6.7%), \textit{Norovirus virus} (10.7%), \textit{Campylobacter spp.} (3%) and \textit{Giardia spp.} (2.5%) giving a prevalence level of EHEC 0157:H7 higher than in this study. However, taking the global prevalence of EHEC 0157:H7 to be about 2% (Cahoon 'et al'.1987), the prevalence level in the study area is higher than the global prevalence.

Using the Z-test statistic to compare global prevalence versus sample prevalence, then, \( Z_{n.1} = 2.4826 \), \( df=301 \), \( p\text{-value}=0.01304 \). The \( p\)-value (0.01304) is less than 0.05 i.e, the \( p\)-value falls within the acceptance region and as such, the null hypothesis is rejected. Statistically this means that there is significant difference between the global prevalence and sample prevalence of \textit{EHEC} 0157:H7. Therefore, Enterohaemorrhagic \textit{Escherichia coli} infection is a major cause of diarrhea in children below five years in Embu District.

4.2.4 \textbf{ANTIBIOTIC SUSCEPTIBILITY PATTERNS}

Multiple anti-biotic resistance associated with bacterial pathogens is becoming a widespread problem in developing countries which can be attributed to frequent use of over -the-counter drugs without proper medical supervision (Hoge 'et al'.1998). In this study, 60% of 12 isolates positive for EHEC 0157:H7 through sero-typing were found to be resistant to Trimethoprim/Sulfamethoxazole (200\( \mu \)g/ml), 50% were resistant to Ampicillin, 33% were resistant to Chloramphenicol, Tetracycline and Cotrimoxazole (25\( \mu \)g/ml) while all the isolates were susceptible to Streptomycin, Kanamycin and Gentamicin. Multi-drug resistance was detected in 75% (9 out of 12) of the EHEC 0157:H7 isolates that were found to be resistant to two drugs, 25% (3 out of 12) that were found to be resistant to three drugs and in 17% (2 out of 12) EHEC 0157:H7 Isolates that were found to be resistant to more than three drugs (Figure 4.11), this picture is consistent with findings in a study conducted in Kenya (Sang 'et al'.2011) in which 84% of the \textit{E. coli} isolates were resistant to Trimethoprim/Sulfamethoxazole but differs significantly in that whereas this study revealed resistance level of 33% against Tetracycline, the study by Sang 'et al' revealed a resistance level of 63% against Tetracycline, 33% versus 5% to
ampicillin. In Kenya, the drugs of choice in treating infectious diarrhea include cotrimoxazole as the first drug of choice, ampicillin, amoxicillin and floroquinolones (Senerwa 'et al'. 1991).

From the results contained in this study, EHEC 0157:H7 isolates had low resistance to chloramphenicol (33%) at 30µg/ml concentration which compares well with 24% resistance in the study by Sang 'et al' sited above. A similar study conducted in Nigeria (Smith et al. 2009) revealed that 90.9% of EHEC 0157:H7 isolates from clinical as well as environmental sources were resistant to Ampicillin and Tetracycline, 10% to cotrimoxazole and 0% to Gentamicin where as the isolates in this study had a 33% resistance to Tetracycline at 25µg/ml concentration (figure 4.11) Tetracycline is not normally used in children below five years in the study area but nonetheless, the obtained levels of antibiotic resistance associated with it are important in that *E. coli* infection is not only a problem in under-five’s but also in the elderly where tetracycline may be in use and also with regard to horizontal gene transfer).

These results give the indication of the emergence of multidrug resistance associated with EHEC 0157:H7 which should be a Cause for concern. The findings further suggest an acquisition of resistant genes horizontally or from the resistance factor I plasmids which are chromosomal elements that carry multiple resistance genes which can be acquired either through conjugation, transduction or transmission (Toma 'et al'. 2003). Another way in which antibiotic resistance could develop is by the fact that most hospitals and clinics in developing countries including Kenya routinely treat diarrheal infections with antibiotics symptomatically without the benefit of definitive laboratory results (Tjaniadi 'et al'. 2003). This therefore calls for more studies to reveal what mediates the development of resistance and the mitigating factors if any.
CHAPTER FIVE

5.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

This study had sought to establish the prevalence as well as anti-biotic susceptibility patterns of EHEC 0157:H7 among children below five years in Embu district through prospective cross-sectional survey, in summary, 302 diarrheal cases were sampled, diarrhea accounted for 7% of all causes of out-patient morbidity among children below five years attending the out-patient clinic at Embu Provincial General Hospital, 75% of the 302 cases of diarrhea were recorded in children below 2 years while 25% of the cases were recorded in ages between 2 and 5 years. 94.4% of the cases had *E. coli* but only 4% of the cases were caused by pathogenic EHEC 0157:H7 after confirmation through sero-typing. Certest gave only 0.7% as the prevalence of EHEC 0157:H7 in the study area. It is worthwhile noting that the two cases identified as true positives of EHEC 0157:H7 through CerTest also produced distinct agglutination upon being sero-typed with the 0157 & H7 anti-sera. They were also confirmed as 95% similar to *E. coli* through biochemical tests i.e. Mini-API ID 32 E. (Table 5.1).

Table 5.1: SUMMARY

<table>
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<th>Organism</th>
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<th>Number of cases</th>
<th>Total cases screened</th>
<th>Percentage</th>
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<td>Cultivation on indicator medium</td>
<td>285</td>
<td>302</td>
<td>94.4%</td>
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<tr>
<td>EHEC 0157:H7</td>
<td>Cultivation on SMAC media</td>
<td>32</td>
<td>201</td>
<td>11%</td>
</tr>
<tr>
<td>EHEC 0157:H7</td>
<td>Sero-typing</td>
<td>12</td>
<td>302</td>
<td>4%</td>
</tr>
<tr>
<td>EHEC 0157:H7</td>
<td>CerTest</td>
<td>2</td>
<td>302</td>
<td>0.7%</td>
</tr>
</tbody>
</table>

Summary of identification steps/results

The age of the child, sources of drinking water at home and the education level of the parent/caretaker appear to be predisposing factors for infection with EHEC 0157:H7 while gender, type of animal reared at home or factors like school attendance, water and milk handling practices have no association with infection with EHEC 0157:H7. 60% of the isolates are resistant to Trimethoprim/Sulfamethoxazole, 50% to Ampicillin while 33% are resistant to Chloramphenicol and Tetracycline. All the EHEC 0157:H7 isolates were found to be susceptible to Kanamycin, Streptomycin and Gentamicin. Multi-drug resistance was also detected.
5.2 CONCLUSION

In conclusion,

- Diarrhea is a big burden particularly in children below five years in Embu District Kenya and accounted for 7% of all causes of out-patient morbidity and mortality in under-five’s at the health facility during the study period. 11% of all the cases of diarrhea were accounted for by diarrheagenic *E. coli* with about 4% being accounted for by Enterohaemorrhagic *E. coli 0157:H7* pathotype. This is an indication that an etiologic agent of acute gastroenteritis could be in circulation in the study area with public health implications since, exposure to even low doses can cause an infection and could cause an epidemic.

- Embu District is a peri-urban region with agricultural activities such as daily cattle farming as well as growing of horticultural crops being the main economic activity and as such, chances of people coming into contact with animals and animal products either directly or indirectly are quite high which suggests that the most possible route of transmission to the defined age group under consideration is person to person transmission which calls for public health promotion measures focusing on the parent/caretaker on the need to maintain high standards of personal hygiene as well as on the dangers of consuming undercooked beef, unpasteurized milk, untreated raw municipal water raw vegetables and unwashed fruits.

- Although Enterohaemorrhagic *Escherichia coli 0157:H7* prevalence level in the study area is not alarmingly high, it could be a major cause of acute renal failure in young children through induction of Haemolytic Uremic Syndrome (HUS) which is difficult to detect unless one is on the lookout for the symptoms. The cases of EHEC 0157:H7 isolated had high levels of resistance (60%) to Tromethoprim/Sulphamezoxazole and 50% to Ampicillin two antibiotics that are widely used in the treatment of bacterial infections in Kenya and this is a cause for concern since it further complicates patient management and as such, antibiotic therapy is not recommended as it only worsens the disease course but supportive therapy, electrolyte replacement and management of complications should be the mainstay in the management of *EHEC 0157:H7* infection.

- The prevalence of EHEC 0157:H7 could possibly have been higher if this study had focused on children presenting with Haemorrhagic colitis and Haemolytic uremic syndrome.

- Children at very young age are the most venerable to infection with EHEC 0157:H7.
The source of drinking water in the study area could be the source of infection with EHEC 0157:H7. while Age of the child and Education level of the parent/Guardian are predisposing factors to infection with EHEC 0157:H7.

The fourth millennium development goal is the reduction of mortality in under-five year old children by 67% by the year 2015. Kenya has done very little towards the realization of this goal (UNICEF, 2011). This goal can be achieved if the issue of diarrhea in under-five’s in the study area is critically addressed.

5.3.1 RECOMMENDED INTERVENTIONAL MEASURES

Given the magnitude of the problem of diarrhea and more so the health implications of having a potentially pathogenic bacteria circulating silently in the general population particularly where children are involved and in a country where many children do not reach Age 5 with diarrhea being one of the major contributors to the mortality rate, it is imperative that the following be done and as a matter of priority:

1. Public health programmes should be initiated aimed at reducing cases of diarrhea as well as mitigating the effects of diarrhea particularly in children below five years. All cases of bloody diarrhea should be confirmed through the laboratories to rule out EHEC 0157:H7 besides introduction of faster presumptive diagnostic tools such as rapid-antigen tests at District as well as Provincial general hospitals, these tools can be used even in the remotest areas of our country since they do not require elaborate laboratory structures.

2. Even though EHEC 0157:H7 has not been classified as a notifiable disease in Africa, it is a notifiable disease in most European countries. There should be a state mandated Cases reporting where EHEC 0157:H7 is detected.

3. Health promotion and education programmes should be instituted aimed at educating the general public and in particular parents/caretaker with children below two years on the need to maintain high levels of hygiene particularly with regard to food stuffs such as milk, fruits, meat as well as drinking water.

5.3.2 RECOMMENDATIONS FOR FURTHER RESEARCH

Surveillance programmes should be started in the study area to establish factors contributing to development of drug resistance with regard to EHEC 0157:H7.
✓ Studies should be accelerated to come up with suitable antimicrobial agents for management of EHEC 0157:H7 infections.

✓ A well structured laboratory based prospective study within this defined population and targeting the whole country should be conducted to ascertain the actual national situation with regard to EHEC 0157:H7 prevalence and establish the risk factors associated with its transmission.
REFERENCES


European Food Safety Authority [EFSA]( 2011). Urgent advice on the public health risk of Shiga-toxin producing *Escherichia coli* in fresh vegetables. 9(6): 2274.


Hhapiro R.L, Kumar L, Phillips-Howard P, Wells J.G, Adcock P, Brooks J,


Public Health Laboratory Surveillance [PHLS](1987). Communicable Disease Surveillance Centre.(report), BMJ.; 295:1545-6


APPENDICES

APPENDIX 1: EQUIPMENTS

The following equipments were used in the project:

Autoclave machine: Make-Asteel-MA240N-2006
   Serial number CLN51567

Incubator: Make-Sanyo incubator MIR 553
   Serial number 60603010

Microscope: Make-OLYMPUS CA21FSI
   Serial number: 4H01956

Refrigerator: Make-Sanyo MPR 513 -4°C
   Serial number 60602660

Freezer: Make-Sanyo MDF-0537D -30°C
   Serial number 60203784
APPENDIX 2: MATERIALS

Sample containers (poly pots)-302
Spatulas (4×60 packets)
Sterile gloves (10×100’s)
Slides (5×72 pieces)
Cover slips (4×100’s)
Wire loops
Sterile cotton swabs
Plastic petridishes-500 pieces
Selective growth media-Sorbitol-MacConkey Agar: Batch number: 060905
XLD agar: lot number-0000006662. Lot number: 0000006662
MacConkey agar with crystal violet Lot number: 00026842
Kovac’s indole reagent: 100ml lot number-0000063738.
Peptone water broth (500g) Lot number: 787719
Mueller Hinton agar (500g) Lot number: 0000039745
0.5 MacFarland standard
Anti-sera 0157 and H7 serotype-Denka Sieken co. Ltd, Tokyo, Japan
Multidrug Culture & sensitivity discs: Lot numbers- Ac10/p (100NH3), JH08/P. (100NH3),
H08/P (100NH3) –Biotec (ltd) England.
Rapid antigen testing kits (CERTEST E. coli 0157:H7 CARD) 3kits Ref: E820001FC, lot
Numbers: E012-Biotec-Spain.
E. coli ATCC 25922
Zefixime & Tellulite (Zheigin-China)
Mini Api ID 32 E strips=12, Ref: 32 400 (bio Merieux® SA)
APPENDIX 3: QUESTIONNIER

SERIAL NUMBER:-EHEC/A...../09

ENTEROHAEMORRHAGIC ESCHERICHIA COLI INFECTIONS AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS AMONG CHILDREN ATTENDING OUT-PATIENT CLINIC AT EMBU PROVINCIAL GENERAL HOSPITAL-KENYA

NAME..................................... AGE................................GENDER......

VILLAGE........................... DISTRICT..........................

1. Highest level of education attained by the parent/guardian. Primary/secondary/tertiary

2. Who stays with the child for most of the time? Parent  house help  others specify

3. Does the child attend school? Yes  No

4. Source of drinking water at home. piped water  bore hole  stream  rain water
   Dug out well  other sources (specify) □

5. Do you boil drinking water? Yes  No

6. Do you treat water for domestic use? Yes  No
   B. If yes how? Water guard  Aqua tab  Other ways, (specify) □

7. Do you have domestic animals at home such as cattle  ? sheep  ? goats□?

8. Do you share the house with these domestic animals? Yes  No

9. Do you clean fruits/vegetables before eating? Yes  No
   If yes how? Under running water  In a wash basin  Other ways, (specify) □

10. Do you boil fresh milk before drinking? Yes□  No□

11. Availability of sanitary facilities at home. Available  Not available

CONTACTS........................................ SIGNATURE..................................
### APPENDIX 4: Annual diarrhea under-five child deaths by country

<table>
<thead>
<tr>
<th>RANK</th>
<th>COUNTRY</th>
<th>Annual diarrhea under-five child deaths by country</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>386,600</td>
</tr>
<tr>
<td>2</td>
<td>Nigeria</td>
<td>151,700</td>
</tr>
<tr>
<td>3</td>
<td>DRC Congo</td>
<td>89,900</td>
</tr>
<tr>
<td>4</td>
<td>Afghanistan</td>
<td>82,100</td>
</tr>
<tr>
<td>5</td>
<td>Ethiopia</td>
<td>73,700</td>
</tr>
<tr>
<td>6</td>
<td>Pakistan</td>
<td>53,300</td>
</tr>
<tr>
<td>7</td>
<td>Bangladesh</td>
<td>50,800</td>
</tr>
<tr>
<td>8</td>
<td>China</td>
<td>40,000</td>
</tr>
<tr>
<td>9</td>
<td>Uganda</td>
<td>29,300</td>
</tr>
<tr>
<td>10</td>
<td>Kenya</td>
<td>27,400</td>
</tr>
<tr>
<td>11</td>
<td>Niger</td>
<td>26,400</td>
</tr>
<tr>
<td>12</td>
<td>Burkina Faso</td>
<td>24,300</td>
</tr>
<tr>
<td>13</td>
<td>Tanzania</td>
<td>23,900</td>
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<tr>
<td>14</td>
<td>Mali</td>
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</tr>
<tr>
<td>15</td>
<td>Angola</td>
<td>19,700</td>
</tr>
</tbody>
</table>

Source: WHO. Global burden of diseases estimates, 2004 updates. 2007
Appendix 5: Serotypes characteristic of the diarrheagenic E. coli categories.

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>SEROGROUP</th>
<th>ASSOCIATED H antigen(s)</th>
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</thead>
<tbody>
<tr>
<td>ETEC</td>
<td>06</td>
<td>H16</td>
</tr>
<tr>
<td></td>
<td>08</td>
<td>H9</td>
</tr>
<tr>
<td></td>
<td>011</td>
<td>H27</td>
</tr>
<tr>
<td></td>
<td>015</td>
<td>H11</td>
</tr>
<tr>
<td></td>
<td>020</td>
<td>NM</td>
</tr>
<tr>
<td></td>
<td>025</td>
<td>H42, NM</td>
</tr>
<tr>
<td></td>
<td>027</td>
<td>H7</td>
</tr>
<tr>
<td></td>
<td>078</td>
<td>H11, H12</td>
</tr>
<tr>
<td></td>
<td>0128</td>
<td>H7</td>
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<tr>
<td></td>
<td>0148</td>
<td>H28</td>
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<tr>
<td></td>
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<td>H10</td>
</tr>
<tr>
<td></td>
<td>0159</td>
<td>H20</td>
</tr>
<tr>
<td></td>
<td>0173</td>
<td>NM</td>
</tr>
<tr>
<td>EPEC</td>
<td>055</td>
<td>H6, NM</td>
</tr>
<tr>
<td></td>
<td>086</td>
<td>H34, NM</td>
</tr>
<tr>
<td></td>
<td>0111</td>
<td>H2, H12, NM</td>
</tr>
<tr>
<td></td>
<td>0119</td>
<td>H6, NM</td>
</tr>
<tr>
<td></td>
<td>0125&lt;sub&gt;oc&lt;/sub&gt;</td>
<td>H21</td>
</tr>
<tr>
<td></td>
<td>0126</td>
<td>H27, NM</td>
</tr>
<tr>
<td></td>
<td>0128</td>
<td>H2, H12</td>
</tr>
<tr>
<td></td>
<td>0142</td>
<td>H6</td>
</tr>
<tr>
<td>EHEC</td>
<td>026</td>
<td>H11, H32, NM</td>
</tr>
<tr>
<td></td>
<td>055</td>
<td>H7</td>
</tr>
<tr>
<td></td>
<td>0111&lt;sub&gt;ob&lt;/sub&gt;</td>
<td>H8, NM</td>
</tr>
<tr>
<td>O113</td>
<td>H21</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>O117</td>
<td>H14</td>
<td></td>
</tr>
<tr>
<td>O157</td>
<td>H7</td>
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**EAEC**

<table>
<thead>
<tr>
<th>O3</th>
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<tbody>
<tr>
<td>O15</td>
<td>H18</td>
</tr>
<tr>
<td>O44</td>
<td>H18</td>
</tr>
<tr>
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<tr>
<td>O127</td>
<td>H2</td>
</tr>
<tr>
<td>O29</td>
<td>H10</td>
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**EIEC**

<table>
<thead>
<tr>
<th>O28&lt;sub&gt;ac&lt;/sub&gt;</th>
<th>NM</th>
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</thead>
<tbody>
<tr>
<td>O29</td>
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<td>O124</td>
<td>H30, NM</td>
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<td>O136</td>
<td>NM</td>
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<tr>
<td>O143</td>
<td>NM</td>
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<td>O144</td>
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</tr>
<tr>
<td>O159</td>
<td>H2, NM</td>
</tr>
<tr>
<td>O164</td>
<td>NM</td>
</tr>
<tr>
<td>O167</td>
<td>H4, H5, NM</td>
</tr>
</tbody>
</table>

*O antigen untypeable by conventional methods.

Source: (James P. Nataro & James B. Kaper, 1998)
### APPENDIX 6: UNDER 5’S YEAR OLD OUT PATIENT DISEASES DISTRIBUTION NOV 2009-MAY 2010

<table>
<thead>
<tr>
<th>Disease/Month</th>
<th>NOV'09</th>
<th>DEC'09</th>
<th>JAN'10</th>
<th>FEB'10</th>
<th>MAR'10</th>
<th>APR'10</th>
<th>MAY'10</th>
<th>TOTAL</th>
<th>PERCENTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>57</td>
<td>134</td>
<td>81</td>
<td>141</td>
<td>74</td>
<td>95</td>
<td>582</td>
<td></td>
<td>7.1%</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Dysentery</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>7</td>
<td></td>
<td>0.08%</td>
</tr>
<tr>
<td>Cholera</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Chicken pox</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>13</td>
<td></td>
<td>0.16%</td>
</tr>
<tr>
<td>Measles</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Infectious hepatitis</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>6</td>
<td></td>
<td>0.06%</td>
</tr>
<tr>
<td>Mumps</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Clinical malaria</td>
<td>203</td>
<td>423</td>
<td>328</td>
<td>332</td>
<td>331</td>
<td>329</td>
<td>1946</td>
<td></td>
<td>23.8%</td>
</tr>
<tr>
<td>Confirmed malaria</td>
<td>2</td>
<td>11</td>
<td>2</td>
<td>15</td>
<td>10</td>
<td>3</td>
<td>43</td>
<td></td>
<td>0.53%</td>
</tr>
<tr>
<td>Urinary tract infections</td>
<td>8</td>
<td>30</td>
<td>21</td>
<td>9</td>
<td>24</td>
<td>10</td>
<td>102</td>
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<td>Typhoid fever</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Bilharzia</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0%</td>
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<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td></td>
<td>0.06%</td>
</tr>
<tr>
<td>Malnutrition</td>
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<td>31</td>
<td>14</td>
<td>10</td>
<td>29</td>
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<td>Anaemia</td>
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<td>15</td>
<td>6</td>
<td>14</td>
<td>8</td>
<td>59</td>
<td></td>
<td>0.71%</td>
</tr>
<tr>
<td>Eye infections</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td></td>
<td>0.12%</td>
</tr>
<tr>
<td>Ear infections</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td></td>
<td>0.09%</td>
</tr>
<tr>
<td>Diseases of respiratory system</td>
<td>450</td>
<td>848</td>
<td>513</td>
<td>798</td>
<td>772</td>
<td>785</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>82</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>Dental disorders</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>Skin diseases</td>
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<td>134</td>
<td>3</td>
<td>63</td>
<td>90</td>
<td>104</td>
<td>459</td>
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<td>1</td>
<td>0</td>
<td>0</td>
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<td>3</td>
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<td>Poisoning</td>
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<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>0.31%</td>
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</tr>
<tr>
<td>Accidents/Injuries</td>
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<td>13</td>
<td>0</td>
<td>18</td>
<td>12</td>
<td>13</td>
<td>56</td>
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<td>Sexual assault</td>
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<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0.02%</td>
<td></td>
</tr>
<tr>
<td>Burns</td>
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<td>5</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>9</td>
<td>0.11%</td>
<td></td>
</tr>
<tr>
<td>Bites: Animals, snakes</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.01%</td>
<td></td>
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<tr>
<td>Diabetes</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.01%</td>
<td></td>
</tr>
<tr>
<td>Other diseases</td>
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<td>154</td>
<td>70</td>
<td>57</td>
<td>111</td>
<td>109</td>
<td>567</td>
<td>6.93%</td>
<td></td>
</tr>
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</table>
APPENDIX 7: CONSENT FORM

CONSENT AGREEMENT FORM

ENTEROHAEMORRHAGIC ESCHERICHIA COLI INFECTIONS AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS AMONG CHILDREN ATTENDING OUT-PATIENT CLINIC AT EMBU PROVINCIAL GENERAL HOSPITAL-KENYA.

A case study of Embu Provincial General Hospital

I take this opportunity to wish you and your child quick recovery. I also wish to kindly request you as the parent/guardian for permission to collect stool from your child who has diarrhea for purposes of further analysis in order to give your child a more comprehensive treatment.

The stool specimen shall be preserved at the hospital laboratory for future reference.

Thank you for your kind assistance

With Regards and Best Wishes,

Ndung‘u D. Wachirah
RESEARCHER

Dr C.M. Muli
MEDICAL SUPERINTENDENT;- EMBU PGH.

I hereby give consent for stool to be collected from my child for further analysis.

NAME OF PARENT/GUARDIAN  SIGNATURE  DATE
APPENDIX 8: RESEARCH PERMIT

NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

P.O. Box 30923, 00100
Nairobi
Website: www.ncst.go.ke

Date: 1st October, 2009

Ref: NCST/5/002/R/913/6

David Wachira Ndung'u
Kenyatta University
P.O. Box 43844
NAIROBI

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on
"Assessment of Enterohaemorrhagic Escherichia coli infections and
antibiotic resistance patterns among children in Embu District, Kenya"
I am pleased to inform you that you have been authorized to undertake
your research in Manyatta, Embu District for a period ending 31st
August 2012.

You are advised to report to the District Commissioner and District
Education Officer, Embu District before embarking on your research
project.

Upon completion of your research project, you are expected to submit
two copies of your research report/dissertation to our office.

[Signature]

PROF. S. A. ABDULRAZAK PH.D, MBS
SECRETARY

Copy to:
The District Commissioner
The District Education Officer
Embu District