PATHOGENIC MULTI-DRUG RESISTANT ESCHERICHIA COLI FROM FOODHANDLERS WORKING IN SELECTED TOURIST HOTELS IN NAIROBI, KENYA

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A thesis submitted in partial fulfilment of the requirements for the award of Master of Science (Medical Biochemistry) degree in the School of Pure and Applied Sciences of Kenyatta University

MAY, 2009
DECLARATION

I declare that this thesis is my own original work and has not been presented for a degree in any other university or any other award.

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To wife Eddeby and my sons Teddy, Timo, Kevin and Don who gave me the inspiration to accomplish this study.
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LIST OF ABBREVIATIONS

API – Appareils et Procedes d’identification
CMR - Center for Microbiology Research
DNA – Deoxyribonucleic acid
DVBD-Division of Vector Borne Diseases
EDTA – Ethylene diamine tetra-acetic acid
GEL - Gelatin
KEMRI – Kenya Medical Research Institute
MIC – Minimum Inhibition Concentration
NaOH – Sodium hydroxide
NAS – NAS Airport Services (Nairobi)
NBT – Nitro blue tetrazolium
NPHLS – National Public Health Laboratory Services
RT – Room temperature
SDS - Sodium dodecyl sulphate
SSC - Sodium citrate
TE - Tris ethylene diamine tetra-acetic acid
Tris HCl – Tris hydrochloric acid
WHO – World Health Organization
ABSTRACT

Tourism, an important resource for socio-economic development in Kenya faces great challenges, by among other factors travelers diarrhoea. Contagious diarrheal diseases contribute significantly in the morbidity and mortality to people of all age groups, race and diverse locations especially in developing countries. *Escherichia coli* (E. coli) strains such as Enteroaggregative *Escherichia coli* (EAEC), Enterotoxigenic *Escherichia coli* (ETEC), Enterohemorrhagic *Escherichia coli* (EHEC) and Enteropathogenic *Escherichia coli* (EPEC) are aetiological associated with traveler’s diarrhea. Consumption of contaminated foods and water are implicated as the main route of transmission. Food handlers in tourist destination hotels could, therefore, play a significant role in the epidemiology of pathogenic *Escherichia coli*. The study sought to determine the prevalence of pathogenic *Escherichia coli* among the 885 consenting food handlers; including waiters, cooks, chefs, barmen, butchers and delivery personnel working in nine selected tourist hotels in Nairobi. In addition antibiotic resistant profiles, toxins and conjugation assays of the isolated *Escherichia coli* were determined. Thirty nine (4.4%) food handlers were infected with 1.8% EAEC, 1.2% ETEC, 1.1% EPEC and 0.2% EHEC. 61.5% and 38.5% of these pathotypes were isolated from diarrheal and non-diarrheal stool respectively. Food handlers (14.4%) from hotel-4 accounted for most of the pathogenic infection than any other hotel (*P* = 0.019). Sexes and ages of food handlers was not significantly associated with infection by pathogenic *Escherichia coli* (*P* = 0.256 and *P* = 0.126 respectively). All the EPEC had eaeA toxin genes, ETEC had STp and LT toxin genes, EAEC had aggR genes and LT toxin type while EHEC the stx2 toxin genes. Similarly 5.1% of the isolated serotypes were the virulent or more transmissible O157:H7 strains. 15.4% *Escherichia coli* isolates were resistant to amoxicillin-Clavulanic acid, 8.7% to ampicillin, 53.8% sulphamethaxazol/trimethroprim and 56.4% tetracycline. While over 61.5% of these isolates were resistant to more than two different drug regimens. A total of 89.7% *Escherichia coli* isolates had plasmids ranging in size from 6-100 MDa of which 87.2% were able conjugate with recipient *Escherichia coli* K12FNA' LA giving plasmids ranging in size from 4.5 to 58 MDa. This study illustrates that food handlers working in some tourist hotels in Nairobi are both symptomatic and asymptomatic carriers of multi-drug resistant and toxin expressing pathogenic *Escherichia coli* and are eminent danger in the transmission of traveler’s diarrhea both to the local and foreign tourist. The government needs to review the Public Health Act and enhance surveillance systems for prompt detection of pathogenic *E. coli*. Further, there is the need to develop rapid kits for detection of these strains and genetically map the untypable isolates in Kenya.
CHAPTER ONE
INTRODUCTION

1.1 Background

Kenya is an example of an African country which has embraced tourism as an important resource for socio-economic development. Tourism development in Kenya is a quick and reliable source of foreign exchange receipts, job creation and economic growth. The industry has contributed to the growth of the country's Gross Domestic Product (GDP), raised the foreign exchange earning capacity, and has created employment opportunities (Akama, 1997). The country's total tourism revenues increased from K£27 million in 1970 to over K£1 billion in the late 1980s, so that for the first time tourism earnings surpassed those from tea and coffee (Kenya's leading export crops). The total tourism earnings represented over 12% of the country's GDP and the industry provided over 120,000 direct jobs for Kenyans during the late 1980s and early 1990s (Sindiga, 1999). Tourism in Kenya however faces threats of decline because of among other factors, food borne diseases.

Over the years and more so now, the main goal of the Kenya government has been to develop the tourism industry by encouraging more tourists to visit the country. Consequently, the country's tourism policy has put major emphasis in the expansion of tourism and hospitality facilities in order to attract increasing numbers of international tourists. Among the Popular Hotels in Nairobi include; Safari Park
The World Tourism Organization estimates world tourist arrivals at 567 million in 1995, and this figure is expected to rise with increase in years. Over the past 200 years, the average distance traveled and the speed of travel has increased 1,000 times while incubation periods for diseases have not changed. As a result, a person can be exposed to a foodborne illness in one country and expose others to the infection in a location thousands of miles from the original source of the infection (WHO, 1996). Depending on their destination, travelers are estimated to run a 20% to 50% risk of contracting a foodborne illness.

Illness due to contaminated food has perhaps been the most widespread health problem in the developed world and an important cause of reduced economic productivity (Kaferstein et al., 1996). Recent data from industrialized countries indicate that annually up to 15.8% or more of the population may have food borne diseases (CDC, 2008). The situation is equally serious in developing countries, where infant diarrhea causes many illnesses and deaths (Raju and Ballal, 2009). In addition to known food borne diseases, public health is being challenged by the emergence of new or newly recognized types of food borne illnesses, often with serious and chronic health consequences. Certain populations (e.g., pregnant
women, the elderly, infants and children, immunocompromised persons, and the undernourished) are particularly vulnerable.

In economic terms, food borne illnesses are very costly for industry, health services, and society as a whole. Among the factors that have contributed to the increase in food borne disease could be inadequate knowledge of food handlers in the tourist destination hotels in Kenya which has contributed to increased contamination of primary foodstuffs in this setting.

Bacterial contamination of food and water is on the rise around the globe. The Center for Disease Control and Prevention (CDC) has estimated that microbial pathogens in food cause 76 million cases of human illness, 325 000 hospitalizations, and up to 5000 deaths in the United States each year (Schwetz et al., 2001). Although prevention efforts are reducing the number of outbreaks, their severity is continuing to rise. Microbial food-borne infections are of particular concern due to rising antibiotic resistance, strain variations and the increased movement of people and food products across the globe.

*Escherichia coli* (*E. coli*) is a predominantly non-pathogenic facultative flora of the human intestine (Nataro et al., 1998; Ombui et al., 2001). However, several strains of *E. coli* have developed the ability to cause disease in humans. Many *E. coli* strains have been etiologically associated with diarrheal illnesses that affect individuals of all age groups and at diverse locations around the world. In
underdeveloped countries, the organisms frequently cause diarrhea in infants and in visitors from industrialized countries.

Surveillance of foodborne diseases particularly caused by pathogenic *E. coli* strains will play an important role in the early detection of foodborne disease outbreaks and their control in Kenya. Early identification of the source of the outbreak is becoming increasingly important as countries move towards industrialization. This study was designed to determine the involvement of food handlers in selected tourist destination hotels in Nairobi in the transmission dynamics of multi-drug resistant pathogenic *E. coli* and whether they also are a reservoir of other bacterial pathogens associated with foodborne diseases.

1.2 Characteristics of *Escherichia coli* infections

Strains of *E. coli* that cause gastroenteritis in humans can be grouped into six categories: enteroaggregative, enterohemorrhagic, enteroinvasive, enteropathogenic, enterotoxigenic, and diffusely adherent *E. coli*. The pathogenic *E. coli* are serotyped on the basis of their O (somatic), H (flagella), and K (capsular) surface antigen profiles (Nataro *et al*., 1998). Each of the six categories has a different mode of pathogenesis and comprises a different set of O: H serotypes (Benenson, 1995).
1.3 Justification

Epidemiologically, 25% of tourists to Kenya suffer traveller’s diarrhoea whose etiological agent is pathogenic *E. coli* (Jiang *et al.*, 2002). These isolates have exhibited multi-drug resistance to common antibiotics available through prescriptions to the public (Chartarjee, 2001; Jiang *et al* 2002). Current examination of food handlers by clinical laboratories based on the requirements of Public Health Act Cap 254 section 15 (b) Laws of Kenya does not determine the presence or absence of these pathogenic multi-drug resistant serotypes. Infection of consumers with such strains of *E. coli* through contaminated food or water served by infected food handlers would consequently result in morbidity among the susceptible including tourists. This may subsequently impact negatively on the high end tourist hotels in Kenya, which are major tourism destination for foreigners who contribute to huge economic growth through foreign exchange.

1.4 Hypotheses

i. Food handlers working in tourist hotels do not harbour multidrug resistant enteropathogenic *E. coli* to consumers.

ii. The *Escherichia coli* isolates from food handlers do not possess toxins that encode for pathogenicity
1.5 Objectives

1.5.1 General objective

To determine the carriage of pathogenic multi-drug resistant *E. coli* among food handlers working in tourist hotels in Nairobi

1.5.2 Specific objectives

i. To isolate and characterize the pathogenic *E. coli* from food handlers in selected tourist hotels.

ii. To determine the antibiotic susceptibility, toxin assay and plasmid profiles of the characterized pathogenic *E. coli*
CHAPTER TWO
LITERATURE REVIEW

2.1 Enteroaggregative *E. coli* (EAEC)

Enteroaggregative *E. coli* is associated with persistent diarrhoea (>14 days), both in developed and developing countries. The incubation period has been estimated to be 20 to 48 hours (Manson-Bahr et al., 1987). The diarrhoea is usually watery, secretory and not accompanied by fever or vomiting (Nataro et al., 1998). EAEC are defined by their ability to adhere to epithelial cells in a characteristic 'stacked-brick pattern' of adherence to cultured human epithelial cells (Nataro et al., 1987).

Genes that contribute to the pathogenicity of EAEC encode adhesions, enterotoxins that lead to exfoliation of enterocytes and induction of a net secretory state (Nataro et al., 1998). The toxins include the Pet cytotoxin, the ST-like toxin EAST 1 and the *Shigella* enterotoxin 1 (ShET1) (Nataro and Kaper, 1998; Villaseca et al., 2000; Vila et al., 2000). Pet is a member of the autotransporter family of secreted proteins that enters the epithelial cells and acts to cleave spectrin-a cytoskeletal protein. This cleavage of spectrin induces cell rounding and may induce intestinal secretion (Villaseca et al., 2000). EAEC infection may comprise mild inflammatory enteritis via the EAEC flagellin, which is a mediator for this clinical effect in the patients (Steiner et al., 2000). EAEC infected individuals can be treated with fluoroquinolones but alternative treatment protocols need to be investigated (Okeke et al., 2001).
2.1.1 Enterohemorrhagic *E. coli* (EHEC)

The main enterohemorrhagic serotype is *E. coli* O157:H7. Other serotypes such as O111:H8 and O104:H21 are diarrheagenic in humans (Honda, 1992). EHEC excretes potent toxins called verotoxins or Shiga toxins (Ballows, 1991). This group of organisms are also referred to as Shiga toxin-producing *E. coli* (STEC). Toxin-producing *E. coli* strains can cause haemolytic-uremic syndrome. About 20% to 50% of all EHEC infections are caused by non-O157 serotypes (Nataro *et al.*, 1998), which appear to be less virulent than the O157 serotype. They are less likely to cause bloody diarrhoea and haemolytic uremic syndrome, which are potentially life-threatening complications of all STEC infections (Griffin and Tauxe, 1991). Human milk (colostrums) strongly inhibits the adhesion of *E. coli* 0111 via a complement-mediated bactericidal activity to some serum-susceptible strains (Ogundele, 1999). The most common non-O157: H7 serotypes associated with disease in humans include O26:H11, O103:H2, O111 and O113:H21 (Nataro *et al.*, 1998). Transmission may occur by ingestion of contaminated food or water. Non-O157 serotypes have been found in ground pork, chicken, cheese and ground beef (Griffin and Tauxe, 1991).

2.1.2 Enteroinvasive *E. coli* (EIEC)

Enteroinvasive *E. coli* strains were first shown to cause diarrhoea in studies conducted on volunteers (DuPont *et al.*, 1971). The organism invades the epithelial
cells of the intestine and causes watery diarrhoea. In minority of patients, EIEC may produce an illness that is similar to shigellosis (Benenson, 1995; Nataro et al., 1998). Dysentery caused by EIEC usually occurs within 12 to 72 hours following ingestion of contaminated food and is self-limiting. The illness is characterized by the appearance of blood and mucus in the stools, abdominal cramps, vomiting, fever, chills and malaise (Honda, 1992). Outbreaks are food borne or waterborne and have been associated with the consumption of contaminated meat hamburger and unpasteurized milk or water (Lindqvist et al., 2001; Oundo et al., 2001).

2.1.3 Enteropathogenic E. coli (EPEC)

Enteropathogenic E. coli is the oldest recognized category of diarrhoeagenic E. coli (Honda, 1992). EPEC causes either watery or bloody diarrhoea by producing a heat-stable cytotoxin, which is invasive. EPEC has been linked to acute diarrhoeal diseases, which account for an estimated 12,600 deaths of children daily in Africa, Asia and Latin America (Nataro et al., 1998). The pathogenicity of the disease causing strains is largely due to virulence factors such as enterotoxins, which are plasmid mediated (Fredrick et al., 1987). Transmission of EPEC is via the faecal-oral route. The incubation period can be as short as nine hours in adults (Benenson, 1995). Achlorhydria (lack of hydrochloric acid in the gastric fluid) may be a risk factor for illness in adults (Nataro et al., 1998).
2.1.4 Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic *E. coli* causes acute cholera-like diarrhoea in both humans and animals. Disease process does not involve tissue invasion (Honda *et al.*, 1984). ETEC causes diarrhoea via two steps of colonization of intestinal epithelium and production of heat-labile, heat-stable enterotoxins or both (Honda *et al.*, 1984). Colonization is a result of colonization factor antigens (CFA) produced by some strains of ETEC isolated from human patients. These CFAs have been characterized as CFA I and CFA II (Evans *et al.*, 1978). The ability of ETEC to produce CFA I or CFA II is plasmid mediated. Genes coding for CFA I and heat-stable (ST) enterotoxins production have been found to be on the same plasmid whereas genes coding for CFA II production have been associated with plasmids coding for both heat-labile (LT) and heat-stable (ST) enterotoxins.

2.1.5 Diffusely Adherent *E. coli* (DAEC)

Diffusely adherent *E. coli* was initially used to refer to any HEp-2-adherent *E. coli* strain that did not form EPEC-like microcolonies. With the discovery of EAEC, most authors now recognize DAEC as an independent category of potentially diarrheagenic *E. coli*. Little is however, known about the pathogenetic features of DAEC-induced diarrhea. Cloning and characterization of a surface fimbria in this strain which mediates the DA phenotype have been described (Bilge *et al.*, 1993). The genes encoding the fimbria can be found on either the bacterial chromosome or a plasmid. DAEC strains have been shown to induce finger-like projections
extending from the surface of infected Caco-2 or HEp-2 cells. These projections embed the bacteria, providing protection against gentamicin but without complete internalization. A role for this phenotype in pathogenesis has yet to be demonstrated (Yamamoto et al., 1994; Cookson and Nataro 1996). Several recent studies have implicated DAEC strains as agents of diarrhea, while other studies have not recovered DAEC strains more frequently from diarrheal patients than from asymptomatic controls. An age-dependent susceptibility may explain this observation, because when populations are stratified by age, the association of DAEC with diarrhea is found only in children older than one year (Baqui et al., 1992; Gunzberg et al., 1993).

2.2 Escherichia coli Infections in International Travelers

*Escherichia coli* strains especially ETEC remains endemic all year round but is highest during the warm season, reflecting the seasonal difference of ETEC and other bacterial enteropathogens in the country visited (Mattila et al., 1992; Shaheen et al., 2003), suggesting that travelers are more vulnerable to the diarrheal illnesses at these times. In travelers, the phenotypes of ETEC strains vary from country to country, e.g., LT-only ETEC was more commonly isolated from visitors to Jamaica (58%), LT/ST ETEC was most often seen in visitors to India (45%) and ST-only ETEC in visitors to Kenya (51%) (Jiang et al., 2002; Shaheen et al., 2003). Thus, strains that are present in the population of a particular country, infecting primarily
children, and contaminating the water and food sources (as well as the hands of the food handlers) may determine the type of ETEC infecting the travelers.

Travelers to such countries do not know the cause of their diarrheal illness since it cannot be identified on site, outside of research studies. The data available suggest that from 20% to 40% of traveler’s diarrhea cases (Black, 1990, Jiang et al., 2002; Shaheen et al., 2003) may be caused by ETEC, and the children resident in those countries have rates of 20% of hospitalized diarrheal episodes caused by ETEC. Thus, ETEC seems to be the most frequent cause of traveler’s diarrhea in North Americans and Europeans visiting developing countries (Ericsson, 2003; Shaheen et al., 2003).

Theoretical and empirical studies also indicate that the morphology and dynamics of the intestinal tract influence the probability that *E. coli* will establish a population in the gut. Further, these same factors, in part, determine the genotype of the strain that successfully establishes. In adult humans, there is significant variation among individuals in the dynamics and morphology of the intestinal tract. The length of the intestine varies between men and women and between people of different ages, with the intestine being longer in men and in young people (Hounnou et al., 2002). A number of studies have demonstrated that gut transit time differs between males and females (Graft et al., 2001). Transit times are, on average, significantly longer in females compared to males and this is true of transit
times in the small intestine and colon. The majority of \textit{E. coli} strains inhabit the colon (Hartl and Dykhuizen, 1984), and in this region, transit times are typically about 50% longer in females compared to males. Gut transit times also change with age in adult humans, although the direction of change depends on the gut region under consideration. Transit times in the small intestine generally decrease with age, but colon transit times' increase.

\textbf{2.3 Isolation and identification \textit{E. coli}}

The assays to identify all categories of diarrheagenic \textit{E. coli} are commercially available however; in many situations it is not necessary to implicate a specific \textit{E. coli} pathogen in a particular patient. Patients with enterotoxigenic \textit{E. coli} (ETEC) traveler's diarrhea, for example, generally resolve their diarrhea long before they come to medical attention for stool culture. Most enteroinvasive \textit{E. coli} (EIEC) isolates will be missed in the clinical laboratory, yet diarrhea generally resolves and patients respond to empirical antibiotics. Culturing stools for most categories of diarrheagenic \textit{E. coli} should be performed in cases of persistent diarrhea, especially in travelers, children and the immunocompromised, as well as in outbreak situations. \textit{Escherichia coli} can be isolated from the stool and sent to a qualified reference laboratory for definitive identification.
2.3.1 Biochemical identification of *E. coli*

*Escherichia coli* is the type species of the genus *Escherichia*, which contains mostly motile gram-negative bacilli within the family *Enterobacteriaceae* (Edwards and Ewing, 1972; Bettelheim, 1995). *Escherichia coli* can be recovered easily from clinical specimens on general or selective media at 37°C under aerobic conditions. *Escherichia 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, 1991). *Enterobacteriaceae* are usually identified via biochemical reactions by tests performed in individual culture tubes or by using test "strips" which are commercially available. Either method produces 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 only 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*.
2.3.2 Serotyping techniques

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 1998, Adam showed by serologic typing those 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 form today. According to the modified Kauffinan scheme, *E. coli* strains are serotyped on the basis of their O (somatic), H (flagellar), and K (capsular) surface antigen profiles (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. A specific combination of O and H antigens defines the "serotype" of an isolate. *Escherichia coli* of specific serogroups can be associated reproducibly with certain clinical syndromes, but in general it is not the serologic antigens themselves that confer virulence.
2.3.3 Phenotypic assays based on virulence characteristics

Serotypic markers correlate, sometimes very closely, with specific categories of diarrheagenic *E. coli*; however, these markers are rarely sufficient to reliably identify a strain as diarrheagenic. In addition to its limited sensitivity and specificity, serotyping is tedious and expensive and is performed reliably only by a small number of reference laboratories. Thus, detection of diarrheagenic *E. coli* has focused increasingly on the identification of characteristics that determine the virulence of these organisms. This may include in vitro phenotypic assays which correlate with the presence of specific virulence traits or detection of the genes encoding these traits (Nataro and Kaper, 1998).

One of the most useful phenotypic assays for the diagnosis of diarrheagenic *E. coli* is the HEp-2 adherence assay first described in 1979 by Cravioto *et al.*, 1990. This assay was and remains the "gold standard" for the diagnosis of EAEC and diffusely adherent *E. coli* (DAEC) (Donnenberg and Nataro, 1995). The HEp-2 assay has been modified often since its first description, including such variations as extending the incubation time to 6 h or changing the growth medium during the incubation. However, collaborative studies have shown that the assay performed essentially as first described provides the best ability to differentiate among all three adherent diarrheagenic categories (EPEC, EAEC, and DAEC) (Vial *et al.*, 1990).
The HEp-2 adherence assay entails inoculating the test strain onto a semi-confluent HEp-2 monolayer and incubating it for 3 h at 37°C under 5% CO₂. After this incubation time, the monolayer is washed, fixed, stained, and examined by oil immersion light microscopy. The three patterns of HEp-2 adherence, localized adherence (LA), aggregative adherence (AA), and diffuse adherence (DA), can be differentiated reliably by an experienced technician (Nataro and Kaper, 1998).

2.4 Molecular detection methods

Molecular methods remain the most popular and most reliable techniques for differentiating diarrheagenic strains from nonpathogenic members of the stool flora and distinguishing one category from another. Substantial progress has been made both in the development of nucleic acid-based probe technologies as well as PCR methods. The use of DNA probes for detection of heat-labile (LT) and heat-stable (ST) enterotoxins in ETEC revolutionized the study of these organisms, replacing cumbersome and costly animal models of toxin detection (Moseley, 1982). Since then, gene probes have been introduced for all diarrheagenic categories.

Two general methods are commonly used for nucleic acid probe specimen preparation. The first entails the inoculation of purified cultures onto agar plates to produce "colony" blots, in which 30 to 50 such cultures are inoculated per plate. After incubation, the bacterial growth is transferred to nitrocellulose or Whatman filter paper for hybridization. The bacterial growth on the paper can be lysed, denatured, and hybridized with the probe in situ, and then a radiographic image is
generated by exposure to X-ray film. However, the use of this method requires that the *E. coli* strain first be isolated from the patient's stool, which introduces the possibility that any number of *E. coli* colonies picked from a stool culture may fail to yield the offending pathogenic strain (Nataro and Kaper, 1998). An alternative to the use of colony blots is the stool blot method. In this technique, stool samples are spotted directly onto nitrocellulose filters that have been overlaid onto an agar plate (Lanata, 1985). The advantages of this technique include (i) that the *E. coli* colonies need not be isolated from the stool and (ii) that there may be increased sensitivity if the pathogenic strain represents a minority member of the flora.

However, the presence of large numbers of other bacteria decreases the sensitivity of this test, and a threshold number (ca. $10^5$ to $10^6$ per g of stool (Nataro et al., 1985) of pathogenic organisms must be present to yield definitive results. In addition, the use of stool blots alone does not result in a pure culture of the pathogen, which may be required for verification of phenotypes.
2.4.1 Nucleic acid-based probes

Nucleic acid-based probes themselves can be of two types: oligonucleotide or polynucleotide (fragment probes). DNA fragment probes may be derived from genes that encode a particular phenotype or may instead be empirical probes which, through extensive testing, are found to be linked with the presence of a phenotype. Although empirical probes have generated useful results (Baudry, 1990), probes which represent the virulence genes themselves are generally superior (Giron et al., 1993).

Oligonucleotide probes are derived from the DNA sequence of a target gene. Annealing temperatures and other conditions of hybridization and washing need to be determined much more precisely than for polynucleotide probes. Moreover, very slight strain-to-strain differences among the virulence genes may generate false-negative results with oligonucleotide probes. Nevertheless, oligonucleotide probes have the advantage of faster and often cleaner results than those generated by polynucleotide methods, a factor that comes into play especially when screening for very small genes.

Whereas the original probe techniques involved radio-nucleotides to detect probe hybridization, non-isotopic methods are becoming more popular. These include several methods for tagging oligonucleotide probes and a smaller number of effective techniques for detection of polynucleotide probes. These nonisotopic
techniques have facilitated the introduction of probe technology into areas where the use of radioisotopes is impractical (Nataro and Kaper, 1998).

2.4.2 Polymerase Chain Reaction based tests

Polymerase Chain Reaction based tests are a major advance in molecular diagnostics of pathogenic microorganisms, including *E. coli*. PCR primers have been developed successfully for several of the categories of diarrheagenic *E. coli*. Advantages of PCR include great sensitivity in situ detection of target templates. However, substances within stools have been shown to interfere with the PCR, thus decreasing its sensitivity (Stein *et al.*, 1996).

Traditional PCR methods require amplification in a thermocycler and product separation by gel electrophoresis followed by hybridization with a probe. This is a time-consuming and laborious process. However, the products of the PCR can also be detected by using a DNA binding dye, such as SYBR Green, or through the use of fluorescent probes. The nonsequence-specific SYBR Green assay is less expensive than the fluorescent-probe-based assays utilizing Taqman probes or molecular beacons. Real-time PCR assays can be automated and are sensitive and rapid. They can also quantify PCR products with greater reproducibility while eliminating the need for post-PCR processing, thus preventing carryover contamination (Stein *et al.*, 1996).
2.5 Prevention and Control

2.5.1 Treatment and Management

The treatment of diarrheal disease due to pathogenic *E. coli* is the same as that for cholera or any other acute secretory diarrheal disease. The correction and maintenance of hydration is always most important. Antimicrobials are useful only when the diagnosis or suspicion of ETEC-related diarrhea or cholera is made. Provision of adequate nutrition is critical in children in the developing world, where all diarrheal diseases are frequent (Qadri *et al.*, 2005).

2.5.1.1 Rehydration

Rapid rehydration using intravenous fluids (such as Ringer's lactate) is required initially for all patients with severe dehydration. After restoration of blood pressure and major signs of dehydration, patients can be put on oral rehydration solutions for the remainder of therapy. For all other patients with lesser degrees of dehydration, therapy with oral rehydration solutions alone can be used until the diarrhea ceases (Alam and Ashraf, 2003).
2.5.1.2 Antimicrobials

Antimicrobials are of definite benefit in the treatment of diarrhea of travelers, a diarrheal syndrome in which the clinical symptom is well recognized and ETEC is known to be the most frequent pathogen. Childhood diarrheas, however, are caused not only by ETEC but also by other bacterial and viral agents, and the clinical presentations are not sufficient to differentiate them (Qadri et al., 2005). It has been difficult to study the effect of antimicrobials in children with ETEC disease and antimicrobials are not used routinely in treatment of childhood diarrhea. It should be noted, however, that antimicrobials used for traveler’s diarrhea will treat not only ETEC but also most of the other known causes (enteroaggregative E. coli, Shigella, and Campylobacter) of the diarrhea (Jiang, et al., 2002).

The antimicrobial treatment of traveler’s diarrhea has changed over the years because of increasing antimicrobial resistance (Ericsson, 2003). When pathogenic E. coli were first recognized, the bacteria were usually highly sensitive to all antimicrobials, including tetracyclines and trimethoprim-sulfamethoxazole (Sack, 1990). However, with time, antibiotic resistance emerged, necessitating the use of newer antimicrobials for treatment of traveler’s diarrhea. Antimicrobials that have been used in effective treatment include doxycycline, trimethoprim-sulfamethoxazole, erythromycin, norfloxacin, ciprofloxacin, ofloxacin, azithromycin, and rifamycin. The new nonabsorbable antimicrobial rifaximin recently became available and effective for treatment of traveler’s diarrhea in
adults, using 200 mg two times a day for 3 days. For children, azithromycin, 10 mg/kg/day for 2 days is empirically recommended as a standard dose (DuPont, 2001).

2.5.1.3 Multidrug resistance patterns

The choice of antibiotics has changed since the late 1970s, when doxycycline and trimethoprim-sulfamethoxazole were the preferred drugs. Due to increasing microbial resistance of pathogenic *E. coli*, newer drugs have been used. A fluoroquinolones such as ciprofloxacin, levofloxacin, or ofloxacin is currently the drug of choice, since no significant resistance to these drugs has yet developed (Ericsson, 2003). A newer nonabsorbed drug, rifaxamin, has also been shown to be as effective as a fluoroquinolone and has only recently been approved for use in the United States (DuPont, 2001). Multidrug resistance is increasing in pathogenic *E. coli* due to the widespread use of chemotherapeutic agents in countries where diarrhea is endemic. Antimicrobial sensitivities, however, have only been studied extensively in international travelers and during common source outbreaks of disease or specific epidemiologic studies in areas where diarrhea is endemic. The primary reason for this is the difficulty of recognizing the organisms.

Due to the high sensitivity of ETEC to doxycycline and since it has a long half-life and high levels in stool, doxycycline was first chosen to study antibiotic prophylaxis among travelers to developing countries (Gorbach *et al.*, 1975). The
first studies of doxycycline prophylaxis were done in Peace Corps volunteers in Kenya (Sack et al., 1978) and Morocco (Sack et al., 1979), who showed high degrees of protection (85%). In the Kenyan study all ETEC strains were sensitive to tetracycline, and only a few were resistant to streptomycin and sulfonamide in the Moroccan study. An interesting finding in these two traveler’s diarrhea studies was that nontoxigenic E. coli strains showed more antimicrobial resistance than ETEC (Sack et al., 1978; Sack et al., 1979). This pattern was also seen in a study of large numbers of ETEC isolated before 1978, suggesting that there may be some protective effect of harboring enterotoxin plasmids; it was also shown that ST-producing strains were more likely to be resistant to antimicrobials than either LT or LT/ST strains.

More recently, studies from Bangladesh and India have also shown multiple antimicrobial resistances of ETEC isolates (Raju and Ballal, 2009). A comparison of the resistance pattern in strains isolated recently with those obtained 30 years back highlights the increase of resistance to commonly used drugs (DuPont et al., 1981). Studies of ETEC strains isolated between 1999 and 2001 show intermediate to complete resistance to multiple drugs and combined resistance to four to six drugs (including erythromycin, ampicillin, cotrimoxazole, tetracycline, streptomycin, and doxycycline); however, not a single strain was found to be resistant to ciprofloxacin. In studies in India, multidrug resistance including
resistance to nalidixic acid and to fluoroquinolones is increasing (Chakraborty et al., 2001).

2.5.2 Nutritional and micronutrient therapy

Zinc supplement to the therapy of diarrhea in children with diarrhea has been shown to shorten duration of illness and a decrease in mortality from diarrhea (Bhandari et al., 2002). Nutritional therapy for all childhood diarrheas, including those due to ETEC, is an integral part of diarrhea treatment. Episodes of diarrhea due to any cause, including ETEC, result in decreased nutritional status and thus inhibit growth in children (Mata, 1992). Attention to providing food, particularly breast milk, early in the course of therapy is essential. Additional food during and following the diarrheal episode will help in catch-up growth (Ahmed et al., 1999).

2.5.3 Vaccine development

Prevention of ETEC infection is clearly related to water and sanitation, including food preparation and distribution. In the developing world, major improvements in such areas will be a long time coming (Qadri et al., 2005; CDC, 2008). It is estimated that it would take US$200 billion to make the improvements necessary to prevent fecally spread diseases in South America alone (Quick et al., 1996). Other methods on a microscale are presently being done, such as, building safe-water tube wells, chlorination / filtration/heating of drinking water, and building and improving latrines. Attempts to block transmission are certainly effective if
implemented but cannot solve the problem quickly. Therefore, there is much interest in the development of vaccines for prevention of ETEC disease.

Based on the great impact of ETEC infections on morbidity and mortality, and probably also on nutritional status, particularly of children in areas where they are endemic, an effective ETEC vaccine is highly desirable (Mata, 1992). Such a vaccine is feasible since epidemiologic evidence and results from experimental challenge studies with human volunteers have demonstrated that specific immunity against homologous strains follows ETEC infection. Furthermore, multiple infections with antigenically diverse ETEC strains seem to lead to broad-spectrum protection against ETEC diarrhea (Cravioto et al., 1990). Experimental studies with animals and indirect evidence from clinical trials suggest that protective immunity against ETEC is mediated by secretory immunoglobulin A antibodies directed against the CFs, other surface antigens, and LT; ST, which is a small peptide, does not elicit neutralizing antibodies following natural infection (Svennerholm and Holmgren, 1995).
CHAPTER THREE

MATERIALS AND METHODS

3.1. Study site

Tourism enterprises in Nairobi province include about 28 tourist hotels, 50 tour operators and 130 curio shops; the number that exceeds those which are located in the other provinces of Kenya. The number of tourists visiting the main Kenya's national parks and reserves are mainly concentrated in Nairobi (Akama, 1997) and provides the justification for the choice of Nairobi province as study site. This study was carried in nine different tourist hotels located in Nairobi province. The identities were concealed for ethical reasons and codes use instead. Selection of the hotels was based on their capacity and preference for accommodations by the majority of tourists on transit or visiting Nairobi (http://travel.yahoo.com/p-travelguide-799631-nairobi_nairobi-i). The hotels routinely seek for medical examination services from CMR-KEMRI for health certification for the workers deployed in kitchens, restaurants, room services and ration stores.

3.2. Study population

This study was conducted among adult population working in the nine tourist destination hotels in Nairobi as food handlers. The food handlers were hotel employees who receive and store, prepare or serve meals and drinks to customers of the respective hotels. Eligibility criteria included age ≥18 years, working as a food handler in the selected tourist destination hotels for past six months prior to
study; have handled food and water at least once during the past two weeks and are able to understand and give informed consent. Vials were issued to the participants for individual collection of stool specimen at the hotels then transported to Centre for Microbiology Research, Kenya Medical Research Institute (KEMRI), Nairobi for culture and further assays.

3.3 Sample size

The minimum sample size of 296 was determined using the formulae by Lwanga and Lameshow (1991) as shown below.

\[ N = \frac{Z^2 P (1-P)}{D^2} \]

Where

- \( N \) = Minimum sample size required
- \( Z \) = 1.96 standard error
- \( P \) = 0.26 (26%) the expected isolation rate of pathogenic \( E. coli \) from food handlers (Nataro and Kaper, 1998).
- \( D \) = 0.05 the interval of 95% confidence limit (allowable error).

Therefore

\[ N = \frac{1.96^2 (0.26) (0.74)}{0.05^2} \]

\[ N = 295.65 \]

\[ N = 296 \]
Thus a minimum sample size of 296 was required for this study. However, a total of 885 food handlers (233 female and 652 male) who met the criteria were subsequently enrolled in the study.

3.4 Sample collection

The food handlers working at various tourist destination hotels were given stool collection vials and asked to self collect the stool samples. On pre-arranged appointment dates, these stool samples were collected from hotel staff and identified using code numbers. They were placed in cool boxes and transported to CMR, KEMRI for processing. On receipt of the stool samples, the identification numbers were verified and details entered into the sample reception log book and stored in a cold room until ready for processing.

3.5 Isolation and identification of organisms

Day one

The stool samples were inoculated on Xylose Lysine Dextrose (XLD), Salmonella-Shigella (SS) Agar and Seletine F broth media. This was done using cotton tipped swab which were dipped into the container and the swab and thereafter spread into one-third of the plate. Using a flame sterilized wire loop, the inoculum was streaked into four quadrants of the plate with flame sterilization. The cotton swab was dropped into sterile Seletine F broth. The inoculated plates and bottles were incubated aerobically at 37°C for at least 18 hours.
Day two

The XLD and SS agar plates were removed from the incubator and examined for non-lactose fermenting, black centred colonies with clear edges indicative of *E. coli* colonies. These colonies were picked and inoculated into Triple Sugar Iron (TSI), Motility Indole Ornithine (MIO), Simon’s citrate agar and Urea agar as follows.

**Tube biochemistry test**

TSI agar was inoculated by touching the top of the colony using a sterilised inoculation wire loop and stabbing to the bottom of the tube with a single up and down motion. The slanted surface of agar was immediately streaked and the tube loosely capped and placed into racks. The Motility Indole Ornithine (MIO) tube was inoculated by stabbing in a single up and down motion in the centre of the agar going three fourths of the way down the tube and keeping the wire vertical. Thereafter the tubes were loosely capped and put into racks. Simon’s Citrate tube was inoculated by streaking the slanted surface of the agar with loose capping and placing into racks. Urea agar tube was inoculated by stabbing 2-3 times into the agar, loosely capping the tube and returning to the rack. All the biochemical test tubes were incubated at 35°C – 37°C overnight. An inoculum from the Seletine F both was sub-cultured onto XLD and SS agar plates and incubated at 37°C for approximately 18-24 hours.
Day three

The XLD and SS subculture plates from Seletine F were examined for non-lactose fermenting colonies. Positive cultures were bio-typed as in day two above. Biotyping media from day two were read for typical reactions.

For TSI agar tube, lactose fermentation was indicated by yellow color in the slant and non-lactose fermentation by red color of the slant. Glucose or sucrose fermentation was indicated by yellow colour of the butt. Gas formation was interpreted by the formation of cracks in the media and the media being pushed up the tube and an empty space left at the bottom of the tube. Production of hydrogen peroxide was indicated by blackening of the medium.

The MIO medium was interpreted as follows:

- Motility indicated by cloudiness in the medium and diffused growth,
- Non-motility indicated by growth only along stab line,
- Indole accumulation was tested by addition of a few drops of Kovac’s reagent into the MIO tube and the development of red colour in the alcohol layer indicated a positive reaction,
- Ornithine decarboxylation was indicated by purple colour in the medium,
- Non-decarboxylation of Ornithine was indicated by yellow colour in the medium.
Citrate utilization was indicated by development of blue colour and visible growth along the slant of Simon's citrate medium tube. The presence of urease was indicated by development of bright pink colour in the urea tube. All the biochemical results were interpreted as per Biochemical chart for Enterobacteriaceae, Aeromonas and Plesiomonas.

3.6 Antimicrobial susceptibility testing

3.6.1 Antibiotic sensitivity discs

The disc diffusion technique of Kirby-Bauer (Bauer et al., 1966) was used to test the efficacy of antibiotics available at clinical laboratory settings. The *E. coli* colonies isolated on Mueller Hinton Agar (M-H A) were picked with sterile wire loop and transferred into sterile normal saline to obtain turbidity visually comparable to that of McFarland 0.5 standard. The mixture was diluted ten times to a density of $10^5$ cfu/ml. A sterile cotton swab was dipped into the inoculum suspension while rotating the swab firmly on the inside wall of the tube to remove excess fluid. The dried surface of M-H agar plate that had been brought to room temperature was inoculated by streaking three times over the entire agar surface, rotating the plate at an approximate angle of 60 degrees to ensure an even distribution of the inoculum. The surface of the agar was allowed to dry before placing antibiotic discs gently and pressing to stick to agar surface by use of sterile forceps after which the inverted plates were incubated at 37°C overnight and the result interpreted according to National Committee for Clinical Laboratory
Standards (NCCLS, 2003). The antibiotics included Ampicillin, Nalidixic acid, Chloramphenicol, Tetracycline, Cefotaxime, Cotrimoxazole, Ceftazidime and Ciprofloxacin. The panel of antimicrobials were chosen because of importance in the treatment of Gram-negative bacterial infection, widespread availability and use for treatment of enteric fever in Kenya.

3.6.2 Determination of minimum inhibitory concentrations (MIC)

The minimum inhibitory concentration (MIC) of antimicrobials against the bacterial isolates was determined by the agar dilution technique as described by the National Committee for Clinical Laboratory Standards, 2003. Pure antimicrobial powder of cotrimoxazole, ampicillin, chloramphenicol, gentamicin and nalidixic acid were used to prepare doubling dilutions of the antibiotics in M-H agar. The concentrations to be tested were determined by the interpretive breakpoints as provided by NCCLS (2003). MICs were taken as the lowest concentration of the antimicrobials to prevent visible growth of the bacteria. The weight of pure powder to be used was calculated as:

\[
\text{Weight (mg)} = \text{Volume (ml)} \times \text{Concentration (µg/ml)}
\]

\[
\text{Assay potency (µg/ml)}
\]

The concentrations were prepared as doubling dilutions from the highest (256µg/ml) concentrations to the lowest (0.032 µg/ml). The stock solution was prepared as X10 strength. Two millilitres of the stock solutions of the drugs was
added to 18ml of sterile M-H agar, cooled to 45°C to give a final concentration of X1 as required. Serial two fold dilutions were then prepared in normal saline and 2 ml added to 18 ml of M-H agar above. The plates were left on the bench overnight to control for media contamination. The isolates were grown in 2 ml of M-H broth at 37°C overnight and 0.5 McFarland turbidity standard prepared for each isolate in sterile normal saline. These were introduced to the multiple inoculating chambers and inoculated into the plates with antimicrobials. The plates were then incubated at 37°C overnight. The next day the plates were removed from the incubator and MIC read as the lowest concentration of the respective antibiotic showing no visible growth.

3.7 Toxin assay using DNA hybridisation test

Enterotoxin assay was done according to the procedures described by Tamatsukuri (1991). Briefly, the test organisms were inoculated into 4ml of Lauri Bertani (LB) broth and incubated at 37°C for 16-20 hours with shaking to achieve maximum aeration of the culture to increase yield. One and a half millilitres of the culture was transferred to a micro tube. This was centrifuged at 15,000 rpm for 30 seconds. The tube was removed from the centrifuge and supernatant discarded completely from the tube. The bacterial pellet was re-suspended in 50µl of sterilised de-ionised water and 5µl spotted on nylon membrane and air-dried. In water bath set at 50°C, 0.5 N NaOH, 1% SDS and 5X SSC were warmed. The nitrocellulose membrane was placed on Whatman 3 mm filter paper soaked with 0.5 N NaOH 1 % SDS.
This was kept for 10 minutes to lyse the cells. The membrane was then transferred to Whatman 3mm filter paper soaked with 1M Tris HCl pH 7.4 and kept for 1 minute to neutralise alkaline pH. This step was repeated twice with timing of 1 minute and 10 minutes to neutralisation and lysis respectively. The cell debris from the membrane was gently rubbed with sponge in 5X SSC 1% SDS. The membrane was dried at room temperature for 1 hour and placed into a hybridisation bag. The hybridisation buffer and 2X SSC 1% SDS were warmed in a water bath at 50°C. Two millilitre of the working hybridisation buffer were prepared by adding 10µl of the probe to 2ml of hybridisation buffer (5µl of probe/ml). The working hybridisation buffer was poured into a hybridisation bag. The bubbles were removed and the hybridisation bag sealed by heat and incubated in a water bath at 50°C for 15 minutes then the hybridisation bag was removed from the water bath, opened and the membrane removed using forceps. The membrane was transferred to 100ml of 2X SSC 1% SDS then incubated at room temperature for 10 minutes with gentle shaking (first shaking). The membrane was transferred to about 100ml of 1X SSC 0.5% Triton X and then incubated at room temperature for 10 minutes with gentle shaking (second wash). The substrate buffer 7.5ml was prepared by adding 33µl of NBT solution and 25µl of BCIP solution to 7.5ml alkaline phosphate buffer. The membrane was then placed into hybridisation bag and the substrate buffer poured into the hybridisation bag. The bubbles were removed and hybridisation bag sealed with heat. The sealed bag was incubated at 37°C for 30-60
minutes to detect positive and negative reactions. The bag was removed from the incubator, opened then the membrane washed with de-ionised water and kept dry.

3.8 Plasmid DNA extraction and detection

Plasmid DNA extraction and profiling was done for all the resistant *E. coli* isolates according to the method described by Birnboin and Doly (1979) in Appendix C. The *E. coli* isolates were inoculated into 4ml of Lauri Bertani (LB) broth and incubated at 37°C for 16-20 hours with a shaking rhythm of 120 cycles per minute. The shaking was used provide maximum aeration to the culture to increase the yield. Solution I (B-1) and solution II (B-11) were prepared fresh before use (Appendix 2). One and a half millilitre of the sample was transferred to an eppendorf microfuge. This was then centrifuged at 15,000 rpm for 30 seconds, removed from centrifuged then supernatant discarded to leave the bacterial pellet at the bottom of the microfuge. The pellet was re-suspended I 100μl of ice-cold solution I (B-1), vortexed and incubated on ice for 10 minutes. A 200 μl of solution II was added, mixed by vortexing and incubated on ice for 5 minutes. A 150μl of 3M-sodium acetate, pH 5.2 (B- III) was added to the microfuge and contents mixed by inverting the microfuge several times before incubating on ice for 10 minutes. The mixture was centrifuged at 15,000 rpm for 10 minutes after which 400μl of the supernatant was transferred to a fresh sterile microfuge leaving a small pellet in the tube to which 1000μl of cold ethanol was added to precipitate the DNA. The content was mixed by inverting the microfuge severally. The tube was incubated at
-80°C for 10 minutes and centrifuged at 15,000 rpm for 10 minutes. The supernatant was discarded and the pellet dissolved in 200µl of Tris ethylene diamine tetra acetic acid (TE) buffer and 100µl of 7.5 M Ammonium acetate added then incubated on ice for 30 minutes. The mixture was centrifuged at 15,000 rpm for 10 minutes and 300µl of the supernatant transferred to a new microfuge. 800µl of cold ethanol added then mixed by inverting the tube severally. The mixture was incubated at -80°C for 10 minutes. The mixture was removed and immediately centrifuged at 15,000 rpm for 10 minutes and supernatant discarded. The DNA pellet was rinsed with 1ml of 80% ethanol and centrifuged at 15,000 rpm for 5 minutes. The supernatant was gently discarded by aspiration. The DNA pellet obtained was dried in a 37°C incubator for 30 minutes and dissolved in 50µl TE buffer by vortexing. It was then stored at -20°C until analysed.

3.8.1 Gel electrophoresis

The DNA was removed from the -20°C and allowed to thaw at room temperature. A 1% agarose gel was prepared by dissolving 1gm of Agarose in 100ml of 1X TE buffer. The Agarose was cooled to about 45°C and poured into the gel container with combs in place. The gel was left to set for 30 minutes. A 1:10 solution of Tris Borate ethylene diamine tetra acetic acid (TBE) electrophoresis buffer was prepared and poured into the electrophoresis tank and the gel introduced. A 10µl plasmid DNA solution and 5µl stop mix was loaded into each well of the gel. Lanes 1 and 14 were loaded with the plasmid DNA of *E. coli* V517 and *E coli* 39R861.
strains (Macrina et al., 1978) respectively, which are plasmid markers with known molecular sizes. The gel was allowed to run for approximately 30 minutes at a voltage of 240V. The gel was stained with ethidium bromide (10mg/ml) in a plastic tray for 5 minutes and washed with running tap water, visualised under UV and photographed with Polaroid instant camera.

3.8.2 Quantification of bacterial DNA

DNA concentration and purity was estimated using ethidium bromide fluorescence by mixing 2 µl of DNA sample with 0.4 µl of gel loading buffer containing bromophenol blue loaded into a slot in a 1.5% ethidium bromide stained agarose gel (0.5µg/ml). Two µl of each of the serial dilutions of uncut lambda DNA (0, 2.5, 5, 20, 30, 40, 50 µl/ml) was each mixed with 0.4µl of the loading buffer and loaded into individual wells of the agarose gel. The gels resolved by electrophoresis (Pharmacia Biotech electrophoresis system) and photographed under ultraviolet (UV) illumination. The intensity of the fluorescence of the unknown DNA was compared with that of the DNA standard to estimate the quantity of sample DNA.

3.8.3 Transconjugation assays

In vitro conjugation tests on transferable antimicrobial resistance were performed according to the method of Walia et al., 1987. Single discreet colonies of each donor bacterial strain and recipient *E. coli* K12 (F<sup>-</sup> Na<sup>+</sup> Lac<sup>+</sup>) strain were sub-cultured into 5ml Tryptic Soy Broth (Difco) and allowed to multiply to the
logarithmic phase by incubating at 37°C for 2 hours on a shaker at 250rpm. The donor and the recipient bacterial broth cultures were diluted 1:10 in fresh Tryptic Soy Broth and mixed in equal proportions to make a 5ml broth culture. The mixture was incubated at 37°C and conjugation allowed to take place overnight without shaking. The culture was flush centrifuged at 15,000rpm for 5 seconds and media discarded. The pellet was washed once with normal saline (0.85% sodium chloride solution in distilled water). This was used to wash out any β-Lactamases, which might have leaked into the media from the bacterial cell thereby giving false resistance. To select transconjugants, 3μl samples were drawn from the overnight culture and plated onto MacConkey agar plate, containing 32μg/ml nalidixic acid and 32μg/ml ampicillin using a multiple inoculator. The agar plates were then incubated at 37°C overnight. In order to determine what antibiotic resistances co-transferred to recipient *E.coli* K12 strain, antimicrobial disk susceptibility tests for each the transconjugants were performed as described on section on antibiotic susceptibility testing.

3.9 Hydrophobic testing

Isolates identified as *E. coli* were grown on Colonization Factor Antigen (CFA) agar plates with bile salts at 37°C for 20 hours. The ‘salting out test’ was performed as described by Tamatsukuri *et al* (1992). Briefly this involved the preparation of the salting out reagent (Appendix 3). The bacterial isolate (10⁹ cells/ml) being tested was suspended in 20 μl of 0.002 M Na₂HPO₄ (pH 6.8) on a clean grease free
glass slide. An equal volume (20 μl) of (NH₄) SO₄ buffer was added onto each suspension. The slide was gently rocked by hand for 2 minutes at room temperature. Visible clumping was taken as a positive reaction for surface hydrophobicity of the bacterial cells (Honda et al, 1983). The following enterotoxigenic *E. coli* strains from Osaka University Research Institute for Microbial Diseases, Japan, were used as standard positive control strains during the various Colonization Factor Antigen (CFA) detection tests: ETEC H10407 (CFA/I), ETEC M424C1 (CS1 and CS3, subtypes of CFA/II), ETEC C91F1 (CS2, subtype of CFA/II), ETEC 31 – 10 (CFA/III), ETEC E17374A (CS4 and CS6, subtypes of CFA/IV), ETEC E17018A (CS5 and CS6, subtypes of CFA/IV). The standards were a generous donation of Dr. Joseph Oundo, CMR-KEMRI.

3.10 Data analysis

Descriptive data such as variation and prevalence of *E. coli* strains among the different sexes, age groups, hotels of isolation and the prevalence of other intestinal parasites were analyzed using chi square in SPSS (Statistical Package for the Social Sciences) version 13.0 to determine the proportions. The sensitivity profiles and the quantitative data such as variation in minimum inhibition concentrations among *E. coli* species were also analyzed using chi square.
CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Prevalence of *E. coli* pathotypes among the food handlers

A total of 885 samples were analyzed in this study. The overall prevalence rate of enteropathogenic *Escherichia coli* among the food handlers was 4.4%. By category of the pathotypes, the prevalence of *E. coli* ranged from 0.2%, 1.1%, 1.2% and 1.8% for Enterohemorrhagic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC) and Enteroaggregative *E. coli* (EAEC) respectively. Other infectious intestinal parasites isolated included *Entamoeba coli* (0.8%) and *Entamoeba histolytica/dispar* (0.1%) as shown in Table 1. This distribution of pathotypes among the 885 sampled population was not significantly different ($\chi^2 = 3540$, df = 3536, $P = 0.478$).

4.2 Prevalence of other intestinal parasites

1.4% of food handlers had intestinal protozoal infections in their faeces. Out of the 1.4%, majority 0.8% of study participants were found to be infected with cysts of *Entamoeba coli* while *Entamoeba histolytica/dispar* accounted for only 0.1% of the intestinal protozoal infections. Comparatively, helminthiasis was detected in only 0.5% of the food handlers examined. The round worms detected included *Ascaris lumbricoides*, Hookworm and *T. trichiura*. The proportions were 0.1% for *A. lumbricoides* while Hookworm and *T. trichiura* 0.2% apiece (Table 1). There was
however no significant different in infection of other intestinal parasites among these food handlers ($\chi^2 = 5310$, df = 5304, $P = 0.474$).

**Table 1: Prevalence of *E. coli* pathotypes and other intestinal parasites isolated from food handlers in Nairobi**

<table>
<thead>
<tr>
<th>Pathotype</th>
<th>% Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong> = 885</td>
<td></td>
</tr>
<tr>
<td>Enteropathogenic <em>E. coli</em> (EPEC)</td>
<td>10 (1.1%)</td>
</tr>
<tr>
<td>Enterotoxigenic <em>E. coli</em> (ETEC)</td>
<td>11 (1.2%)</td>
</tr>
<tr>
<td>Enteroaggregative <em>E. coli</em> (EAEC)</td>
<td>16 (1.8%)</td>
</tr>
<tr>
<td>Enterohemorrhagic <em>E. coli</em> (EHEC)</td>
<td>2 (0.2%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>39 (4.4%)</strong></td>
</tr>
</tbody>
</table>

**Other intestinal parasites**

<table>
<thead>
<tr>
<th>Pathotype</th>
<th>% Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Entamoeba coli</em></td>
<td>7 (0.8%)</td>
</tr>
<tr>
<td>Hookworm</td>
<td>2 (0.2%)</td>
</tr>
<tr>
<td><em>Trichuris trichiura</em></td>
<td>2 (0.2%)</td>
</tr>
<tr>
<td><em>Entamoeba histolytica / dispar</em></td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>13 (1.5%)</strong></td>
</tr>
</tbody>
</table>
4.3 Distribution of *E. coli* pathotypes among the sexes

The overall prevalence rate of enteropathogenic *E. coli* among the male and females are as follows; Out of the 652 males sampled 5.2% were infected with enteropathogenic *E. coli* and out of the 5.2% infected men majority were 2.3% EAEC, followed by 1.5% ETEC, 1.1% EPEC and 0.3% EHEC (Figure 1). Similarly, of the 233 female food handlers sampled only 2.1% were found to be infected with enteropathogenic *E. coli*. These included 1.3% EPEC, 0.4%) EAEC and 0.4% ETEC (Figure 1). There was however, no significant difference among the pathotypes infecting men and female food handlers sampled ($\chi^2 = 4.187$, df = 8, $P = 0.840$).

![Figure 1: Prevalence of enteropathogenic *E. coli* by sex](image-url)
4.4 Distribution of *E. coli* pathotypes among the age groups

Out of the 885 sampled food handlers only 86.3% gave their actual ages. In the age group 18 – 23 years 3% were infected with enteropathogenic *E. coli*. These were 2% ETEC and 1% EAEC. In the age group 24 – 29 years, 4.6% were infected with 0.7% ETEC, 2.4% EAEC, 0.3% EHEC and 4.6% EPEC. In the age group 30 -35 years, 4.2% were infected with 1.2% ETEC, 2.4% EAEC and 0.6% EPEC. Among the age group 36 - 41 years, 4.9% were infected with 2.5% ETEC, 1.7% EAEC and 0.8% EPEC. Among the age group 42 - 47 years, 4.8% were infected with 1.6% ETEC and 3.2% EPEC. In the age group 48 - 53 years, 35% were infected with 5% ETEC, 10% EAEC, 5% EHEC and 15% EPEC (Table 2). There was no significant difference in the distribution of pathotypes among these age groups of sampled food handlers ($\chi^2 = 141.422$, df = 184, $P = 0.991$).
Table 2: Age distribution of *E. coli* pathotypes among the study population

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Sample size</th>
<th>Pathogenic E. coli</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EAEC ETEC EPEC EHEC</td>
<td></td>
</tr>
<tr>
<td>18 - 23</td>
<td>100</td>
<td>1 (1) 2 (2) 0 0</td>
<td>3 (3)</td>
</tr>
<tr>
<td>24 - 29</td>
<td>287</td>
<td>7(2.4) 2(1.2) 3(1) 1(0.3)</td>
<td>13 (4.6)</td>
</tr>
<tr>
<td>30 - 35</td>
<td>167</td>
<td>4(2.4) 2(1.2) 1(0.6) 0</td>
<td>7 (4.2)</td>
</tr>
<tr>
<td>36 - 41</td>
<td>121</td>
<td>2 (1.7) 3(2.5) 1(0.8) 0</td>
<td>6 (4.9)</td>
</tr>
<tr>
<td>42 - 47</td>
<td>63</td>
<td>0 1(1.6) 2(3.2) 0</td>
<td>3 (4.8)</td>
</tr>
<tr>
<td>48 - 53</td>
<td>20</td>
<td>2(10) 1(5) 3(15) 1(5)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>&gt; 53</td>
<td>6</td>
<td>0 0 0 0</td>
<td>0</td>
</tr>
</tbody>
</table>
4.5 Distribution of *E. coli* pathotypes among the sampled hotels

Food handlers were sampled from a total of nine hotels identified by concealed codes. These were 53 samples for Hotel-1; 60 samples for hotel-2; 185 samples for hotel-3; 74 samples for hotel 4; 126 samples for code 5; 53 samples for hotel 6; 82 samples for hotel-7; 45 samples for hotel 8 and 207 samples for hotel-9. In general, 14.9% of the food handlers sampled from hotel-4 had *E. coli* infections. Others include 7.1% at hotel-5; 5.7% at hotel-1; 4.9% at hotel-7; 3.8% at hotel-3; 2.2% at hotel-8 and 1.9% at hotel-9. None of the food handlers sampled from hotels-2 and-6 were found to be infected with *E. coli* infections (Table 3). The distribution of food handlers infected with pathogenic *E. coli* was not significant ($\chi^2 = 44.831$, df = 32, P = 0.066).
Table 3: Distribution of pathogenic *E. coli* among food handlers from different hotels

<table>
<thead>
<tr>
<th>Hotel type</th>
<th>Sample size</th>
<th>No. Infected</th>
<th>EAEC</th>
<th>ETEC</th>
<th>EPEC</th>
<th>EHEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hotel 1</td>
<td>53</td>
<td>3 (5.7)</td>
<td>2 (3.8)</td>
<td>1 (1.9)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hotel 2</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hotel 3</td>
<td>185</td>
<td>7 (3.8)</td>
<td>3 (1.6)</td>
<td>0</td>
<td>4 (2.2)</td>
<td>0</td>
</tr>
<tr>
<td>Hotel 4</td>
<td>74</td>
<td>11 (14.9)</td>
<td>4 (4.9)</td>
<td>3 (4.2)</td>
<td>3 (4.2)</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Hotel 5</td>
<td>126</td>
<td>9 (7.1)</td>
<td>5 (3.9)</td>
<td>2 (1.6)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Hotel 6</td>
<td>53</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hotel 7</td>
<td>82</td>
<td>4 (4.9)</td>
<td>0</td>
<td>4 (4.9)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hotel 8</td>
<td>45</td>
<td>1 (2.2)</td>
<td>0</td>
<td>1 (2.2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hotel 9</td>
<td>207</td>
<td>4 (1.9)</td>
<td>2 (1.6)</td>
<td>0</td>
<td>2 (0.9)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>885</td>
<td>39</td>
<td>16</td>
<td>11</td>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>
4.6 Prevalence of enteropathogenic *E. coli* serotypes

Among the 39 characterized enteropathogenic *E. coli* among these food handlers, 71.8% were untypable. *Escherichia coli* 0157 and *E. coli* 0128 were identified in 5.1% each. Other serotypes identified included *E. coli* 18, *E. coli* K88, *E. coli* O115, *E. coli* O119, *E. coli* O126, *E. coli* O159 and *E. coli* O63 each were identified in 2.6% *E. coli* pathotype (Table 4). Serotypes *E. coli* 0157 and *E. coli* 0128 were significantly common than the other serotypes ($\chi^2 = 1636.665$, df = 40, $P = 0.0001$).

4.7 Distribution of toxins among the *E. coli* pathotypes

All the 39 characterized enteropathogenic *E. coli* among these food handlers were carriers of different toxin types (Figure 2). All the 25.6% EPEC isolated were carriers of eaeA toxin type and were obtained from food handlers with loose stool types. Toxin types STp 15.4% and LT 12.8% were found in the ETEC, 17.9% were from formed stool type while 7.7% and 2.6% from loose and mucoid stool types respectively. Similarly 38.5% of EAEC had aggR toxin type while 2.6% had LT toxin type. 12.8% of the EAEC were isolated from formed stool type while 20.5% and 7.7% were from loose and semi-formed stool type respectively. All the 5.1% EHEC had toxin type stx2 and were from 2.6% mucoid and 2.6% loose stool type (Table 4). The toxin type aggR was significantly abundant than the other toxin types ($\chi^2 = 3333.87$, df = 20, $P = 0.001$).
Figure 2: Nitrocellulose membrane blot paper assay illustrating the enterotoxins identified from *E. coli*.
Table 4: Distribution of toxin and serotypes from the characterized pathotypes among food handlers in Nairobi (2007)

<table>
<thead>
<tr>
<th>Stool type</th>
<th>Prevalence</th>
<th>Toxin type</th>
<th>Prevalence</th>
<th>Serotypes</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathotype</td>
<td>N = 39</td>
<td>N = 39</td>
<td>N = 39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loose</td>
<td>10 (25.6)</td>
<td>eaeA</td>
<td>10 (25.6)</td>
<td>Untypable</td>
<td>7 (17.9)</td>
</tr>
<tr>
<td>EPEC</td>
<td></td>
<td></td>
<td></td>
<td>O63</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>O157</td>
<td>2 (5.1)</td>
</tr>
<tr>
<td>Formed</td>
<td>7 (17.9)</td>
<td>LT</td>
<td>5 (12.8)</td>
<td>Untypable</td>
<td>5 (12.8)</td>
</tr>
<tr>
<td>Loose</td>
<td>3 (7.7)</td>
<td>STp</td>
<td>6 (15.4)</td>
<td>Untypable</td>
<td>4 (10.3)</td>
</tr>
<tr>
<td>ETEC</td>
<td></td>
<td></td>
<td></td>
<td>K88</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>Mucoid</td>
<td>1 (2.6)</td>
<td>aggR</td>
<td>15 (38.5)</td>
<td>Untypable</td>
<td>12 (30.8)</td>
</tr>
<tr>
<td>EAEC</td>
<td></td>
<td></td>
<td></td>
<td>O128</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>O128</td>
<td>2 (5.1)</td>
</tr>
<tr>
<td>Formed</td>
<td>5 (12.8)</td>
<td>LT</td>
<td>1 (2.6)</td>
<td>O119</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Loose</td>
<td>8 (20.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-formed</td>
<td>3 (7.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EHEC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucoid</td>
<td>1 (2.6)</td>
<td>stx2</td>
<td>2 (5.1)</td>
<td>Untypable</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Loose</td>
<td>1 (2.6)</td>
<td></td>
<td></td>
<td>O159</td>
<td>1 (2.6)</td>
</tr>
</tbody>
</table>
4.8 Distribution of hydrophobicity among the food handlers

Out of the 885 food handlers stool samples assessed, 40.5% were hydrophobic while 59.5% were not (Table 5). Stool samples from 40.8% males and 39.5% females were hydrophobic. The distribution of hydrophobicity between sexes was significantly different ($\chi^2 = 6.229, \text{df} = 2, P = 0.044$). Among the 39 pathotypes identified 25.6% were hydrophobic while 74.4% were not. This was significantly different ($\chi^2 = 13.692, \text{df} = 4, P = 0.008$). Overall, out of the 30 different serotypes identified 16.7% were hydrophobic while 88.3% were not and there was no significant different among these serotypes ($\chi^2 = 12.692, \text{df} = 10, P = 0.253$). Out of the 39 toxins identified in the pathotypes from these food handlers 28.2% were hydrophobic while 71.7% were not and this distribution was significantly different ($\chi^2 = 14.742, \text{df} = 5, P = 0.012$).
Table 5: Distribution of hydrophobicity by sex, pathotypes, serotypes and toxin types

<table>
<thead>
<tr>
<th>Attribute</th>
<th>N</th>
<th>Hydrophobic</th>
<th>Non Hydrophobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>652</td>
<td>266 (40.8)</td>
<td>386 (59.2)</td>
</tr>
<tr>
<td>Female</td>
<td>233</td>
<td>92 (39.5)</td>
<td>141 (60.5)</td>
</tr>
<tr>
<td>Total</td>
<td>885</td>
<td>358 (40.5)</td>
<td>528 (59.5)</td>
</tr>
<tr>
<td>Pathotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPEC</td>
<td>10</td>
<td>0</td>
<td>10 (100)</td>
</tr>
<tr>
<td>ETEC</td>
<td>11</td>
<td>7 (63.6)</td>
<td>4 (36.4)</td>
</tr>
<tr>
<td>EAEC</td>
<td>16</td>
<td>3 (18.8)</td>
<td>13 (81.2)</td>
</tr>
<tr>
<td>EHEC</td>
<td>2</td>
<td>0</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>10 (25.6)</td>
<td>29 (74.4)</td>
</tr>
<tr>
<td>Serotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O63</td>
<td>1</td>
<td>0</td>
<td>1 (100)</td>
</tr>
<tr>
<td>O157</td>
<td>2</td>
<td>0</td>
<td>2 (100)</td>
</tr>
<tr>
<td>K88</td>
<td>1</td>
<td>0</td>
<td>1 (100)</td>
</tr>
<tr>
<td>18</td>
<td>1</td>
<td>0</td>
<td>1 (100)</td>
</tr>
<tr>
<td>O126</td>
<td>1</td>
<td>1 (100)</td>
<td>0</td>
</tr>
<tr>
<td>O128</td>
<td>2</td>
<td>0</td>
<td>2 (100)</td>
</tr>
<tr>
<td>O119</td>
<td>1</td>
<td>0</td>
<td>1 (100)</td>
</tr>
<tr>
<td>O159</td>
<td>1</td>
<td>0</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Untypable</td>
<td>20</td>
<td>4 (20)</td>
<td>16 (80)</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>5 (16.7)</td>
<td>25 (83.3)</td>
</tr>
<tr>
<td>Toxin type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eaeA</td>
<td>10</td>
<td>0</td>
<td>10 (100)</td>
</tr>
<tr>
<td>LT</td>
<td>6</td>
<td>6 (100)</td>
<td>0</td>
</tr>
<tr>
<td>STp</td>
<td>6</td>
<td>2 (33.3)</td>
<td>4 (66.7)</td>
</tr>
<tr>
<td>aggR</td>
<td>15</td>
<td>3 (20)</td>
<td>12 (80)</td>
</tr>
<tr>
<td>stx2</td>
<td>2</td>
<td>0</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>11 (28.2)</td>
<td>28 (71.7)</td>
</tr>
</tbody>
</table>
4.9 Antimicrobial sensitivity profile for enteropathogenic *E. coli* isolates

Out of the 39 characterized pathotypes, 4 (10.4%) were resistant to chloramphenicol while 35 (89.7%) were sensitive. Significantly pathotypes were more resistant to chloramphenicol ($\chi^2 = 923.781$, df = 8, $P = 0.0001$). Six (15.4%) pathotypes were resistant to amoxicillin-Clavulanic acid while 30 (76.9%) and 3 (7.7%) were sensitive and intermediate respectively and the distribution was significant ($\chi^2 = 1019.704$, df = 12, $P = 0.0001$). Six (15.4%) pathotypes were resistant to gentamicin while 36 (92.3%) and 1 (2.6%) were sensitive and intermediate respectively and the distribution was significant ($\chi^2 = 1019.704$, df = 12, $P = 0.0001$). Three (7.7%) pathotypes were resistant to ciprofloxacin while 35 (89.7%) and 1 (2.6%) were sensitive and intermediate respectively and the distribution was significant ($\chi^2 = 1019.704$, df = 12, $P = 0.0001$). Eighteen (46.2%), 20 (51.3%) and 1 (2.6%) pathotypes were resistant, sensitive and intermediate respectively to ampicillin and the distribution was significant ($\chi^2 = 944.728$, df = 12, $P = 0.0001$). Twenty (51.3%) and 19 (48.7%) pathotypes were resistant and sensitive respectively to sulphamethaxazol/trimethroprim and the distribution was significant ($\chi^2 = 957.546$, df = 8, $P = 0.0001$). Four (10.3%) and 35 (89.7%) pathotypes were resistant and sensitive respectively to cefuroxime and the distribution was significant ($\chi^2 = 990.978$, df = 8, $P = 0.0001$). One (2.6%), 35 (89.7%) and 3 (7.7%) pathotypes were resistant, sensitive and intermediate respectively to cefotaxime and the distribution was significant ($\chi^2 = 990.978$, df = 8, $P = 0.0001$). Twenty one (53.8%), 17 (43.6%) and 1 (2.6%) pathotypes were
resistant, sensitive and intermediate respectively to tetracycline (Figure 2) and the distribution was significant ($\chi^2 = 1022.674$, df = 12, P = 0.0001).

![Figure 3: Antibiotic sensitivity profile among isolated pathotypes. CHLOR- Chloramphenicol, AMC- Amoxicillin-Clavulanic acid, GENT- Gentamicin, CIP - Ciprofloxacin, AMP- Ampicillin, SXT- Sulphamethaxazol/trimethoprim, CXM- Cefuroxime, TET- Tetracycline and CXT- Cefotaxime.]

4.9.1 Distribution of resistant types among *E. coli* pathotypes

We identified different mono and multiple antibiotics resistant types (Table 6). Dual resistant types included CIP/TE, AMP/TE, SXT/TE and AMP/SXT. Those *E. coli* pathotypes that were resistant to five different antibiotics included CHLOR/AMC/AMP/SXT/TE and CHLOR/GENT/AMP/SXT/TE.
Table 6: Distribution of resistance to antibiotics by pathotypes

<table>
<thead>
<tr>
<th>Resistant type</th>
<th>Isolates</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMC, AMP, SXT</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>AMC, AMP, SXT, TE</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>AMC, CIP, TE</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>AMP, CXM, CTX</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>AMP, SXT</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>AMP, SXT, TE</td>
<td>5</td>
<td>12.8</td>
</tr>
<tr>
<td>AMP, SXT, TE</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>AMP, SXT, TE</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>AMP, TE</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>CHLOR, AMP, SXT, TE</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>CHLOR, AMC, AMP, SXT</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>CHLOR, AMC, AMP, SXT, TE</td>
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<td>2.6</td>
</tr>
<tr>
<td>CHLOR, GENT, AMP, SXT, TE</td>
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<td>2.6</td>
</tr>
<tr>
<td>CIP, AMP, SXT, TE</td>
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<td>2.6</td>
</tr>
<tr>
<td>CIP, TE</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>GENT, AMP, SXT, TE</td>
<td>1</td>
<td>2.6</td>
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<tr>
<td>SXT, CXM, TE</td>
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<td>2.6</td>
</tr>
<tr>
<td>SXT, TE</td>
<td>2</td>
<td>5.1</td>
</tr>
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4.10 Minimum inhibitory concentration for enteropathogenic *E. coli*

The MIC (mg/ml) range for all pathotypes isolated from the food handlers was 2 – 128 (Mean = 11.24, STD Dev = 29.63) against chloramphenicol and this MIC range varied significantly among the tested pathotypes ($\chi^2 = 51.235$, df = 2, $P = 0.0001$). The MIC range was 1 – 16 (Mean = 7.93, STD Dev = 5.82) against amoxicillin-clavulanic acid which varied significantly among the tested pathotypes ($\chi^2 = 18.39$, df = 4, $P = 0.001$). The MIC range was 1 – 4 (Mean = 2.05, STD Dev = 0.74) against gentamicin, which varied significantly among the tested pathotypes ($\chi^2 = 33.122$, df = 2, $P = 0.0001$). All the tested pathotypes had similar MIC values of 0.125 against ciprofloxacin. The MIC range was 2 – 64 (Mean = 7.42, STD Dev = 13.45) against ampicillin, which varied significantly among the tested pathotypes ($\chi^2 = 22.667$, df = 4, $P = 0.0001$). The MIC range was 2 – 16 (Mean = 9.67, STD Dev = 5.43) against trimethoprim, which was not significantly different among the tested pathotypes ($\chi^2 = 1$, df = 2, $P = 0.607$). The MIC range was 128 – 1024 (Mean = 554.67, STD Dev = 449.128) against sulfamethoxazole, which was not significantly different among the tested pathotypes ($\chi^2 = 0.0001$, df = 2, $P = 1$). The MIC range was 2 – 128 (Mean = 12.767, STD Dev = 21.86) against cefuroxime, which varied significantly among the tested pathotypes ($\chi^2 = 60.615$, df = 6, $P = 0.0001$). The MIC range was 1 – 64 (Mean = 18, STD Dev = 24.94) against tetracycline, which varied significantly among the tested pathotypes ($\chi^2 = 24.615$, df = 6, $P = 0.0001$). The MIC range was 2 – 128 (Mean = 20.42, STD Dev = 36.07).
against cefotaxime, which varied significantly among the tested pathotypes ($\chi^2 = 20.947, \text{df} = 4, P = 0.002$).
Table 7: Minimum Inhibitory Concentration (mg/ml) range among the *E. coli* pathotypes

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>N</th>
<th>Mean MIC</th>
<th>Std. Dev</th>
<th>MIC range</th>
<th>Trend</th>
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<td>40</td>
<td>11.24</td>
<td>29.63</td>
<td>2</td>
<td>128</td>
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<tr>
<td>AMC</td>
<td>40</td>
<td>7.93</td>
<td>5.82</td>
<td>1</td>
<td>16</td>
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<td>GENT</td>
<td>40</td>
<td>2.05</td>
<td>0.74</td>
<td>1</td>
<td>4</td>
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<tr>
<td>CIP</td>
<td>40</td>
<td>0.125</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMP</td>
<td>40</td>
<td>7.42</td>
<td>13.45</td>
<td>2</td>
<td>64</td>
</tr>
<tr>
<td>TRIM</td>
<td>40</td>
<td>9.67</td>
<td>5.43</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>SULF</td>
<td>40</td>
<td>554.67</td>
<td>449.52</td>
<td>128</td>
<td>1024</td>
</tr>
<tr>
<td>CXM</td>
<td>40</td>
<td>12.767</td>
<td>21.86</td>
<td>2</td>
<td>128</td>
</tr>
<tr>
<td>TET</td>
<td>40</td>
<td>18</td>
<td>24.94</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>CTX</td>
<td>40</td>
<td>20.42</td>
<td>36.07</td>
<td>2</td>
<td>128</td>
</tr>
</tbody>
</table>

MIC - Minimum Inhibitory Concentration, Std. Dev - Standard deviation

CHLOR- Chloramphenicol, AMP- Ampicillin, GENT- Gentamicin, CIP- Ciprofloxacin, AMC- Amoxicillin-Clavulanic acid, CXM- Cefuroxime, CTX- Cefotaxime, TET- Tetracycline, TRIM- Trimethoprim and SULF- Sulfamethoxazole
4.11 Plasmid types among the \textit{E. coli} pathotypes
All the 39 isolated \textit{E. coli} pathotypes were analysed for the presence of different plasmid types. Samples 4, 6, 8 and 9 had different plasmid of varying fragment sizes. Heavy and light fragment plasmids were identified with fragment size ranging from 6 to $>100$ and 46 Mega Dalton (MDA). The most common plasmid profile types observed was 56 MDA accounting for 6 (12.8%). The least frequent plasmid types had fragment sizes 51, 44, 17 and 58, 47 MDA which were observed only once (2.6%). Four (10.3 \%) \textit{E. coli} isolates had no plasmid detected.

4.11.1 Plasmid sizes of transconjugant \textit{E. coli} K12 F$^-$ NA$^+$ LA
All the 39 \textit{E. coli} isolates were subjected to conjugation using standard strain (\textit{E. coli} K12 F$^-$ NA$^+$ LA). Samples 1 - 12 had different plasmid of varying fragment sizes. Six (15.4\%) \textit{E. coli} isolates transconjugated with the \textit{E. coli} plasmids of fragment sizes 49, 6 MDA as shown in Figure 5. The lightest common plasmid had the fragment size ranging from 4.5, 4.9 to 6 MDA with frequency of 1. The medium sized plasmid with frequency of 4 had fragment size of 47 MDA, while the heaviest common plasmid had 56 MDA fragment size with a frequency of 8. Ten isolates were not conjugated.
Table 8: Frequency of plasmids among the *E. coli* isolates and the *E. coli* K12 F⁻ NA⁻ LA conjugates.

<table>
<thead>
<tr>
<th>Pathogenic <em>E. coli</em> plasmids</th>
<th><em>E. coli</em> K12 F⁻ NA⁻ LA</th>
</tr>
</thead>
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<tr>
<td>Size (MDA)</td>
<td>N (%)</td>
</tr>
<tr>
<td>6</td>
<td>4 (10.3)</td>
</tr>
<tr>
<td>7</td>
<td>3 (7.7)</td>
</tr>
<tr>
<td>7.2, 6</td>
<td>2 (5.1)</td>
</tr>
<tr>
<td>46</td>
<td>4 (10.3)</td>
</tr>
<tr>
<td>51, 7.2</td>
<td>2 (5.1)</td>
</tr>
<tr>
<td>51, 42, 7.2</td>
<td>3 (7.7)</td>
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<tr>
<td>51, 44, 17</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>51, &gt; 100</td>
<td>4 (10.3)</td>
</tr>
<tr>
<td>56</td>
<td>5 (12.8)</td>
</tr>
<tr>
<td>58, 47</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>&gt; 100, 41</td>
<td>3 (7.7)</td>
</tr>
<tr>
<td>&gt; 100, 46</td>
<td>3 (7.7)</td>
</tr>
<tr>
<td>None</td>
<td>4 (10.3)</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
</tr>
</tbody>
</table>

MDA - Mega Dalton
4.12 Discussions

This study has revealed that the food handlers in the nine tourist hotels in Nairobi, Kenya are carriers of *E. coli* pathotypes. None of the food handlers were co-infected with more than one *E. coli* pathotypes or had a co-infection between *E. coli* pathotypes and other identified intestinal parasites. EAEC was the most abundant pathotype while EHEC was the least common. A study done in Tanzania (Via *et al.*, 1999) found a higher prevalence of EAEC than other diarrhoegenic *E. coli*. Various studies however, recognize ETEC as the more difficult to identify among the *E. coli* pathotypes and is therefore, not often appreciated as being the major cause of infantile diarrhoea or cholera like diarrhoea and travellers diarrhoea in the developing countries (Black, 1990; Ericsson, 2003). In most studies in the developing world, ETEC isolates have been shown to be the most common bacterial enteric pathogen, accounting for approximately 20% of cases (Black *et al.*, 1981; Abu-Elyazeed *et al.*, 1999; Sooka *et al.*, 2004; Qadri *et al.*, 2005). EHEC was the least common, which is in agreement with other studies where EHEC was absent (Nataro and Kaper, 1998), or present in much lower proportions (Vila *et al.*, 1999) such as 8.8% among children sampled from Kenya (Saidi *et al.*, 1997) and 4% in Taiwanese children (Teng *et al.*, 2004).

In this study 5.2% men and 2.1% female food handlers were infected with pathogenic *E. coli* which was not significantly different. The age distribution ranged from 3% in the age group 18 – 23 years to 35% in the age group 48 – 53
years which was not significantly different. Other studies have shown males as being more prone to infestation than their female counterparts (2003; Gordon et al., 2005). The results by Gordon et al., (2005) from a similar study suggest that the morphological, physiological and dietary differences that occur among human individuals of different sex or age may influence the distribution of *E. coli* genotypes. However, this study did not focus on the above parameters as potential variants between gender and age.

From this study; no significant differences in the prevalence of pathogenic *E. coli* was observed among food handlers from nine different hotels, though high prevalence of *E. coli* pathotypes were isolated from those hotels. This high prevalence especially in hotel-4 which is ranked among the high-end hotels in Nairobi is of major concern and every effort should be made by the government to establish reliable surveillance systems that would minimize the revenue loss from the eminent stigma to be associated with travelers diarrhea transmitted from these hotels.

EAEC expressing *aggR* genes were isolated from 18.7% semi-formed, 31.3% formed and 50% loose stool types. All the EPEC and EHEC were isolated from food handlers with loose stools while 63.6% ETEC isolated from formed stool, 27% and 9.1% from loose and mucoid stool types respectively. Keskimaki et al., (2000) found a ratio of 35% and 26% EAEC in diarrhoeic and non-diarrhoeic stool
respectively. In Nigeria 1.6% EPEC isolates were isolated from non-diarrhoeic stool while 20.4% and 6.4% EHEC were from diarrhoea and non diarrhoea stools respectively (Teng et al., 2004). In Bangladesh, Finland and Tanzania 14%, 62% and 27.5% of ETEC were isolated from diarrhoeal stool samples respectively while 8%, 33% and 4% were also isolated from non-diarrhoea stools respectively from these studies (Qadri et al., 2000; Keskimaki et al., 2000). In the current study not only were pathogenic E. coli isolated from diarrhoeagenic stools but a significant number were isolated from diarrhoeal asymptomatic food handlers. This demonstrates that a clear likelihood exists that these food handlers could pass these pathotypes to tourists when they unhygienically handle foods especially salads and cold cuts. Infection transmission could also be exuberated by the continuous use of chopping boards for both meats and vegetables.

Significantly in this study, E. coli 0157 and E. coli 0128 serotypes were more commonly isolated than other serotypes which included E. coli 18, E. coli K88, E. coli O115, E. coli O119, E. coli O126, E. coli O159 and E. coli O63 each of which, were isolated from 2.6% of the food handlers. Most outbreaks of EHEC infection have been caused by EcO157:H7 strains, suggesting that this serotype is more virulent or more transmissible than other serotypes. Nevertheless, other serotypes of Stx-producing E. coli have been implicated in both sporadic disease and outbreaks, and the incidence of disease due to other serotypes is considered to be on the rise (Johnson et al., 1999). E. coli strains belonging to over 200 serotypes can express
Stx, but within most serotypes, both Stx-positive and Stx-negative strains can be found (Johnson et al., 1999). More than 50 non 0157:H7 serotypes have been associated with bloody diarrhoea or Haemolytic Uremic Syndrome in humans. The most common non-O157:H7 serotypes associated with human disease include O26:H11, O103:H2, O111 and O113:H21 (Griffin and Tauxe, 1991). The isolation of *E. coli* 0157 in Kenya has been very rare with the only existing report documented by Sang et al., (1996). This study documents the most recent presence of this EHEC O157:H7 that is an important human pathogen and is predominantly associated with hemorrhagic colitis and the more severe complication of hemolytic uremic syndrome.

Overall, all the enteropathogenic *E. coli* isolated in this study were carriers of different toxin types. EPEC had *eaeA* genes, ETEC had *STp* and *LT* genes, EAEC had *aggR* genes and LT toxin type while EHEC the *stx2* genes. Studies have shown that once diarrheagenic *E. coli* strains establish colonization, they exhibit remarkable variety in pathogenetic strategies. Three general paradigms have been described by which *E. coli* may cause diarrhea; these are entero-toxin production by ETEC and EAEC, invasion by EIEC, and/or intimate adherence with membrane signalling utilized by EPEC and EHEC (Nataro and Kaper, 1998). The versatility of the *E. coli* genome is conferred mainly by two genetic configurations: virulence-related plasmids and chromosomal pathogenicity islands.
All six categories of diarrheagenic *E. coli* have been shown to carry at least one virulence-related property upon a plasmid. EIEC, EHEC, EAEC, and EPEC strains typically harbour highly conserved plasmid families, each encoding multiple virulence factors (Nataro *et al.*, 1987; Hales *et al.*, 1994; Yamamoto and Echeverria, 1996). Of great concern therefore, is that the food handlers working in tourist hotels in Nairobi are important carriers of multi-drug resistant *aggR, eaeA, STp* and *LT* and *stx2* expressing diarrheagenic *E. coli* that could cause diarrhoea, food poisoning and a more severe disease to visiting tourists who could have acquired it through the faeco-oral route from these carriers.

*Escherichia coli* isolates from this study exhibited high drug resistant rates to both single and multiple antibiotics commonly prescribed over the counters for management of diarrhoea. Among the single antibiotics that exhibited high drug resistance included Amoxicillin-Clavulanic acid, ampicillin, sulphamethaxazol/trimethoprim and tetracycline while more than half of the pathogenic isolates were resistant to more than two different drug regimens. Most of these antibiotics are widely available and commonly used to the extent that it would easily lead to therapy failure in situations where they are administered to patients who would need them. This agrees with studies done in Nigeria, where resistant patterns to ampicillin, tetracycline and chloroamphenical were observed (Okeke *et al.*, 2000).
In Tanzania resistance rates of 83.1% ampicillin, 57% chloroamphenical, 87.7% tetracycline and 90.8% co-trimoxazole were determined (Vila et al., 1999). These figures are comparable to findings of this study. Of major concern now is that while all drugs which were more resistant are inexpensive and widely available and could have been abused. Drugs such as ciproflaxin and cefotaxime, which are reserve antibiotics in Kenya, showed considerable increasing resistance. This is a pointer to the abuse of these and other class of antibiotics that would have major implications on the treatment of diarrheagenic *E. coli* and drug policies in Kenya and other developing and developed countries.

Antibiotic resistant bacterial strains have been isolated from diverse environments and the resistance has been shown to be plasmid mediated (Borrego et al., 1991). This study supports a high possibility that some of the drug resistance conferred by diarrheagenic *E. coli* were plasmid mediated as evidenced by the ease of transfer of resistance to *E. coli* K12 by conjugation. Findings by McKeon et al., (1995) and Adam et al., (1998) showed high evidence of plasmid conferring multidrug resistance in enterobactericiae. Plasmid fragments of molecular weight of 58 mega Dalton and less were present in both the donor and the transformant diarrheagenic *E. coli* and could be implicated as the plasmids conferring multi-drug resistance in this study.
CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

i. Food handlers working in tourist hotels in Nairobi are important carriers of pathogenic multi-drug resistant-aggR expressing EAEC strains that could cause diarrhoea.

ii. This study has failed to accept the Null hypothesis.

iii. In Kenya, it is very difficult to routinely test for diarrhogenic E. coli and there is severe scarcity of relevant data in-country.

iv. Increase in the antibiotic resistant diarrhogenic E. coli could affect the choice of drugs to be used in future in the management of traveller’s diarrhoea.

v. Previously unrecognized novel diarrheagenic E. coli serotypes, which could not be serotyped in this study, may be circulating in the population.

5.2 Recommendations

i. The government and other global health development agencies should upgrade Public Health Infrastructure and build capacity for advanced molecular or appropriate techniques that will be applicable in the clinical microbiological laboratories for prompt detection of different infectious pathogens from food handlers in various geographic regions of Kenya.
ii. There is need to review the Public Health Act so as to expand the scope of laboratory parameters and redefine the medical examination requirements for food handlers to include mandatory routine detection of pathogenic *E. coli*.

iii. The isolation of multi-drug resistant EAEC, ETEC, EPEC and EHEC from non-diarrhoeal stools of (apparently healthy) food handlers is indicative that an elaborate epidemiological surveillance system for veterinary and human drugs should be put in place in all sectors of food chain for local and export markets.

iv. Enhanced surveillance programmes for behavioural communication change targeting hygienic practices among food handlers both in formal and informal hotel industry should developed and implemented by public health authorities.

v. Further research is necessary to develop rapid diagnostic kits for detection of diarrheagenic *E. coli* in the stools of food handlers.

vi. Further research needs to be conducted for genetic mapping on the serologically non-typable *E. coli* isolates from the handlers.

vii. Future studies should seek to understand the role of food handlers in transmission dynamics of pathogenic serotypes of *E. coli* in Kenya for prevention strategies.
REFERENCE


(Mombasa), India (Goa), or Jamaica (Montego Bay). *Journal of Infectious Diseases*. 185:497–502.


Qadri F, Svennerholm A, Faruque AS, Bradley G, Sack R. 2005. Enterotoxigenic Escherichia coli in Developing Countries: Epidemiology,


**Stein M, Kenny B, Stein MA, Finlay BB.** 1996. Characterization of EspC, a 110-kilodalton protein secreted by enteropathogenic *Escherichia coli* which is homologous to members of the immunoglobulin A protease like family of secreted proteins. *Journal of Bacteriology.* 178:6546–6554.


**APPENDICES**

Appendix 1: 20 NE READING TABLE

<table>
<thead>
<tr>
<th>TESTS</th>
<th>SUBSTRATES</th>
<th>REACTIONS/ENYMES</th>
<th>RESULTS</th>
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<th>POSITIVE</th>
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<td>Potassium nitrate</td>
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<td>pink - red</td>
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<td></td>
<td></td>
<td>Reduction of nitrates to nitrogen</td>
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<td>colorless</td>
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<td>OX / 1 – 2 min</td>
<td>OX / 1 – 2 min</td>
</tr>
</tbody>
</table>
Appendix 2: Reagent preparation for plasmid extraction and characterization

1. LB broth

   Bacto Tryptone          10 g
   Bacto yeast extracts    5 g
   Sodium Chloride         10 g
   Distilled water         1000 ml

   The media was distributed into 4 ml portions in large test tubes and sterilized by autoclaving at 121°C for 15 minutes.

1. 10 M NaOH was made by dissolving 80 g sodium hydroxide pellets in 200 ml distilled water.

2. 20% Sodium Dodecyl Sulphate (SDS) was prepared by dissolving 40 g SDS in 160 ml distilled water.

3. 0.5M EDTA, pH 8.0 was prepared by adding 37.22 g Na₂EDTA·2H₂O to 160 ml distilled water. pH was adjusted to 8.0 with 4 g sodium hydroxide pellets and made to 200 ml. This was sterilized by autoclaving at 121°C for 15 minutes.

4. 20% Glucose was prepared by dissolving 40 g anhydrous dextrose in 200 ml distilled water and which was then sterilized by filtration through a 0.22 μm disposable filter. This was then stored at 4°C until use.
5. 1 M Tris-HCl, pH 8.0 was prepared by dissolving 121.1g Tris base in 800 ml distilled water. pH was adjusted to 8.0 with HCl and made up to 1000ml with distilled water. This was sterilized by autoclaving.

6. 3 M Sodium acetate, pH 5.2 (B-III) was prepared by dissolving 204.12 anhydrous sodium acetate in 400 ml distilled water. pH was adjusted to 5.2 with 100 ml acetic acid and made to 500ml with distilled water and sterilized by autoclaving.

7. 7.5M Ammonium acetate was prepared by dissolving 289.05 ammonium acetate in 500ml distilled water and sterilised by autoclaving.

8. 20 X TE buffer (1X is 10mM Tris, 1mM EDTA) was made by dissolving 24.11 g Tris base and 7.45g Na₂EDTA.2H₂O in 800 ml distilled water. The pH was adjusted to 8.0 with HCl and made to 1000 ml with distilled water.

9. Stop mix (0.07% BPB, 7% SDS and 20% Ficoll) was prepared by dissolving 70mg bromophenol blue, 7g SDS and 20g Ficoll type 400 in 80 ml distilled water. This was warmed to dissolve the content, mixed and made to 100 ml with distilled water.

10. 5 X TBE electrophoresis buffer (used as 1:10 solution in the bath) (1X is 89 mM Tris, 89 mM boric acid and 2.8 mM EDTA) was prepared by dissolving 53.89 g Tris base, 27.51g boric acid and 5.21g Na₂EDTA.2H₂O in 1000ml distilled water.

11. 10 mg/ml ethidium bromide was prepared by dissolving 0.5 g ethidium bromide in 50 ml distilled water in a pre-sterilized bottle. The mix was stored at 4°C.
until ready for use. Ethanol (80%) was prepared by mixing 160ml ethanol with 40 ml sterilized distilled water in a pre-sterilized bottle.
### Solution I (B-I)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>20 mg</td>
<td>2 mg/ml</td>
</tr>
<tr>
<td>1M Tris-HCl, pH 8.0</td>
<td>0.25 ml</td>
<td>25 mM</td>
</tr>
<tr>
<td>0.5M EDTA, pH 8.0</td>
<td>0.20 ml</td>
<td>10 mM</td>
</tr>
<tr>
<td>20% Glucose</td>
<td>0.45 ml</td>
<td>50 mM</td>
</tr>
<tr>
<td>Distilled water</td>
<td>9.10 ml</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>10.00 ml</td>
<td></td>
</tr>
</tbody>
</table>

### Solution II (B-II)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 M NaOH</td>
<td>0.4 ml</td>
<td>0.1 N</td>
</tr>
<tr>
<td>20% SDS</td>
<td>1.0 ml</td>
<td>1.0%</td>
</tr>
<tr>
<td>Distilled water</td>
<td>18.6 ml</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>20.00 ml</td>
<td></td>
</tr>
</tbody>
</table>
Appendix 3: Reagent preparation for colony hybridisation test

1. LB Broth

**Bacto Tryptone** 10 g
- Bacto Yeast extracts 5 g
- Sodium Chloride 10 g
- Distilled Water 1000 ml

The media was distributed into 4 ml portions in large test tubes and sterilized by autoclaving at 121°C for 15 minute.

1. 0.5 N NaOH was prepared by dissolving 10 g sodium hydroxide pallets and 5 g Sodium Dodecyl Sulphate (SDS) in 500 ml deionised water.

2. SSC was prepared by dissolving 175 g sodium chlorides and 88 g sodium citrate dehydrates in 1 litre-deionised water.

3. 0.5% Triton X-100 was prepared by taking 0.5 ml into 9.5 ml of Triton X-100, fill up to 100 ml with deionised water.

4. Tris HCL PH 7.4 was prepared by dissolving 121.1 g Tris base in 800 ml and at adjusting PH to 7.4 with HCL.

5. Hybridisation buffer was prepared by dissolving 2.5 g BSA 2.5 g polyvinyl pyrrocidone and 5 g SDS in 500 ml distilled water.

6. Alkaline Phosphates buffer was prepared by dissolving 6.1 g Tris base 6.8 mg Zinc Chloride and 0.25 g Sodium azide in 400 ml distilled water and adjusting the PH to 8.5 with HCL.
7. Nitro blue tetrazolum solution was prepared by dissolving 100 mg NBT in 1.33ml 75% N-dimethyl formamide.

8. BCIP (5-bromo-4-chloro-3-idolyl phosphate tohidine salt) solution was prepared by dissolving 100 mg BCIP in 2 ml N, N-dimethyl formamide.

9. Alkaline phosphate linked oligonucleotide probe was prepared by adding oligonucleotide probe.
Appendix 4: Protocols for media preparation

**Sulphur-indole motility decarboxylase medium (sim oxoid cm 435)**

SIM Medium, Dehydrated 30 g

Distilled water 1000 ml

Boil to dissolve completely. Dispense in 4ml aliquots into 13 x 100 mm screw cap tubes. Sterilize by autoclaving at 121°C for 15 min and store at 4°C until use.

**Triple sugar iron (TSI) agar (oxoid cm 277)**

TSI Agar, dehydrated 65 g

Distilled water 1000 ml

Boil to dissolve completely. Dispense into test tubes and autoclave at 121°C for 15 min. Slant tubes to form deep butts and moderate slants. Allow to harden completely and store at 4°C until use.

**CONTROLS**

*Escherichia coli* ATCC 25922

*Proteus vulgaris* ATCC 13315

*Salmonella enteritidis* ATCC 13076
Tryptone soya broth glycerol medium (15%)

Tryptone soya broth base (OXOID CM 129) 30 g
Glycerol (BDH 284546F) 150 ml
Distilled water 850 ml

Dispense in 1ml amounts in cryovial and autoclave at 121°C for 15mins. Cool and store at 4°C until use.

Urea agar (oxoid cm 53)

Urea agar base, dehydrated 2.4 g
Distilled water 95 ml

Boil completely to dissolve, sterilize by autoclaving at 121°C for 15 min. Cool to 50-55°C. Aseptically add 5ml sterile 40% urea solution SR 20 (OXOID). Mix well and dispense into 2ml aliquots in sterile containers and allow setting in slope position. Store at 4°C.

CONTROLS

Positive control- *Proteus vulgaris* ATCC 13315
Negative control- *Escherichia coli* ATCC 25922

Blood agar (oxoid cm 331)

Blood agar base, dehydrated 40 g

Distilled water 1000 ml

Sterile whole blood, (horse, sheep, human and rabbit)
Boil to dissolve completely. Sterilize by autoclaving at $121^\circ C$ for 15 minutes. Cool to $45-50^\circ C$ and add 7% sterile blood. Mix in gentle rotation and pour onto sterile petri dishes.

**CONTROLS**

**Positive control** - *Staphylococcus aureus* ATCC 25923

**Negative control** - Uninoculated media.

**MacConkey agar** *Tween 80* (Oxoid cm 7)

MacConkey agar dehydrated 52 g

Distilled water 1000 ml

Boil carefully to dissolve completely. Autoclave at $121^\circ C$ for 15 minutes. Cool to $50^\circ C$ and *Tween 80* aseptically, dispense into sterile petri dishes. Allow to solidify completely and store at 2-8$^\circ C$ until use.

**Positive control** - *Escherichia coli* ATCC 25922

**Negative control** - *Enterococcus faecalis* ATCC 29212

**McFarland standard no 0.5**

Add 0.5 ml of a 1.175% solution of barium chloride dihydrate (BACL₂₂H₂O) to 99.5 ml of 0.36 N (1%) sulphuric acid. Dispense 5 ml aliquots into screw cap bijou bottles and seal with cap. The turbidity standard can be stored in the dark room temperature for 6 months or more, provided the bottle is sealed to prevent
evaporation. The standard must be thoroughly mixed just before use, preferably on a vortex mixer.

**MRVP medium (oxoid cm43)**

MRVP dehydrated 15 g  
Distilled water 1000 ml  
Suspend the medium in distilled water. Mix well and distribute into final containers and sterilize by autoclaving at 121°C for 15 minutes. Store at 4°C until required.

**CONTROLS**

<table>
<thead>
<tr>
<th>MR POSITIVE</th>
<th>Escherichia coli</th>
<th>ATCC 25922</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR NEGATIVE</td>
<td>Klebsiella pneumoniae</td>
<td>ATCC 13883</td>
</tr>
<tr>
<td>VP POSITIVE</td>
<td>Enterobacter cloaceae</td>
<td>ATCC 23355</td>
</tr>
<tr>
<td>VP NEGATIVE</td>
<td>Escherichia coli</td>
<td>ATCC 25922</td>
</tr>
</tbody>
</table>

**Mueller-Hinton agar (oxoid cm337)**

Mueller-Hinton dehydrated medium 38 g  
Distilled water 1000 ml  
Suspend in distilled water. Boil to dissolve. Autoclave at 121°C for 15 minutes and allow to cool up to 50°C. Pour into sterile petridishes. Store at 4°C until required.
CONTROL

POSITIVE  *Escherichia coli*  *ATCC 25922*

POSITIVE  *Staphylococcus aureus*  *ATCC 25923*

NEGATIVE  *Uninoculated media*

**Simmons citrate agar (oxoid cm 155)**

Simmons Citrate agar  23 g
Distilled water  1000 ml

Boil carefully to dissolve completely. Aliquot 2 ml into 13 mm x 100 mm tubes. Sterilize by autoclaving at 121°C for 15 minutes, set to cool in a slanted position.

Store at 4°C.

Positive Control  *Citrobacter freundii*

Negative Control  *E. coli*  *ATCC 25922*

**Alkaline peptone water**

Bactopeptone medium dehydrated  10 g
Distilled water  1000 ml

Suspend the medium in distilled water. Adjust pH with sodium hydroxide. Mix well, distribute into final containers and sterilize by autoclaving at 121°C for 15 minutes. Store at 4°C until required.
Positive Control \( \textit{Vibrio cholerae} \text{ ATCC 14035} \)

Negative Control \( \textit{E. coli} \text{ ATCC 25922} \)

**Thiosulphate citrate bile salt sucrose (biotec)**

TCBS Agar, dehydrated \( 88 \text{ g} \)

Distilled water \( 1000 \text{ ml} \)

Suspend 88 g in 1000ml of distilled water and bring to the boil to dissolve the agar.

Cool to 47\(^\circ\)C and pour into petri dishes. Keep at 4\(^\circ\)C until needed for use.

Positive Control \( \textit{Vibrio cholerae} \text{ ATCC 14035} \)

Negative Control \( \textit{E. coli} \text{ ATCC 25922} \)