ANTIMICROBIAL SUSCEPTIBILITY PROFILES AND GENOTYPIC CHARACTERIZATION OF SELECTED *ENTEROBACTERIACEAE* STRAINS ISOLATED FROM FOOD HANDLERS IN NAIROBI, KENYA

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Antimicrobial susceptibility profiles and genotypic characterization of selected *Enterobacteriaceae* strains isolated from food handlers in Nairobi, Kenya

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DECLARATION

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

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This thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION

To my Family

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LIST OF ABBREVIATIONS AND ACRONYMS

AMP	Ampicillin
AMC	Amoxicillin/Clavulanic acid
AT CC	American Type Culture Collection
bla	beta-lactamase gene
CAZ	Ceftazidime
CIP	Ciprofloxacin
CN	Gentamicin
CTX	Cefotaxime
C	Chloramphenicol
CTX-M	CefoTaXimases 'Munich'
CMR	Center for microbiological research
CDC	Centre for Disease Control
CI	Confidence Interval
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic acid
ESBL	Extended spectrum beta-lactamases
E. coli	*Escherichia coli*

EHEC	Enterohemorrhagic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
F AO	Food and Agricultural Organization
FOX	Cefoxitin
FEP	Cefepime
KEMRI	Kenya Medical Research Institute
kb	Kilobase
MDR	Multi-drug resistance
MHA	Mueller-Hinton agar
NA	Nalidixic acid
O.R	Odds Ratio
OXA	Oxacillinase Enzymes
PCR	Polymerase Chain reaction
SERU	Scientific Ethical Review Unit
S	Streptomycin
ST	Sequence Type
SXT	Sulfamethoxazole/Trimethoprim
spp.	Species

SHV	Sulfhydrl Variable Enzymes
TBE	Tris-borate-EDTA
TEM	Temoneira Enzymes
UTIs	Urinary tract infections
UNICEF	United Nations Children's Fund
WHO	World Health Organization

ABSTRACT

Increased urbanization, industrialization as well as population growth in cities has led to an increase in consumption of food in public eating-places. Over 40% of Nairobi residents consume foods made outside their homes, and this raises possibilities of food contamination and dissemination of multi-drug resistant (MDRs) strains which confer resistance to multiple antimicrobial drugs such as β-lactams which are a unique class of antibiotics used for treatment of various infections. A cross-sectional study was conducted among 323 food-handlers in Nairobi and their socio-demographic profiles obtained using a structured questionnaire. Susceptibility profiling and phylogenic relatedness of isolates obtained from the participants of different socio-demographic characteristics were analyzed using Chi-square, culture and molecular strategies. Ethical clearance was sought from KEMRI/ IRB and a written informed consent obtained from all participants. There was near parity in the proportions of males (51%) and females (49%) recruited. Majority of the participants (75%) were between 18-30 years of age and working in middle class hotels. High resistances were recorded against Sulfamethoxazole/Trimethoprim (70%), Ampicillin (44.6%), Streptomycin (42%) and Tetracyclines (41%) while the Imipenem and Cefepime were effective against 99% of the isolates. A third 97(30%) of all isolates were multi-drug resistant (MDR), 11(3.4%) were ESBLs and 44.6% exhibited resistance to narrow spectrum β-lactams (NSBLs). The bla_{TEM} gene was the most prevalent at 37%, bla_{SHV} at 25%, $bla_{\text{CTX-M}}$ at 12% and bla_{OXA} gene at 7%. The inhibitor resistant TEM phenotype, Complex mutant TEMs phenotypes and the plasmid-mediated ampicillin β-lactamases phenotypes accounted for <2%. In conclusion, the observed resistances pose a major therapeutic challenge since the isolates were obtained from apparently healthy food handlers who are capable of passing them to the general public. The study recommends proper and safe food handling practices among food handlers and the launch of surveillance studies on β-lactamase-producers in developing countries.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

A food-handler is anyone who works in a food business and who either handles food directly or indirectly (Davies *et al*., 2014). Foodborne diseases are diseases commonly transmitted through food consumption (WHO, 2009). Illness resulting from consumption of contaminated or poorly prepared food from hotels and restaurants is a serious public health problem worldwide (Lam *et al*., 2013). Increased incidences of foodborne illnesses have renewed interest in the hygiene and cleanliness of public eating places (Rane, 2011; Omemu & Aderoju, 2008).

Gastrointestinal tract infections in humans occur through ingestion of contaminated water or animal food products, most often eggs, poultry and raw meat (Karshima *et al*., 2013: Smith *et al*., 2012). Following ingestion of the organisms, the likelihood of infection developing as well as the severity of infection is directly related to the dose and virulence of the organism in question or its strain and the status of host's defense mechanisms (Moro *et al*., 2014; Payment & Riley, 2002). Biological contaminants such as bacteria, viruses, fungi, protozoa and helminths are the major causes of food poisoning which range from mild to chronic, sometimes life-threatening conditions such as cholera, campylobacteriosis, *Escherichia coli* gastroenteritis, Salmonellosis, Shigellosis, Typhoid fever, brucellosis and amoebiasis (Weaver *et al*., 2010).

Many foodborne disease incidents are reported every year in Africa with numerous factors contributing to high incidents rates (Havelaar *et al.,* 2015). Although typhoid fever is a major global public health problem, data on the relative risk of contracting travel-associated enteric fever is grossly inadequate in developing countries due to poor surveillance mechanisms (Crump *et al*., 2004). Vending of street foods, particularly in

urban areas, is a growing global phenomenon since they serve as sources of daily meals for massive urban populations (Khairuzzaman *et al*., 2014).

Food poisoning and food safety were declared a major public health concern by international health agencies due to their impact on health (Moro *et al*., 2016; WHO, 2006). Increase in urbanization, industrialization as well as population growth in cities has led to a sharp increase in consumption of food in collective eating-places thereby increasing the importance of these eateries in the urban settings (Khairuzzaman *et al*., 2014).

Dissemination of multi-drug resistant (MDRs) strains that are resistant to β-lactam antibiotics by foodhandlers to the general public attracts a special interest since β-lactam antibiotics are heavily relied upon in the treatment of various bacterial infections in both hospital and community settings, hence may result in therapeutic drawbacks. Continued increase in antimicrobial resistance especially from members of the *Enterobacteriaceae* family has led to drastic deteroriation in the management of infectious diseases that no longer respond to conventional prescribed antimicrobial drugs. Moreover, rising incidences of ESBLs among the *Enterobacteriaceae* family conferring resistance to β-lactams is of increasing concern in Kenya (Kiiru *et al*., 2012).

Screening food-handlers and implementing public awareness programs has been recommended as an intervention to control transmission of enteric pathogens (Onyango *et al*., 2009). Therefore, food-handlers play a significant role in public health since this group of individuals alone influences the life and health of thousands of people daily especially if the foods are not prepared, preserved or served hygienically. Several factors such as mishandling of food and the disregard of hygienic practices and environmental sanitation enable pathogens to contaminate food and, in some cases, survive and multiply to cause illnesses in clients who patronize these eateries (Käferstein, 2003).

1.2 Statement of the Problem

Infectious foodborne diseases present a serious challenge to public health in both developed and developing countries like Kenya (Oundo *et al*., 2008). Despite the government effort to screen food-handlers for transmissible infections, possibility of outbreaks emanating from sick foodhandlers remains high. Limited studies have been carried out to determine the potential of food-handlers as sources of infections to the general public. There is a concern that foodhandlers may act as focal points for disease outbreaks and such outbreaks may be caused by highly resistant strains. Moreover, food-handlers may be potential carriers for ESBL-producers making it difficult to treat infections arising from such strains with β-lactam antibiotics (Kiiru *et al*., 2012).

The patterns of antimicrobial use among foodhandlers is rarely investigated yet misuse of antimicrobials in this group can pose a risk to their customers who may get a foodborne pathogen that has already acquired high-grade resistance to various classes of antimicrobials. The resistance profiles of strains encountered among food handlers in Kenya is poorly investigated and the prevalence of ESBL-producers remains unknown. This absence of data from properly formulated studies impedes the preparedness of public health experts to deal with outbreaks emanating from food handlers.

1.3 Justification

This study sought to fill the missing knowledge gaps regarding factors that may contribute to carriage of highly resistant strains using *E. coli* as a model organism. The data generated identified various risk factors associated with carriage of MDR strains especially those that are ESBL-producers. The study also investigated and determined the genetic basis of resistance to various important classes of antimicrobials and also determined phylogenetic relatedness of the strains obtained.

This data will not only inform policy makers on strategies to reduce cross coinfections between the food handler and their patrons but will also help public health officials

identify some risk factors for carriage of MDR strains. The data also revealed that some strains may be spreading between and among foodhandlers and this could further suggest that similar clonal expansions may be observed for other enteric pathogens such as *Salmonella* and *Shigella.*

1.4 Research questions

In order to formulate succinct objectives for this study, the following research questions were set.

1. What are the antimicrobial susceptibility profiles of *E. coli, Salmonella* and *Shigella* strains isolated from food-handlers in Nairobi County?
2. What proportion of *E. coli, Salmonella* and *Shigella* isolates obtained from food-handlers in Nairobi County are ESBL producers?
3. What is the prevalence of selected ESBL genes-*TEM, OXA, SHV* and *CTX-M* in *E. coli, Salmonella* and *Shigella* isolates obtained from food-handlers in Nairobi County?
4. What are the socio-demographic factors that are associated with carriage of resistant *E. coli, Salmonella* and *Shigella* strains among food-handlers in Nairobi County?
5. Is there genetic relatedness of isolates obtained from food-handlers in Nairobi County?

1.5 General objective

To determine the antimicrobial susceptibility profiles, molecular basis of resistance, genetic relatedness and factors associated with carriage of selected resistant *Enterobacteriaceae* strains isolated from food-handlers in Nairobi County.

1.5.1 Specific objectives

1. To determine the antimicrobial susceptibility profiles of *E. coli, Salmonella* and *Shigella* isolates obtained from food-handlers in Nairobi County.
2. To determine the proportion of isolates exhibiting the ESBL phenotype among food-handlers in Nairobi County.
3. To determine the presence of selected ESBL genes (*bla*$_{TEM}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{CTX\text{-}M}$) in *E. coli, Salmonella* and *Shigella* isolates from food-handlers in Nairobi County.
4. To determine the socio-demographic factors associated with carriage of antimicrobial resistant *E. coli, Salmonella* and *Shigella* strains among food-handlers in Nairobi County.
5. To determine the genetic relatedness of isolates obtained from food-handlers in Nairobi County.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Enterobacteriaceae* and enteric bacterial infections

The *Enterobacteriaceae* is a large family of Gram-negative bacteria that includes harmless commensals and symbionts as well as familiar pathogens, such as *Salmonella, Escherichia coli, Yersinia pestis, Klebsiella* and *Shigella.* Other disease-causing bacteria in this family include *Proteus, Enterobacter, Serratia,* and *Citrobacter* among others (Elikwu *et al.,* 2015). Members of *Enterobacteriaceae* family are incriminated in most of the infectious diseases incidences worldwide with most of them causing acute gastroenteritis (Singh, 2010).

The most common pathogenic enteric strains include *E. coli, Salmonella* and *Shigella*. For example*, Shigella dysenteriae* causes bacillary dysentery while *E. coli and Salmonella enterica,* causes food poisoning and acute gastroenteritis. Strains in this family are the major cause of high mortality associated with devastating outbreaks of diarrhoea (Todar, 2012). According to the World Health Organization, more than 16 million deaths are due to infectious diseases, out of these deaths, enteric bacterial diarrhoea accounts for over 3 million deaths annually (WHO, 2009). Diarrhoea is defined as the passage of three or more loose or watery stools per day (WHO, 2009). Diarrhoea associated with bacterial, viral or parasitic infections is the most common infectious illness experienced by millions of children under five years in developing countries like Kenya, and also the major cause of disease in international travelers (Mosquito *et al*., 2011; Roy *et al*., 2010;WHO, 2009).

Globally, diarrhoea caused by members of the *Enterobacteriaceae* family is the second highest cause of mortality in children under five years of age (WHO/UNICEF, 2016). According to WHO Global Burden of Disease estimates, diarrhea accounts for nearly 3

million deaths per year in developing countries. Examples of diarrheal diseases associated with bacteria are cholera, dysentery, typhoid and diarrhoea due to Enterotoxigenic *Escherichia coli* (ETEC) (Bryce *et al*., 2005).

2.2 Transmission of enteric pathogens

Enteric disease has been and continues to be a major cause of morbidity and mortality worldwide. In both the developed and developing world, food and water contamination are the principal modes of transmission in which enteric pathogens are often implicated (Eisenberg *et al*., 2012). Transmission of enterics and their subsequent cause of diarrhoea illnesses in developing countries is attributed to unsafe water, food, inadequate sanitation such as lack of (clean) toilets and poor personal hygiene practices (Fewtrell *et al*., 2005). Moreover, gastrointestinal pathogens are also transmitted primarily by the fecal-oral route, although contaminated surfaces, hands or food may also serve to transmit infection in some cases. *Musca domestica* (common housefly) is considered as a nuisance pest and a potential vector for the transmission of variety of pathogenic microorganisms including enterics (Dennehy, 2000).

2.3 Diversity of *Escherichia coli, Salmonella* and *Shigella* strains

Escherichia coli are the most abundant facultative anaerobic bacteria of the human intestinal flora. Whereas, *E. coli* usually appears to be harmless commensals, in other circumstances, *E. coli* strains can be pathogenic, and it's the most common cause of urinary tract infections (UTIs). Several pathotypes of *E. coli* exist with each associated with an array of illnesses depending on the site of colonization (Kotloff *et al*., 2013).

Phylogenetically *E.coli* strains are categorized into group A, B1, B2 and D. The most virulent extra intestinal pathogenic *E. coli* strains belong to group B2 and Group D. Generally, isolates designated as group A or B1 are less likely to cause infections (Carlos *et al*., 2010; Boerlin, 2005). The dissemination of fluoroquinolone-resistant subclone of *E. coli* sequence type 131 (ST131) is one of the most dramatically expanded

antimicrobial-resistant *E. coli* lineage (Nicolas *et al*., 2014). Several studies have used *E.coli* as a model organism for antimicrobial resistance due to its umbiquitos distribution and possible association with diarrhea. *E. coli* remains an important study organism to monitor transmission routes of infections pathogens acquired through the fecal-oral route.

The genus *Salmonella* comprises of two major species, *Salmonella bongori* and *Salmonella enterica,* with the latter further subdivided into six subspecies: *S. e. enterica, S. e. salamae, S. e. arizonae, S. e. diarizonae, S. e. houtenae,* and *S. e. indica* (Agbaje *et al*., 2011). This taxonomic group contains over 2500 serotypes (serovars) defined by the somatic O (lipopolysaccharide) and flagellar H antigens as per the Kauffman–White classification (Grimont & Weill, 2007). *Salmonella enterica* subspecies are found worldwide in all warm-blooded animals and in the environment whereas, *Salmonella bongori* is restricted to cold-blooded animals, particularly reptiles (Eng *et al*., 2015). These strains of *Salmonella* are responsible for a variety of illnesses such as typhoid fever and paratyphoid fever. Moreover, infections with nontyphoidal serotypes of *Salmonella* generally results in food poisoning (Eng *et al*., 2015). Most cases of invasive nontyphoidal salmonella infection (iNTS) are caused by *S. typhimurium* or *S. enteritidis* (Tennant *et al.,* 2016).

Shigella organisms are also members of the family *Enterobacteriaceae* and are generally grouped into four species: *Shigella dysenteriae, Shigella flexneri, Shigella boydii* and *Shigella sonnei,* also known as groups A, B, C, and D, respectively (Taneja & Mewara, 2016). *Shigella* is one of the leading bacterial causes of diarrhea worldwide causing moderate-to-severe diarrhea in both developed and developing countries. *Shigella* infection is typically by ingestion of contaminated food, water, formites or hands (Fletcher *et al*., 2013).

Salmonella, E.coli and *Shigella* strains pose increasing clinical challenge in both community and hospital settings due to emerging antimicrobial resistance, most notably to fluoroquinolones and extended-spectrum cephalosporins, with the latter mediated mainly by extended-spectrum beta-lactamases (ESBLs), such as *CTX-M-*$_{15}$ (Talan *et al*., 2016; Croxen, 2010; Johnson *et al*., 2010).

2.4 Global epidemiology of enteric bacterial diseases

Historically, pathogenic bacteria have been the most common food safety hazard worldwide (Newell *et al*., 2010). These pathogens cause disease of varying severity, some of which may result in mortality. Microbial sources account for up to 95% of all reported foodborne disease outbreaks (Gould *et al.,* 2013). Members of the *Enterobacteriaceae*, particularly *Salmonella* serovars, enteropathogenic *E. coli* and *Shigella* species and members of the Campylobacteraceae are responsible for the majority of foodborne bacterial illnesses (Johnson, 2003).

Shigella infection due to contaminated foods is the third most common cause of bacterial gastroenteritis in the United States, after *Campylobacter*, *Salmonella* and *E. coli* O157 infections. The global burden of shigellosis has been estimated at 165 million cases per year, of which 163 million are in developing countries giving rise to more than one million deaths yearly due to *Shigella* infection (Lee *et al*., 1991). In developing countries, *S. flexneri* is the leading cause of shigellosis whereas *S. dysenteriae type* 1 is a frequent cause of epidemic throughout the world (Yang *et al*., 2005). Bacillary dysentery due to *Shigella dysenteriae type* 1 contributes to a substantial degree of bloody diarrheal illness based on reports from Kenya, Egypt, Ethiopia and Nicaragua (John *et al.,* 2003; Vilchez *et al*., 2009). Enterotoxigenic *Escherichia coli* (ETEC) is responsible for more than 650 million diarrheal episodes and two million deaths annually in children living in developing countries (Vilchez *et al*., 2009; Wenneras & Erling, 2004).

Salmonella enterica subspecies *enterica* includes over 2,400 serotypes found in humans and other warm-blooded animals. Non-Typhi *Salmonella* (NTS) is among the most common pathogens causing bacterial bloodstream infections in adults and children in sub-Saharan Africa (Shaw *et al.,* 2008). Estimates of the burden of invasive *Salmonella* infections in sub-Saharan Africa are limited by the scarcity of regional data. The global burden of typhoid fever was estimated at 26.9 million cases in 2010 (Buckle *et al*., 2012). In 2000, typhoid fever-related deaths were estimated to be 210,000 from about 22 million cases reported with only Egypt and South Africa contributing to this estimate for the African continent (Crump *et al*., 2004).

Within Africa, surveillance from Kenya, Tanzania, Malawi, and South Africa has shown marked regional variation in the incidence and age-specific patterns of typhoid fever (Breiman *et al*., 2012; Feasey *et al*., 2012). Invasive salmonelloses are a major cause of morbidity and mortality in Africa, but the incidence and case fatality of each disease vary markedly by region (Muthumbi *et al*., 2015). In Kenya, invasive nontyphoidal *Salmonella* (iNTS) disease causes severe bacteremic illness among adults with human immunodeficiency virus (HIV) and especially among children <5 years of age coinfected with HIV or malaria, or who are compromised by sickle cell disease or severe malnutrition. The incidence of iNTS disease in children ranges from 166 to 568 cases per 100 000 persons per year (Kariuki & Onsare, 2015). Comorbidity with malaria has also been closely associated with increased incidence of iNTS disease in studies in Kenya (Brent *et al*., 2006; Berkley *et al*., 2009) and elsewhere in Africa including Malawi (Bronzan *et al*., 2007) , The Gambia, and Democratic Republic of Congo (Phoba *et al*., 2012).

2.5 Disease burden, risk factors, and implications on management of enteric diarrheal diseases in Sub-Saharan Africa

In sub-Saharan Africa including Kenya, increase in urbanisation has led to rapid expansion of cities and creation of overcrowding in urban slums characterised by low socio-economic conditions and poor sanitation especially poor waste disposal mechanisms which in turn have raised the occurrence of infectious diseases including diarrhoea caused by enteric pathogens (Patel *et al.,* 2009).

Diarrhoea is one of the most common causes of morbidity and mortality among children in sub-Saharan Africa, and one of the main causes of hospital admissions in Kenya (Sang *et al*., 2012). The challenge with diarrhea is that most causative pathogens are transmissible and are therefore likely to be transferred from foodhandlers to their unsuspecting clients. Food safety is a major issue that is drawing increasing concern in Kenya (Hodges & Gill 2010). Unfortunately, the current food safety system in Kenya faces increasing challenges due to ineffective enforcement of laws required to reduce the number of foodborne illnesses and the contradictions in food regulations and inspection procedures (Nyamari, 2013).

Few studies have been carried out in Kenya to estimate the health impact of foodborne diseases. The 2004 reports by the Ministry of Health showed that among the ten leading causes of outpatient visits to health institutions were all forms of diarrheal diseases and intestinal parasites which were related to food directly or indirectly (Gieseker, 2004).

2.6 Food handlers as potential transmitters of pathogens

Food-handlers are the most important sources for the transfer of microbial pathogens to food either from their hair, skin, hand, digestive systems, respiratory tracts, or from contaminated food prepared and served by them (Gun & Satu, 2007). Improper food handling practices increase the chances of food contamination and consequently foodborne diseases which pose a potential risk to public health (Campos *et al*., 2009).

Also, food-handlers may harbour microorganisms either during their course of gastrointestinal illness or after convalescence, when they no longer have symptoms. Several studies have indicated that various bacteria, amongst others *Staphylococcus, Escherichia coli* and *Salmonella* species can survive on hands and surfaces for hours or even days after initial contact with the microorganisms (Kusumaningrum *et al*., 2002).

Foodborne diseases represent a persistent global health burden, and food-handlers play a vital role in their transmission. Even though the sources of food contamination are diverse, food handlers serve as an important source of food contamination either as carriers of pathogens or through poor hygienic practices. Mishandling of food and the disregard of hygienic practices enable pathogens to contaminate food and, in some cases, survive and multiply to cause illnesses in clients. Poor personal hygiene and environmental sanitation are among the key factors in the transmission of foodborne diseases (Käferstein, 2003).

The spread of infections via food-handlers is a common and persistent problem worldwide with poor personal hygiene among food-handlers being the most commonly reported food preparation malpractice contributing to these illnesses (Andargie *et al*., 2008; Lillquist *et al*., 2005). Studies conducted in different food establishments in Ethiopia showed that poor sanitary conditions of catering institutions and poor personal hygiene of food-handlers are the primary sources of foodborne illnesses caused by pathogenic organisms such as *Campylobacter, Salmonella, Staphylococcus, Bacillus cereus* and *E. coli* (Aklilu *et al*., 2015).

A study on pathogenic *E. coli* among food-handlers working in luxurious hotels in Nairobi, Kenya indicated that food-handlers harboured strains of resistant *E. coli* pathotypes that could potentially cause an outbreak if not treated in advance (Onyango *et al*., 2009). Due to its umbiquitos distribution and possible association with diarrhea, *E. coli* remains an important study organism to monitor transmission routes of infectious pathogens acquired through the fecal-oral route.

2.7 Management of gastroenteritis

Gastroenteritis is usually an acute and self-limiting infection that does not require medication. The preferred treatment in those with mild to moderate gastroenteritis is oral rehydration therapy (Guarino *et al*., 2014). The primary treatment of gastroenteritis in both children and adults is rehydration. Drinks especially high in simple sugars, such as soft drinks and fruit juices, are not recommended for children under five years as they may increase diarrhoea (Gregorio, 2016; Guarino *et al*., 2014).

Although most of the gastroenteritis infections are self-limiting, antibiotics are prescribed if symptoms are particularly severe or if a susceptible bacterial cause is isolated or suspected. Macrolide such as azithromycin is preferred over fluoroquinolones due to higher rates of resistance to the latter (Christopher *et al.,* 2010). Considering the fact that most diarrhea caused by *E. coli* do not require treatment with antibiotics (excpet in dysentric cases), emergence of antimicrobial resistant *E. coli* strains is in its self a good estimator of the existing selective pressure that can lead to emergence of resistant strains belonging to other species.

2.8 Antimicrobials and Antimicrobial Resistance

Antimicrobials refer to all agents that act against microbial organisms. These antimicrobials are classified based on their spectrum of activity, effect on bacteria and mode of action (Kaufman, 2011). The discovery of penicillin by Alexander Fleming in 1928 and the subsequent administration of its first dose in clinical practice in 1941 marked a radical shift in patient care. The success of antimicrobials against disease-causing microbes is among modern medicine's greatest achievements of all time (Peach *et al*., 2013; Kimang'a, 2012).

The first arrival of a sizable shipment of penicillin at the North African Theatre of Operations for USA military use in 1943 was a landmark that turned a new chapter of antibiotic use in Africa. To date, antibiotics are widely prescribed drugs in hospitals. In

Africa, of all those who use antibiotics, 31.7 % do not receive a prescription from clinicians and about 26.4% obtain antimicrobials from an informal dispenser (Kimang'a, 2012). Evidently, antibiotics are widely and inappropriately used in Africa resulting in increased antibiotic resistance (Kimang'a, 2012; Vialle-valentin *et al*., 2012). In Kenya, surveillance on antimicrobial resistance has been conducted only at the institutional levels, with limited sharing of information; as a result, the actual scale of regional or national antimicrobial drug resistance is not well defined (Sang *et al*., 2012).

Antimicrobial resistance is one of the world's perilous public health problems, many of the microbes (bacteria, viruses, protozoa) that cause infectious disease no longer respond to conventional prescribed antimicrobial drugs. Ineffectiveness of antibiotics is as a result of selective pressure brought about by increased use and misuse of these antibiotics (WHO, 2014; Frost, 2010). Several *Enterobacteriaceae* strains, for instance, some *K. pneumoniae* strains have been isolated which are resistant to carbapenems the last line of antibiotic defense against resistant organisms (Taneja *et al*., 2004). Moreover, rising cases of ESBLs among the *Enterobacteriaceae* family conferring resistance to third and fourth generation cephalosporins is of increasing concern in Kenya, and this has been facilitated by weak enforcement of laws governing the antimicrobial usage in both humans and agriculture especially in animal husbandry (Okeke *et al*., 2010).

Studies have revealed that limited access to medical care and effective treatments, the common practice of self-medication and the availability of counterfeit drugs have contributed to the emergence and spread of drug resistance in the developing world. Resistance to this antimicrobials can be intrinsic, conferred by naturally occurring characteristics of the bacteria, or acquired (Maltha *et al*., 2014; Vialle-valentin *et al*., 2012; Frost, 2010).

Bacteria can acquire resistance through mutations of preexisting genes or transfer of resistance determinants from other bacteria (horizontal gene transfer). The horizontal transfer occurs much more commonly than de novo development of resistance through mutation (White *et al*., 2008). It is through horizontal gene transfer that resistance genes, alone or in groups, can spread within bacterial populations and even to other bacterial species. This has led to the emergence of Extended-spectrum β-lactamases (ESBLs) among the *Enterobacteriaceae* strains which confer resistance to β-lactams. Thus, very broad antibiotic resistance extending to multiple antibiotic classes is now a frequent characteristic of ESBL-producing enterobacterial isolates. As a result, ESBL-producing organisms pose a major problem for clinical therapeutics (Kiiru *et al*., 2012).

2.8.1 Antimicrobials used in management of common enteric infections

The basics of treatment of *Enterobacteriaceae* strains infections depend on the site of infections and are mostly managed using broad-spectrum antimicrobials whose mechanism of action are summarized in **Table 2-1**.

Table 2-1: Mechanisms of action of antimicrobial agents

Mechanism	Examples of Antimicrobial(s) used
Interference with cell wall synthesis	β-lactams:Penicillins,Cephalosporins, Carbapenems,Monobactams. Glycopeptides: Vancomycin, Teicoplanin
Protein synthesis inhibition	
• Bind to 50S ribosomal subunit	Macrolides, Chloramphenicol,
• Bind to 30S ribosomal subunit	Clindamycin. Aminoglycosides, Tetracyclines.
Interference with nucleic acid synthesis	
• Inhibit DNA synthesis	Quinolones.
• Inhibit RNA synthesis	Rifampin
Inhibition of metabolic pathway	Sulfonamides, folic acid analogues
Disruption of bacterial membrane structure	Polymyxins, Daptomycin.
Interference with Efflux pumps	*β*-lactams, fluoroquinolones, Chloramphenicol

Adapted from (McDermott *et al*., 2003)

2.8.2 Extended Spectrum Beta-Lactamases (ESBLs) in *Enterobacteriaceae*

Extended-spectrum β-lactamases (ESBLs) are a group of diverse, complex and rapidly evolving enzymes that mediate resistance to extended-spectrum cephalosporins and other classes of antimicrobials. These enzymes act on β-Lactam which is a group of antibiotics acting on the cell wall of a bacterial cell (Shaikh *et al*., 2015; Ghafourian *et al*., 2014; Rawat & Nair, 2010).

These enzymes (β-lactamases) are commonly classified according to two general schemes: the Ambler molecular classification and the Bush–Jacoby–Medeiros functional classification (Bush *et al*., 1995; Ambler, 1980). The Ambler scheme classifies β-lactamases into four classes according to the protein homology of enzymes. The β-lactamases of class A, C, and D are serine β-lactamase while class B enzymes are metallo-β-lactamases. The Bush–Jacoby–Medeiros functional scheme is based on functional properties of enzymes, that is; the substrate and inhibitor profiles (Bush & Jacoby, 2010).

One of the mechanisms of resistance towards β–lactams is production of β-lactamases that hydrolyze the β-lactam ring and render the antibiotic inactive before it reaches the penicillin-binding protein (PBP) target mainly mediated by (Temoniera-1) TEM-1 and SulfHydryl Variable -1 (SHV-1) enzymes (McDermott *et al*., 2003). These antibiotics include penicillins, cephalosporins, carbapenems and monobactams. They bind to and inhibit the activities of carboxypeptidases and transpeptidases leading to weakening of the cell wall structure, hence cell lysis. ESBLs producing *Enterobacteriaceae* are posing a major therapeutic challenge today in the treatment of hospital and community acquired- infections (Kiiru *et al*., 2012).

ESBL-producing microorganisms also exhibit co-resistance to many other classes of antimicrobials, resulting in limited therapeutic options (Livermore & Paterson, 2006). *Enterobacteriaceae*, especially *Klebsiella* producing ESBLs such as SHV and TEM-types, have been confirmed as primary sources of hospital-acquired infections since the

early 1980s. However, during the late 1990s, several community-acquired strains that commonly cause urinary tract infections and diarrhoea have also been found to be ESBL producers. These include *Escherichia coli, Salmonella, Shigella* and *Vibrio cholera* (Nguyen *et al*., 2016). Mutations arising from the amino acid substitutions or the re-arrangements of the omega loop of parent TEM-1 and SHV-1 enzymes give rise to a variety of β- lactamases. They are encoded by the many genes among them are *blaSHV*, *blaTEM*, and *blaCTX-M*.

2.8.3 TEM and SHV *bla* genes

Narrow Spectrum β-Lactamase (NSBL) phenotypes contain *blaTEM-1* or *blaSHV-1* or both (Kiiru *et al*., 2012). TEM and SHV are the most common variants of ESBLs. TEM encodes for β- lactamases with extended spectrum with TEM-1 been responsible for up to 90% Ampicillin and penicillin resistance in *E. coli* as well as resistance in *H. influenza, N. gonorrhoea* and *K. pneumoniae* (Kiiru *et al*., 2012). The TEM-type ESBLs are derivatives of TEM-1 and TEM-2. It is the most commonly encountered β-lactamase among Gram-negative bacteria. Currently over 100 TEM-type β-lactamases have been described (Rawat & Nair, 2010). SHV refers to the SulfHydryl variable.

The SHV types of ESBLs have been detected in a wide range of *Enterobacteriaceae* and outbreaks of SHV – producing *Pseudomonas* and *Acinetobacter* species have been reported (Rawat & Nair, 2010). The SHV-1 β-lactamases are most commonly found in *K. pneumoniae* and are responsible for up to 20% of the plasmid-mediated Ampicillin resistance in this species. Moreover, the prevelance of these genes among isolates obtained from food handlers in Kenya is yet to be determined.

2.8.4 CTX-M and OXA bla genes

CTX-M is derived from "CefoTaXimase Munich" family and constitutes a complex and non-homogeneous group of enzymes (Gutkind *et al*., 2013). The *CTX-M* is a recently described family of ESBLs; these enzymes hydrolyze cephalosporins especially Cefotaxime with high efficiency (Alobwede *et al*., 2003). Since the initial identification of CTX-M enzymes, at least five genetically distinct groups based on their amino acid identities and alignment of the amino acid sequence have been identified from isolates around the world, including over 160 representatives, both plasmid-borne and chromosome-encoded enzymes. CTX-M type has been reported in most parts of the world, and it is believed that it might be the most frequent type of ESBLs in the world. More than 113 CTX-M varieties are currently known (Bonnet, 2004).

In Kenya, the predominant ESBL genotype is *blaCTX-M*, most of which is isolated from isolates obtained from the urinary tract (Maina *et al*., 2012), and it's the most important ESBL gene among *E. coli* and in *Salmonella enteric serovar typhimurium* (Wang *et al*., 2012). It's believed that *blaCTX-M* was acquired from Genus *Kluyvera* via mobile genetic elements and strains producing such enzymes should be monitored closely in hospital and community settings (Kiiru *et al*., 2012). To date, *CTX-M*-15 and *CTX-M*-14 enzymes are the most predominant types of ESBLs with *CTX-M*-15 showing global distribution (Lahlaoui *et al*., 2014).

The OXA β-lactamases were among the earliest β-lactamases detected; however, these class D β-lactamases were originally relatively rare and always plasmid-mediated with their substrate profile limited to the penicillins, but some became able to confer resistance to cephalosporins as early as from the 1980s onwards. *Acinetobacter baumannii* was one of the earliest strains to show resistance to the carbapenems due to the availability of plasmid-encoded β-lactamases (OXA-23, OXA-40, and OXA-58).

Recently, the carbapenem-resistant OXA β-lactamases (OXA-48) have migrated into the *Enterobacteriaceae* and are now becoming a significant cause of carbapenem resistance. The emergence of OXA enzymes that can confer resistance to third generation cephalosporins and carbapenems, particularly in *Enterobacteriaceae*, has transformed these β-lactamases from a minor hindrance into a major problem set to demote the clinical efficacy of the carbapenems and cephalosporins (Santillana *et al*., 2007; Gutkind *et al.,* 2013).

2.9 Methods of ESBL detection

Several approaches are used to screen and confirm the presence of Extended Spectrum β –lactamase (Pitout *et al*., 2008). These methods can differ between countries and clinical microbiology laboratories. The most common ones are:

2.9.1 Double-disk synergy test

In this test, disks of third generation cephalosporins and amoxicillin/clavulanic acid (Augmentin) are kept 30 mm apart, centre to centre, on inoculated Mueller-Hinton Agar. A clear extension of the edge of the inhibition zone of cephalosporin towards augmentin disk is interpreted as positive for ESBL production (Rawat & Nair, 2010; Jarlier *et al*., 1988).

2.9.2 Three-dimensional test

The three-dimensional analysis gives phenotypic evidence of ESBL-induced inactivation of extended-spectrum cephalosporins or Aztreonam without relying on demonstration of inactivation of the β-lactamases by a β-lactamase inhibitor. The presence of β-lactamase–induced drug inactivation is visualized as a distortion or discontinuity in the usually circular inhibition zone or as the production of discrete colonies in the vicinity of the inoculated slit.

2.9.3 E-test

The E-test ESBL strip (AB Biodisk, Solna, Sweden) carries two gradients: on the one end, Ceftazidime; and on the opposite end, Ceftazidime plus clavulanic acid. MIC is interpreted as the point of intersection of the inhibition ellipse with the E-test strip edge. A ratio of Ceftazidime MIC to Ceftazidime-clavulanic acid MIC equal to or greater than 8 indicates the presence of ESBL (Paterson & Bonomo, 2005).

2.9.4 Molecular detection of ESBLs

These include DNA probes, PCR, oligotyping, PCR-RFLPs and nucleotide sequencing. Molecular methods can detect different ESBL variants but they can be labor intensive and expensive to be adopted as conventional methods (Bonnet, 2004).

2.10 Genetic fingerprinting of bacterial isolates

Genetic fingerprinting is a technique used to determine genetic relatedness of bacteria of the same species from the same source to study diversity and dynamics of microbial communities (Tenover *et al.,* 1995). There are several gel-based methods of determining relatedness of bacterial isolates namely; Restriction fragment length polymorphism (RFLP), Random amplified polymorphic DNA (RAPD), Repetitive extragenic palindromic (REP), Enterobacterial repetitive intergenic consensus (ERIC), $(GTG)_5$ and Pulsed-Field Gel Electrophoresis (PFGE). The $(GTG)_5$ technique was chosen on the basis that it's a fast, reliable, efficient and less resource intensive method as per recommendations from a previous study (Mohapatra *et al*., 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

This study was carried out among eligible food-handlers working in various food establishments within the Nairobi County and who personally sought medical certification at the Centre for Microbiology Research (CMR). The hotels were conveniently classified based on their food prices, the diversity of their facilities and services rendered. Low- class hotels (LCHs) were conveniently categorised as food stalls along the roadside majorly made of makeshifts structures and selling food and offering no extra social or recreational facilities. In this LCH, the average cost of tea and a baked snack costs less than a dollar.

The Middle-class hotels (MCH) included restaurants and cafeterias operating in permanent structures including those of up to the three-star category, and in these hotels the prices ranged from more than a dollar for the same kind of snacks and tea while the high-class hotels (HCH) comprised of hotels rated from four-star and above. The areas where the participants resided were conveniently categorised into the four cardinal directions East, West, North and South based on the geography of Nairobi.

3.2 Approval of the study

This study involving human samples was approved by Scientific and Ethical Review Unit of Kenya Medical Research Institute. All approved procedures conformed to the required standards **(Appendix I).**

3.3 Study design

This was a cross-sectional study.

3.4 Target population

The target population was the food handlers currently working in food establishments in Nairobi County in particular those seeking medical certification at CMR-KEMRI.

3.5 Sample size determination

The sample size was determined based on a prevalence of 30% of bacterial diarrhea in Kenya among food handlers (Oundo *et al*., 2008). The sample size was calculated using the Naing (2003) formula as shown below.

$$n=Z^2 p(1-p)/d^2$$

Where **n** is the sample size,

z = the confidence interval at 95% (1.96)

d = the margin of error at 5% (0.05)

p=anticipated prevalence used 30% (Oundo, 2008)

$$\frac{1.96^2\ 0.3(1-0.3)}{0.05^2} = 323$$

The minimum sample size of clinical specimens of 323 was used.

3.6 Inclusion and Exclusion criteria

Before the study participants were recruited, they had to meet the required criteria which was as follows ;

3.6.1 Inclusion criteria

All adult food handlers who gave consent to participate and were working in food establishments within Nairobi County and who personally sought medical certification at CMR-KEMRI were recruited.

3.6.2 Exclusion criteria

All food handlers who consented to participate in the study but did not provide a stool sample were excluded.

3.7 Ethical consideration and recruitment of participants

Enrollment to the study was on voluntary basis whereby informed consent was sought from the participants and information collected kept confidential. There were no monetary gains for those who participated in the study and there were no penalties for those who declined participation, **(Appendix 1I).**

3.8 Sampling method

The simple random sampling technique was used whereby every third individual presenting at CMR-KEMRI for medical certification was recruited. Thirty-two samples were collected per week over a period of 10 weeks.

3.9 Sample collection

Data regarding socio-demographic and predisposing factors was collected using a structured questionnaire **(Appendix III)**.

The study participants collected their own stool in privacy following instruction from the principal investigator. Fecal specimens were collected in sterile containers labeled with a unique identifier rather than the name of the participant.

3.9.1 Microbiological analysis

Stool samples were macroscopically examined for colour and consistency then followed by microbiological analysis. A gram of stool specimens were enriched into 9 ml of Buffered peptone water overnight at 37°C. A pea-sized stool sample was transferred into 9 ml Selenite F broth (Oxoid, UK), and incubated overnight at 37°C. A loopful from each of the enrichment medium was then subcultured onto MacConkey agar (Oxoid, UK), for the detection of lactose fermentaters (for example, *E. coli*) and non-lactose fermentors (for example, *Salmonella*). Another set of plating was done on Xylose Lysine Deoxycholate (XLD) agar (Oxoid, UK) for isolation of *Shigella* and *Salmonella* species. All plates were incubated for 24 hours at 37°C. Gram staining was carried out for pure isolates and biochemical tests performed for further identification of the isolates. Results obtained from cultural and biochemical reactions were used for identification of strains to species level as previously described (Cheesebrough, 2006) (**Appendix IV**).

3.10 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done on Mueller-Hinton agar plates (Oxoid). In the first plate, the following antibiotics were used: Ampicillin (AMP, 10µg), Imipenem (IPM, 10µg) Ceftazidime (CAZ, 30µg), Cefotaxime (CTX, 30µg), Aztreonam (ATM, 30µg), Cefoxitin (FOX, 30µg), Cefepime (FEP, 30µg). In order to detect the zone of synergy for ESBL-producers, Amoxicillin-Clavulanic acid disk (AMC, 30µg) was

placed at the centre of this plate at a distance of 15-20 mm from each of the β-lactam antimicrobials.

In the second plate, the following antibiotics were used: Tetracycline (TE, 30µg), Sulfamethoxazole/trimethoprim (SXT, 23.75µg/1.25µg), Streptomycin (S, 10µg), Gentamicin (CN, 10µg), Chloramphenicol (C, 30µg), Ciprofloxacin (CIP, 5µg) and Nalidixic acid (NA, 10µg). The plates were incubated at 37°C for 18–24 hours. These antibiotics were chosen on the basis of their use in the management of enteric bacterial infections. The inocula for susceptibility testing were compared against the McFarland 0.5 turbidity standards. The *E. coli* ATCC 25922 strain was used as the test standard for quality control of media and disc potency. The interpretation of inhibition zone diameters (potency) results into susceptible, intermediate or resistant was done as per the Clinical and Laboratory Standards Institute guidelines (CLSI, 2015) as seen in (**Appendix V).**

3.10.1 Detection and confirmation of ESBLs using disc diffusion test

Genotypic characterization was done on 22 *E.coli* isolates that were resistant to cephalosporins and tested for ESBLs production using the double disk synergy test following the CLSI (2015) guidelines. Only isolates showing synergy zones between amoxicillin/clavulanic and one or more third generation cephalosporins were identified as ESBL-producers (Rawat & Nair, 2010; Jarlier *et al.*, 1988). Enlargement or distortion of the inhibition zones to form a keyhole appearance/ghost inhibition zone between the cephalosporins discs and the Amoxicillin/Clavulanate disc was interpreted as an ESBL enzyme production phenotype.

3.10.2 DNA extraction and amplification

DNA extraction was done using the boiling method. The heating block was set at a temperature of 95^{o}C and the inoculum heated for a maximum of 12 minutes before centrifuged at 26000 RPM for 5 minutes (Sepp *et al.*, 1994). The resulting supernatant

was stored at 4° C for short-term use and at -80° C for long term storage. PCR amplification reactions were performed in volumes of 20 µl containing 4 µl of 5x Firepol ready to use master mix (SOLIS BIODYNE), 0.4 µl concentrations of each primer, 12.2 µl of PCR water, BSA 1 µl and 2 µl of DNA template. The cycling parameters were as follows: an initial denaturation at 94°C for 5 min; followed by 35 cycles at 94°C for 30 s, annealing depeneded on the primer temperature was done for between 30 seconds and 1 min, before a short extension step at 72°C for 1 min. A final extension temperature was set for 72°C for between 10 min for short fragments and 20min for longer fragments. The amplified PCR products were subjected to electrophoresis in 1.0% agarose gel in 1X TBE buffer and stained with ethidium bromide. A 1kb DNA ladder was used as a standard and visualized under the UV transillumination.

3.11 Determination of phylogenetic relatedness of strains

Thirty *E.coli* isolates representing major phenotypes and from participants with diverse social-economic and clinical backgrounds were randomly selected for genetic diversity and relatedness using the $(GTG)_5$ low-resolution typing by use of primers indicated in **Table 3-1** following the described steps; initial denaturation at 95°C for 2minutes, annealing at 40°C for 1minute, a short extension step at 65°C for 8 minutes and then a final extension at 65°C for 8 minutes.

Table 3-1: Nucleotide sequences of PCR primers used to amplify ESBL genes

Gene	Primer sequence	Expected Size(bp)	Annealing Temp(°C)
bla_{TEM}	F=5'GCGGAACCCCTATTTG 3'	999 bp	52
	R=5'TCTAAAGTATATATGAGTAAACTTGGTCTGAC 3'		
bla_{SHV}	F=5' TTCGCCTGTGATTATCTCCCTG 3'	851bp	60
	R=5' TTAGCGTTTGCCAGTGYCG 3'		
bla_{CTX-M}	F=5'ATGTGCAGYACCAGTAARGTKATGGC 3'	593bp	60
	R=5'TGGGTRAARTARGTSACCAGAAYCAGCGG 3'		
bla_{OXA}	F=5'ATGAAAAACACAATACATATCAACTTCGC 3'		
	R=5' GTGTGTTTAGAATGGTGATCGCATT 3'	920bp	52
$(GTG)_5$ Primers	5′-GTG GTG GTG GTG GTG-3′		

Table 3-1 shows nucleotide sequences of PCR primers used to amplify ESBL genes with their respective expected band sizes (base pairs) and annealing temperature; bp-base pairs; F-forward primer; R-reverse primer.

3.12 Disposal of bio-hazard material

All infectious materials were taken to KEMRI incinerator for proper disposal. No bio-hazard materials were released or transported without proper packaging and inspection by the laboratory bio-safety personnel.

3.13 Data management and analysis

The data on socio-demographic factors and test samples were recorded in Microsoft Excel, and checked for consistency before analysis. Data was kept confidential by use of unique identifiers and not participants names in a password protected database and interpreted in form of tables and charts. Antibiotic susceptibility patterns were presented in the form of means and histogram. A Chi-square statistical test was used to analyze bivariate data and all results were interpreted at 95% CI and presented in tabular and graphical formats.

3.14 Dissemination of findings

The study findings were sent for publication in East African Medical Journal, a peer-reviewed journal.

CHAPTER FOUR

RESULTS

4.1 Socio-demographic profiles of the study participants

In terms of gender distribution, there was near parity in the proportions of males (51%) and females (49%) recruited. Majority of the participants (75%) were between 18-30 years of age. A high proportion (76%) of the participants worked in middle-class hotels while the rest were either in low-class or high-class hotels. More than half of the participants (55%) had a tertiary education with only a small proportion (<1%) being partially illiterate (not able to read and/or write) while 3% had a primary school education with over (90%) having worked for less than five years in the hotel industry**, Figure 4.1.**

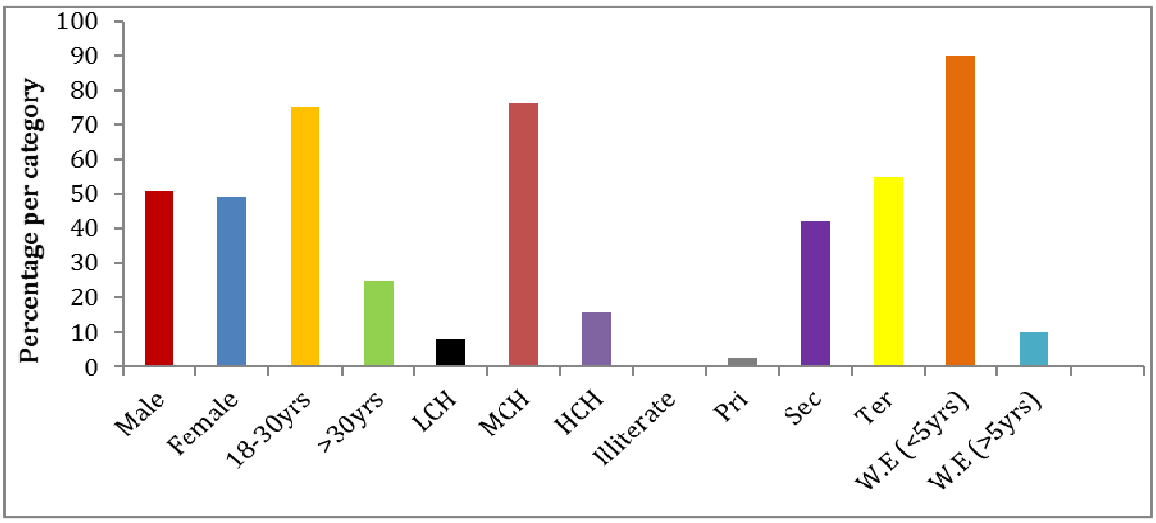

Figure 4.1: Non-clinical socio-demographic profile of apparently healthy food-handlers working in various food establishments within Nairobi County based on gender, age, type of hotel, level of education and the number of years worked within the food establishment.

*LCH- Low Class Hotel; MCH-Medium Class Hotels; HCH-High Class Hotel; Pri-Primary; Sec-Secondary; Ter-Tertiary; W.E-Work Experience.

The study also noted that a high proportion of the participants (76%) normally accessed (bought) antibiotics from local chemists with over 50% being those who self-medicate. Moreover, 11% did not complete the prescribed dosage as required by the clinicians. Over 60% of the recruited participants lacked a foodhandlers' medical certificate with 29% having not trained in either proper food hygiene or safety. About 20% of the participants did not wash their hands regularly while handling or preparing foodstuffs. About 24% of the participant reaveled that their facilities (hotels) lacked a cold-storage facility (fridge) for food preservation and continous supply of piped treated municipal water, **Figure 4.2.**

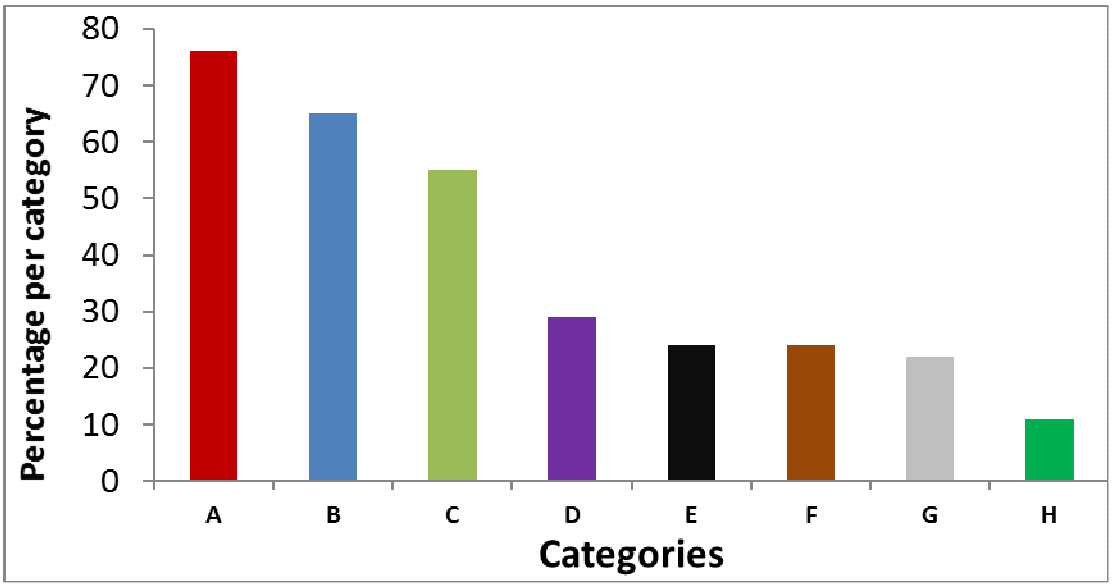

Figure 4.2: Non-clinical socio-demographic profile of apparently healthy food-handlers working in various food establishments within Nairobi County based on personal hygiene, training in food hygiene, clinical history, food preservation mechanism, medical certification and water accessibility.

*__A__-Proportion of individuals accessing antibiotics from chemists ; __B__-Proportion of individuals without foodhandlers' certificate ; __C__-proportion of individuals practising self-medication ; __D__-Proportion of foodhandlers who were not trained in food hygiene and safety ; __E__-Proportion of hotels that lacked a cold-storage facility ; __F__-Proportion of

hotels that lacked a continous municipal water supply ; **G**-Proportion of foodhandlers who did not wash their hands regularly ; **H**-Proportion of foodhandlers who did not complete the prescribed dosage.

4.2 Microorganisms isolated from the study participants

The study sought to isolate *E.coli*, *Salmonella* and *Shigella* species among food-handlers through culture of fecal samples. However, only *E.coli* isolates (323 *E.coli* isolates) were recovered from the 323 study participants recruited and neither *Salmonella* nor *Shigella* was isolated.

4.3 Antimicrobial susceptibility profiles of *E. coli* isolates

Following susceptibility testing of the 323 *E.coli* isolates recovered from the foodhandlers, high resistances were recorded for Sulfamethoxazole/Trimethoprim (SXT, 70%), Ampicillin (AMP, 44.6%), Streptomycin (S, 42%) and Tetracyclines (TE, 41%); whereas Imipenem (a Carbapenem) and Cefepime (a fourth generation cephalosporin) were effective against 99% of the isolates. Among quinolones, resistance to ciprofloxacin was significantly lower (7%) compared to that of Nalidixic acid (16%) (p: 0.001, OR 0.367 CI 0.198-0.675). Resistance to cephalosporins was observed among twenty two (22) *E.coli* isolates recording a prevalence of 6.8%. It was notable that out of the 323 isolates, only 3% exhibited a combined resistance to third generation cephalosporins and Ciprofloxacin, **Figure 4.3.**

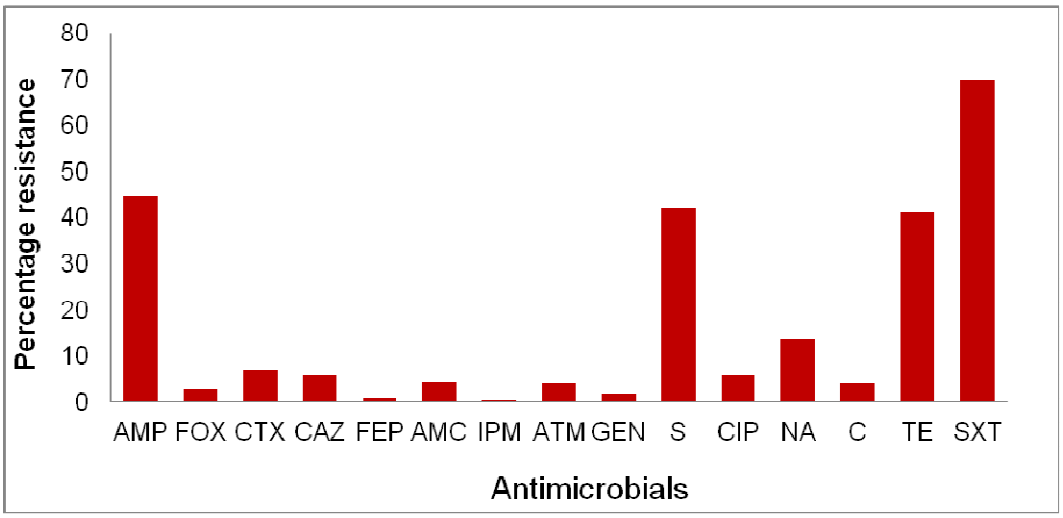

Figure 4.3: General Resistance pattern of *E. coli* isolates recovered from apparently healthy food-handlers working within Nairobi County on selected antibiotics.

*AMP-Ampicillin; FOX-Cefoxitin; CTX-Cefotaxime; CAZ-Ceftazidime; FEP-Cefepime; AMC-Amoxicillin-Clavulanic acid; IPM-Imipenem; ATM-Aztreonam; CN-Gentamicin; NA-Nalidixic acid; S-Streptomicin; SXT-Sulfamethoxazole/Trimethoprim; CIP-Ciprofloxacin; Chloramphenicol; TE-Tetracycline.

4.3.1 Distribution patterns of inhibition zones against Tetracycline, Sulfamethoxazole-Trimethoprim combination and Chloramphenicol

Tetracycline potency was low with majority 99(31%) of isolates investigated recording zone diameters of 6 mm (the zone of extreme resistance). Even those that were susceptible, majority clustered in the zone diameter ranging between 23 and 25 mm indicating that these values are close to the intermediate zone, **Figure 4.4A.** Extreme resistance to SXT antimicrobial was evident in 196(61%) of the isolates that recorded zone diameters of 6mm. Isolates susceptible to this antimicrobial were scattered across zone diameters of between 16 mm and 24 mm indicating a high possiblibity of increase of the resistance prevalence towards this antimicrobial, **Figure 4.4B**.

Chloramphenicol exhibited minimal resistance with only 2% of the isolates recording inhibition resistance zones of 6 mm. Majority of the strains (>90%) clustered in the zone diameters ranges of between 24mm and 26mm (medium to high susceptibility) with a few isolates recording zone diameters of >30 mm (extreme susceptibility). These results therefore suggest that this antimicrobial will remain potent for a high proportion of isolates for much longer, **Figure 4.4C**.

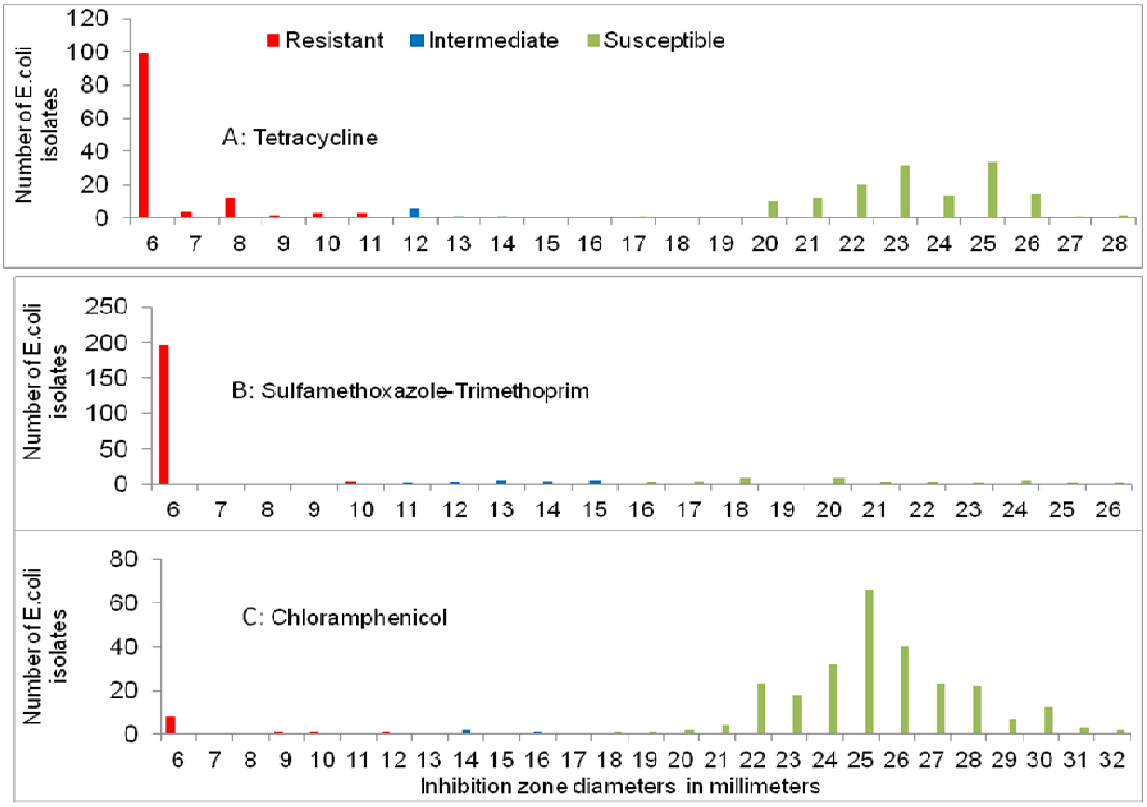

Figure 4.4: Zone diameters against Tetracycline, Sulfamethoxazole-Trimethoprim combinations and Chloramphenicol among *E. coli* isolates recovered from food-handlers.

*Resistant-Isolates that were not inhibited by the actions of the antimicrobial and showed reduced inhibition zone diameters *Intermediate-Isolates that were partially inhibited by the antimicrobial (they were neither resistant nor sensitive to the antimicrobial) *Susceptible-Isolates that were sensitive to the actions antimicrobial and showed high inhibition zones.

4.3.2 Distribution patterns of inhibition zones of *E. coli* isolates against Ampicillin

High proportion 109(34%) of isolates showed extreme resistance towards Ampicillin with majority of these isolates recording zone sizes of 6 mm. Isolates that were susceptible clustered between zone sizes of 20 mm and 21 mm indicating the possibility of shifting to the resistant spectrum, **Figure 4.5**.

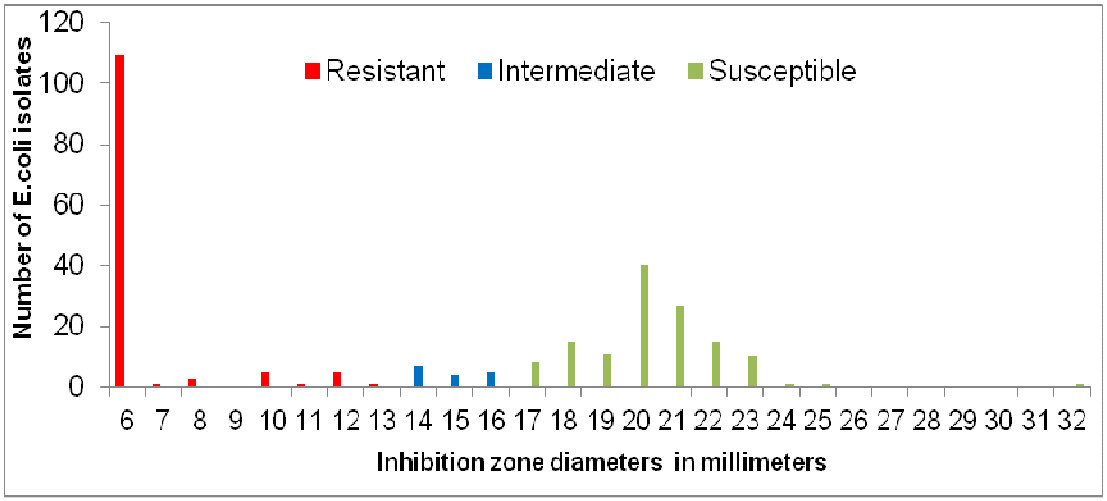

Figure 4.5: Distribution patterns of zone diameters of *E. coli* isolates recovered from food-handlers against Ampicillin antimicrobial.

*Resistant-Isolates that were not inhibited by the actions of the antimicrobial and showed reduced inhibition zone diameters *Intermediate-Isolates that were partially inhibited by the antimicrobial (they were neither resistant nor sensitive to the antimicrobial) *Susceptible- Isolates that were sensitive to the actions antimicrobial and showed high inhibition zones.

4.3.3 Distribution patterns of inhibition zone diameters of *E. coli* isolates against selected third and fourth generation cephalosporins

Two of the third generation cephalosporins (Cefotaxime and Ceftazidime) showed high potencies towards most isolates investigated (95%). Ceftazidime compared to Cefotaxime was more potent because only 2% of the isolates clustered within the

resistant zone ranges from 11 mm to 17 mm compared to 5% for Cefotaxime. The potency of the fourth generation cephalosporins (Cefepime) was higher than that of the third generation cephalosporins (Cefotaxime and Ceftazidime) with only less than 1% (<1%) of isolates clustering within the resistance range, **Figure 4.6.**

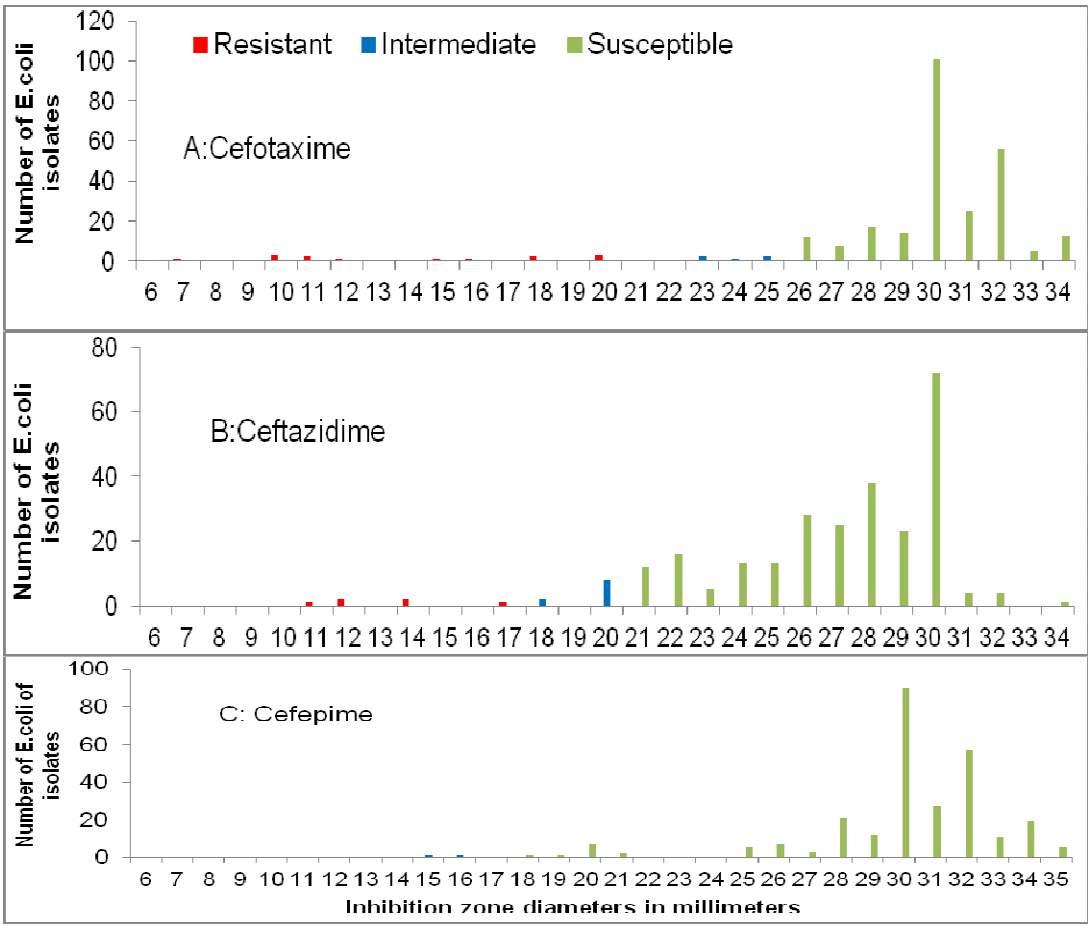

Figure 4.6: Zone diameters of *E. coli* isolates recovered from apparently healthy food-handlers against third generation cephalosporins Cefotaxime , Ceftazidime and Cefepime, a fourth generation cephalosporin.

*Resistant-Isolates that were not inhibited by the actions of the antimicrobial and showed reduced inhibition zone diameters *Intermediate-Isolates that were partially inhibited by the antimicrobial (they were neither resistant nor sensitive to the antimicrobial) *Susceptible-Isolates that were sensitive to the actions antimicrobial and showed high inhibition zones.

4.3.4 Distribution patterns of inhibition zone diameters of *E. coli* isolates against Amoxicillin-Clavulanic acid

A large proportion (>90%) of the isolates were susceptible to the β-lactamase inhibitor (Amoxicillin-Clavulanic acid, AMC) with only a (4.3%) showing intermediate to total resistance. None of the isolates recorded an extreme resistance zone of 6 mm indicating that resistance to this antimicrobial is low compared to third generation cephalosporins (Cefotaxime and Ceftazidime). Due to its high potency, this antimicrobial inhibited majority of the isolates, **Figure 4.7**.

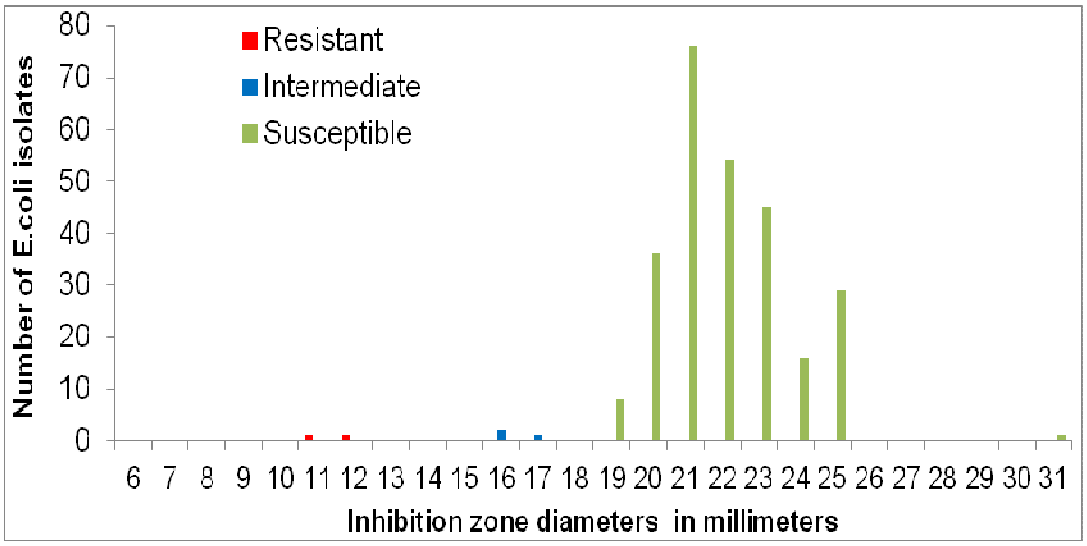

Figure 4.7: Zone diameters of Amoxicillin-Clavulanic acid on *E. coli* isolates recovered from apparently healthy food-handlers working in various hotels within Nairobi county Zone diameters of E. coli isolates recovered from apparently healthy food-handlers against.

*Resistant-Isolates that were not inhibited by the actions of the antimicrobial and showed reduced inhibition zone diameters *Intermediate-Isolates that were partially inhibited by the antimicrobial (they were neither resistant nor sensitive to the antimicrobial) *Susceptible-Isolates that were sensitive to the actions antimicrobial and showed high inhibition zones.

4.3.5 Distribution patterns of inhibition zone diameters of *E. coli* isolates recovered from food-handlers on monobactams, cephamycins and carbapenems

In this study, only one (1) isolate showed extreme resistance recording zone inhibition of 6mm towards Aztreonam. Majority (>90%) of the isolates clustered in the susceptible region of between 30 mm and 32 mm with a few isolates recording zone diameters of >32 mm. These large inhibition zones suggest that this antimicrobial will remain potent against a large proportion of the isolates for much longer.

Among β-lactams, Cefoxitin and Carbapenems registered the highest potency. Only about 3% of all isolates were resistant to Cefoxitin and less than 1% was resistant to Imipenem. Majority of strains clustered in susceptible zones ranges of between 25 mm and >32 mm. Imipenem and Cefoxitin are therefore likely to remain potent aganist most of the strains encountered in this population for long, **Figure 4.8**.

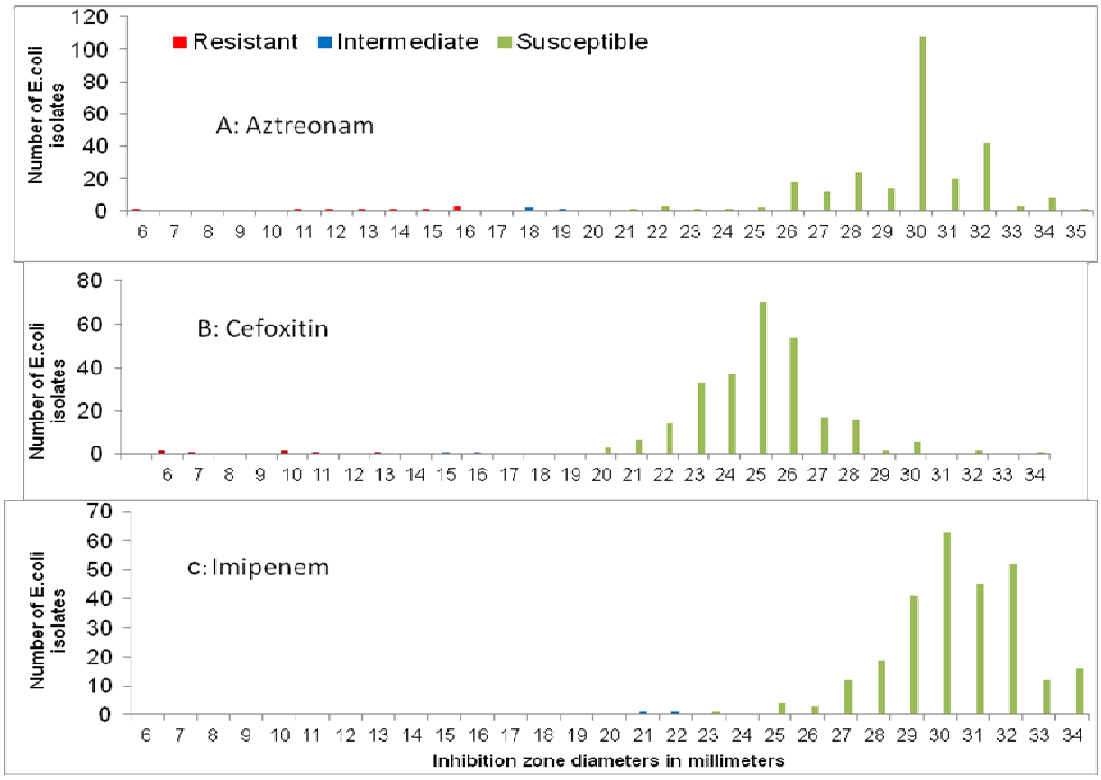

Figure 4.8: Distribution patterns of inhibition zone diameters of *E. coli* isolates recovered from apparently healthy food-handlers working in various hotels within Nairobi County on aztreonam (monobactams), cefoxitin (cephamycin) and imipenem (carbapenems).

Resistant-Isolates that were not inhibited by the actions of the antimicrobial and showed reduced inhibition zone diameters Intermediate-Isolates that were partially inhibited by the antimicrobial (they were neither resistant nor sensitive to the antimicrobial)* Susceptible-Isolates that were sensitive to the actions antimicrobial and showed high inhibition zones.

4.3.6 Distribution patterns of inhibition zone diameters of selected aminoglycosides among *E. coli* isolates recovered from food-handlers

Among aminoglycosides, Gentamicin was more potent than streptomicin because only one isolate clustered within the resistant zone range of 6mm compared to 45 isolates for streptomycin. Even among those that showed susceptibility to Streptomycin, majority (>42%) clustered in the intermediate zones of between 15 mm and 18 mm further showing that this agent has a low potency against these *E.coli* strains. Majority of the susceptible isolates to Gentamicin clustered between zone ranges of 16 mm to 20 mm, **Figure 4.9.**

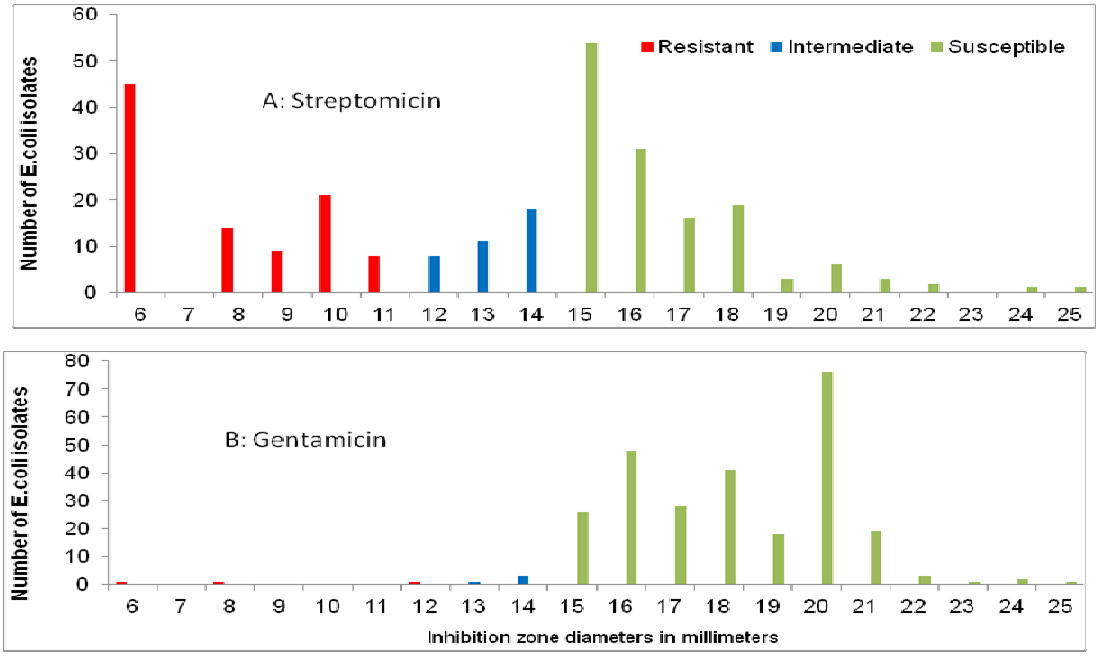

Figure 4.9: Distribution of zone diameters of *E. coli* isolates recovered from apparently healthy food-handlers working in various hotels within Nairobi County on against selected aminoglycosides (Gentamicin and Streptomycin).

Resistant-Isolates that were not inhibited by the actions of the antimicrobial and showed reduced inhibition zone diameters Intermediate-Isolates that were partially inhibited by the antimicrobial (they were neither resistant nor sensitive to the antimicrobial); *Susceptible-Isolates that were sensitive to the actions antimicrobial and showed high inhibition zones.

4.3.7 Distribution patterns of inhibition zone diameters of quinolones among *E. coli* isolates recovered from food-handlers

Ciprofloxacin was more potent with only 3% of all isolates showing disc zones sizes of 6mm compared to 7% of strains that had the same zone size for Nalidixic acid.

Majority of the susceptible isolates recorded inhibition zone sizes of 25mm and >32mm for Nalidixic acid and Ciprofloxacin respectively indicating that this class of antimicrobials will remain useful in the treatment of infections in this population for much longer, **Figure 4.10**.

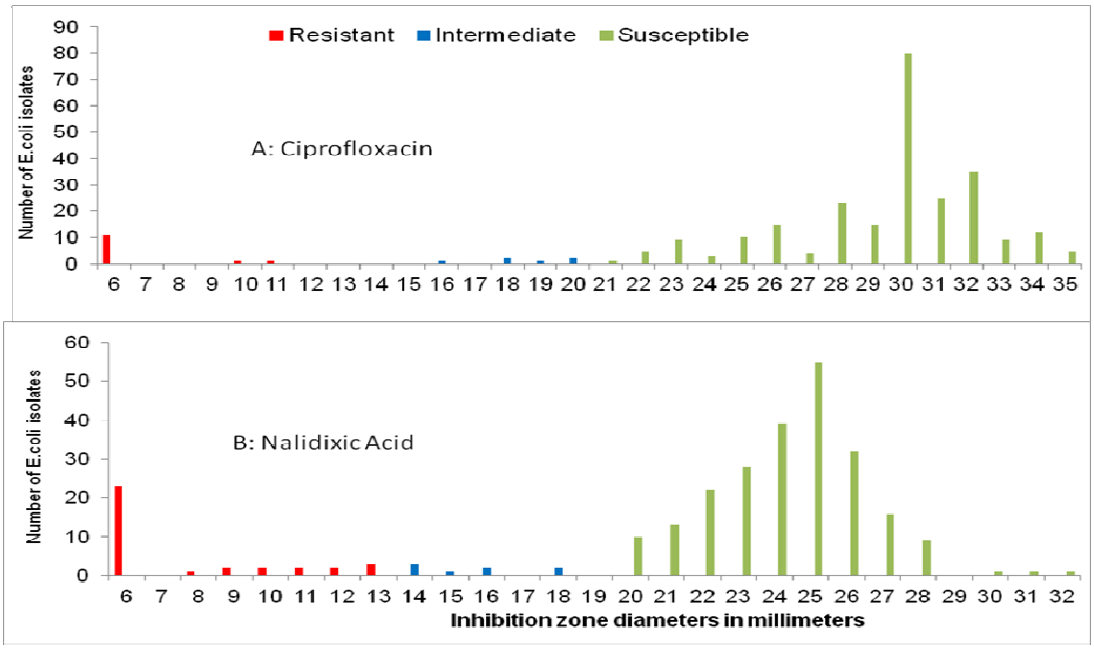

Figure 4.10: Distribution of zone diameters of quinolones (Nalidixic acid and Ciprofloxacin) among *E. coli* isolates recovered from food-handlers working in various hotels within Nairobi County.

Resistant-Isolates that were not inhibited by the actions of the antimicrobial and showed reduced inhibition zone diameters Intermediate- Isolates that were partially inhibited by the antimicrobial (they were neither resistant nor sensitive to the antimicrobial) *Susceptible- Isolates that were sensitive to the actions antimicrobial and showed high inhibition zones.

4.4 Comparions of resistance prevalence based on various socio-demographic and clincial profiles

Some selected socio-demographic and clinical profiles of the participants were analyzed to determine the overall trend of antimicrobial resistance.

4.4.1 Resistance patterns based on gender

At least 70% of isolates from both sexes were resistant to Sulfamethoxazole/ Trimethoprim (SXT) and over 40% to Ampicillin (AMP), Streptomycin (S) and Tetracycline (TE). Low resistance (< 10%) was recorded for Aztreonam (a monobactam), Chloramphenicol, Ciprofloxacin, Cefoxitin (cephamycin) and to cephalosporins (Cefotaxime, Ceftazidime and Cefepime) for either gender. Low resistance (<2%) was also recorded for Gentamicin and Imipenem in all isolates regardless of the gender of the source participant, **Figure 4.11.** Generally, there was no significant differences observed between the two genders in terms of resistances observed for Multi-drug resistant strains (p:0.205; OR 0.711; CI 0.418-1.206).

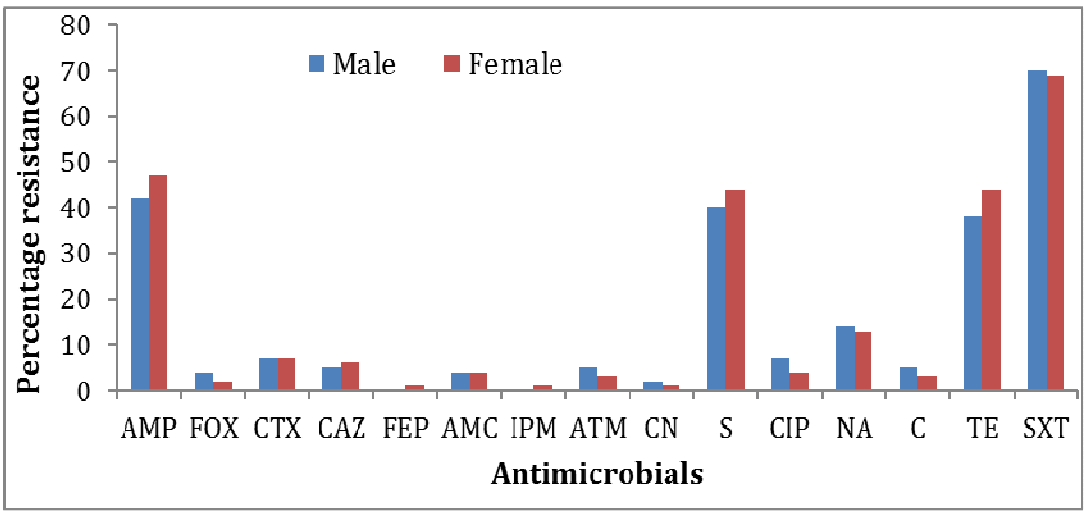

Figure 4.11: Resistance pattern of *E. coli* isolates based on gender recovered from apparently healthy food-handlers working within Nairobi County on selected antibiotics.

*AMP-Ampicillin; FOX-Cefoxitin; CTX-Cefotaxime; CAZ-Ceftazidime; FEP-Cefepime; AMC-Amoxicillin-Clavulanic acid; IPM-Imipenem; ATM-Aztreonam; CN-Gentamicin;NA-Nalidixicacid;S-Streptomycin;SXT-Sulfamethoxazole/Trimethoprim;CIP-Ciprofloxacin;Chloramphenicol; TE-Tetracycline.

4.4.2 Resistance patterns based on Age

For easy analysis of resistance patterns, the ages of the study participants were conveniently grouped into two; 18-30 years of age and those who were above 30 years. A significant number of isolates obtained from individuals aged between 18 and 30 years were resistant to Ampicillin (AMP, 34%), Sulfamethoxazole/Trimethoprim (SXT, 54%), Streptomycin (S, 30%) and Tetracyclines (TE, 29%). In contrast, only a few isolates (<1%) from individuals above the age of 30 years were resistant to third generation cephalosporins, Gentamicin and Ciprofloxacin, **Figure 4.12**. Generally, there was no significant differences observed between the two age groups in terms of resistances observed for SXT (p:0.129; OR 1.706; CI0.804-3.595) and S or TE (p:0.104, OR 0.630, CI 0.346-1.144).

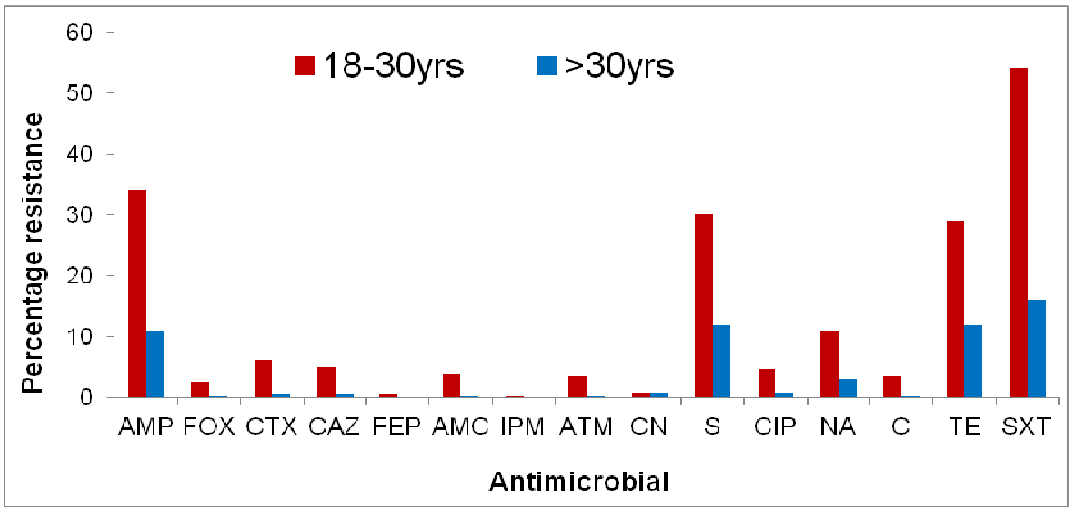

Figure 4.12: Resistance pattern of *E. coli* isolates based on age recovered from apparently healthy food-handlers working within Nairobi County on selected antibiotics.

*AMP-Ampicillin; FOX-Cefoxitin; CTX-Cefotaxime; CAZ-Ceftazidime; FEP-Cefepime; AMC-Amoxicillin-Clavulanic acid; IPM-Imipenem; ATM-Aztreonam; CN-Gentamicin; NA-Nalidixic acid;S-Streptomycin;SXT-Sulfamethoxazole/Trimethoprim;CIP-Ciprofloxacin;Chloramphenicol; TE-Tetracycline.

4.4.3 Resistance patterns based on hotel type

Regardless of the participants' place of work (hotel type), high resistances (≥ 40%) were recorded against Sulfamethoxazole/Trimethoprim, Ampicillin, Streptomycin and Tetracyclines. Isolates from any of the hotel types exhibited low resistance of less than 10% towards carbapenem, monobactams, and β-Lactams. Similar low resistances were observed for both Gentamicin (aminoglycoside) and Chloramphenicol. It was noted that only isolates from middle class hotels exhibited resistance to Imipenem (a carbapenem) and Cefepime, a 4th generation cephalosporin.

Resistance to Ampicillin among the individuals working in low-class hotels was 59%, 42% for middle-class hotels and 47% for high-class hotels. Similarly, a high proportion of SXT-resistant strains were found to be more common among those obtained from participants working in high-class hotels (74%), than in low-class hotels (67%), **Figure 4.13**.

Carriage of MDR-resistant strains among those who worked in high-class hotels was (5.3%), middle-class hotels (22%) and in those who worked in low-class hotels, 2.8%. Despite these varying proportions there were no significant differences in the carriage of MDR among isolates in MCH and those from LCH hotels (p: 0.659; OR 1.225; CI 0.483-3.053) or between those from MCH and those from HCH hotels (p: 0.614; OR 0.816; CI 0.410-1.635).

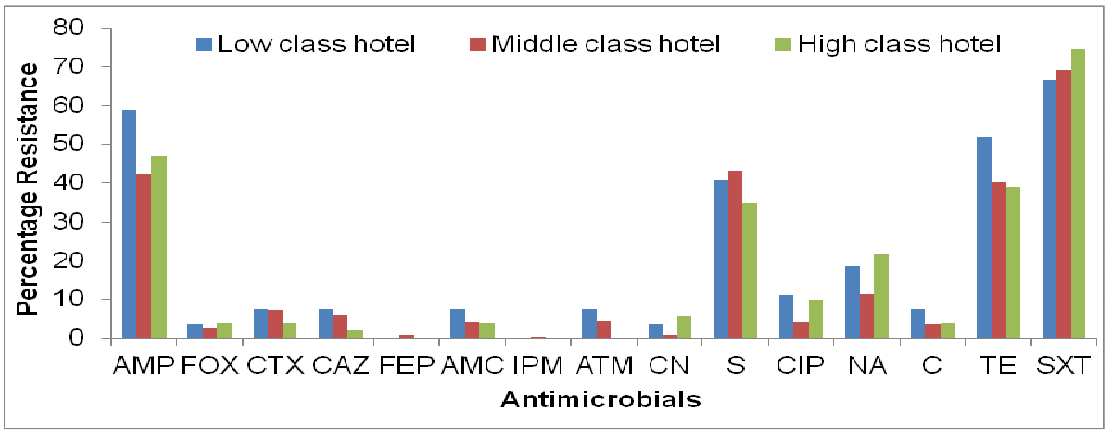

Figure 4.13: Resistance pattern of *E. coli* isolates based on hotel type of apparently healthy food-handlers working within Nairobi County on selected antibiotics.

*AMP-Ampicillin; FOX-Cefoxitin; CTX-Cefotaxime; CAZ-Ceftazidime; FEP-Cefepime; AMC-Amoxicillin-Clavulanic acid; IPM-Imipenem; ATM-Aztreonam; CN-Gentamicin; NA-Nalidixic acid;S-Streptomycin;SXT-Sulfamethoxazole/ Trimethoprim; CIP-Ciprofloxacin;Chloramphenicol; TE-Tetracycline.

4.4.4 Resistance patterns based on level of education of the participant

A relative high proportion 77(24%) of isolates from individuals with secondary and tertiary education showed total resistance towards Sulfamethoxazole/Trimethoprim, Ampicillin, Streptomycin and Tetracycline. However, isolates obtained from persons with a primary level of education showed no resistance towards Cefoxitin, Imipenem, Cefepime, Chloramphenicol and Gentamicin, **Figure 4.14**.

MDR strains accounted for 33% among individuals with basic primary education, 29% among those with secondary education and 31% among those with tertiary education. Generally, there were no significant differences in the carriage of MDR among isolates obtained from individuals with only primary education and those from individuals with secondary education (p: 0.725; OR 1.200; CI 0.224-5.783) or between those with secondary education and those with tertiary education (p: 0.901; OR 0.949; CI 0.565-1.592).

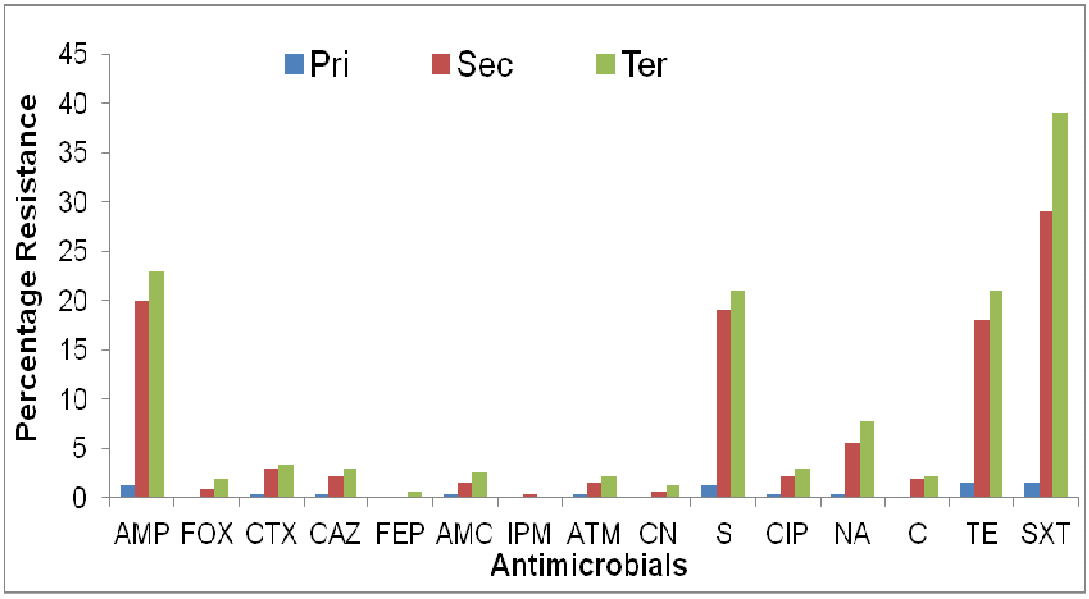

Figure 4.14: Resistance pattern of *E. coli* isolates based on different levels of education of apparently healthy food-handlers working within Nairobi County on selected antibiotics.

*AMP-Ampicillin; FOX-Cefoxitin; CTX-Cefotaxime; CAZ-Ceftazidime; FEP-Cefepime; AMC-Amoxicillin-Clavulanic acid; IPM-Imipenem; ATM-Aztreonam; CN-Gentamicin; NA-Nalidixic acid;S-Streptomicin;SXT-Sulfamethoxazole/Trimethoprim;CIP-Ciprofloxacin;Chloramphenicol; TE-Tetracycline.

4.5 Prevalence of Multi-drug resistant strains

Following antimicrobial testing of all the 323 *E.coli* isolates recovered from the study participants, a third 97(30%) of the isolates were multi-drug resistant (MDR) with near parity in the carriage of this phenotype in both genders (males, 27% and females 33%). Prevalence of MDR strains among individuals within the age category of 18-30years was 22%. The highest proportion of MDR strains among the three hotel types were obtained from those working in middle-class hotels (22%). Moreover, the prevalence of this phenotype was <1%, among those with only a basic primary education compared to 29% among those with post primary education, **Figure 4.15**.

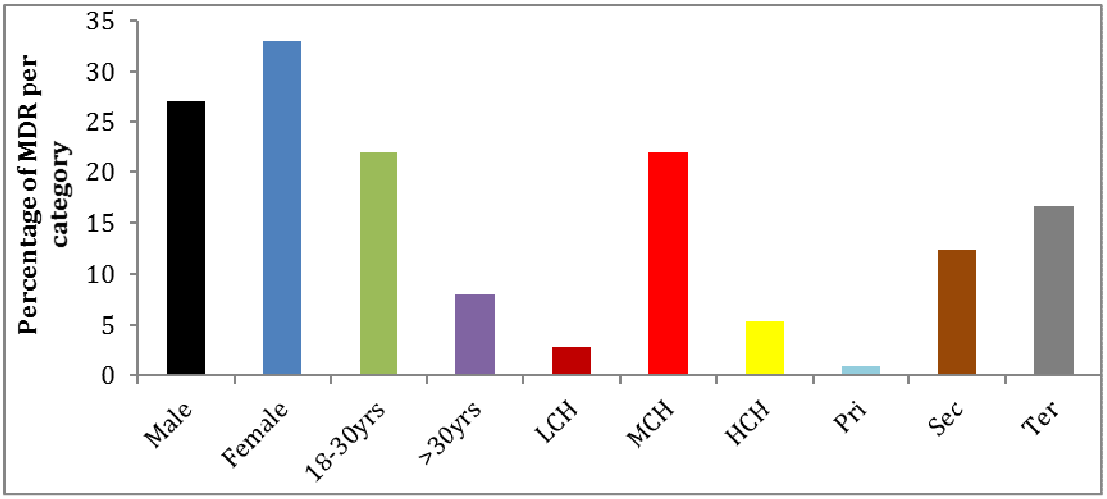

Figure 4.15: Carriage of Multi-drug resistant isolates among apparently healthy food-handlers working in various food establishments within Nairobi County based on gender, age, type of hotel and level of education.

*LCH- Low Class Hotel; MCH-Medium Class Hotels; HCH-High Class Hotel; Pri-Primary; Sec-Secondary; Ter-Tertiary

4. 6 Prevalence of β-lactamase phenotypes

The β-lactamase phenotypes observed in different isolates were defined based on a scheme published before (Kiiru *et al*., 2012). Briefly, the Narrow Spectrum β-lactamases (NSBLs) phenotype (characterized by susceptibility to cephalosporins and other advanced classes of β-lactams but exhibiting significant resistance to Ampicillin was the most common β-lactamase phenotype 144(44.6%). The classical ESBL phenotype (characterized by resistance to cephalosporins with concomitant susceptibility to at least a β-lactamase inhibitor, cephamycins and carbapenems) was only in 11(3.4%) of the isolates. The inhibitor-resistant TEM phenotype (IRT phenotype) characterized by resistance to β-lactamase inhibitors (Amoxicillin-Clavulanic acid) and ampicillin but with concomitant susceptibility to all other classes of β-lactams was only evident in 3(<1%) isolates. Advanced β-lactamase phenotypes (pAmpC and CMT) characterized

by resistance to multiple cephalosporins, and even cephamycins were noticeable in less than 2% of the isolates as indicated in **Table 4-1**.

Table 4-1: Diversity of β -lactamase phenotypes among the *E.coli* isolates recovered from apparently healthy foodhandlers working in various food establishments in Nairobi County

Antibiotics to which isolates were resistant							
Penicillin	Third generation cephalosporins/ monobactam *e.g* Cefotaxime	Fourth generation cephalosporin cefepime	β- lactamase inhibitor (AMC)	Cephamycin (Cefoxitin)	Carbapenem e.g.imipenen	Most probable phenotype	Total n(%) of 323
AMP	none	none	-none	-none	- none	NSBL	144(44.6)
AMP	none	-none	AMC	-none	- none	IRT	3(<1)
AMP	CTX[b] /CAZ[b]	-none	-none	-none	- none	ESBL	11(3.4)
AMP	CTX,CAZ,ATM	FEP	AMC	-none	- none	CMT	4(1.2)
AMP	CTX,CAZ	FEP	AMC	FOX	- none	pAmpC	1(<1)

*AMP-Ampicillin; CTX-Cefotaxime; CAZ-Ceftazidime; ATM-Aztreonam; FEP-Cefepime; AMC-Amoxicillin-clavulanic acid; FOX-Cefoxitin; NSBLs-Narrow spectrum β-lactamases; IRT-Inhibitor resistant TEM phenotype; ESBL-Extended Spectrum β-lactamases; CMTs- Complex mutant TEMs phenotypes; pAmpC-plasmid-mediated ampicillin β-lactamases phenotypes; b-appearance of zones of synergy between a given cephalosporin and amoxicillin-clavulanic acid(AMC); None-Isolate that was susceptible to a given class of antibiotics.

4. 7 Detection of selected *bla* genes among the *E.coli* isolates analyzed

Among all the 323 *E.coli* isolates analyzed, 37% tested positive for bla_{TEM} and 25% for bla_{SHV}. Other advanced classes of β-lactamase genes such as $bla_{\text{CTX-M}}$ and bla_{OXA}

accounted for 12% and 7% respectively. A high proportion (91%) of ESBLs isolates were obtained from individuals working in middle-class hotels, whereas 9% were isolates obtained from participants working in high-class hotels. In contrast, none of the isolates from those working in low-class hotels exhibited the classical ESBL phenotype, **Plate 4-1; Plate 4-2**.

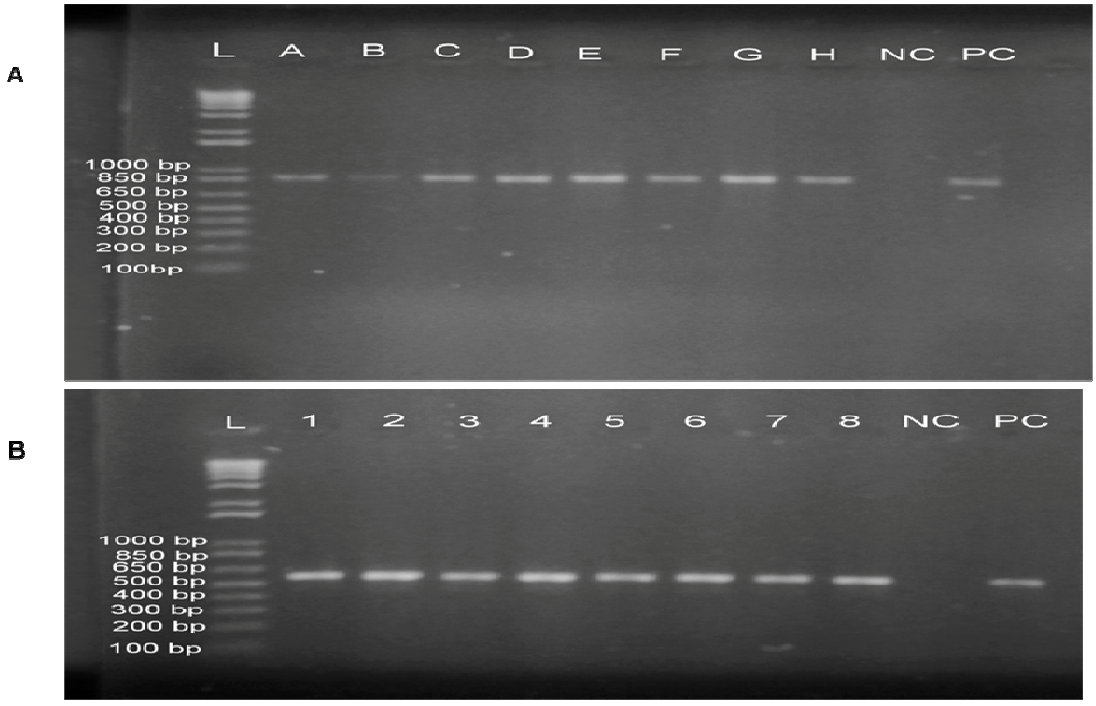

Plate 4-1: Electrophoresis gel results for *bla*$_{\text{TEM}}$ and *bla*$_{CTX\text{-}M}$ genes.

Plate A: *bla*$_{TEM}$ gene (999 bp); **Plate B:** *bla*$_{CTX\text{-}M}$ gene (593 bp); L-Molecular Ladder; NC-Negative Control; PC-Positive Control**;** bp-base pairs.

*Numbers at the top represent random DNA numbers of the isolates.

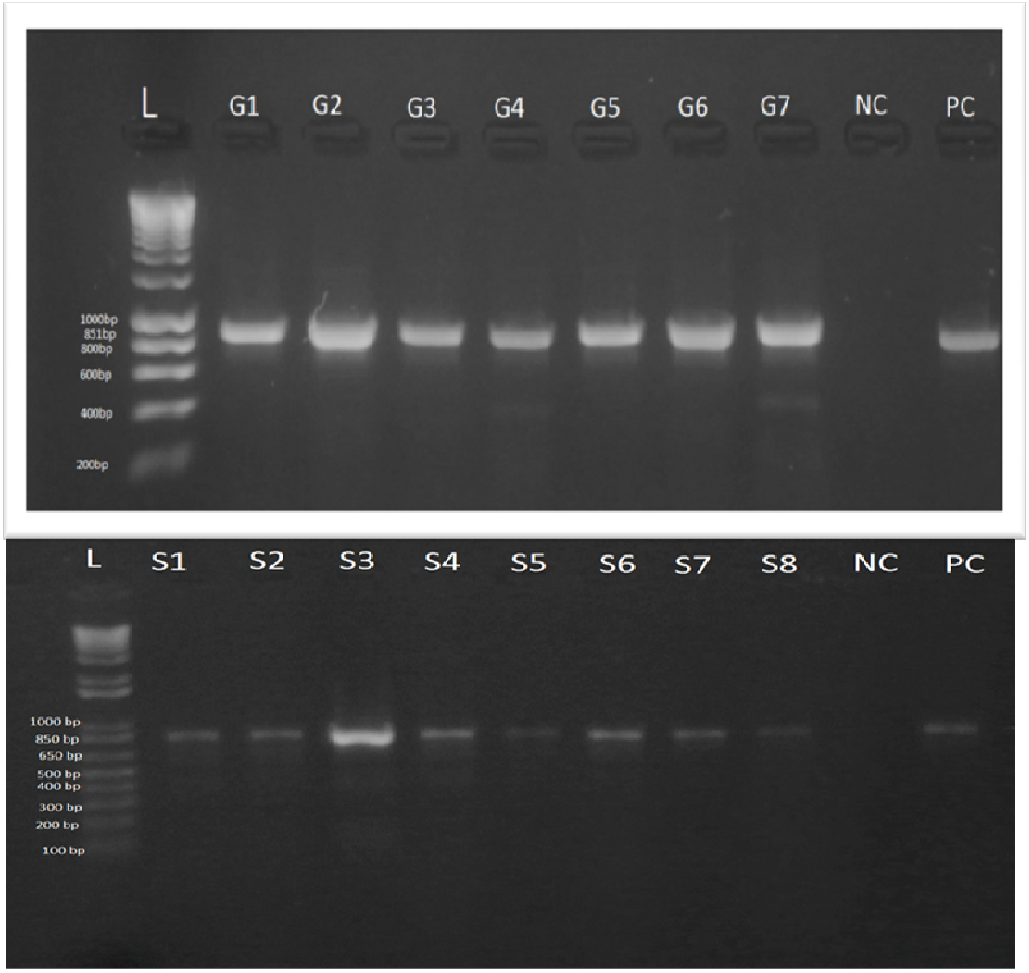

Plate 4-2: Electrophoresis gel results for *bla*$_{SHV}$ and *bla*$_{OXA}$ genes.

Plate C: *bla*$_{SHV}$ gene (851 bp); **Plate D:** *bla*$_{OXA}$ gene (820bp); L-Molecular Ladder; NC-Negative Control; PC-Positive Control; bp-base pairs.

*Numbers at the top represent random DNA numbers of the isolates.

4.8 Possible factors associated with carriage of resistant *E.coli* isolates among apparently healthy food-handlers

The study found that among those sampled, majority 177(55%) of the food-handlers revealed that they regularly buy medications (antibiotics) or use alternative medicines when sick without consulting a clinician (self-medication). Of these, 35% of the isolates obtained from self-medicating individuals were MDRs. High resistances were recorded against Sulfamethoxazole/Trimethoprim (SXT, 87%), Ampicillin (AMP, 51%), Streptomycin (S, 48%), Tetracycline (TE,50%) and Amoxicillin-Clavulanic acid (AMC, 4%) from isolates obtained from self-medicating individuals.

Another 29(9%) of those recruited indicated that they did not complete the prescribed antibiotic dosage when sick as required. Of these, 28% of isolates exhibited the MDR phenotype and the isolate showing resistance to imipenem (a carbapenem) was from this category of participants who did not complete their dose. Moreover, 65% of the participants had no valid medical license to operate as food-handlers within the capital, whereas 29% had not received any formal training regarding food hygiene and safety. Isolates from these categories of participants showed resistances towards otherwise potent antimicrobials such as third generation cephalosporins (Cefotaxime and Ceftazidime); Cephamycin, Amoxicillin-Clavulanic acid, Aztreonam and Ciprofloxacin, **Figure 4.16.**

Statistically, there were no significant differences observed in the resistance patterns for third generation cephalosporins (CTX and CAZ) among individuals who self-medicated versus those who did not (p: 0.287; OR 0.542; CI 0.183-1.613) or between those who completed their dosage and those who did not (p: 0.396; OR 0.505; CI 0.123-2.388). Moreover, there were no significant differences observed in those who had valid medical license versus those who lacked it (p: 0.080; OR 2.458; CI 0.812-7.294) or between those who had been trained and those who were not trained (p: 1.000; OR 1.081; CI 0.361-3.363) in terms of carriage of MDR strains.

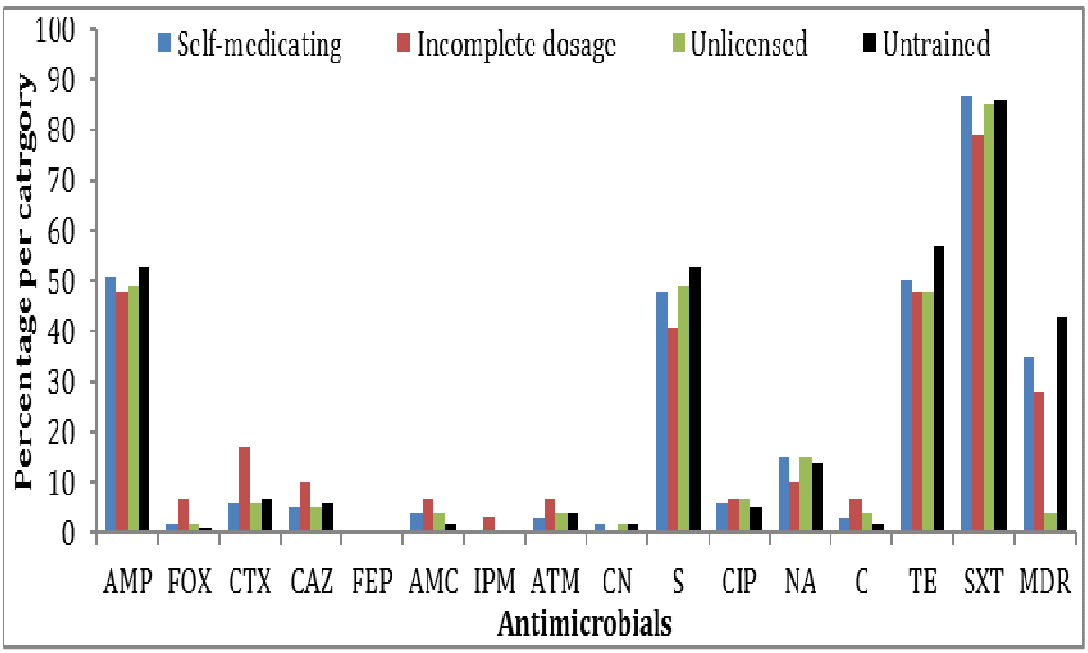

Figure 4.16: Common factors associated with carriage of resistant *E. coli* isolates among apparently healthy food-handlers working within Nairobi County on selected antibiotics.

*AMP-Ampicillin; FOX-Cefoxitin; CTX-Cefotaxime; CAZ-Ceftazidime; FEP-Cefepime; AMC-Amoxicillin-Clavulanic acid; IPM-Imipenem; ATM-Aztreonam; CN-Gentamicin;NA-Nalidixicacid;S-Streptomicin;SXT-Sulfamethoxazole/Trimethoprim;CIP-Ciprofloxacin;Chloramphenicol; TE-Tetracycline.

4.9 Genetic relatedness of *E.coli* isolates

The results showed that at 65% phenotypic similarity level, all the thirty (30) randomly selected *E.coli* isolates analyzed fit into 11 sub-clusters. Isolates from clusters 1 and 2 had near homogenous characteristics including resistance phenotypes, the type of hotel, gender, education level, clinical and location of the source participant. The two sub-clusters comprised of MDR strains, 60% of which were isolated from young females (<30years of age) who had a history of self-medication. Majority (80%) of isolates in these two sub-clusters were obtained from people who had a secondary education and were only obtained from people working in middle-class hotels. In contrast, isolates making sub-cluster 3-6 were of heterogeneous characteristics in terms of type of hotel

the participant worked in, their gender, the level of education, and even clinical profile of the source participant. Isolates in these four sub-clusters were distributed almost equally among people of either gender, and were not restricted by the level of education of the source, or the type of hotel they worked in. Clinical history and self-medication did not influence the clustering in this sub-group.

Sub-clusters 7-11 comprised of 86% of isolates from persons working in the middle-class hotels and majority (67%) of these strains were isolated from people who had a tertiary education. About 50% of isolates were from individuals residing within the Eastern region of Nairobi while the rest came from people living in other areas of the county, **Figure 4.17**.

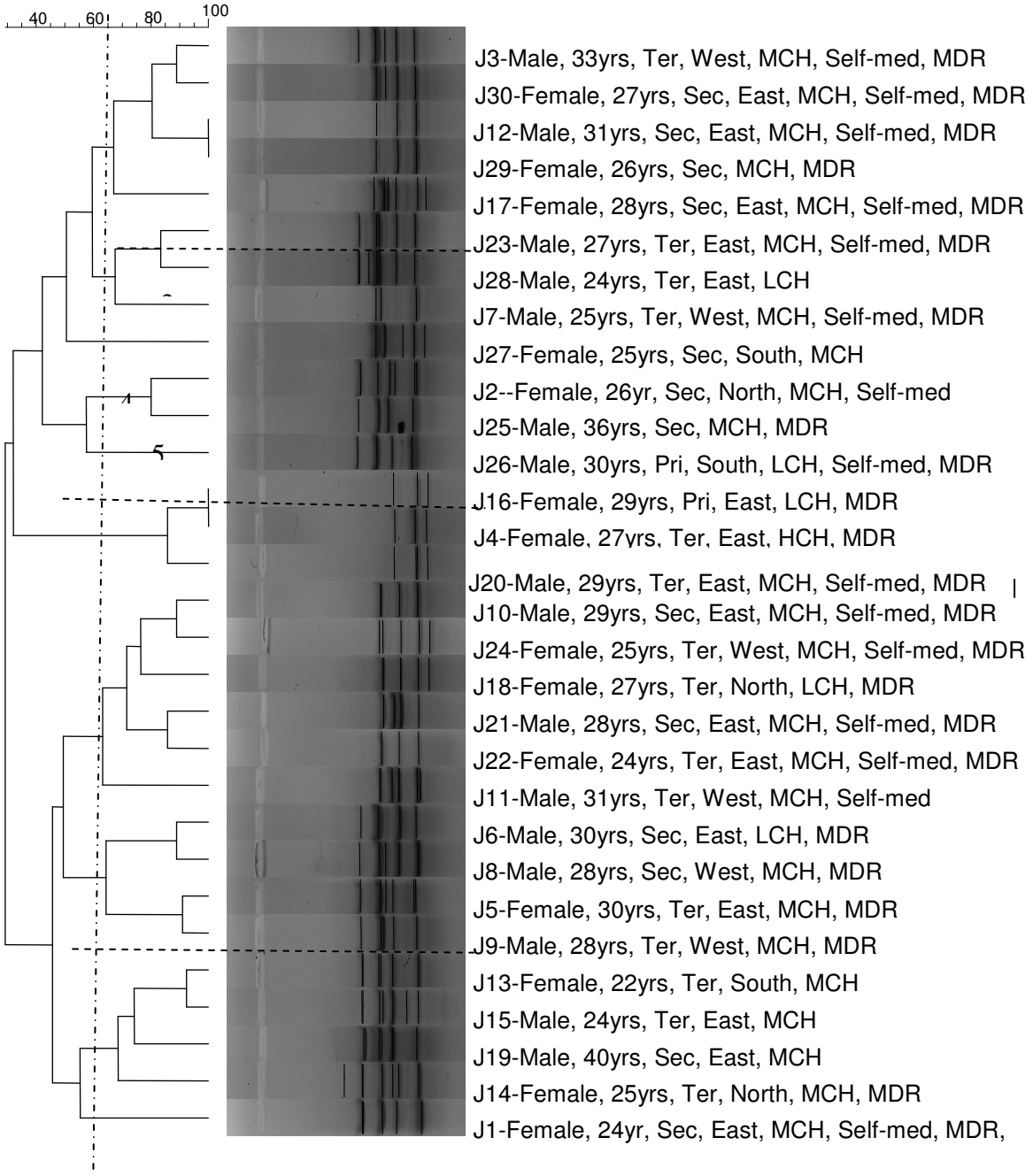

Figure 4.17: Dendrogram showing the demographic and the resistance profiles of *E.coli* isolates recovered from apparently healthy foodhandlers working in different food establishments within Nairobi County.

*MDR-Multi drug resistant isolates; Pri-Primary level of education; Sec-Secondary level of education; Ter-Tertiary level of education; LCH-Low-Class Hotels; MCH-Middle-Class Hotels; HCH-High-Class Hotel. Geographically the participants working within the capital were conveniently grouped as per their area of residence/locations (East, West, North or South).

CHAPTER FIVE

DISCUSSION

5.1 Introduction

Food-handlers in urban settings are important players in the health sector because they handle food consumed by more than 50% of the urban population. Whether illegally in the job market system or not, unhygienic or sick food handlers can be a potential public health risk to the unsuspecting clients who consume foodstuffs prepared by them and which may culminate into severe outbreaks resulting in high proportions of morbidity and mortality rates.

5.2 Social demographics of the study participants

The demographics of those surveyed indicated that there was near parity in the numbers of participants based on gender with males and females accounting for 51% and 49% respectively, a clear indication that public eating places in Nairobi County are not predominantly an occupation of one gender only. However, this study contradicts similar studies by Musa and Akande in 2003 and Santos *et al.,* (2008) conducted in Nigeria and Portugal in which both showed that majority (>90%) of the hospitality industry workers were women. This suggests that strategies to curb disease transmission through the food chain should focus on both genders in Kenya.

A large proportion (75%) of the participant recruited in this study was between 18-30 years of age. This contradicts a study done by Akabanda *et al.,* (2017) in Ghana which indicated that majority of food-handlers were above the age of 30. These results suggest that young individuals are the key driving force in the hospitality industry within the capital. Since hygienic and clinical demographics may be largely be affected by gender and age, new strateties should be put in place to educate the relatively young workforce in the food handling business in Nairobi.

The literacy level among food-handlers was high in that 97% had at least a secondary education. These findings were in contrast to a similar study done by Prabhu and Shah, (2014) in Maharashtra, India which observed that close to 90% of the food-handlers were illiterate. It has been documented that individuals with no or low literacy levels are more negligent especially on basic hygienic practices thereby increasing the possibility of food contamination due to unhygienic practices which may culminate into severe foodborne illnesses in food establishments (WHO, 2002). However, other studies showed that improved knowledge in food hygiene practices does not necessarily result in the required transformation in food handling behavior (Howes *et al.,* 1996).

In Kenya, food-handlers are required to undergo medical examinations after which they are issued with a certificate in order to work in the hospitality industry. In this study, neither *Salmonella* nor *Shigella* was isolated from fecal samples, only *E.coli*. This could be attributed to the fact that *E.coli* is a normal flora in the gastrointestinal tract, and also the study participants were apparently healthy individuals who majorly did not present any clinical signs of disease. These findings are in agreement with a similar study conducted by Kusolsuk *et al*., (2008) in Thailand among food-handlers working in tourist hotels and institutional cafeterias which reported that neither *Salmonella* nor *Shigella* species was isolated from the study participants.

However, it differs from other related studies conducted among food-handlers. For instance, a study conducted by Mama and Alemu, (2016) in South of Ethiopia among food-handlers in a learning institution (Arba Minch University) reported that *Salmonella* and *Shigella* accounted for 6.9% and 3% respectively of all bacterial isolates obtained. Whereas, Omemu and Oloyede, (2014) reported that *Salmonella* species was isolated in 5.5% of the food-handlers working in small businesses in an urban area of Abeokuta, Nigeria. This disparity in findings may be due to differences in their personal hygiene, knowledge and practice of food hygiene or the health status of the food-handlers.

The study noted that 65% of those recruited did not have a food handler's certificate. These findings concur to those reported by Assefa *et al.,* (2015) in Ethiopia and Okojie *et al*., (2005) in Nigeria which showed that high proportions of the interviewed food-handlers lacked medical certification thereby increasing the chances of lateral transmission of foodborne infections to the clients since their clinical health status are unknown. This study also found a number of characteristics among handlers that could potentially increase the possibility of disease transmission. Such practices included lack of training on proper food hygiene and safety (29%) and poor personal hygiene (e.g. not washing hands after visiting the toilet or when handling foodstuffs).

In terms of personal hygiene, about 22% of the study participants revealed that they did not wash their hands regularly while handling or preparing foodstuffs, further increasing the possibilities of food contamination and transmission of foodborne infections due to poor personal hygiene. Related studies conducted among food handlers in Nigeria and Iranian food-handlers which found that food handlers with poor personal hygiene such as not washing their hands especially after visiting the toilet pose the risk of carrying high loads of microbes such as *E. coli* and *S. aureus* on their hands thereby increasing the chances of food contamination and subsequent outbreak of food-related illnesses (Shojoei *et al.,* 2006; Okojie *et al.,* 2005).

Another study conducted in USA suggested that poor personal hygiene and improper food handling practices contributed to approximately 97% of all foodborne illnesses in food-service establishments and homes (Howes *et al.,* 1996). Therefore based on these findings, it is evident that training and health education on good food and personal hygiene practices among food handlers is critical in minimizing the possible risks of food contamination with pathogenic strains.

This study also noted that 24% of the hotels the participants worked in did not have a cold-storage facility such as a fridge to preserve foodstuffs under cold temperatures so as to minimise food spoilage. Lack of a proper cold chain has previously been identified as a risk for accelerating of foodborne infections/diseases in hotels and related facilities (FDA, 2015; Kadariya *et al*., 2014). A study conducted in Italy revealed that lack of knowledge regarding the right temperature for food storage as one of the key factors that increase the likelihood of contracting a foodborne illness (Angellilo *et al.,* 2001). It is therefore possible that lack of proper cold chain facilities may accelerate the proliferation of microorganism culminating in foodborne outbreaks that could lead to illness or death of victims.

Several studies have reported that in most developing countries, lack of access to safe treated water, inadequate sanitation or unhygienic practices are some of the major risks for diarrheal illnesses especially among young children (Ogunleye *et al*., 2013). The current study revealed that 24% of the hotels did not have continued supply of piped municipal water due to either water shortages or rationing hence purchased water from water vendors who did not disclose the source of the water they sold or whether the water was treated or not. This raises the possibility of occurrence of water-related outbreaks such as cholera, typhoid and bacillary dysentery among these food establishments. Therefore, these results suggest the need to increase access to potable water to the hotels as well as to the residence of the workers so as to minimize the chances of occurrence of water-borne diseases. All these characteristics involving poor personal hygiene, lack of proper food storage facilities and inaccessible potable water have been found to predispose food handlers to carriage and transmission of pathogens (Assefa *et al.,* 2015).

5.3 Antimicrobial susceptibility profiles and genotypic characterization of *E.coli* isolates

This study revealed that a high proportion (>40%) of the *E.coli* isolates were resistant to Ampicillin (AMP), Sulfamethoxazole-Trimethoprim (SXT), Streptomycin (S), and Tetracycline (TE) but low resistances of between <1% and 3% were recorded towards Imipenem (carbapenems), Cefoxitin (cephamycin), Cefepime (a fourth generation cephalosporin) and Gentamicin (an aminoglycoside). These results are in agreement to those published in a related study conducted in Kenya among food-handlers working in luxurious tourist hotels which noted that clinical *E.coli* were more resistant to Ampicillin (>40%) and Sulfamethoxazole-Trimethoprim (>50%) (Onyango *et al.,* 2009). It appears that the resistances values to these antimicrobials among *E. coli* isolates from Kenya are higher than those recorded in developed countries like Japan (Bii *et al*., 2005). These differences may be as a result of the difference in antimicrobials use practices and probably, the difference in genetic diversity of circulating strains (Sonda *et al* 2016; Vialle-Valentin *et al.,* 2012).

In order to demonstrate the spectrum of resistance category for each of the antimicrobial, the sizes of antimicrobial inhibition zones for each strain was measured and a frequency graph generated. Such distribution curves provide an insight on possible future trends of resistance. For example, resistance to SXT was observed in >65% of isolates and zone distribution frequencies confirmed that majority of the resistant strains clustered within the zone of extreme resistance (6mm-10mm) as demonstrated in **Figure 4.4B.**

Thus, it is evident that antimicrobials such as AMP and SXT may no longer be effective against strains circulating among these apparently healthy participants and would therefore not find significant application in the treatment of clinical infections arising from such strains. This is particularly worrying because SXT is heavily relied upon as a prophylaxis against opportunistic infections among individuals infected with HIV in

Kenya (Malwal *et al.,* 2016). With increased travel and globalization, there is a possibility of dissemination of such resistant strains to even other countries.

Among aminoglycosides, Gentamicin was more potent than Streptomycin because only one (1) isolate clustered within the resistant zone range of 6 mm compared to 45 isolates for Streptomycin. Aminoglycosides are an important class of antimicrobials that may be used together with β-lactams and also with fluoroquinolones aganist invasive strains. Most aminoglycosides are adminstered through injections and may therefore be protected against the degradative effect of the GIT as is common with other antimicrobials administered orally. Due to their mode of administration, it is more unlikely that this class of antimicrobials are misused and are therefore more amenable for treatment of hospitalized patients especially those in ICU.

Moreover, among the quinolones, resistance to Ciprofloxacin was low in that only 3% of the isolates clustered within the resistant zone range of 6 mm compared to 7% for Nalidixic acid. Based on these results it is evident that both Ciprofloxacin and Gentamicin are still potent antimicrobials as replacer antimicrobials for treatment of infections caused by ESBL producers and majority of MDR strains. The apparent increase in fluoroquinolone-resistant strains in Kenya and the world could be attributed to its wide use of these antimicrobial against ESBL-producers and in the treatment of UTIs and respiratory infections. Other studies have hinted that overuse of fluoroquinolones in human and veterinary medicine could also breed resistance (Webber, 2001).

Based on the resistance profiles of the isolates investigated, 3.4% of all isolates were ESBL-producers and majority of such strains were encountered among middle-class hotels. Another 4.3% of isolates were also resistant to Amoxicillin-Clavulanic acid (a β-lactamase inhibitor) heavily relied upon for treatment of ESBL-producers and a further 1% of isolates recovered from individuals working in middle-class hotels showed resistances to a combination of some of the most potent antimicrobials (third generation

cephalosporins, Ciprofloxacin and Gentamicin). About 30% of isolates were MDR, but only 5% and <1% were resistant to cephamycins and carbapenems respectively. Though these percentages are low, it is worrying to note that apparently healthy food-handlers carry resistance to these high-end β-lactams thus a clear indication that their proliferation is only set to increase the challenge of access to the already existing empiric therapeutic options. Resistance to cephamycins is normally attributed to the increase in the prevelance of AmpC genes especially through CMY-2 β-lactamases (Philippon *et al*., 2002).

Recently, other studies by Nakane *et al*., (2016) and Zhang *et al.,* (2015) reported a high prevalence of ESBL-producing *E. coli* among healthy individuals in Southeast Asia, including China (65.0%) and a range of between 58.2%–69.3% in Thailand (Sasaki *et al.,* 2010), 51% in Vietnam (Le *et al.,* 2015) and also among healthy tourists traveling from Netherlands to East and South Asia (Paltansing *et al.,* 2013). This global increase in the prevalence of ESBL-producing strains is particularly worrisome and this situation might be accelerated by globalization, tourism and/or migration of individuals who are likely to aid in the spread of these resistant strains worldwide making the containment of such strains difficult especially in hospital and community settings.

In Kenya, studies have reported on the increasing antimicrobial resistant pattern among food-handlers and yet few mitigation actions have been undertaken to curb this situation despite being one of the critical areas of public health concern since majority of the urban population consume foods made outside their homes (Onyango *et al.,* 2009; Hussein *et al*., 2007). As expected, majority (37%) of the *E.coli* isolates tested positive for bla_{TEM} (a NSBL) but were negative for other advanced classes of β-lactamases such as $bla_{\text{CTX-M}}$ and the bla_{OXA}. This $bla_{\text{TEM-1}}$ encodes for β- lactamases responsible for up to 90% of ampicillin and penicillin resistance in *E. coli* as well as resistance in *H. influenza*, *N. gonorrhoea* and *K. pneumoniae* and it is the most commonly encountered β-lactamase gene among gram-negative bacteria (Kiiru *et al.,* 2012).

The other NSBLs gene SulfHydryl Variable β-lactamases (*bla*$_{SHV}$) accounted for 25% of all the isolates analyzed. The *SHV*-1 β-lactamases are most commonly found in *K. pneumoniae* and is responsible for up to 20% of the plasmid-mediated ampicillin resistance among *Enterobacteriaceae* species. Thus, there is dare need to closely monitor the spread of NSBLs among this apparently healthy population since mutations arising from the derivatives of NSBLs are responsible for the exponential increase in antimicrobial resistance worldwide.

Although sequencing experiments of the selected genes detected was beyond the scope of the current study, it was most likely that the CTX-M gene types are implicated in the occurrence of the classical ESBL phenotype (12%). This possibility arises due to the fact that majority of the ESBL isolates were resistant to Cefotaxime, a known indicator for carriage of *bla*$_{CTX-M}$ genes. Moreover, among the CTX-M genes, the *bla*$_{CTX-M-15}$ gene is the most important because is associated with the globally disseminated multi-drug resistant clone of *E. coli* ST131 strains frequently associated with urinary tract and bloodstream infections in both community and clinical settings (D'Andrea *et al.,* 2013; Coque *et al.,* 2008).

Many ST131 strains exhibit resistance to multiple antimicrobials with key resistance to fluoroquinolones (Price *et al*., 2013). Based on the current results, there is a basis to suspect that some of the isolates carrying the *bla*$_{CTX-M}$ and exhibiting concomitant resistance to fluoroquinolones are members of this clonal complex because resistance to fluoroquinolones with or without the production of ESBL is the primary indicator of members associated with this sequence type (Johnson *et al.,* 2013; Price *et al*., 2013). Previous studies in Kenya have also reported ST131 strains bearing the *bla*$_{CTX-M-15}$ and/or Ciprofloxacin resistance among clinical isolates from hospitalized and non-hospitalized patients (Kiiru *et al.,* 2012). Thus, these strains are likely to present drastic negative health implications in both community and hospital settings since infections arising from these strains are associated with high probabilities of treatment failure.

The inhibitor-resistant TEM phenotype (IRT phenotype) was only recorded in 3(<1%) isolates. Whereas advanced β-lactamase phenotypes (pAmpC and CMT) were recorded in less than 2% of the isolates. The study suggests that the resistance observed towards AMC in this study is largely attributable to carriage of $bla_{\text{OXA-}1}$ gene. This hypothesis is partially based on findings from a study conducted in Kenya that described $bla_{OXA\text{-}1}$ enzymes in *Salmonella* strains that contain promoter mutations and confer resistance to β-lactamase inhibitors (Boyle *et al.,* 2011). Amoxicillin-clavulanic acid (a β-lactamase inhibitor) is one of the commonly used antimicrobials for the treatment of infections caused by ESBL-producers and any increase in the resistance due to a combination of ESBL and the OXA-1 enzymes would jeopardize the clinical significance of this antimicrobial.

The current findings on various β-lactamase phenotypes among apparently healthy food-handlers vary from those reported by Kiiru *et al.,* (2012) among hospitalised and non-hospitalised patients which found that the NSBLs and ESBL phenotypes accounted for 30.5% and 27% respectively, whereas advanced β-lactamase phenotypes (CMT, IRT and pAmpC) accounted for 24%, 8% and 10% respectively. This disparity in findings suggests that clinical isolates are more likely to be more resistant than those obtained from healthy participants probably due to a stronger selective pressure associated with the hospital environment.

Resistance of pathogenic organisms to countenance antibiotics has become a worldwide problem with serious consequences on the treatment of infectious diseases. The heightened use/misuse of antibiotics in human medicine, agriculture and veterinary is primarily contributing to the phenomenon (Chantziaras *et al*., 2013). Some of the risky behaviors observed in this study population that may favor emergence of resistant strains included self-medication and accessing antibiotics over the counter without a prescription among 55% of those interviewed and not completing the prescribed required dosage in 11%. These are known key risk factors for the emergence of antimicrobial resistance as well as colonisation with MDR strains (Vialle-Valentin *et al.,*

2012). Findings from this study are in agreement with other related studies that found out that in African settings over-the-counter sale of antimicrobials or self-medication, consumption of counterfeit drugs, improper dosage and non-adherence are very common and that such malpractices fuel the rate of the emergence and spread of resistance to commonly prescribed antimicrobials (Sonda *et al.,* 2016; Kimang'a, 2012).

Although self-medication and not completing the prescribed dosage alone may not explain the high resistances observed in this population, other confounding factors in conjunction with indiscriminate antimicrobial usage may be responsible for the emergence and spread of resistant strains among the study participants. All these clinical characteristics and poor health-seeking behaviour have the potential to create a strong antimicrobial selection pressure that would, in turn, promote emergence and spread of resistant strains. These observed resistances are likely emerging and spreading possibly due to the over reliance of these antimicrobials to treat different infections without adequate laboratory confirmation, a situation brought about by the lack of enforcement of the policies pertaining to antibiotic usage in the country. It is also possible that the practice of issuance of antibiotics without prescription, lack of culture and susceptibility testing and other yet to be identified factors may be driving the buildup of selective pressure that favor proliferation of these strains resistant to β-lactams.

Based on the resistant profiles observed, potent antimicrobials such as cephalosporins and carbapenems are the only tenable antimicrobials that can be used to treat infections arising from such strains. However, these antimicrobials are costly and are not readily available in most resource-constrained countries. The data from this study is already worrying because one of the isolates obtained from an individual working in middle-class hotel and who had a history of not completing the prescribed dosage was resistant to carbapenem. Thus, the study speculates that if such strains continue to increase among apparently healthy individuals bearing in mind that carbapenems are the last line defense antimicrobials against severe infections, then the buildup of selective pressure to these antimicrobials would have drastic negative health implications in both community and

hospital settings since infections arising from these strains are associated with high probabilities of treatment failure and mortality rates (Kempf *et al*., 2012; Sosa *et al*., 2010).

5.4 Genetic relatedness of *E.coli* isolates

Genetic analysis using the $(GTG)_5$ technique suggested that isolates from this study clustered based on medication history and practices of the participants (especially those who self-medicate) and the type of hotel the individual worked in. The study also demonstrated that *E.coli* isolates recovered from individuals working in middle-class hotels were more likely to cluster together indicating close genetic relatedness. Although the $(GTG)_5$ typing may not be as robust as Multilocus sequence typing (MLST-typing), these results suggest a likelihood of cross-transfer of clonally related strains among people working in the middle-class hotel facilities. There is also a likelihood that some clones are expanding among these apparently healthy subjects who work in close quarters and come into contact with a large population of patrons who may carry such strains including the MDR strains to their families. Thus, there is need to monitor the clonal expansion of these strains among these hotels in order to minimize their spread among these apparently healthy individuals working in food establishments as well as to preserve the efficacy of the β-lactams and carbapenems.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

1. This study demonstrated that a significant proportion of apparently healthy food-handlers do not only carry MDR strains but also strains that are already resistant to most important classes of antimicrobials such as carbapenems, fourth generation cephalosporins, fluoroquinolones and aminoglycosides. Although the proportion of strains resistant to Ciprofloxacin, cephamycin (Cefoxitin) and carbapenem (Imipenem) was still low, there is a possibility that resistance to these antimicrobials may increase in the near future if their gross misuse continues or their over-reliance in treatment of infections.
2. Although the proportion of isolates exhibiting the ESBL phenotype was 11(3.4%), this prevalence of ESBL-producing strains in these apparently healthy individuals is particularly worrisome and this may make the containment of such strains difficult especially if they find their way into hospital and community settings.
3. The heightened use/misuse of antibiotics among the study participants may accelerate the spread of NSBLs among this apparently healthy population. Moreover, mutations arising from the derivatives of NSBLs may lead to an exponential increase in antimicrobial resistance worldwide due to production of complex β-lactamase phenotypes such as CMT, IRT and pAmpC which may downgrade the efficacy of β-lactams as therapeutic agents.
4. The study also found a possible relationship of carriage of resistant strains with a history of self-medicating, not adhering to prescription requirements (not completing the required dosage) and working without medical certification. Poor personal hygiene, self-medicating, poor storage mechanisms of foods, lack of potable water to prepare food and not complying with certification requirements

due to negligence are some of the practices that are likely to promote the exchange of isolates including the pathogens, which may result in the onset of severe outbreaks among these hotels.

5. Genetic cluster analysis of isolates in this study suggested a possible exchange of strains between those working in middle-class hotels. While advanced techniques are recommended to ascertain whether these exchanges are due to clonal expansion or not, since if such strains gain resistance determinants, then the potential for an increase in the proportion of resistant strains is very high in the near future and their subsequent dissemination to the general public is inevitable.

6.2 Recommendations

1. This study advocates that treatment of all individuals should be guided by proper laboratory investigations and prescriptions should only be issued after confirmation of the causative agent by laboratory reports. This will help in curbing the common practice of self-medication.
2. The study also recommends that efforts be made to ensure that all food-handlers have access to potable water for both personal and hotel use and proper food hygiene practices such as handwashing and use of personal protective equipment when handling foodstuffs should be encouraged. It is also important to ensure that all workers are examined appropriately and regularly before certification, and that infected workers should not handle food until the infection clears. This will help in minimising the chances of lateral transmission of infectious agents to fellow hospitality employees and cross contamination of foods.
3. Finally, the study recommends that advanced techniques such as whole genome sequencing be used in future studies to elucidate the genetic and population structure of MDR strains circulating among food-handlers in the country and special emphasis should be put on MDRs, ESBLs and carbapenem resistant strains which are capable of down grading even the most current potent antimicrobials.

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APPENDICES

Appendix I: Ethical approval

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI - Kenya
Tel: (254) (020) 2722541, 254 (020) 2713349, 0722-205901, 0733-400003 Fax (254) (020) 2720030
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KEMRI/RES/7/3/1 **August 12, 2015**

TO: **JUMA ARNOLD,**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DIRECTOR, CMR,**
NAIROBI

Forwarded 19/8/15

Dear Sir,

RE: **KEMRI/SERU/CMR/P0018/3084 (*RESUBMITTED INITIAL SUBMISSION*): ANTIMICROBIAL SUSCEPTIBILITY PROFILES AND GENOTYPIC CHARACTERIZATION OF SELECTED ENTROBACTERIACEAE STRAINS ISOLATED FROM FOOD HANDLERS IN NAIROBI COUNTY.**

Reference is made to your letter dated 28th July, 2015. KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on August 03, 2015.

This is to inform you that the Committee notes that the issues raised during the 240th C meeting of the KEMRI/Ethics Review Committee (ERC) held on 25th June, 2015 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **12th August, 2015** for a period of one year. Please note that authorization to conduct this study will automatically expire on **August 11, 2016.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **June 30, 2016**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

FOR

PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

In Search of Better Health

Appendix II : Consent seeking and information form for study participants

Title of Research: Antimicrobial susceptibility profiles and genotypic characterization of selected *Enterobacteriaeaceae* strains obtained from food handlers in Nairobi County, Kenya

Investigators: Juma Onani JKUAT, Dr Kiiru-CMR-KEMRI, Dr Ngugi, JKUAT

Purpose of the study

We aim to obtain information on molecular basis of antimicrobial resistance and factors associated with carriage of ESBL-producers among selected Enterobacteriaceae species obtained from food-handlers in Nairobi County. We would like to obtain a stool sample (approximately a spoonful) from participants in order to culture and isolate DNA (the chemical basis of life) from which we will analyze for genes (information that define the characteristics inherited from parents) related to this resistance. By analyzing these genes, we will establish the molecular basis of antimicrobial resistance among the food handlers within Nairobi County. In doing so, we will be able to get information that will help us develop better ways for treating and preventing the spread of multidrug resistant strains. The study will recruit adult food handlers aged above 18years working in food establishments within Nairobi County. The selection of food handlers as the target group is justified by the fact they handle food consumed by the public and may therefore contaminate these foods and pass pathogens to their clients. The stool sample will be collected by the participants and taken to KEMRI-CMR laboratory for culture, isolation and DNA extraction.

We are requesting you to consent (agree) to do the following:

To allow us interview you concerning information that can help us determine factors associated with carriage of antimicrobial resistant enteric bacteria among food handlers.

To provide us with stool sample for laboratory analysis. We would also like to disclose to you the following:

That the process of obtaining stool will cause a little embarrassment and discomfort.

That there are no monetary gains for participating in this study.

That there are no penalties for declining participation in this study.

That you can withdrawal from this study at any time.

That you may decline to answer any of our questions.

Your part in the research

The participant will be investigated for the presence of antimicrobial resistant enteric bacteria as well as factors associated with their spread. In order to investigate this, we ask you to provide us with a signed consent. Once you grant us the consent, you will be requested to provide us with less than a tea spoonful of your stool specimen for laboratory testing.

What additional procedures will there be for participants who help with research?

In order to collect stool specimen the participants will collect their own specimen after clear explanation from the Principal Investigator**.** The stool samples will be collected in a sterile wide mouthed universal container with a tightly fitting lid. The stool must not be contaminated with urine. The bottle will be labeled for specimen identification and then be taken to the laboratory for examination within two hours.

Risks

Some of the anticipated risk will include some little discomfort and embarrassment while in the process of collecting the stool sample as well as time consumption and delays due to answering the questionnaire

What will happen after the study?

The data obtained from this study will highlight the emerging threat of antimicrobial resistant enteric bacteria among food handlers and this will form a basis for decision making, policy formulation and planning towards the prevention and control of enteric infections in Kenya.

Your benefit in the study

There are no monetary gains for participating in this study. In case the study finds relevant resistant strains of *E.coli, Salmonella* or *Shigella* in participants, such participants will be contacted and together with resident doctor given guidance on proper medication in order to prevent the spread of resistant enteric bacteria.

What if I change my mind about helping with this research?

If you agree to participate in this study and later change your mind, you are free to withdraw at any time. You will not be discriminated against in any way in the future due to your decision to withdrawal or to decline to participate.

Who will read or hear about information collected from me?

The information collected from those who help with this research will be stored using codes so that each individual cannot be recognized. Coded information will be held on computers protected by passwords known to the research team only.

Do you have any questions that you would like me to answer now?

If you would like to know more details about the research or have any issues that needs to be discussed in the future you can contact any of the following people

Contact information

All the information and queries can be asked through the Principal investigator Juma Onani. Contacts are; **0728859611**. In case of any issues concerning your rights for participation you may contact; Secretary KEMRI/S.E.R.U P.O Box54840-00200, Nairobi; Telephone no; 020-2252541; 0722205901; 0733400003.

Consent Form

I confirm that I understand the information provided for the above study and have had the opportunity to ask questions. I understand that participation is voluntary and that I am free to withdraw from the study at any time without giving reasons, without my medical care or legal rights affected

Name of consenting adult: _____ Sign _____ Date _____

I certify that the above was explained verbally to me, and that I understand the nature and purpose of the study and consents to participate in the study. I have given them opportunity to ask questions which have been answered satisfactorily.

Witness' signature: _____ Date_____

Witness' name: _____

Signature or left thumbprint of participant: _____ Date: _____

Researcher's Signature _____ Date_____

Researcher's Name: _____

Appendix III: **Questionnaire**

Anonymous study on knowledge, attitude and practice of food handlers towards food-borne diseases and food safety

Kenya Medical Research Institute (KEMRI) is implementing a study on social demographical factors associated with foodborne illnesses due to enterics among food handlers in Nairobi to help plan better policies on their prevention and control. This study is strictly anonymous and voluntary. An independent scientific institute will analyze the information. Your personal results will not be disseminated to anyone.

Gender Male…….. Female……

How old are you……

Marital status Single…….

Married……..

Divorce……..

Others……..

Place of residence: …….

Level of education:

No formal education……

Primary school……

Secondary school…….

Diploma……

Degree……..

Do you have a valid Medical license to operate as a food handler in Nairobi County? Yes…….NO……

For how long have worked in food establishment?

<6 months…..

>6 months to 1 year……..

>1 year to 5 years……..

>5 years to 10 years……..

>10 years……

Type of premise: Five star hotel…….

Restaurant

Food stall/Kiosk

Others

Are you trained in Food hygiene and Safety? NO……. YES……

What do you use to wash hands with before handling foodstuffs?

Soap……

With Plain water only…….

Others…….

Do you have washrooms in your food establishments? YES……NO……..

When do you often wash hands? ……

After handling dirty dishes……

After visiting the washrooms……

Before serving food to customers……..Others……

Last episode of Diarrhea……

Last month….

Two weeks ago….

One week ago…..

Few days ago…

Others….

What caused the diarrhoea? .....

Food poisoning….

Contaminated water….

Don't know…

Others…..

Last time antibiotic was used? .....

Last Month…

Last week….

Few days ago….

Can't remember….

Where did you get the antibiotic from? ……

Bought from the local Chemist…

Health care facility….

Others…….

Was it a doctor's prescription? YES…….NO…….

How long was antibiotic used? .....

Till the dosage was complete……

For a few days and stopped after feeling well…

Others…

Did you finish the antibiotic treatment? YES.....NO.....

Which is your main source of water?

Nairobi Water Company……

Dug Boreholes……..

Others.....

Do you experience water shortage/rations? YES.....NO…….

How frequent is the water shortage? ……

No water at all…

Once in a month….

No water shortage

Others….

What other alternatives of water supply do you have? .....

Stored water in tanks….

Purchase from water vendors……

No other alternatives…..

Others…….

What storage conditions do you store your foods? .....

In Fridges…

In Containers…

Others…

How is food leftovers disposed in your establishment?

In dust bins…

Disposing in a designated area…

Others…….

How often do you wash your apron?

Once a week…

Daily…..

Others…

Appendix III: Procedures

Citrate test

Citrate utilization test is commonly used to distinguish between members of the Enterobacteriaceae family based on their metabolic by-products. Citrate utilization test is used to determine the ability of bacteria to utilize sodium citrate as its only carbon source. Simmons Citrate Agar was inoculated lightly on the slant by touching the tip of a needle to a colony then incubated at 37^{o}C for 18-24 hours. Then media was observed for the development of blue color; denoting alkalinization. *Escherichia coli* isolates tested negative since the media retained its original colour.

Triple Sugar Iron Test

The Triple Sugar Iron or TSI test is a microbiological test roughly named for its ability to test microorganism's ability to ferment sugars and to produce hydrogen sulfide. A sterile needle was used to pick a pure isolates from an 18-24 hour culture a single colony which was used to stab into the medium up to the butt of the TSI tube, and then streaked on the slant and was incubated at 37^{o}c for 18 to 24 hours and observed for hydrogen sulfide production, gas production and colour change in the media. The *E.coli* was presumably identified by the following reactions: Acid slant/ Acid butt (A/A); Yellow/Yellow with gas production but no production of hydrogen sulfide.

Urease Test

Urea is a diamide of carbonic acid. It is hydrolyzed with the release of ammonia and carbon dioxide. The urease enzyme is able to split urea in the presence of water to release ammonia and carbon dioxide. The ammonia combines with carbon dioxide and water to form ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow color to bright pink. The broth medium was inoculated with a loopful of a pure culture of the test organism and

incubated at 37 ^{o}C for 18-24 hours. Then the media was observed for colour change. *E.coli* tested negative since there was no colour change in the media

SIM (Sulfur, Indole Motility) Test

The formulation of SIM Medium is designed to allow the detection of sulfide production, indole formation and motility. The SIM Medium was inoculated by stabbing the center of the medium then incubated aerobically at 35ºC for 18-24 hours. H_2 S production and motility was observed in the medium before adding three drops of Kovacs Reagent. The formation of a red ring denotes a positive test. The *E.coli* isolates were negative for sulfur production but positive for both indole and motility.

DNA Extraction by Boiling Method

The heating block was set at a temperature of 95^{o}C then pure colonies for DNA isolation were Identified and labeled. Approximately 1ml of molecular grade water was added to 2 ml Eppendorf tubes and marked with a number corresponding to the isolate been analyzed. Using a sterile swab, a pea-sized amount of inoculum was scrapped from the culture and transferred to the corresponding tube. The tubes were then placed in the heating block and left to heat for a maximum of 12 minutes. The tubes were then transferred to a table-top centrifuge. The contents were centrifuged at maximum speed for 5-6 minutes. The supernatant was transferred to a clean vial and then stored at -80^{o}C.

Appendix V: Clinical and laboratory standards institute (2015) guidelines on Performance Standards for Antimicrobial Susceptibility Testing

	Interpretive Categories and Zone Diameter Breakpoints (nearest whole mm)		
Antibiotic	Sensitive	Intermediate	Resistant
Ampicilin (AMP,10µg)	≥17	14-16	≤13
Imipenem (IPM, 10µg)	≥23	20-22	≤19
Cefotaxime (CTX, 30µg),	≥26	23-25	≤22
Ceftazidime (CAZ, 30µg),	≥21	18-20	≤17
Aztreonam (ATM, 30µg),	≥21	18-20	≤17
Cefoxitin (FOX, 30µg),	≥18	15-17	≤14
Cefepime(FEP,30µg),	≥25	19-24	≤18
Amoxicillin-Clavulanic(AMC,30µg)	≥18	14-17	≤13
Ciprofloxacin(CIP,5µg)	≥21	16-20	≤15
Nalidixic acid(NA,30µg)	≥19	14-18	≤13
Tetracycline(TE,30µg),	≥15	12-14	≤11
Gentamicin(CN,10g)	≥15	13-14	≤12
Sulfamethoxazole/Trimethoprim (SXT,23.75µg/1.25µg),	≥16	11-15	≤10
Streptomicin(S,10µg)	≥15	12-14	≤11
Chloramphenicol (C, 30µg).	≥18	13-17	≤12