ANTIBIOTIC SUSCEPTIBILITY PROFILES OF *ESCHERICHIA COLI* ISOLATED FROM FOOD-HANDLERS IN NAIROBI, KENYA

By

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(HND)

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DECLARATION

This thesis is my original work and has not been presented for an award of degree or any other award in any other university.

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DEDICATION

I wish to dedicate this work to my supervisors at Kenyatta University (Dr. Rekha R. Sharma (Department of Ziological Sciences) and Dr. Michael F. Otieno (Department of Clinical Medicine), as well as Dr. Yeri Kombe at Kenya Medical Research Institute (KEMRI). I also wish to express my sincere gratitude to Dr Joseph Oundo and other staff at Centre for Microbiology Research (CMR) who provided laboratory facilities and guidance with some of the laboratory work. Lastly I thank my family for being patient while I studied.

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ABBREVIATIONS AND ACRONYMS

ARS	Antibiotic Resistance Surveillance
CBS	Centre for Bureau Statistics
CDC	Centre for Disease Control
CMR	Centre for Medical Research
EAEC	Enteroaggregative *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
FDA	Food and Drug Act
HB	High –Budgets
HBH	High-Budget Hotel
KEMRI	Kenya Medical Research Institute
MDR	Multiple Drug Resistance
MOH	Ministry Of Health
NTS	Non Typhoidal Salmonella
OTC	Over The Counter
ROK	Republic Of Kenya
WHO	World Health Organization
LB	Low Budget
LBH	Low Budget Hotel
TD	Traveller's Diarrhoea

DEFINITION OF TERMS

Drug resistance: Is a process where by a drug is rendered ineffective as a result of microbes changing in ways that reduce or eliminate the drug potency.

Food-handler: Is that person who gets into contact with food for commercial purposes in the process of preparing and serving it.

Hotel: These are eating houses whereby the premises are licensed to prepare and serve food for commercial purposes as well as providing other social amenities e.g. accommodation, conferences, etc.

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ABSTRACT

Antibiotic resistance represents a serious problem for clinicians, veterinarians, community and government at large. This involves cost implications as far as treatment is concerned. Multiple drug resistance as a result of empirical treatment have rendered most drugs ineffective.. In view of the above, this descriptive cross-sectional study, aimed at isolating resistant entero-bacteria *E. coli* from food-handlers working in both high and low budget hotels. Specimens in the form of stool were collected from 297 food-handlers in their respective hotels were considered and analyzed for resistant *E.coli* bacteria at KEMRI-Centre for Microbiological Research (CMR), in Nairobi. Besides, questionnaires were administered in order to assess health-seeking behaviour of the study subjects prior to medical examination. Antibiotics used in this study were amoxicillin-clavulanic acid, co-trimoxazole, ampicillin tetracycline, kanamycin, gentamycin, cefuroxime and chloramphenicol. These antibiotics were chosen on the basis of their use in the management of enteric bacterial infections. The results from high budget hotels (i.e. three, four and five stars) and low budget hotels (i. e two stars and below) indicates high frequency of resistance. The highest level of resistance in both hotel categories was seen in co-trimoxazole. 66.3% in high budget hotels and 66.4% in low budget hotels. Resistance difference between high budget hotels and low budget hotels was not statistically significant (P=0.56681). This was followed by ampicillin showing resistance of 55% in high budget hotels and 46% in low budget hotels, (P=0.8235). Tetracycline showed a resistance level of 34% in high budget hotels and 58% in low budget hotels, (P=0.2835) and co-amoxyclav showed resistance of 37% in high budget hotels and 38% in low budget hotels, (P=0.5074). The P-value results clearly indicate no significant relationship between taking medication by food-handlers prior to medical examination and the pattern of antibiotic resistance. Comparatively, the resistance pattern for the antibiotics tested is somehow the same for low and high budget hotels, indicating that, irrespective of the hotel category, if those working in hotels are potential carriers of drug resistant *E. coli*, then the category of hotel is irrelevant and either has a potential of transmitting resistant *E. coli*.

CHAPTER ONE

INTRODUCTION

1.1 Antimicrobial resistance: A historical perspective

The discovery of penicillin by Fleming in 1928 and the subsequent publication of his discovery ushered in an era of antimicrobial chemotherapy that revolutionalised the lives of patients. Antibiotics in general have useful therapeutic properties in clinical medicine when used appropriately. However, the indiscriminate use of these compounds may encourage the selection and proliferation of antibiotic resistant bacteria. Drug resistance is the result of microbes changing in ways that reduce or eliminate the effectiveness of drugs, chemicals, or other agents to cure or prevent infections (CDC, 2001).

The Centre for Diseases Control and Prevention (CDC) for instance, estimates that more than one-third of prescribed drugs are unnecessary. Over 40% of the antibiotics produced in the United States are used in agriculture, where they are freely administered in livestock feed to promote better growth. Bacteria that develop resistance in the environment can easily find their way into the food supply and present a threat to humans.

The increase in the pool of antibiotic resistant bacteria reduces the number of available choices of antibiotics for therapeutic intervention. Bacteria apply various protective mechanisms in order to survive in the unfavourable environment of antibiotics. Both gram- positive and gram-negative bacteria

such as *Staphylococci, Klebsiella* respectively produce β-lactamase enzymes that inactivate antibiotics such as the β-lactam group (Pelczar *et al.*, 1997). Resistance to antimicrobial agents is affecting the entire approach to chemotherapy of infectious diseases.

Bacteria have evolved mechanisms to withstand the attack of antibiotics and in parallel, mechanisms for the spread of these characters to other bacteria by means of exchange of genetic material (Ombui *et al.*, 2000). The increasing prevalence of antibiotic resistance is a cause of serious concern for public health officers. The significantly high carriage of MDR *E. coli* by apparently healthy individuals has been demonstrated in a study in Nigeria (Okeke *et al.*, 2004) and Kenya (Kariuki *et al.*, 1997).

The current knowledge on resistance is that, it can be transmitted horizontally between strains, despite being commensal representative found in the intestinal flora of man and animals, certain strains of *E. coli* are highly pathogenic. Faecal contamination of food and drinking water is the major route of infection for humans (Kuhnert *et al.*, 2000). This has remained a global concern that calls for a continuing research to be able to identify these strains and impose rational use of antibiotics in order to minimize resistance level both locally and internationally.

The primary driving force behind rising resistance is antibiotic use and misuse, which leads to selection pressure favoring the emergence and spread of drug resistant microbes. Antibiotics are freely available over-the-counter (OTC) and many sufferers self-medicate to save the time and money needed to consult a

healthcare provider (Bimal *et al.*, 2003). The development of resistance to commonly used antimicrobials is of particular concern when it occurs in pathogenic organisms that cause invasive diseases. This has implications on morbidity and mortality of infections and will also result in an increase in the cost of care due to the use of alternative antimicrobials which are often more costly.

1.2 Statement of the problem and justification

Antibiotic usage is considered the most important factor in promoting the emergence, selection and dissemination of antibiotic-resistant micro-organisms in both veterinary and human medicine. Resistance is not only in pathogenic bacteria but also in the endogenous flora of exposed individuals (animals and humans) or populations (Van den and Stobberingh, 2001).

Efficacy of antibiotics is increasingly being compromised by the development of bacterial resistance to the drugs currently available for therapeutic use. The ideal strategies of the treatment of infectious diseases guided by microbial diagnosis and resistance pattern are violated in most developing countries leading to excessive use of antibiotics and development of resistance (Aseffa *et al.*, 1997). Infectious diarrhoea caused by *E. coli* continues to be a major cause of morbidity and mortality especially in the developing countries where the burden of disease has been estimated at between 500 million and 1.5 billion episodes annually (Obi *et al.*, 2004) and mortality rate of 12,600 children daily in Africa, Asia and Latin America (Iijima *et al.*, 2004). It has been estimated that about 26% of all tourists coming to Kenya suffer from travellers' diarrhoea and that most of these episodes are from consumption of contaminated foods

and drinks. It has also been shown that a large proportion of the causative agents are multi-drug resistant enterotoxigenic *E. coli* (Gomi *et al.*, 2001).

The non-prescribed use of antibiotics will contribute to the development of antibiotic resistant bacteria, which is an issue of global concern. The study therefore provides information on the level of drug resistant *E. coli* isolated from food-handlers in Nairobi. These data can be helpful to the Ministry of Health (MOH) and other respective bodies, where projects can be initiated to combat resistant bacteria at national level and formulate policies regarding antimicrobial use and misuse. The increasing prevalence of antibiotic resistance is a cause of serious concern and requires an international approach to its management.

1.3 Research questions

How prevalent is antimicrobial resistant *Escherichia coli* isolated from food-handlers in the city of Nairobi?

What is the pre-medical health-seeking behavior among food-handlers in Nairobi hotels?

1.4 Null Hypothesis

Food- handlers working in Nairobi Hotels are not an important reservoir of multi-drug resistant *Escherichia coli.*

1.5 Objectives

General objective

To determine the existence and extent of antibiotic resistant *Escherichia coli* isolated from food-handlers undertaking medical examination in Nairobi.

1.5.2 Specific objectives

To determine the antimicrobial susceptibility profiles of the isolated *Escherichia coli* from the food-handlers.

ii) To determine the pre-medical examination health-seeking behavior among food-handlers in Nairobi hotels, (i.e. High and Low budget hotels)

1.6 Significance of study and anticipated output

In Africa, the customs of casually self-prescribing antibiotics further raises the risk of resistance. Ready access to these drugs is a fact of life. Many of the species that cause outbreaks of hospital acquired infections are part of the normal flora of healthy carriers. Currently WHO recommends prudent use of antibiotics as a global strategy for containment of antibiotic resistance and to conserve the drug efficacy. In many developing countries, the boom in food service establishments is not matched by effective food safety education and control. Unhygienic preparation of food is source of many food-borne diseases which pose a considerable threat to human health and the economy of individuals, families and nation.

The normal intestinal flora for instance, *Escherichia coli* is a useful indicator of antimicrobial resistant bacteria in the community. Studies on *Escherichia coli* are of particular interest since these species can efficiently exchange genetic material with pathogens such as *Shigella, Yersinia,* and *Vibrio* species, as well as pathogenic *E. coli* (Iruka and Susan, 2000).

Bacterial infections are a major cause of morbidity and mortality in developing countries including Kenya (Malonza *et al.*, 1997). Easy availability and indiscriminate use of antimicrobials by health providers and the general population have resulted in emergence of resistant organisms. Resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries. The increasing frequency of drug resistance has been attributed to a combination of microbial characteristics, selective pressure of antimicrobial use and societal factors that enhance the transmission of drug resistant organisms. The emergence of antibiotic resistant bacteria has generally correlated with the rise and fall of specific antibiotic use in clinical practice (Mbori *et al.*, 1997). However, studies currently focus on reducing the need for antibiotics by developing vaccines and probiotics and implementing public health strategies. One major concern of health services has been to ensure the availability of safe and effective drugs of acceptable qualities at a reasonable cost and to promote the rational use of antibiotics. Hence the information from the study will form the basis for future research to be undertaken by the MOH to come up with proper data showing the magnitude of the problem and implement policies to curb the spread of resistant bacteria. Studies suggest that, antimicrobial-resistant strains are more virulent than susceptible bacterial strains (Tomkin, 2002).

CHAPTER TWO

LITERATURE REVIEW

2.1 A global perspective of drug resistance

The emergence of resistance to anti-microbial agents among previously susceptible organisms, continues to be an important obstacle to the successful treatment of bacterial infections, (O'Brien, 2002). The currently used antibiotics are less effective against bacterial infection, making it more difficult to treat bacterial illness due to decreasing options available. The infection with *Escherichia coli* 0157:H7 was first described in 1982. Subsequently, it has emerged rapidly as a major cause of bloody diarrhea and renal failure. Outbreaks of infection, generally associated with beef, have been reported in Australia, Canada, Japan, United States, in various European countries, and in southern Africa. In 1996, an outbreak of *Escherichia coli* 0157:H7 in Japan affected over 6,300 school children and resulted in two deaths. This is the largest outbreak ever recorded for this pathogen. Changes in the human population are expanding world-wide because of ageing, malnutrition, HIV infections and other underlying medical conditions. These factors make one highly vulnerable to food-borne diseases since their immune system is weakened and succumb to infections like *Salmonella, Campylobacter, Listeria* and *Cryptosporidium*. Multi-drug resistant (MDR) strains of a number of bacteria are known, including *Salmonella* sp., *Streptococcus pneumonia, Enterococcus* sp., *Klebsiella pneumonia* and *Campylobacter jejuni* (Cowen *et al.*, 2002).

2.2 Antibiotic susceptibility profiles of the isolates

The natural Antibiotic Resistance Surveillance (ARS) programme, revealed that in 2000 alone, *Salmonella typhimurium* isolated in Malaysian government hospitals were 10.6% and 8.5% resistant to ampicillin and chloramphenicol respectively. The resistance rates with non-typhi *Salmonella*, recorded against ampicillin, chloramphenicol and co-trimoxazole were 12%, 6.3% and 13.2% respectively (Lim, 2002).

In Taiwan community, quinolone use in animal feeds has rapidly selected for ciprofloxacin – resistance isolates which were identified as *Escherichia coli* and *Salmonella* species, Po-Ren (2004). A study carried out in Spain in the year 2001, revealed high frequency of resistant *E.coli* to several antibiotics i.e. ampicillin 58.46%, co-trimoxazole 32.91%, ciprofloxacin 17.19% and gentamicin 6.39% of isolates respectively. However, most isolates of *Shigella flexineri* were resistant to ampicillin and co-trimoxazole and over 80% resistance to chloramphenicol (Oteo *et al.*, 2001).

In Dhaka –Bangladesh, studies revealed that, *Nisseria gonorrhoeae* strains resistant to the commonly used antimicrobial agents have increased, and consequently penicillins and tetracycline can no longer be recommended for the treatment of gonorrhoea. Ciprofloxacin may soon approach the end of its utility as a first- line drug for treatment of gonorrhoea in Bangladesh, where 11.7% of *N. gonorrhoea* isolates are currently resistant , while tetracycline is approaching 60% resistance level (Bahar *et al.*, 1999).

Currently in Bangladesh large numbers of patients are being seen with cholera-like diarrhoea solely caused by ETEC (Qadri *et al.*, 2005).

In the Netherlands, studies indicate that 300,000 kg of antibiotics are used yearly on veterinary prescription in animals, of which 10% is used in poultry. These exposures to therapeutic antibiotics per year have increased selection of antimicrobial resistance. Further, this has been worsened by overcrowding and poor sanitation. These factors are typical of intensive poultry farming and explain the high prevalence and degree of resistance in faecal *E. coli*. However, during rearing of poultry, antibiotics are commonly used. In the avian intestinal tract resistant *E. coli* may persist for a long time (Medders *et al.*, 1998).

2.3 Antibiotic resistance in Kenya

In developing countries where prevalence of bacterial infections remains high and laboratory facilities are limited, the WHO has recommended that a syndromic approach based on clinical symptoms and signs be used. However, the emergence of MDR especially in *S.enterica serotype typhimurium* is associated with frequent therapeutic failure (Kumar *et al.*, 1995). In Kenya, medical-examination is carried out as a requirement of the laws of Kenya Public Health Act Cap 254 (ROK, 1992).

These medical examination activities are on-going at the Kenya Medical Research Institute Centre for Microbiology Research (CMR). This has to be complemented by a vigorous and comprehensive public education programme. Efforts are being made by the CDC, the National Institute of Health and the

Food and Drug Administration (F D A) to implement an action plan to control further emergence of antibiotic resistant bacteria (Woteki and Kineman, 2003).

Bacteria have a remarkable ability to develop resistance to many of the anti-microbial agents (Kakai *et. al.*, 2002). Ciprofloxacillin has been recommended for the treatment of severe infections caused by MDR Salmonella in Kenya (Dutta *et al.*,1995). Considering that in Kenya, *E. coli* is an important cause of bacteraemia in nosocomial infection and a significant public health problem, and that antibiotics are widely used in clinical practise, the need to avert the spread of resistance is important (Gakuya, 2001). Resistance to antimicrobial agents in bacteria may occur due to a spontaneous mutation or may be acquired through transmission from other resistant bacteria.

Comparing *E. coli* from a poultry processing plant in Kenya and isolates from children with diarrhoea living in close contact with poultry, a study has revealed that differences in antibiotic resistance patterns was observed and in the level of multi-drug resistance. Both human and poultry isolates carry two distinct pools of resistance plasmids (Kariuki *et al.*, 1997). A study carried out in Nairobi slums, i.e. Kibera, Kabete and Kawangware revealed that rats harbour resistant *E. coli*. Resistance was only transferred in five (22%) of ampicillin resistant *E. coli* isolates to *E.coli* K 12. In addition this study showed that rats carry antimicrobial resistant *E.coli* and their plasmids pose a public health hazard (Gakuya , 2001). On a separate account another study of antibiotic sale behavior in chemists within Nairobi, revealed that about 64% of chemists sell antibiotics without appropriate prescription and most of them sold under-dose drugs and according to the request

of a patient (Indalo, 2002). In Kiambu, a study carried out with meat purchased from the City of Nairobi, showed high frequency of *Staphylococcus aureus* resistance among the consumers, for instance lincomycin was 67.7%, penicillin 66.7% and co-trimoxazole 51% (Ombui, 2000). A 4-year retrospective study was undertaken at the Kilifi District Hospital along the Coast of Kenya to determine the antibiotic susceptibility patterns and genotype of non-typhoidal salmonella (NTS) isolates from children. The study revealed that over half (56.8%) of the total NTS isolates are multi-drug resistant i.e. *S. enterica serotypes* Typhimurium and *S.enterica serotypes* Enteridis, of micro-epidemic nature that have been previously unrecognized in Kilifi District on the Coast of Kenya (Oundo *et al.*, 2000).

2.3.1 Pathogenesis of *Escherichia coli*

Escherichia coli are gram-negative, facultatively anaerobic bacteria in the family of enterobacteriaceae. They are found in the normal intestinal flora of man and animals, but can also be an important cause of enteric illness. The enteric pattern causing acute diarrhoea in developing countries are largely the same that are encountered in developed countries but their proportions are different (Vesikari, 2001) . Colonization of the intestinal tract with resistant *E. coli* from chicken has been shown in human volunteers. Evidence that animals are reservoir for *E. coli* found in humans was published in 1970's. These resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora. *Escherichia coli* from normal intestinal flora are harmless to the host but can as well become a threat especially to those individuals with impaired immune defences who are not able to contain these commensals in their natural barriers between the gut and

other sites of the body. *E. coli* is a bacterium which is strongly adapted to humans and many animals where it either lives in beneficial symbiosis or creates severe disease. The presence of non-pathogenic *E. coli* in water and food is a clear sign of bad hygiene (Kuhnert *et al.*, 2000). *Escherichia coli* cause diarrhoea by at least five distinct mechanisms. Enterotoxigenic *E. coli* produces a secretory small bowel diarrhoea and are a major cause of travellers diarrhoea. Enteroinvasive *E. coli* cause inflammatory large bowel diarrhoea similar to *Shigella*. Enteropathogenic *E. coli* produce non-inflammatory diarrhoea by destroying the brush-border. Enterohaemorrhagic *E.coli* such as 0157, are recently evolved pathogens which cause haemorrhagic colitis and haemolytic uraemic syndrome. The newly described Enteroaggregative *E.coli* produce chronic bloody diarrhoea particularly in children (Hart, 1998).

2.4 Impact of bacterial food contamination on tourism

Tourism is one of the most important sources of foreign exchange in Kenya and anything that adversely affects it should be addressed and appropriate corrective action taken. Tourists travel to a country to spend money and if they fall sick, then they are forced to change their travel plans and itineraries, hence end up not spending the money they may have allocated for the visit. The people in direct contact with tourists in their daily activities are the hospitality workers. These workers are responsible for many activities including preparation of food in hotels that are consumed by tourists. They can, therefore, be an important source of contamination of the food consumed by tourists resulting in illnesses such as diarrhoea. Contamination of food by faecal material could be as a result

of poor personal hygiene on the part of the workers. Previous studies have concentrated on the causative agents of travellers diarrhoea in affected tourists without concomitant studies on the local population of the workers in the tourist service sector (Paredes *et al.*, 2000, Gomi *et a.*, 2001, Pugh *et al.*, 2001, Jiang *et al.*, 2002a). For instance in a multi-country study done in India, Kenya and Jamaica, it was found that enterotoxigenic *Escherichia coli* was the most common pathogen (25%) among tourists in the three locations, followed by *Shigella* species, Rotavirus, *Salmonella* species, and *Campylobacter* species. However, there are no data on the common pathogens isolated from food handlers working in the tourist hotels.

The current tourist arrivals in Kenya are about 800,000 per year with approximately US$ 300 million generated by the industry annually. The tourist arrivals are projected to increase to about 1,500,000 arrivals annually by 2006 (Jiang *et al,* 2002). About 100,000 people are directly employed in the tourist industry with about four times more depending on it indirectly. This figure will also rise significantly with the rise in tourist arrivals. Currently, tourism is only second to tea as a direct foreign exchange earner for Kenya.

CHAPTER THREE

MATERIALS AND METHODOS

3.1 Study site

The study was conducted in the City of Nairobi which is located 480km inland north-west of the Indian Ocean, with an area of 684 sq. km. It lies at an altitude of 1,670m; the longitude is $36^{0}50'$ east and latitude $1^{0}17'$ south, just 140 km south of the equator. Nairobi lies close to the equator, but being 1,700m above sea level, temperatures are altitude- modified tropical, warm. The months of July and August are distinctly cool. The Mean Annual Temperature is $19^{0}C$ and the Mean Daily Maximum and Minimum are $25^{0}C$ and $14^{0}C$ respectively. Mean Annual Rainfall is 1,080mm falling in two distinct seasons: the long rains, March to May, and the short rains, mid -October to December, (Moss, 2000). The City of Nairobi is conspicuously cosmopolitan, with over 2.5 million inhabitants: at independence the total population was only 350,000. Nairobi is divided into 8 administrative divisions of Dagoretti, Embakasi, Kasarani, Westlands, Kibera, Pumwani, Central and Makadara (CBS, 1999) (Appendix 4).

3.2 Research design

This was a cross-sectional study in which data were collected both quantitatively and qualitatively. The design was primarily interview survey, where the food handlers were given Questionnaires to fill and determine their socio-demographic characteristics and health-seeking behavior towards the use of drugs prior to medical examination, while laboratory tests were conducted on stool specimens to assess the prevalence of E. *coli bacterial* resistance among the commonly used antibiotics.

3.3 Target population

All study subjects were food-handlers working in selected hotels within the City of Nairobi. The respondents in this study were defined as any person involved in any way with handling, processing and serving food, such as waiters, cooks, chefs and delivery people.

3.4 Inclusion criteria

The subjects involved in this study were food-handlers working in selected hotels of Nairobi. They were involved in the preparation, processing and serving of food. They included chefs, cooks, waiters who served for at least six months.

3.5 Exclusion criteria

The food-handlers who did not consent to participate in the study.

3.6 Sample size determination

A total of 297 food handlers were recruited after obtaining individual informed consent from the study participants. The sample size was determined according to the method as used by Fisher *et al.*, 1998.

$$n = \frac{z^2pqD}{d^2}$$

Where n= minimum sample size required

Z=is normal standard deviate (1.96) which corresponds to 95% confidence level

p=the proportion in the target population estimated to have a particular characteristic prevalence which is 20% isolation rate for *E.coli* in Kenya (Jiang *et al.*, 2002).

d= 0.05 (5% absolute precision).

Hence;

$n = \frac{1.96^2 \times 0.2 \times 0.8 \times 1}{0.05^2} = 246$ (the minimum sample size expected for the survey).

NB: - In this particular study the sample size was 297, which is more than the estimated minimum sample by 51. The minimum sample size was purposively raised by 21% to cater for data incompleteness during the survey. This was a prudent measure to ensure that finally we get at least 246 of completed records without any missing response on key variables.

3.7 Sampling method

3.7.1 Hotels sampled

Various hotels within Nairobi were included in the study and were broadly classified as High-Budget Hotels (HBH) and Low-Budget Hotels (LBH). The sampling frame was an existing database monitored by CMR. Hotels already captured in the database among them HBH and LBH were listed. The ratio of HBH to LBH was are distributed all over Nairobi. Random sampling was used in selecting the hotels, only 20 of them were selected due to time and financial constraints. Using the distribution ratio of HBH to LBH as earlier indicated, 9 of the selected were HBH while 11 were LBH. A total of 297 food handlers were selected from the 20 hotels. Due to unequal distribution of the workers in HBH and LBH, the numbers of food handlers selected were as follows; 128 from HBH and 169 from LBH. Food-handlers in every of the sampled hotel were listed to establish their total number. Based on how many they were and considering the

required sample size in every hotel category, the number to be picked was determined. Randomisation was used in selection of workers as well.

3.7.2 Low-Budget Hotels (LBH)

These categories of hotels receive their daily clients locally. They offer meals and rarely accommodation services. Basically they are located in the City centre although most of them are found in the outskirts of Nairobi. Most of these hotels have lunch as their main menu and a few may provide breakfast and dinner.

3.7.3 High- Budget Hotels (HBH)

The High Budget Hotels (HBH) rating was from 3 to 5 star while Low Budget Hotels (LBH) rating was from 2 star and below. HBH, additionally offer services such as, Social amenity like Casino, Swimming Pool, Golf Course and Fitness Centre, Conference Centre, Hostel, Guest house and Bed and Breakfast. The hotels in this category were well built with permanent modern structures. They are basically located in town although some were away from the City centre. They receive their clients both locally and internationally. Most of them are tourists from abroad. They provide three courses meals as well as outside catering. They also have accommodation services.. The food-handlers prepare and serve food to these visitors who are less likely to be immune to the prevailing tropical diseases than the local people. This can result in serious outbreaks if not checked or controlled especially the resistant strains of virulent organisms.

Table 1 Categorization of the Study Hotels Within Nairobi.

HBH	Star Rating	LBH	Star Rating
A	5	J	2
B	5	K	2
C	5	L	1
D	5	M	1
E	4	N	1
F	4	O	2
G	5	P	2
H	3	Q	1
I	5	R	2
		S	1
		T	2

Key (i) A-I: Codes represent names of the hotels. This information is strictly confidential.

(ii) 1-5: Star ratings which were as provided by the hotels and as evaluated by the Ministry of Tourism.

3.8 Data Collection

Data were collected by the Principal investigator and several research assistants. Training of the research assistants was done before the process. Data were coded, cleaned, processed and entered as collected. The principal investigator will be instructing, conducting and organising tests in the laboratory while research assistants will be feeling the findings as well as questionnaires.

3.8.1 Questionnaires

Socio-demographic data (i.e. age, sex and period of working in the establishment) was collected using structured questionnaires (Appendix 3). Pre-testing of research instruments was carried out at CMR and Mbagathi District Hospital to enhance the validity of the instruments and improve on the questionnaires to obtain qualitative data from each individual

3.8.2 Stool sample collection

The study subjects were provided with sterile wide mouth containers (poly pots) and asked to provide freshly voided stool specimen (without urine). This was done at the hotel premises. Stool samples were only collected during the day between 8am and 5pm. Samples were collected after obtaining an informed consent from the study subjects. The samples were later transported in chilled cool-boxes to the laboratory at the Centre for Microbiology Research, Kenya Medical Research Institute, in Nairobi and processed within one hour of collection.

3.8.3 Stool processing

The stool samples were plated onto MacConkey, XLD and TCBS media and enriched in Selenite F broth (all the media from Oxoid, Basingstoke, UK) and incubated for 24 hours aerobically at 37^0 C. Five pink/red lactose fermenting colonies were picked at random from each plate and biotyped using Triple Sugar Iron (TSI), Lysine Indole Motility (LIM), Simon's Citrate (SC), Methyl Red-Vorges Proskauer (MRVP) and Urea media (all the media from Oxoid, Basingstoke, UK). The isolates were confirmed as *Escherichia coli* by API system (Appendix 2).

3.8.4 Antimicrobial susceptibility testing

Antibiotic susceptibility testing was performed on the isolates using the Kirby-Bauer disc diffusion technique (Bauer *et al.*, 1966). The following drugs were tested: Ampicillin – (AMP) 10 μg, Chloramphenicol – (CH) 30 μg, Gentamicin – (CN) 10 μg, Co-trimoxazole – (SXT) 25 μg, Cefuroxime – (CXM) 30μg, Co-amoxyclav – (AMC) 30 μg, Tetracycline – (TE) 10 μg, Kanamycin – (KN) 10μg. These antibiotics were chosen on the basis of their use in the management of enteric bacterial infections. 18 to 24 hour old isolates of *E. coli* cultures were suspended in normal saline to match McFarland turbidity standard 0.5. The suspension was inoculated on Mueller Hinton agar plate after which the antibiotic disks were applied to test for antibiotic susceptibility. *E. coli* ATCC 25922 with known susceptibility values of each antimicrobial agent was included in each test run as a control for antimicrobial potency. The zone diameter interpretive standards and equivalent minimal inhibitory concentration (MIC) break points were interpreted according to criteria set by NCCLS (2002).

3.9 Data management/analysis

Data was collected using questionnaires and latter entered in to a computer database which was designed using MS-Access. Data cleaning and validation was done to minimize data entry error. A clean set of data was archived using CD-Rom and a flash disk to ensure electronic data backup. The questionnaires were serially arranged and filed in folders which were then properly kept in drawers under lock and key to safeguard the data and ensure confidentiality. A clean set of MS-Access file was exported to a Statistical Package for Social Sciences (SPSS) format for data processing and analysis. Since majority of the variables were categorical, Pearson Chi-square test of significance was used to assess differences in distribution of resistance between high budget hotels and low budget hotels, there by establishing the relationship between susceptibility patterns with various antibiotics and hotel categories where the food handlers are working. Cross tabulation technique was used for every antibiotic susceptibility pattern by hotel category. This technique was able to show the level of resistance for every antibiotic in high budget hotel versus low budget hotels.

3.9.1 Ethical consideration

Permission was sought from the Ministry of Education, Science and Technology, Kenyatta University and the administration of the same hotels in order to conduct the research and was approved by the Ministry of Health, (Appendix 1). Consent from the study subjects was sought and those unwilling to participate did not participate in the study. Confidentiality of information was strictly maintained by the interviewers.

CHAPTER FOUR

RESULTS

4.1 Demographic profiles of study subjects:

The demographic data and other characteristics of the study subjects are shown in Table 2 and 3. A total of 297 food handlers sampled comprising 240 (80%) males and 57 (20%) females were selected. Sex distribution across hotel categories differed significantly (P=0.003). In low budget hotels, there were 126 (74.6%) females and 43 (25.4%) males while in high budget hotels, there were 113 (88.3%) females and 15 (11.7%) males. Mean age for the total sample population was 32.4 years ranging from18 to 56 years. There was a difference in mean age between hotel categories. In low budget hotels, mean age was 29.6 years ranging from 18 to 53 years while in high budget hotels, mean age was 35 years ranging from 22 to 56 years.

The employment period of the study subjects ranged from 6 months to over 4 years with those having served for 4 years or more being 48.1% of the total study population. The high budget hotels had a steadier workforce (those who had worked for 4 years and more) of 87.5% compared to the low budget hotels who had only 18.3%. The difference was statistically significant (P=0.001).

Provision of health services for workers in low budget hotels and high budget hotels was comparable. 42% of workers in low budget hotels are provided with health services by their employer compared to 52.3% of workers in high budget hotels. The difference was not statistically significant (P=0.080). There is agreement between seeking external health services and provision of services within the workplace as shown in Table 3. Workers in both low budget and high

budget hotels are over 50% compliant to medical examination every six months. Remarkably those ones in high budget hotels are 100% compliant compared to 68% in low budget hotels. The difference was statistically significant (P=0.001). The trend was similar in the provision of sick leave 91.4% of workers in high budget hotels were given sick leave compared to 78.1% in low budget hotels. The difference was statistically significant (P=0.002).

Table 2 Demographic data and other characteristics of the food-handlers

VARIABLE	LBH	HBH
Food handlers sampled	169	128
Males	126	113
Females	43	15
Age range in years	18 – 53	22 – 56
Mean age	29.6	35
Mode	23	25

(N=297)

Table 3: Employment data and other characteristics of the food handlers.

Employment period	4 years 143 (48.1%) 1 – 4 years 96 (32.3%) 6 months 58 (19.5%)	
Employment period by hotel category over (4 years)	**LBH** ≥ 18.3%	**HBH** ≥ 87.5%
Provision of Health services within the establishment	71 (42%)	67 (52.3%)
Proportion of workers seeking external health services	54.4%	44.5%
Proportion of workers undergoing medical examination every six months	116 (68.6%)	128 (100%)
Medicine use in the last 14 days	8.9%	5.4%
Provision of sick leave	114 (78.1%)	117 (91.4%)

(N=297)

4.2.1 Antibiotic Resistance Profile

Antibiotic resistance profiles of the *Escherichia coli* isolated from food handlers working in low budget hotels were as indicated in figure1. Antibiotic resistance was seen in all the antibiotics tested. The resistance ranged from 3% for

cefuroxime, ampicillin 46%, tetracycline 58% and 66% for cotrimoxazole. There were varying levels of antibiotic resistance between the study subjects who had taken medication and those who had not taken any medication before medical examination. The level of resistance to various antibiotics among those who took medication vs. those that did not take medication are as follows;

kanamycin: 4.8% vs. 2.0% (P=0.405), gentamycin: 0.0% vs. 5.5% (P=0.614), cotrimoxazole: 71.4% vs. 62.8% (P=0.489), ampicillin: 47.6% vs. 48.6% (P=1.000), cefurixume: 4.8 vs. 2.0% (P=0.405), amoxy-clav: 28.6% vs. 33.6% (P=0.811), chloramphenicol: 9.5% vs. 7.1% (P=0.661), tetracyclycline: 57.1% vs. 43.1% (P=0.257). However, the level of resistance was not significantly different in those that used medication compared to those that did not use. These results were as indicated in figure 5. Antibiotic susceptibility experiments exhibited high resistance rate against co-trimoxazole for both HBH and LBH as shown in figure 1 and 2.

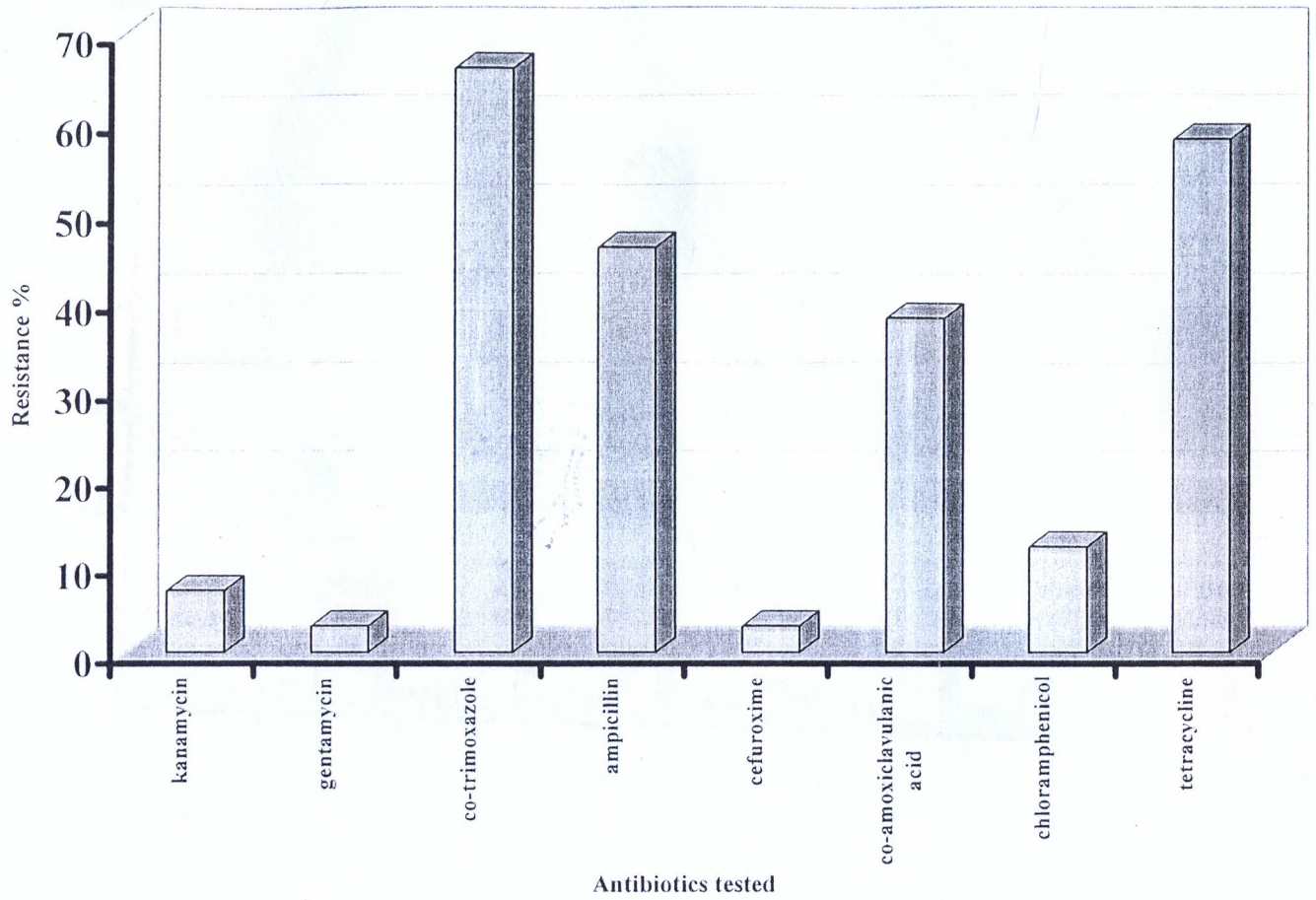


Figure1: Antibiotic resistance profiles of the *Escherichia coli* isolated from food handlers working in LBH

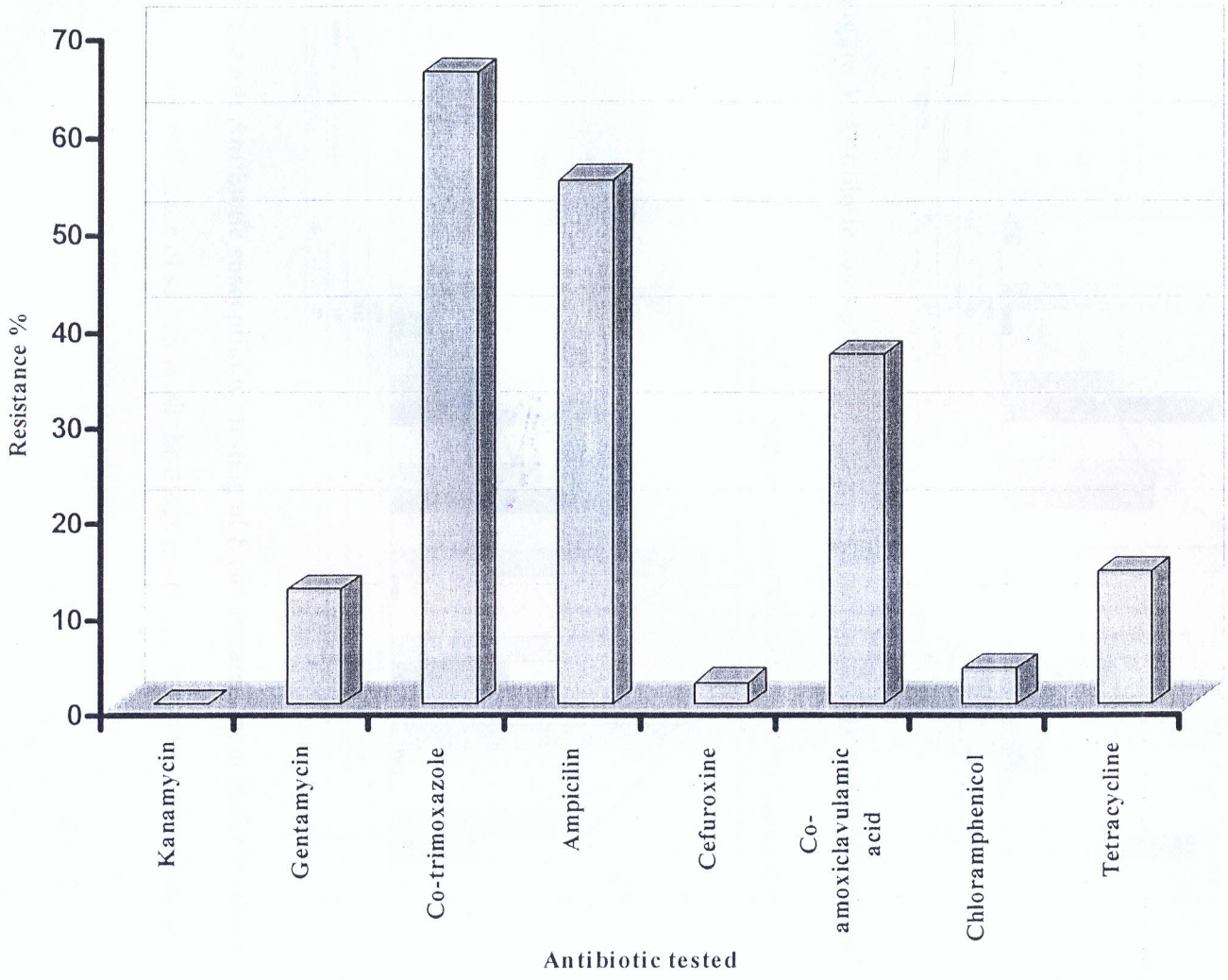
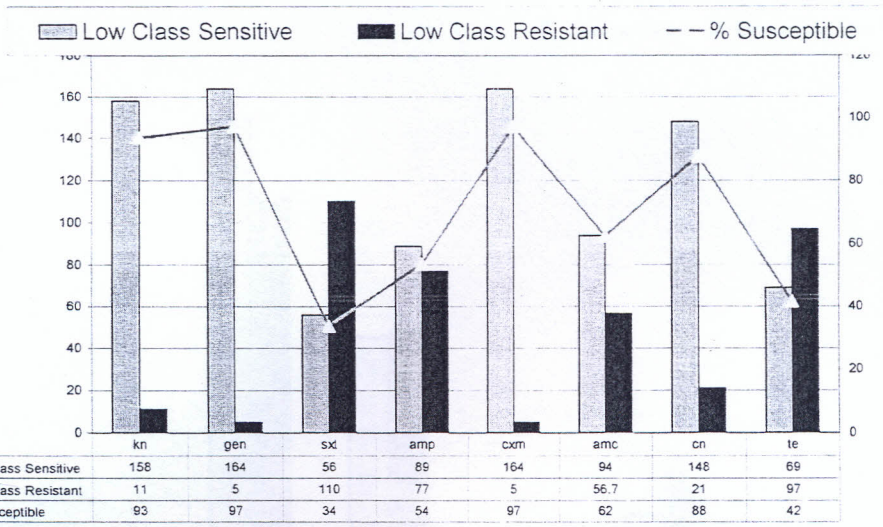
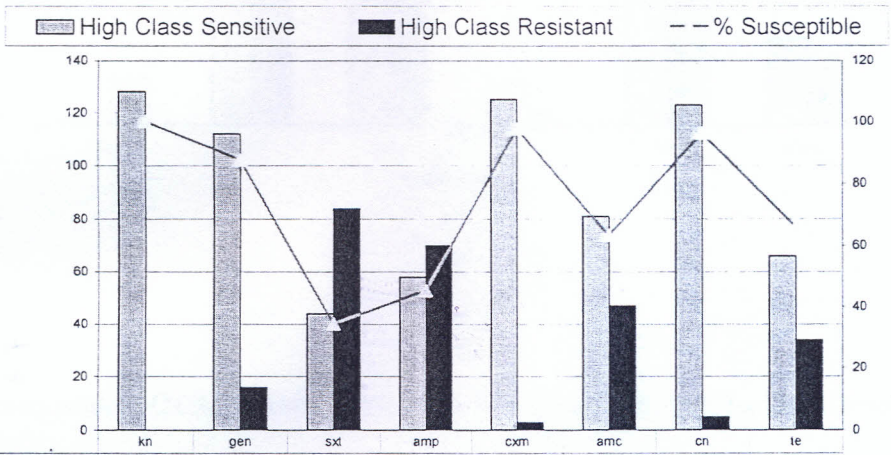


Figure2: Antibiotic resistance profiles of the *Escherichia coli* isolated from food handlers working in HBH



	kn	gen	sxt	amp	cxm	amc	cn	te
Low Class Sensitive	158	164	56	89	164	94	148	69
Low Class Resistant	11	5	110	77	5	56.7	21	97
% Susceptible	93	97	34	54	97	62	88	42

Figure 3: Antibiotic susceptibility testing of *E.coli* isolated from LBH Workers



	kn	gen	sxt	amp	cxm	amc	cn	te
High Class Sensitive	128	112	44	58	125	81	123	66
High Class Resistant		16	84	70	3	47	5	34
% Susceptible	100	88	34	45	98	63	96	66

Figure 4: Antibiotic susceptibility testing of *E.coli* isolated from HBH Workers

Antibiotic resistance observed in all the drugs tested varying between cxm3%, gen 3%,kn 7%, cn 12%, amp 46%, amc 38%, te 58% and sxt 66%.

Key-

kn-kanamycin

gen-gentamycin

sxt-cotrimoxazole

amp-ampicillin

amc- amoxy-clav

cn-chloramphenicol

te-tetracyclycline

cxm -cefurixume

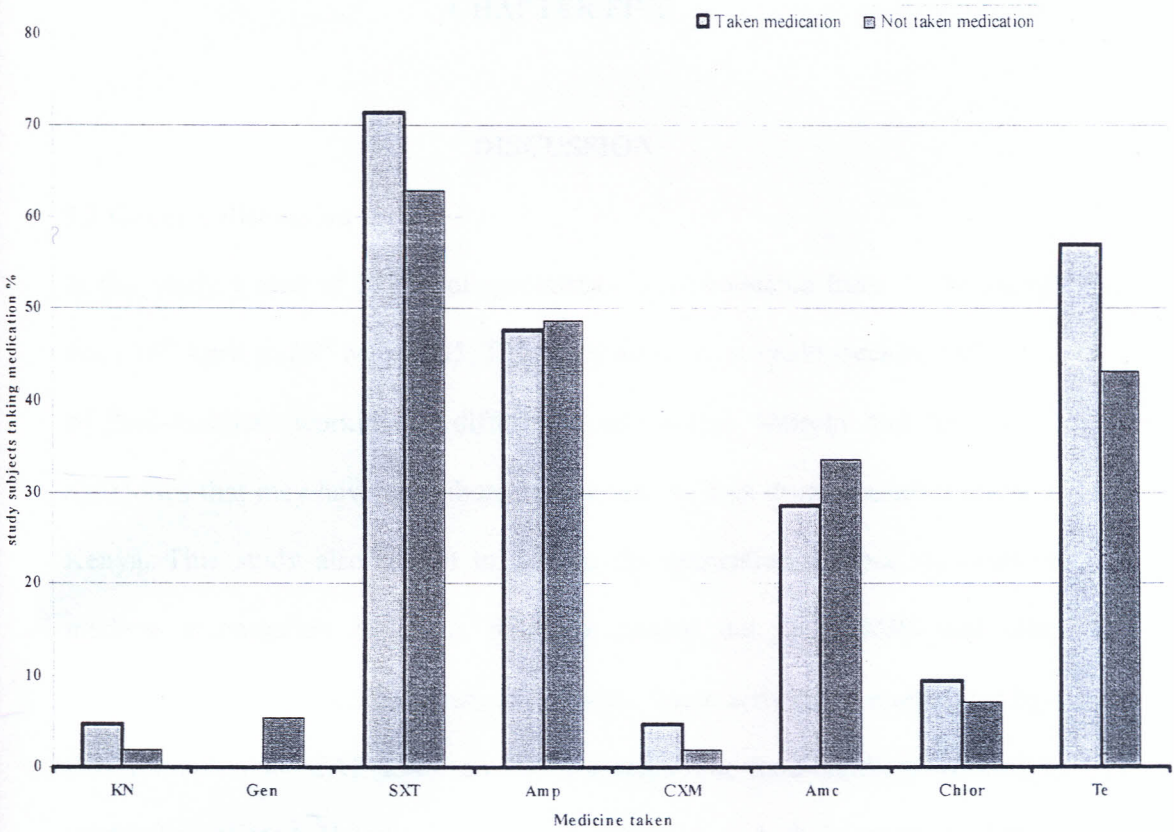


Fig 4 Comparison Of Antibiotic Resistance Patterns Of *E. coli* Isolated From Study Subjects Who Took Medication And Those Who Did Not Take Medication Prior To Examination

CHAPTER FIVE

DISCUSSION

5.1 General discussion

In this study a total of 297 stool specimens were collected from food-handlers from 16th April to 23rd May 2005. This study focused on health-seeking behaviour of food-handlers working in different hotels within Nairobi and the use of antibiotics that may have contributed to the current high drug resistance levels in Kenya. This study also sought to address the perception of food handlers on medical examination which is routinely carried out at KEMRI and other Government health facilities every six months. These activities are regulated by a Government Act CAP 254 on Public Health. The food-handlers working in randomly selected Nairobi hotels were recruited and their stool specimens examined for *E. coli* isolates. These *E. coli* are the predominant species forming normal flora of the intestine. Most strains are non-pathogenic, but some have acquired a diarrhoegenic disease-producing ability and cause infections in the human intestine (Honda, 1992). The ability of multi-drug resistant *E. coli* isolates to transfer resistance to other *E. coli* has been reported to range from 26% to 50% in isolates from humans, hence these plasmids were responsible for transfer of resistance (Niljesten and Stobbering, 1996).

The finding of MDR *E. coli* has become a world wide problem especially as it impacts on the choice of available antimicrobials for treatment of diarrhoeal and other diseases. It was therefore, important to determine the extent of carriage of any MDR *E. coli* by food handlers in a Kenyan setting. Food handlers are a

possible important source of transfer of MDR *E. coli* capable of transferring resistance to other enteric bacteria.

The irrational use of antibiotics due to easy availability over-the- counter (OTC), self medication and use of antibiotics in animal industry has lead to the rise of antibiotic resistance worldwide (Nys *et al.*, 2004). Studies have also shown that about 64% of chemists in Nairobi sell antibiotics as OTC drugs and upon request by individuals (Indalo, 2002).

In this particular study, male respondents were the majority comprising 80%, while females were only 20%. This ratio of 8:2 could be explained by a possible preference of employers for male employees. The reason for this preference for male employees is not known. In the high budget hotels, up to 87.5% of the food handlers have maintained their jobs for over four years in the same department compared to 18.3% in low budget hotels. This high turnover of employees may lead to inexperienced employees with little knowledge to hygienic standards required hence pose a risk of transmitting the bacteria to the consumers. The high prevalence of diarrhoeal diseases in many developing countries suggests a major underlying food safety problems (WHO, 2002).

More than a half of the respondents from high cost hotels (52.3%) were provided with in-house medical service compared to Low budget hotels (LBH) who were 42%. This possibly means that employers in the High budget hotels category are more concerned about workers health status. Despite this, most of the food-handlers from LBH, i.e. 54.4% and HBH 44.5% sought additional health services outside their establishment. However, these percentages were confirmed verbally

through questionnaires and there was no documentation to show this. This trend is worrying as the food handlers could possibly be seeking health services from unqualified health practitioners leading to the observed high levels of antibiotic resistance.

Six monthly medical examination was observed to occur for upto 100% of the respondents in the HBH while it was only 68.6% from LBH workers. The percentages of respondents admitting to have taken medicine prior to medical examination ranged from 5.5% to 8.9%. No studies were done to detect any residual antimicrobials in their urine to confirm this. This would have helped confirm the figures for prior antibiotics usage. Even though these figures may seem low, the resistance levels of *E. coli* bacteria were found to be high. A similar study revealed that drug resistance could be due to horizontal and vertical transfer of resistance plasmids, (Houndt and Ochman, 2000).

Most of the food-handlers from LBH (94.5%) and HBH (91.1%) indicated that they never took any medicine before or after medical examination. However, the resistance levels for co-trimoxazole, tetracycline and ampicillin were high. It has been suggested that the ingestion of antibiotics provides selective pressure on bacteria ultimately leading to a higher prevalence of their resistance, even among persons who have not taken antibiotics (Oteo *et al.*, 2001). It is therefore a very worrying situation if these food handlers were to pass these MDR organisms not only to the clients they serve but also to the general population. Studies carried out on children in South-Western Nigeria revealed high rates of resistance of 80.9% for ampicillin, 95.4% for tetracycline, and 46.5% for chloramphenicol.

This resistance has been demonstrated to be transferable and linked to virulence of EAEC and are carried on the same plasmid (Laminkara *et al.*, 2000).

Up to 91.4% and 78.1% of the respondents from both HBH and LBH respectively, indicated that they were allowed sick leave. This indicates that employers considered the health status of their employees seriously.

High rate of antibiotic resistance was observed especially against the commonly used antibiotics. The findings in this study showed that both aminoglycoside and cephalosporins group of antibiotics have good in vitro activities against *E.coli*. From the antibiotic susceptibility experiments, high resistance rate was exhibited against co-trimoxazole which showed resistance level for both HBH and LBH to be 66 % , while ampicillin for 46 % and 55%, and tetracycline to be 34% and 58% respectively. These results are in agreement with other work done in Kenya, where resistance was slightly higher against tetracycline at 70.7%, co-trimoxazole at 68.3% and ampicillin at 65.9%, (Bii *et al.*, 2005). Similarly, in a study of antibiotic resistance of faecal *E. coli* from healthy volunteers from eight developing countries exhibited resistance to tetracycline to be at 92% and ampicillin at 89%, it had also revealed that resistance to ciprofloxacin ranging from 1 to 63%, more than 20% for gentamicin (Nys *et al.*, 2004). In Tanzania a resistance rate of 83.1% for ampicillin, 57% for chloramphenicol, 87.7% for tetracycline, and 90.8% for co-trimoxazole was recorded (Vila *et al.*, 1999). The most important issue is the observed resistance to ciprofloxacin and which is a reserve antibiotic in this country. This could be a pointer to the abuse/misuse of this antibiotic and others of its class, which has major implications on treatment and drug policy not only in Kenya but throughout the developing world.

The afore mentioned facts on antibiotic resistance is supported by several studies which have shown that major factors selecting for antimicrobial resistance in bacteria is antibiotic use, crowding and poor sanitation. These three factors are typical in poultry farming in south Netherlands and account for high prevalence of resistance in faecal *E. coli* (Ojeniyi, 1989). Poor sanitation as a factor for transferring resistance has also been confirmed (Okeke *et al*, 1999), i.e. an upsurge of resistant *E. coli* isolates could have been from the fact that rats get into contact with these antimicrobials through various sources in the environment, for example food, water and sewer system (Gakuya, 2001). In this study no significant relationship has been demonstrated between taking medication by food-handlers prior to medical examination and the pattern of antibiotic resistance (co-trimoxazole, $p = 0.56681$, ampicillin, $p = 0.8235$ tetracycline, $p = 0.2835$, amoxycillin-clavulanic acid $p = 0.5074$). From these findings it can be postulated that food-handlers working in these hotels are important carriers of multi-drug resistance *E. coli* since the use of drugs did not influence the pattern of antimicrobial resistance. This study therefore concurs with findings from other studies done indicating that *E. coli* isolates have the ability to transfer resistant genes ranging from 26% to 50% in human isolates (Niljesten and Stobbering 1996). It has also been suggested that the selective pressure ultimately leads to a higher prevalence of resistant bacteria, even among persons who have not taken antibiotics (Oteo *et. al.*, 2001).

CHAPTER SIX

6.1 CONCLUSIONS

i) Resistance level was high in both HBH and LBH. the following antibiotics for instance, co-trimoxazole (66%) , tetracycline (34% to 58%) and ampicillin (46% to 55%) respectively. This can lead to therapy failure in about 30% to 60% of the population. This is clearly shown by the level of percentages in the level of resistance for the above antibiotics i.e.34% and 66% for tetracycline and cotrimoxazole respectively.

ii) The antibiotic resistance was about the same for both LBH and HBH indicating that the class of the hotel did not influence drug resistance.

iii) Intensive food safety campaign should be carried out to curb the spread of food-borne diseases. Their control requires a concerted effort on the part of the three principal partners, namely government, the food industry and consumers.

6.2 RECOMMENDATIONS

i) There is need to review policies on antibiotic prescription practise to limit the spread of resistance. This will reduce the widespread use of antibacterial drugs especially the (OTC) drugs and lessens the emergence of resistant strains and eventually reducing morbidity and mortality rates of the population. This will also reduce the cost of patient medical care.

ii) The health certificate now only requires food handlers to be tested for typhoid and the presence or absence of intestinal parasites in their stool samples. There is need for change of policy in the testing of food-handlers for the purpose of issuance of health certificates to include culture and sensitivity testing of bacteria. This will serve as a baseline data of prevalence of drug resistance for this country in future.

iii) Lastly, there is need for a surveillance network for antibiotic resistance in Kenya to monitor changes over time which can act as a frontal guard in the fight against rising antibiotic resistance.

6.3 Suggestions for further research

i) To study antibiotic resistance for other drugs that were not covered in this study including anti-malarials, so as to combat drug resistance and to minimise both morbidity and mortality rates in this country in future.

ii) There is an urgent need for studies to review policies regulating drug dispensing and purchasing for instance, review of Public Health Act Cap 242 and Food, Drug and Chemical Substances Act Cap 254, which have been regulating measures for the purpose of food safety in the country.

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Appendix 1: Ministry of Health

en replying please quote

No. and date MED/46/A Vol.I/185

REPUBLIC OF KENYA

MINISTRY OF HEALTH

DEPARTMENT OF STANDARDS AND
REGULATORY SERVICES (DSRS)
Afya House, Cathedral Road,
P.O. Box 30016, Nairobi, Kenya.
Tel: 254-2-717077 Fax: 254-2-722986
E-mail:dsrs@africaonline.co.ke

5th July 20

Hussein Ahmed Abdulrahman
MOH Resident
Kenyatta University
P.O. Box 43844
NAIROBI.

RE: YOUR APPLICATION TO CONDUCT RESEARCH.

This is to acknowledge that your request to conduct research on "Antibiotic resistant enteric bacterial among food handlers undertaking Medical/Examination in Nairobi" has been received and approved.

You are required to observe all ethical requirement pertaining to this research. Upon completion of the study you are required to give a copy of your findings and recommendation to the Director of Medical Services, the provincial Medical Officer of Health, Nairobi and the Medical Officer of Health, Nairobi City Council before publishing it.

Dr. Tom Mboya Okeyo
Head
Department of Standards and Regulatory Services

Appendix 2: Reactions Interpretation Table for API 20 E

TEST S	SUBSTRATES	REACTIONS/ENZYMES	RESULTS	
			NEGATIVE	*POSITIVE*
ONPG	Ortho-nitro-phenyl-galactoside	Beta-galactosidase	Colourless	Yellow (1)
ADH	Arginine	arginine dyhydrolase	Yellow	Red / orange (2)
LDC	Lysine	Lysine decarboxylase	Yellow	Orange
ODC	Ornithine	Ornithine decarboxylase	Yellow	Red / orange (2)
CIT	Sodium citrate	Citrate utilization	Pale green/Yellow	Blue-green / green (3)
H_2S	Sodium thiosulfate	H_2S production	Colourless/greyish	Black deposit / thin line
URE	Urea	Urease	Yellow	Red / orange (2)
TDA	Tryptophane	Tryptophane desaminase	**TDA** / immediate: Yellow	Dark brown
IND	Tryptophane	Indole production	**JAMES** Reagent / immediate or IND / 2 min: **JAMES** Pale green-yellow IND Yellow ring	**JAMES** Pink IND Red ring
VP	Sodium pyruvate	Acetoin production	**VP 1 + VP 2** / 10 min: Colourless	pink / red
GEL	Kohn's gelatin	Gelatinase	No diffusion of black pigment	Diffusion of black pigment

GLU	GLUCOSE	Fermentation / oxidation (4)	Blue / blue-green	Yellow
MAN	MANNITOL	Fermentation / oxidation (4)	Blue / blue-green	Yellow
INO	INOSITOL	Fermentation / oxidation (4)	Blue / blue-green	Yellow
SOR	SORBITOL	Fermentation / oxidation (4)	Blue / blue-green	Yellow
RHA	RHAMNOSE	Fermentation / oxidation (4)	Blue / blue-green	Yellow
SAC	SUCROSE	Fermentation / oxidation (4)	Blue / blue-green	Yellow
MEL	MELIBIOSE	Fermentation / oxidation (4)	Blue / blue-green	Yellow
AMY	AMYGDALIN	Fermentation / oxidation (4)	Blue / blue-green	Yellow
ARA	ARABINOSE	Fermentation / oxidation (4)	Blue / blue-green	Yellow
ox	ON FILTER PAPER	CYTROCHROME OXIDASE	OX / 1-2 min Colourless ——— Violet	

A very pale yellow should also be considered positive

An orange colour after 24 hours of incubation must be considered negative

Reading made in cupule (aerobic)

Fermentation begins in the lower portion of the tubes, oxidation begins in the cupule.

Appendix 3: Food Handlers Questionnaire

Name of establishment ..... Classification.....

Date of visit .....

Age.....

Sex.....

Department .....Designation.....

1. How long have you been in this establishment?.....

   a)< 6 months ((b) 1 – 2 years (c) 2 – 4 years (d) 4 – 6 years

2. Does this establishment provide health services? Yes or No

3. Do you seek health services outside the establishment? Yes or No

4. How regular is medical examination for certification in this establishment?

   a) 6 months (b) 1 years (c) 2 years (d) 3 years

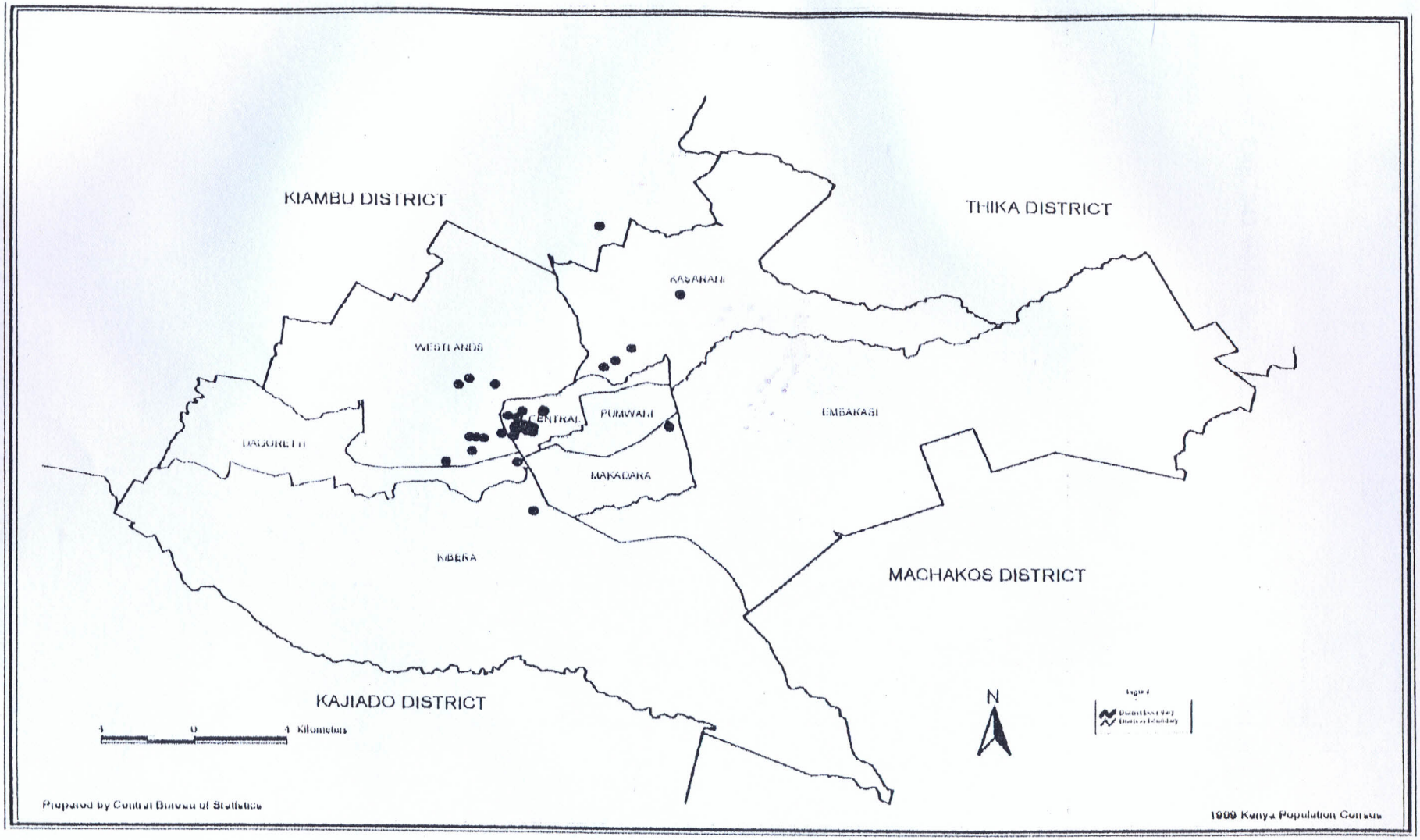
5. Did you use any medicine before 2weeks of medical examination?

   Yes or No

6. What happens if you are found sick?

Nothing happens (b) terminated from work (c) allowed sick off (d) allowed light duties.

Appendix 4 Map of Nairobi



MAP OF NAIROBI ADMINISTRATIVE DIVISIONS SHOWINGS LOCATION OF MAJOR HOTELS

Appendix 5 Food Handling Regulatory Measures

The Food Drugs and Chemical Substances Act **Cap.254** Laws of Kenya(Food Hygiene) in which regulations spell out the hygienic requirements to be observed in food establishments.

The Public Act **Cap. 242** which deals with sanitary measures to be observed in food premises.