ISOLATION, MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL PROFILE OF FOOD BORNE BACTERIAL PATHOGENS FROM MONEY AND CELLPHONES OF FOOD HANDLERS FROM SELECTED FOOD OUTLETS IN NAIROBI

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Isolation, Molecular Characterization and Antimicrobial Profile of Food Borne Bacterial Pathogens from Money and Cellphones of Food Handlers from Selected Food Outlets in Nairobi

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A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Molecular Medicine of the Jomo Kenyatta University of Agriculture and Technology

2021

DECLARATION

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

Signature ………………………………………… Date ……………………….

Gertrude Gati Kisang

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

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DEDICATION

This thesis is dedicated to God first, who gave me strength, favour and provision to enable me see this output. To my beloved husband Eng. Moses Kipkirui Tanui who has tirelessly prayed, encouraged and supported me financially throughout the process of doing this work, my daughter Tyffany, for her patience and understanding despite the loneliness occasioned by my absence, and my mother for her constant prayers.

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LIST OF ABBREVIATIONS AND ACRONYMS

API	Analytic Profile Index
BHI	Brain Heart Infusion
CCD	Charcoal Cefoperazone Deoxycholate
CDC	Centre for Disease Control and Prevention
XLD	Xylose Lysine Desoxycholate agar
FAO	Food and Agriculture Organization of the United Nations
HISP	Hospitality Industry Support Programme
MAC	MacConkey Agar
MIL	Motility Indole lysine
MOH	Ministry of Health
MS	Microsoft
NA	Nutrient Agar
PCR	Polymerase Chain Reaction
SIM	Subscriber Identity Module
SMS	Short Messages Service
SPSS	Statistical Package for Social Scientists
TSI	Triple Sugar Iron
WHO	World Health Organization

ABSTRACT

Currency is used for every type of commerce while mobile phone is used for communication and plays an important role in the life of a food handler in busness. However, the combination of its widespread use and constant exchange of money make them a likely agent for disease transmission but this data in food handles is limited The aim of this study was to isolate, characterize and determine the antimicrobial susceptibility profiles of foodborne bacterial pathogens isolated from money and cellphones of food handlers in Nairobi, Kenya. A total of 384 food handlers were recruited in the study and from each one bank note, coin and cell phone swab was sampled and cultured in 10% Sheep blood agar plate, Xylose Lysine Desoxycholate agar and MacConkey agar (Oxoid, Basingstoke, UK).Biochemical tests were used for identification of the isolates and antimicrobial susceptibility testing was done on the identified bacterial foodborne pathogens. Characterization of toxigenic strains of *E.coli* and determination of the presence of *mec*A gene in *Staphylococci aureus* for antimicrobial resistance was determined by polymerase chain reaction. Data analysis was done using SPSS version 19.0 (CDC, Atlanta, USA). Descriptive analysis was used to obtain simple frequencies and proportions of foodborne pathogens and antimicrobial patterns. Out of the 384 participants, 56.3% (216/384) were males and 46.5% (179/384) were females. 18.8% of the money tested had foodborne bacterial pathogen which included *Staphylococci aureus* (18.4%) and *E.coli* (0.4%) the cell phones had *Staphylococci aureus* (14%), and *E.coli* (7%). Six strains of *E.coli* in this study had a combination of genes coding for the following pothotypes; EPEC, EHEC and EIEC. *E.coli* showed highest antimicrobial resistance with ampicillin (16%) and sulphamethoxazole (15%). 12 % MRSA were resistant to oxacillin, with the highest resistance of *Staphylococci aureus* was seen in cefuroxime and erythromycin (21%). The study established that the foodborne bacterial pathogens were present on money handled by food handlers (18.8%) which were *E.coli* (0.4%) and (18.4%) *Staphylococci aureus*.The cell phones (21.3%)were also contaminated with foodborne bacterial pathogens which included *Staphylococci aureus* (14.3. %) and *E.coli* (7%).The *E.coli* characterized had virulent genes coding for ETEC,EHEC,EAEC,EPEC and EIEC and *Staphylococci aureus* were positive for *mec* A gene. *E.coli* isolates also showed antimicrobial resistant to ampicillin(16%) and sulphamethoxazole(15%) and (12.5%) isolates of MRSA which were all resistant to oxacillin, the highest antimicrobial resistance was observed in cefuroxime and erythromycin (21%) for the *Staphylococci aureus* .This findings will inform the government policy makers and food establishments on the need to formulate and enforce guidelines on the handling of money and phones when processing food.The general public should also be sensitized on the risks involved in handling food after touching money and mobile phones.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Foodborne illness is an important cause of morbidity and mortality worldwide. The global burden of foodborne disease recently published indicate that one in every ten people are affected worldwide each year and the causes being bacterial, viral, parasitic and of chemicals origin.The burden is greater in sub-Saharan Africa and South East Asia (WHO, 2015). Food safety appears to be a challenge not only in developing countries but also in the developed countries despite their developed food chain monitoring systems (Yeleliere *et al*., 2017). Foodborne diseases arise usually as a result of ingesting contaminated food caused by a range of microorganisms and may also arise due to contamination with hazardous chemicals. The source of food contamination can be from the environment where the food is handled, poor personal hygiene of the food handler, poor or lack of basic amenities, inadequate knowledge on food safety, management tools and lack of policies or its implementation (Muinde and Kuria, 2005; WHO, 2015). This makes it difficult to establish what factors contribute most to outbreaks of foodborne diseases. There is paucity of data on the estimation of health impact of foodborne illnesses in Kenya and the incidences are not easy to estimate (Kariuki & Orago, 2017).

Money in the form of paper and coins is the most commonly used medium of exchange for payments of goods and services in Kenya (Kuria *et al.,* 2009).The worth of money being exchanged is in terms of the value of the goods and/ or services being sold. Money is the most sought after and is a measure of wealth by majority of people irrespective of nationality, race and social class. Despite the rapidly changing lifestyles where credit cards and other payment platforms such as mobile money transfers are replacing the use of hard cash, it appears bank notes and coins are still the most commonly used medium of exchange for most small and medium enterprises in Kenya hence "cash is king" still applies in our country (Amayo, 2020).

As observed different people have different ways of keeping money especially when travelling or walking in the streets for fear of losing it. Some keep their money in purses, bags, pockets, socks, undergarments, shoes, and some even place coins in the ear(this is a common practice seen with touts). These practices may be a source of contamination in cases where the person handling the money has ear infections, skin lesions or soiled underwear. Cell phones commonly known as “mobile phones” have largely replaced the traditional public and private land lines (fixed telephone lines) that were initially used for communication in the major cities and rural towns of Kenya. Handsets are commonly shared between close contacts and it is possible that the handsets may pick bacteria and other microorganisms from the hands because of poor hygiene standards.The cellphones have now become affordable and accessible compared to when they were first introduced in the country. In 1999 service provider Safaricom predicted that the cellphone market in Kenya would reach three million subscribers by the year 2020;by 2009 Safaricom already had 14 million subscribers (Aker & Mbiti, 2010). It has been reported that the use of cellphones in Kenya is widespread with 42 mobile subscriptions per 100 people. The ownership rate of those surveyed nationally was found to be 60%; personal phone ownership was claimed by 71% of urban respondents versus 55% of rural dwellers (Bowen & Goldstein, 2010).

In the national economic survey conducted in 2019, mobile telephony subscription has been on a steady increase since 2014.The subscription increased by 15.6% from 42.8 million in 2017 to 49.5 million in 2018.Total money transfers also grew by 9.5% (Kenya Bureau of Statistics Economic Survey, 2019).

1.2 Statement of the Problem

It is estimated that 1 in 10 people fall ill after eating contaminated food world wide Every year 220 million children contract diarrhoeal diseases and 96,000 die. Unsafe food creates a vicious cycle of diarrhoea and malnutrition, threatening the nutritional status of the most vulnerable (WHO, 2015; WHO food safety, 2020). The sources of food contamination are vast and may range from environmental where the food is handled, poor personal hygiene of the food handler, poor or lack of basic sanitation amenities to inadequate knowledge on food safety among others (Kariuki *et al*., 2017). It is difficult to establish what factors contribute most to outbreaks of foodborne diseases that is why it was important to find whether these indispensable accessories –money and phone habour foodborne bacterial pathogens that contribute to food contamination and so possible cause of foodborne disease outbreaks.

1.3 Justification

Foodborne diseases are common in Kenya because of poor food handling practices, lack of hygiene among other many factors (Kariuki *et al.,* 2017). The Data on the causes and burden of foodborne disease is limited, estimates of the health and economic costs of foodborne diseases in Kenya exists only at the national level and even then it is under reported (Hoffmann *et al*., 2019).Cell-phones and money are possible vectors of disease with pathogenic bacteria being isolated from the two systems in other populations. Money and cellphone are handled in close contact if not together with food in the industry especialy after the introduction of the mobile money transfer technology in the country. There is paucity of data on colonization of foodborne bacterial pathogens on money and cellphones among food handlers in Kenya. It is, therefore, important to establish whether the cellphones and money habor foodborne bacterial pathogens which can pose as risk to public health.

1.4 Objectives

1.4.1 General Objective

To isolate, characterize by molecular and genotypic methods and determine the antimicrobial susceptibility profiles of foodborne bacterial pathogens isolated from money and cellphones of food handlers in selected food outlets in Nairobi, Kenya.

1.4.2 Specific Objectives

i. To isolate foodborne bacterial pathogens from money and cellphones of food handlers from selected food outlets in Nairobi, Kenya.
ii. To characterize foodborne bacterial isolates from money and cellphones of food handlers by genotypic and molecular methods.
iii. To determine the antimicrobial susceptibility profiles of the isolated foodborne bacterial pathogens from money and cellphones of food handlers

1.5 Research Questions

i. What foodborne bacterial pathoges are found on money and cellphones used by food handlers in Nairobi?
ii. Are foodborne bacterial pathogens present in money and cellphones of food handlers toxigenic?
iii. How is the antibiotic susceptibility profile of the foodborne bacterial pathogens isolated from money and cellphones of food handlers?

1.6 Scope

This study covered only foodborne bacterial pathogens.

1.7 Limitations

This study did not test for other microoganisms as well as association between the isolated foodborne pathogen on the money and phone of foodhandlers and disease outbreaks.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Foodborne infection is caused by ingestion of contaminated food containing viable bacteria such as *Salmonella* or *Escherichia coli (E.coli*) which then multiply in the host, resulting in illness. Foodborne toxicoinfections result when bacteria present in food, such as *Clostridium perfringens*, are ingested and subsequently produce a toxin in the host. The presence of pathogens in our environment, the ability of some of them to survive and/or proliferate under harsh conditions and for some pathogens, the low number necessary for causing disease are the challenges faced in implementing control measures (Noor, 2019). Foodborne illness is under reported globally nonethelss it is estimated that about 30% of the foodborne illness cases are due to bacteria (WHO, 2008).

Foodborne diseases are still a problem in Kenya, 70% of diarrheal episodes are attributed to ingestion of either contaminated food or water (Oloo, 2010) .There is increase of food vendors in Nairobi who sell both raw and cooked food due to an increase in food demands and the need for income sources. This has strained the limited city resources such as water, sanitation, and also increased the risk of foodborne outbreaks because most of the street food vendors are untrained in food hygiene and sanitation, and work under poor sanitary conditions (Kariuki *et al.,* 2017).

Street food vending has grown and has provided affordable, vast variety of food in the urban setup, this industry has also provided a source of livelihood to many Kenyans in Nairobi. Despite the advantages this sources of food is accompanied with potential health risk to consumers because of the poor hygiene under which the food is prepared and handled (Imathiu, 2017).

A food handlers can be defined as any person involved in any way with handling, processing, and serving of food such as waiters, cooks, chefs, barmen, butchers, and delivery people (Oundo *et al.*, 2008).

E. coli is found as normal flora of the human gut normally found in faces, therefore the presence of E. coli in food types is an indication of faecal contamination at one stage of preparation or from the materials used. The overall occurrence of food contamination in a study done in Kenya was 34.9%. This was based on the total aerobic plate count (APC), Enumeration of total coliforms *E .coli* contamination was observed in 25.2% of the food samples in this study (Kariuki *et al*., 2017).

A study was carried out to establish the role of food workers in foodborne outbreaks, the findings indicate that the workers were not always aware of their infection status either because they were asymptomatic or at incubation period .The pathogen likely to be transmitted is of viral and bacterial origin (Greig *et a*l., 2007).Investigations further reveal that although direct contact of food by hands should be prevented by the use of barriers such as gloves, food handles ignore or use them wrongly. Money and ready-to-eat foods should also be handled as two separate operations, preferably by two workers .Contaminated hands of food handlers has been implicated in foodborne disease outbreaks and hand washing practice recommended as the most convenient way of removing pathogens from hands (Todd *et al*., 2010).

2.2 Foodborne Pathogens from Money

Several studies have been done on microbial contamination of money for many years and in several countries. A review of these studies revealed that contamination of money is a potential cause of sporadic foodborne illness and that the age of the notes and the material used in making the notes influenced the number of microbial contamination (Agersew, 2014).Money in form of coins was collected from different populations in Kenya including food vendors from greengrocers, butcheries, food kiosk/restaurant attendants, and roast maize vendors. The coinage were found to have potentially

pathogenic microbes including food borne bacteria such as *Escherichia coli*, Serratia, Salmonella, Staphylococcus and *Bacillus cereus.*

There is need for further studies to assess the currency notes and establish whether they harbor pathogenic bacteria that is a potential health risk. It is also important to establish whether these isolates could transmit antimicrobial resistant strains through the food chains by the food handlers which again would be a public health concern. (Kuria *et al*., 2009). 400 naira notes were collected and tested for microbial contamination. All the 400 were contaminated with 14 different microbial species predominantly *Escherichia coli* (25%) and *Staphylococcus aureus* (12%). The study shows that Naira notes circulating in Bauchi metropolis were heavily contaminated with microbes (Usman *et al*., 2021).

A study done on food sellers in Ghana revealed that 98.6% of the currency notes were contaminated with pathogenic microorganisms that can cause serious human diseases. Bacteria isolated included acid fast bacilli, *Bacillus* species, Coagulase-negative Staphylococci, *Staphylococcus aureus*, *Enterococcus faecalis*, *Citrobacter freundi*, *Klebsiella pneumoniae*, *Shigella dysenteriae* and *Escherichia coli* (Patrick and Michael, 2010).In a similar study a comparative analysis of bacterial contamination of Nigerian currency notes of different denominations from local food vendors was done. The results showed that about 81.7% of currency notes were contaminated with either *Escherichia coli*, Klebsiella spp. or Staphylococcus spp in varying degrees (Ofoedu *et al*., 2021).

These bacteria are transferred from one person to another by simply touching money. When the food handler receives contaminated money, he or she receives the bacteria as well. If no hand sanitation is practiced before handling food, the bacterium is passed onto the food. The bacteria from contaminated hands of a food handler are transferred to the food prepared and/or handled and as a result the food maybe contaminated with bacteria that can cause food borne disease.

2.3 Cellphones as Possible Vector of Bacteria and Pathogens

A study on the contamination rates of cellphones in Bayer University, Kano in Nigeria indicated 100% and 80% bacterial contamination of public and personal cellphones respectively. The isolates found were *Staphylococcus aureus* and *Streptococcus* species. These organisms may probably have contaminated the phone through the skin and hand to hand mechanism .The isolated bacteria are subset of the normal micro biota of the skin. The duration, location and possible number of users determine the type of the colonizing bacterial and the bacterial load. (Yusha'u *et al.,* 2010).

A prospective cross-sectional study of bacterial contamination in the healthcare environment revealed that of the 70 bed-control handsets that were sampled, 95.7% had bacterial growth with an average of 1.5 different bacterial species identified per hospital bed-control handset. Bacteria causing nosocomial infection were isolated in 41.4% of the handsets while, 31.4% handsets grew *Enterococcal* species, 12.9% Methicillin resistant *Staphylococci aureus* (MRSA), 2.9% grew coliforms, and 1.4% had anaerobic bacteria (Brady *et al.*, 2007).

Cell phones of students in Malaysia were tested for prevalence of microbial contamination and antimicrobial susceptibility pattern. All the phones were contaminated with either single or mix bacterial agents and oxacillin resistance was seen in 5.9% of *S. aureus* isolate (Hikmah, 2020).

2.4 Bacterial Identification methods

Bacterial identification has evolved over the years and is broadly delimited into genotypic techniques and phenotypic techniques.

2.4.1 Phenotypic techniques used in bacterial identification

The phenotype of a microbe can be defined as the appearance of how that microbe looks like, the proteins and enzymes that it produces and expresses. Phenotypic technique is

based on profiling either an organism's metabolic attributes or some aspect of its chemical composition and involves a combination of more than one method. Phenotypic techniques are limited in specificity and sensitivity and can also be tedious, subjective, and inconclusive when conflicting results are obtained. Phenotypic methods will include preliminary tests such as staining, growth characteristics in culture media/specific nutritional requirement, testing for production of certain enzymes and mobility which will help in placing the organism in a general group. Further biochemical and serological tests will then be used to type for genus and a few species types (Sánchez, 2015).

2.4.1.1 Gram stain technique

The gram staining technique is used to differentiate organisms into two groups the gram positive or gram negative and this is the most commonly used method in classifying bacteria in-vitro. This staining technique has been modified by many scientists over time since its discovery but the principle remains the same and its importance unquestionable. The use of this staining method is important as a preliminary step in the initial characterization and classification of bacteria (Thairu, 2014).

2.4.1.2 Bacterial culture

To culture means to grow; bacteria is grown in an artificial media in-vitro which allows provision of nutritional and environmental conditions for the growth of the bacteria being sought. There are different types of culture media some are for general purpose whereas some are selective for specific bacteria. Bacteriological culture media can be prepared as a liquid, a solid, or as a semi-solid. Different bacteria give rise to colonies that may be quite distinct to the bacterial species hence a useful preliminary step in identifying bacteria (Berkowitz, 2016).

2.4.1.3 Biochemical Identification Tests

To identify the isolated bacteria after culture, a series of biochemical and /or serological tests are done. Every bacterial species has a known biochemical profile which can then

be compared with profile of known species. The inoculum from a pure bacterial culture is inoculated into a broth medium containing particular substrates, bacteria metabolizing a substrate by oxidation or fermentation will changes the pH of the medium which is visualized by a P^h indicator that changes the color of the medium. Examples of these biochemical tests include sugar fermentation test (Melter, 2019).

2.4.2 Genotypic methods of bacterial identification

The genotype of a microbe is the genetic material that the microbe is carrying, the DNA in the bacteria. Genotypic methods are based on profiling an organism's genetic material primarily it's DNA (Emerson *et al*., 2008). These methods have the advantage over phenotypic methods in that they are independent of the physiological state of an organism; they are not influenced by the composition of the growth medium or by the organism's phase of growth. Genotypic methods are a common part of clinical diagnostic procedures today (Melter, 2019).

Genotypic microbial identification methods can be divided into two broad categories: pattern- or fingerprint-based techniques and sequence-based techniques. Sequence based methods have proved effective in establishing broader phylogenetic relationships among bacteria at the genus, family, order, and phylum levels, whereas fingerprinting-based methods are good at distinguishing strain- or species-level relationships or genus level (Agulto *et al*., 2008).

2.4.2.1 Polymerase Chain Reaction (PCR)

PCR is an enzyme-driven process that allows amplification of short target regions of DNA in vitro. These sequences of the region of interest must be known before the test and are used to design the oligonucleotide primers that hybridize specifically to the target sequences during the PCR run. Three major steps are employed in PCR; denaturation of template DNA into single-stranded DNA, primers annealing to their complementary target sequences and extension of primers through DNA polymerization

to generate new copy of the target DNA. After which the newly synthesized DNA act as new template for the next cycle. When this is repeated many times then exponential amplification of the target DNA occurs (Usman & Ali, 2009).

Traditional methods of bacterial identification in most of the laboratories in Kenya rely on phenotypic identification using culture method, gram staining, biochemical tests and serological typing. These conventional methods of diagnosis delay the release of results and the clinicians are forced to commence treatment using preliminary results as they await confirmation. The other challenge of the conventional methods is the fact that some of the microbes are slow/poor growers or may not grow in artificial media at all. PCR technique has overcome these, also they can detect the genes coding for virulence factors that cause particular diseases or syndromes, e.g. toxins, and genes coding for antimicrobial resistant except that it is expensive and may not be affordable to many people in this region . (Berkowitz, 2016).

Identification of different diarrheagenic *E. coli* pathotypes is not routinely performed because it is cumbersome and the techniques are not readily available. The challenge with this technique is that the primers must have the same melting temperature, must not interact with each other and the detection of products fragments of same length band or less quantity of amplified product can be difficult (Dinesh *et al*., 2009). Multiplex PCR employs simultaneous amplification of more than one locus for rapid detection of multiple microorganisms in a single reaction. Several specific primer sets are combined into a single PCR assay .The design of the primers is key in the development of a multiplex PCR assay to avoid interaction between the multiple primer sets (Zhao *et al*., 2014). Unlike the other enteropathogens it is difficult to differentiate the pathotypes of *E.coli* using conventional methods except by PCR. Multiplex PCR is a technique in which more than one pair of primer is used to amplify different fragment of the target gene simultaneously with the aim of saving time and resources (Müller et al., 2006).

2.5 Antimicrobial Susceptibility Testing

Antimicrobial resistance is a serious public health concern that threatens the effective prevention and treatment of an ever-increasing range of infections caused by microbes no longer susceptible to the common medicines used to treat them. Over several decades, to varying degrees, bacteria causing common or severe infections have developed resistance new antibiotic coming to market (Prestinaci *et al*., 2015).

The major contribution to the spread of antimicrobial resistant bacteria is the use of antimicrobial agents in human and livestock medicine therapy .Resistant bacteria from domestic animals can be transmitted to man indirectly via the food chain resulting in food-borne illness in humans that is less responsive to treatment with conventional antimicrobial drugs (Kikuvi *et al*., 2013). Antimicrobial resistance in Escherichia coli isolates from faeces and carcass samples of slaughtered cattle, swine and chickens in Kenya. A study carried out in Kenya revealed that food handlers working in tourists hotels in Nairobi, Malindi and Diani are important carriers of multidrug resistant EAEC which is a public health risk (Oundo *et al.*, 2008).

A study was carried out to determine antimicrobial resistance in *Escherichia coli* isolates from feces and carcass samples of slaughtered cattle, swine and chickens in Kenya. This study revealed that multi-drug resistant *E. coli* isolates are prevalent in cattle, pigs and chickens in Kenya and with a considerable proportion of E. coli isolates from fresh cattle and pig carcasses being resistant to a variety of antimicrobial agents (Kikuvi *et al*., 2013).

CHAPTER THREE

METHODOLOGY

3.1 Research design

Cross-sectional study design was used where samples from the target population in selected food outlets under the KEMRI-HISP Hospitality Industry Support Program (HISP) were collected and taken to the laboratory for analysis. Food handlers in this study were persons handling food in food processing firms,farms,dairies ;cooks and weiters in hotels, schools and colleges; persons packaging all types of food for export, and bakers working in bakeries and supermarkets.

3.1.1 Inclusion and exclusion criteria

3.1.1.1 Inclusion criteria

i. The food handlers meeting the following conditions were included in the study:-
ii. All food handlers over 18 years of age working in food outlets recruited in KEMRI- HISP
iii. In possession of a coin, bank note and cellphone at the time of sampling
iv. Consenting to participate in the study.

3.1.1.2 Exclusion criteria

Excluded participants were:-

i. Children
ii. Foodhandlers with either a coin, bank note or cellphone at the time of sampling
iii. Those who did not consent to be included in the study were excluded.
iv. All food outlets that were not recruited under the HISP were excluded in the study.

3.2 Study area

This study was carried out in selected food outlets in Nairobi County which has a population of 4,397,053 people according to the Kenya national census (2019). She seats at 5525 feet above sea level on latitude -1.292066 and longitude 36.821946 (Latitude, Longitude and GPS coordinates of Nairobi City). It has 703.9 km sq with a settlement of 6247 people per sq km (KPHC 2019, vol 1).

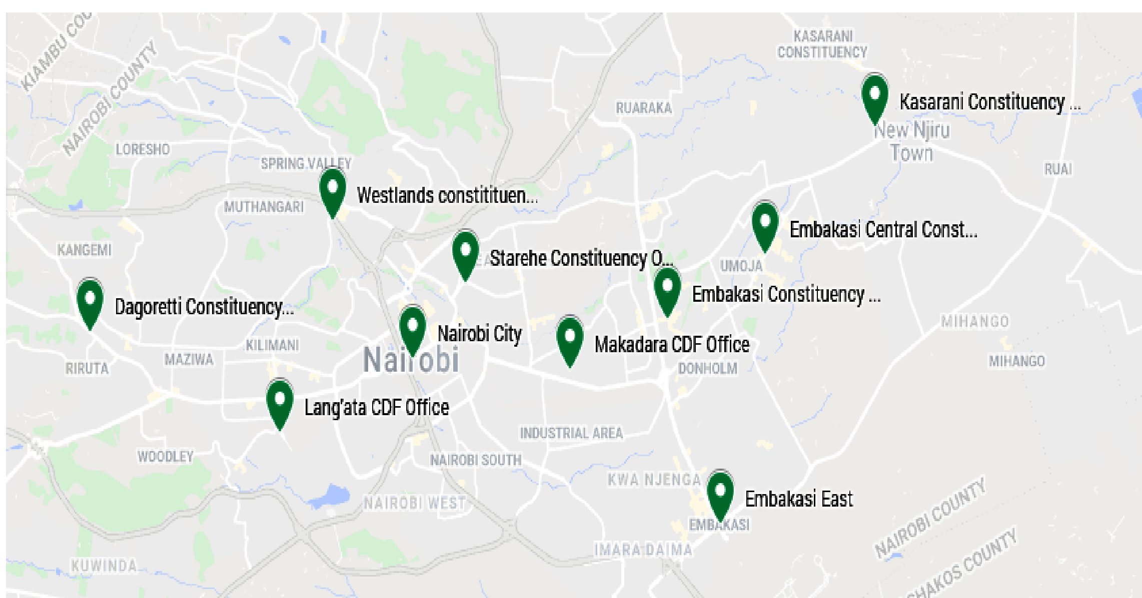


Figure 3.1: Map of the study site

3.3 The target population

The study population consisted of waiters, cooks, bakers, staff packaging food for export and dairy milk handlers in processing firms recruited in the HISP programme.

3.4 Sampling techniques and illustrations

Sample size determination

Assuming a prevalence rate of 50% of foodborne bacterial pathogen (*S.aureus* and *E.coli*) on currencies (Vriesekoop *et al.,* 2 010) and on cellphones (Ulger *et al*., 2009)

and 5% level of significance and using the formula by Naing *et al.,* (2006) the minimum sample size was calculated as below:

$$n=\frac{Z^2pq}{d^2}$$ (Naing *et al.,* 2006)

Where

N=minimum sample size required

Z=1.96 standard error from the mean

q=1-p

d=0.05(5% absolute precision)

P=0.5 –prevalence rate.

Money

$$N = \frac{1.96^2 \text{ x } (0.5\text{x}0.5)}{0.05^2} = 384.16$$

Cell phones

$$N = \frac{1.96^2 \text{ x } (0.5\text{x}0.5)}{0.05^2} = 384.16$$

The minimum sample size used was 384 food handlers.

3.4.1 Sampling method

The food outlets were selected by cluster sampling method where 29 outlets were picked proportionately according to the number of food establishments within the Nairobi subcounties. The total population size was determined using randomised list of participants from each selected outlet before every sampling exercise was done.A selected participant in possession of a bank note,coin and a mobile phone was sampled(ratio of 1:1:1).

3.4.2 Consenting of study participants

Before commencement of the sampling exercise a brief explanation of the study was given to the prospective participants at the sampling center. This was followed with individual discussion and question time with the intrested participants after whom they were consented. All the participants encountered were able to read and write in either English or Kiswahili.

3.4.3 Sample Collection and Transportation

Money and phone swabs were collected aseptically into a sterile plastic disposable test tube which were then labelled and placed in a test tube rack in an upright position and transported to laboratory in a cool box.

3.4.3.1 Collection of money swabs

Using sterile cotton wool swabs moistened in Brain heart infusion broth the entire surface of a participant's coin was aseptically swabbed and a minimum area of about three quarter of a participant's bank note surface was swabbed. The swabs were then dropped back into their individual labelled sterile tubes and transported to the lab within 4 hours for processing.Each swab was treated independently.

3.4.3.2 Collection of cellphone swabs

Sterile wet swabs moistened in brain heart infusion broth was used to swab the cellphone handset availed and each was treated independently. For uniformity the key pad area and the ear piece were aseptically swabbed. The swabs were then dropped back into their individual labelled sterile tubes and transported to the lab for processing.

3.5 Laboratory procedures

3.5.1 Bacterial isolation and identification

Each batch of culture media prepared was first incubated aerobically at 37° C (Aerobic – Memmert) overnight before inoculation to check for sterility and followed by inoculation with appropriate ATCC control organisms to ensure optimal function (*Staphylococcus aureus* 25923, *E. coli* 25922 ,*Salmonella typhimurium* 14028 and *C. jejuni* 29428).

Sample swabs were then used to inoculate the surface of freshly prepared and quality controlled 10% Sheep blood agar (BA) plate, Xylose Lysine Desoxycholate agar (XLD) and MacConkey (MAC) agar (Oxoid, Basingstoke, UK). This was followed by successive periods of streaking the surface of the solid agar plate with a sterile wire loop while turning the plate through 45°, and allowing the wire to cool before proceeding with the aim of achieving single colonies. The plates were then incubated aerobically at 37° C (Aerobic – Memmert) for 24 hours. After inoculation into the solid media, the swabs were inoculated into 10 ml BHI and incubated for 72 hours before subculture into Charcoal Cefoperazone Deoxycholate (CCD) and incubation done 5% CO_2 incubator (CO_2 – Memmert) at 42° C for up to 48 hours. All the primary tubes were then stored at 4° C until the laboratory process was complete.

3.5.2 Examination of growth and selection of isolates

After 24 hours of incubation on BA, XLD and MAC and 72 hours on CCD, the culture plates were observed for visible colonial growth. The plates with no growth observed after 24 hours and 72 hours for CCD were reported as negative. Growth observed along the line of inoculums was counted and colonial appearance noted and recorded. Plates with mixed growth were purified by sub culturing into Mueller Hinton agar (Oxoid, Basingstoke, UK) and colonies of interest were also inoculated into Mueller Hinton (Oxoid, Basingstoke, UK) for maintainance before identification and sensitivity was done. Colonies which were haemolytic on blood agar (showing clear zones or partial clearing zones around the colonies) yellow, cream or white were picked aseptically and smears were prepared for gram staining and subcultures were done on Mannitol salt agar. Lactose fermenters on MAC and non lactose fermenters on XLD were also picked aseptically and smears prepared for gram staining.

3.4.3 Identification of foodborne bacterial pathogens from money and cellphones

3.5.3.1 Gram staining of bacterial isolates from Swabs

To prepare the smear, one drop of sterile normal saline was placed aseptically on to a clean dry grease free labeled slide. A colony of interest was then identified, picked by touching with a sterile cooled wire loop and emulsified on a drop of the sterile normal saline; the loop was moved in an oval spiral outward rotation from the centre until a smooth suspension of the smear was formed covering approximately 2x2 cm. The slide was then allowed to air dry and it was fixed by passing it over the flame 3 times. The quality of the smear was assesed by ensuring that typed print could be read through the stained smear. Too thick smears were repeated.

The stains were tested daily before commencing staining using a known gram positive and gram negative bacteria. The prepared slides with fixed smears were arranged on the staining rack chronologically then flooded with crystal violet dye for 1 minute. The

slides were then rinsed using tap water, Gram's iodine was added to it and kept for 1 minute, followed by decolorisation with 1% acid-alcohol for 4-5 seconds. Counterstaining was done by 0.5% Safranine Bhattacharyya (2015) and the slides were placed on the rack to air dry before examining using the oil immersion objective. When reporting, the staining reaction morphology and cell arrangements were recorded

3.5.3.2 Biochemical characterization and reporting of foodborne bacterial pathogens isolated

3.5.3.2.1 Catalase test

Catalase test (Reiner, 2010) was done on all colonies from Mannitol salt agar plate that produced yellow colonies (mannitol fermenters) and stained Gram positive cocci in clusters. Catalase test was done by introducing a good growth of the suspect organism into 1 ml of 3% hydrogen peroxide solution in a sterile clean test tube using a sterile applicator stick. Immediate bubbling was recorded as positive catalase test reaction but absence of bubbling was recorded as negative catalase reaction. None hemolytic gram positive bacilli and yeast cells were recorded in the lab book but no further identification tests were done.

3.5.3.2.2 Coagulase test

Coagulase test (Katz, 2010) was done on all catalase positive isolates. This was done by emulsifying a colony of the test organism into a drop of plasma on a clean grease free slide to make a thick suspension. Clumping within 2 seconds was recorded as positive coagulase test and no clumping was confirmed by the tube method where plasma was diluted 1:9 and three drops of diluted plasma was dropped aseptically in a sterile test tube, 1 colony of intrest was inoculated and the tube was incubated at 37° C for 30 minutes and 1 hour, thereafter the tubes were observed for clumping before discarding as negative coagulase test.

3.5.3.2.3 Motility Indole lysine, MR-VP, Citrate testing and TSI

Gram negative bacilli isolates was identified by use of biochemical test modified after Ewing, (1986).Each batch of above culture media prepared was first incubated aerobically at 37° C (Aerobic – Memmert) overnight before inoculation to check for sterility and followed by inoculation with appropriate ATCC control organisms to ensure optimal function as instructed by the manufacturer.

One lactose fermenter colony, gram negative from MAC was inoculated into Motility Indole lysine (MIL), MR-VP broth, Simmons citrate agar media (Hi media, India) and Triple sugar iron agar (TSI). Positive results for *E. coli* were read as TSI: acid butt, acid slant, with gas and without hydrogen peroxide; in MIL: positive motility, production of indole after addition of kovacs reagent and positive lysine decaboxylation (media remains purple); Simon's citrate negative (media remains green).Confirmation of difficult isolates were further identified using API E20 (BioMerieux)

All that were confirmed as *E. coli* and *S.aureus* were stocked in Tryptic soy broth (TSB) with 15% glycerol and frozen at -81° C (Freezer - Sanyo Ultra Low) awaiting multiplex PCR for pathotype detection.

3.5.3.2.4 Oxidase testand urease test

Oxidase test was also done Shields, (2010) on all gram negative none lactose fermenters without black centers on XLD agar .This was done by asceptically touching the suspect colony with a sterile applicator stick and making an impression on a wet oxidase disc. Immediate formation of a purple color was reported as oxidase positive and these were presumptively identified as Pseudomonas species. The oxidase negative colonies were then inoculated into urea broth Brink, (2010) and Triple iron sugar. All alkaline/acid with gas and hydrogen sulphide and split urea within 4 hours were presumptively identified as Proteus species. All that produced acid /acid with no or little gas and no indole in MIL and citrate positive as well as split urea after 8 hours was presumed to be

klebsiella species.All these isolates were only recorded and no forther tests were done because they were not foodborne pathogens.

3.6 Molecular characterization of foodborne bacterial pathogens isolated from swabs of money and phones

3.6.1 *DNA Extraction* from E. coli and S.aureus

Extraction was done by simple boiling technique modified after Sambrook *et al.* (1989). The *E.coli* and oxacillin resistant *Staphylococci aureus* isolated from the phones and money of food handlers was grown on Brain Heart Infusion broth (BHI) overnight then centrifuged at 10,000 rpm (Beckman) for 5 minutes at room temperature. The supernatant was discarded and the sediment cells re-suspended in 1 ml of TE buffer and vortexed (Vortex Genie 2). After this, 200µl was transferred to a new sterile tube and boiled for 30 minutes to release the DNA. The suspension was centrifuged at 15,000 rpm for 10 minutes and the supernatant used as template DNA for PCR.20µl of the extracted DNA was confirmed by gel electrophoresis (Mupid electrophoretic tank) in 1.% agarose (TAKARA) after staining with ethidium bromide and visualized under ultraviolet light - White Ultraviolet Transilluminator (UVP) against a standard molecular base pair (1000kb plus) ladder.

3.6.2 Molecular characterization of *E.coli* pathotypes by multiplex PCR

The optimized protocol of modified multiplex PCR Pass *et al.* (2000) was carried out by use of Pure Taq Ready-To- Go PCR beads (Amersham biosciences Buckinghamshire, UK).A total reaction volume of 26.4µl, was used in the run; template DNA was 2.0µl; with 24.4 µl of the master mix prepared by adding 2.4 µl of each of the forward and reverse primer set with 22 µl of sterile distilled water. The PCR conditions were set in the thermocycler(Eppendorf, USA) as follows initial denaturation step of 5 minute at 94°C followed by a further 1 minute of denaturation at 94°C; annealing step at 61° C for 30 seconds, and extension at 72°C for 1 minutes -seconds for 35 cycles, finally 72 for 10

min and cooling at 4ºC (Eppendorf, USA).The PCR products(10µl) were separated by gel electrophoresis (Mupid electrophoretic tank) in 1.5% agarose (TAKARA) and visualized under ultraviolet light - White Ultraviolet Transilluminator (UVP) against a standard molecular base pair (100kb) ladder after staining with ethidium bromide (Fermentas inc.,Maryland USA). Target genes and target primer sequence are shown in the table 3.1.

Table 3.1: Target Primer Sequence

Primer	Target gene	GenBank/EMBL accession no. or reference	Primer sequence	Amplicon size (bp)
LT	*eltB*	S60731	5′-TCTCTATGTGCATACGGAGC-3′	322
			5′-CCATACTGATTGCCGCAAT-3′	
ST	*estA*	M34916	5′-GCTAAACCAGTA$^{G}{}_{A}$GGTCTTCAAAA-3′	147
			5′-CCCGGTACA$^{G}{}_{A}$GCAGGATTACAACA-3′	
VT1	*vt1*	AF461172	5′-GAAGAGTCCGTGGGATTACG-3′	130
			5′-AGCGATGCAGCTATTAATAA-3′	
VT2	*vt2*	AY143337	5′-ACCGTTTTTCAGATTTT$^{G}{}_{A}$CACATA-3′	298
			5′-TACACAGGAGCAGTTTCAGACAGT-3′	
Eae	*eaeA*	AE005595	5′-CACACGAATAAACTGACTAAAATG-3′	376
			5′-AAAAACGCTGACCCGCACCTAAAT-3′	
SHIG	*Ial*	12	5′-CTGGTAGGTATGGTGAGG-3′	320
BfpA	*BfpA*	U27184	5′-CCAGGCCAACAATTATTTCC-3′	367
			5′-TTCTTGGTGCTTGCGTGTCTTTT-3′	
			5′-TTTTGTTTGTTGTATCTTTGTAA-3′	
EA	Pcvd	X81423	5′-CTGGCGAAAGACTGTATCAT-3′	630
			5′-CAATGTATAGAAATCCGCTGTT-3′	
Eae	*bfpB*	U27184	5′-GATAAAACTGATACTGGGCAGC-3′	811
			5′-AGTGACTGTTCGGGAAGCAC-3′	

Table 3.2: Ecoli strains and Target genes for for PCR

Pathotype	Target gene(s)
ETEC	eltB, estA
EHEC	vt1, eaeA
EHEC	vt2, eaeA
EPEC	eaeA, bfpA
EIEC	*ial*
EAEC	pCVD432-harboring strain
E. coli (negative control)	No virulence gene

3.6.3 PCR assay for Identification of *mec*A gene

PuRe Taq Ready-To- Go PCR beads (Amersham biosciences) with a total reaction volume of 25µl, was used in the PCR run using the following primer sets F` AAAATCGATGGTAAAGGTTGGC -3`= 0.2µl; R- 5` AGTTCTGGAGTACCGGATTTGC -3`= 0.2µl; the template DNA = 1.0µl; with sterile distilled water of 23.6µl. The PCR conditions were set in the thermocycler (Eppendorf, USA) as follows: initial denaturation step of 3 minute at 94^0C followed by a further 30-second of denaturation at 94^0C; annealing step at 55^0C for 30-seconds and extension at 72^0C for 30-seconds for 35 cycles. The PCR products(10µl) were separated by gel electrophoresis (Mupid electrophoretic tank) in 1.5% agarose (TAKARA) and visualized under ultraviolet light - White Ultraviolet Transilluminator (UVP) against a standard molecular base pair (1kb) ladder after staining with ethidium bromide (Fermentas inc.,Maryland USA).

3.7 Antibiotic susceptibility testing of the foodborne pathogens isolated from money and phones

Antimicrobial susceptibility testing was determined by Disk diffusion technique (Stokes & Ridgeway, 1980) on Mueller Hinton media (OXOID) and interpreted based on CLSI, (2008). Four colonies of the same type from pure plates of the isolated foodborne pathogen was picked using a sterilized cooled wire loop, and inoculated into 5ml sterile normal saline and turbidity adjusted until it was approximately equivalent to that of the McFarland 0.5 turbidity standard. A sterile swab was then used to inoculate the surface of Mueller Hinton media by moistening with the inoculum and spreading on the whole surface, while turning the plate through 45° while spreading then again to about 90° and streaking the surface again until the entire surface was covered.

Sterilized drug dispenser was used to dispense the antimicrobial disks on the surface of the inoculated plate. The plates were then incubated for 18 hours and diameters of sensitivity zones were measured and the results interpreted as per CLSI standard as either, sensitive, intermediate or resistant. The choice of drugs used was based on the commonly used antibiotics for both gram positive *S. aureus* and Gram negative Enteropathogens. The drugs included chloramphenical 30µg, ampicillin 10µg, co-trimoxazole-25µg, cefuroxime-30µg, gentamycin-10µg, cefotaxime-30µg, amoxicillin-clavulanate-20µg, erythromycin 15 µg, oxacillin 1µg, vancomycin 30 µg, chloramphenicol 30µg, ceftriaxone 30µg based on the CLSI, 2008. The ATCC *Staphylococcus aureus* 25923, *E. coli* 25922 and *C. jejuni* 29428 were used as quality control organisms.

3.8 Data management

3.8.1 Data storage and analysis

The questionnaires were entered and keep in a research workbook, password protected computer Microsoft word and excel software. Hard copies of filed data forms, consent

forms and the lab book were locked for safety and privacy. The data was organized and managed using computer software EPI INFO version 3.5.3 (CDC, Atlanta, USA). Data cleaning was done by running all the frequencies and filling all the gaps. Validation was done by use of Standard Operation Procedure (SOPS) in all the stages, including of the correct quality control, doing double entry of every participant and by going through all the questions and confirming all the entries were correctly done. Back up was done by storing the details in several storage devices (hard disk, flash disc, and CDs). Data analysis was done using SPSS version 19.0 (CDC, Atlanta, USA). Descriptive analysis was done to obtain simple frequencies and proportions of foodborne pathogens from money and phones, to describe the antimicrobial susceptibility patterns of *E. coli and Staphylococci aureus*.Data was presented using statistical summarized Tables and graphs.

3.8.2 Ethical issues

This study was approved by Board of post graduate studies of Jomo Kenyatta University of Agriculture and Technology (JKUAT), the scientific steering committee (SSC), KEMRI (Appendix I) and the National Ethical Review committee (ERC) through the Centre for Microbiology Research, KEMRI (Appendix II). Informed written consent (Appendix III) was soughed from the study participants using a standard consent form designed for the study. The permission was granted by the foodoutlet management. All the information was treated with confidentiality, with all responses to the questionnaires (Appendix IV) kept under lock and key and made available to only the authorized persons when needed.

CHAPTER FOUR

RESEARCH RESULTS

4.1 General characteristics of the study population

A total of 384 food handlers from a total of 29 food outlets were sampled in Nairobi County. Out of the 384 participants, 56.3% (216/384) were males and 46.5% (179/384) were females. Their mean age was 29.0±7.31 years; the minimum age was 16 years whereas the maximum age was 63 years and the standard error was ±0.368.

4.2 Food borne pathogens from Money and cellphones of food handlers

4.2.1 Foodborne pathogens isolates from money of food handlers

In this study 18.8% of foodborne pathogens were isolated from money,*E.coli* (0.4%) and *Staphylococci aureus* (18.4%) (Table 4.1) .The 20 shilling coin and 100 shilling note carried the highest proportion of the foodborne bacterial pathogens (15%) and (28%) respectively as is shown in figure 4.1 below.

Table 4.1: Foodborne Bactrial Pathogens from Money

Pathogens	Money denominations										Total
	1	**5**	**10**	**20**	**40**	**50**	**100**	**200**	**500**	**1000**	**Freq / %age**
E-Coli	0	0	0	0	0	0	0	0	0	1	1(0.4%)
Staph aureus	1	1	7	7	0	8	13	5	2	1	45(18.4%)
Total	1 (2%)	1 (2%)	7 (15%)	7 (15%)	0 (0%)	8 (17%)	13 (28%)	5 (11%)	2 (4%)	2 (4%)	46(18.8%)

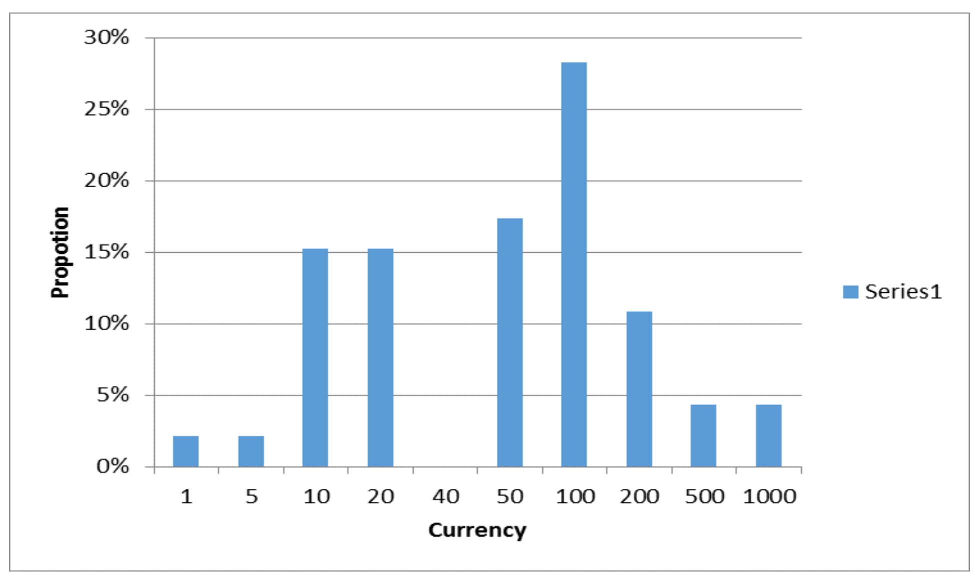


Figure 4.1: Distribution of Foodborne Pathogens Found on Money per denomination

4.2.2 Foodborne bacterial pathogens from isolated food handlers cellphones

21.3% of the foodhandlers phones were contaminated with bacterial foodborne pathogens which included *Staphylococci aureus* (14.3. %) and *E.coli* (7%).

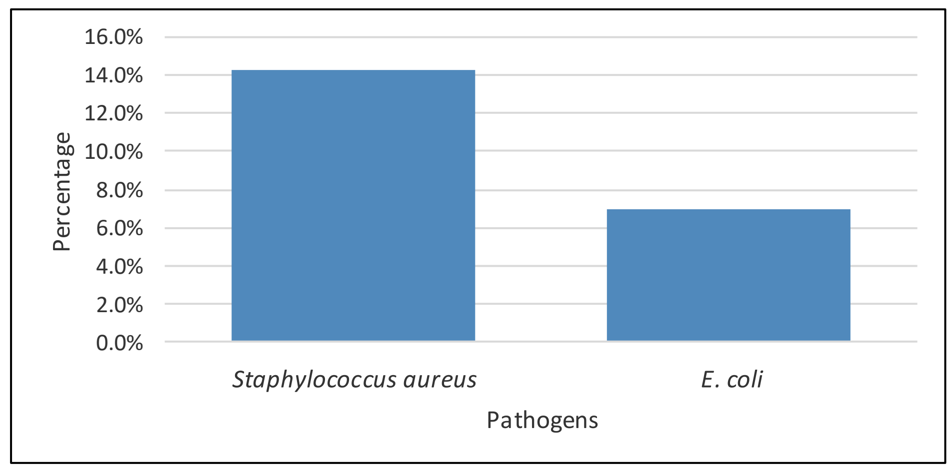
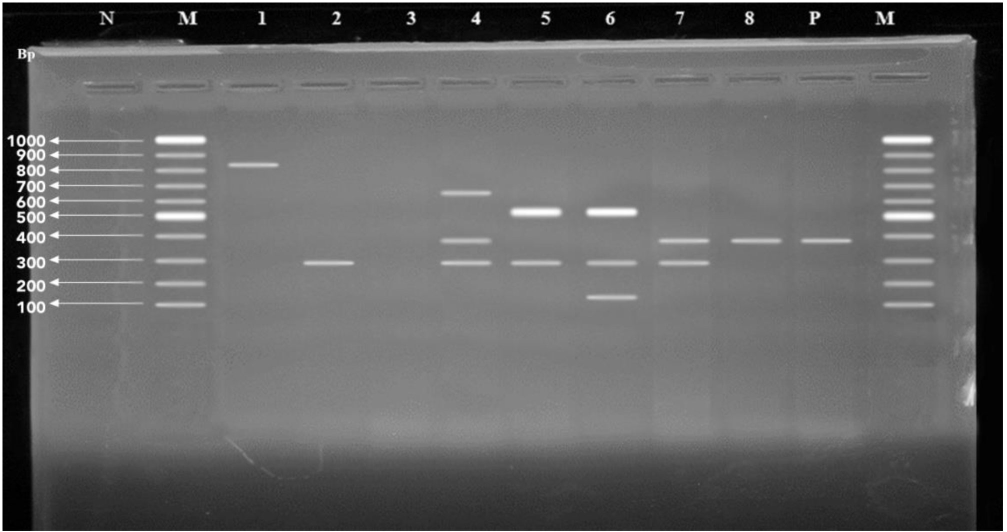


Figure 4.2: Prevalence of foodborne pathogens on phones

4.3 Gel electrophoresis of *E.coli* pathotypes isolated from money and phones

Fiure 4.3 is a gel electrophoresis photo of *E.coli* pathotypes isolated from money and phones of food handlers. Lane 4, 5, 6 and 7 isolates had a combination of 3:2:3 genes respectively coding for EPEC, EHEC and EAEC pathotypes.Lane 1, 2, and 8 had one gene.



Lane M is a molecular ladder of 100 bp; N is a negative control (*E. coli* ATCC 11775) while lane P is a positive control of *E. coli* (ATCC 25922) with st_2 virulence markers of 322 base pairs. Lane 1, 2, 4, 5, 6, 7 and 8 are the *E. coli* with virulence factors}.

Figure 4.3: Virulence gene of E coli Isolates

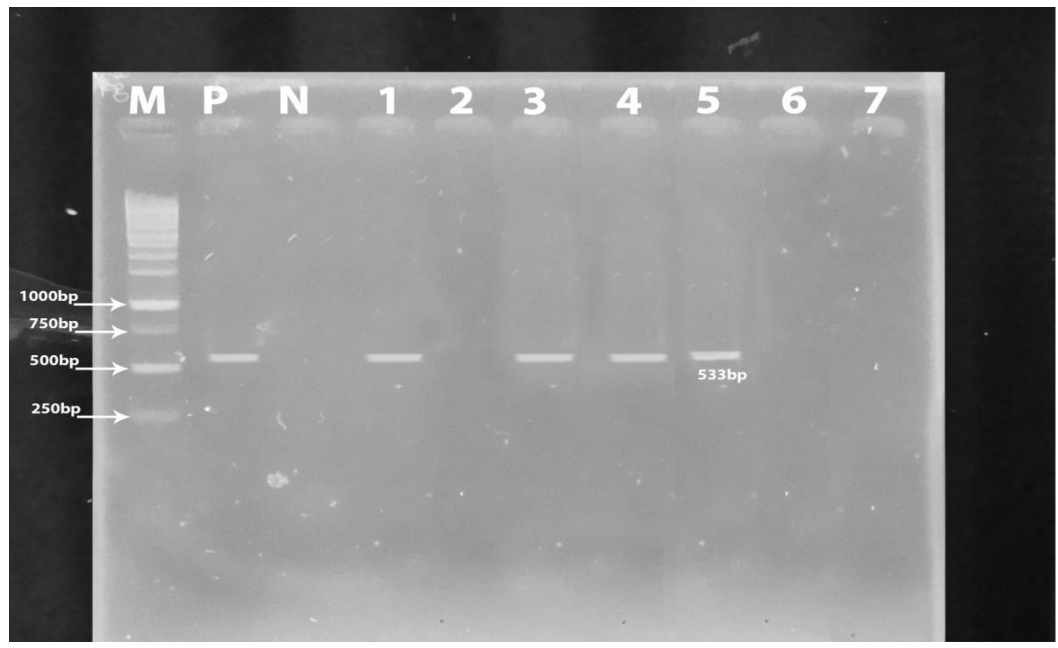
Table 4.2: Summary Table showing identified Escherichia coli pathotypes as shown on the gel photograph

Lane Number	Base pairs	Pathotypes
N	-	Negative control
M	100bp	Molecular marker
1	811	EPEC
2	298	EHEC
3	-	Negative
4	298, 322, 630	EHEC, ETEC,EAEC
5	298, 517	EHEC,EPEC
6	130, 298, 517	EHEC,EHEC, EPEC
7	322, 298	ETEC, EHEC
8	322	ETEC
P	322	Positive control
M	100pb	Molecular marker

Key: EPEC-Enteropathogenic *E. coli*, ETEC-Enterotoxigenic *E. coli,* EHEC-Enterohemaragic *E. coli,* EAEC-Enteroaggregative *E.coli*

4.4 Gel electrophoresis of mecA gene solated from Staphylococcus aureus from money and phones of food handlers

Figure 4.4 is a gel electrophoresis photo of oxacillin resistant *Staphylococci aureus* positive for *mec*A gene that was isolated from money and phones of food handlers.7 samples that were oxacilin resistant were tested and were positive for *mec A* gene .



Lane 1, 3, 4 and 5 show positive match for *mec*A while 2, 6 and 7 are negative for mecA}. Lane M is a molecular ladder of 1000 Bp; P is a positive control *S. aureus* ATCC 49476 for mecA, N is a negative control *S. aureus* strains ATCC 29213).

Figure 4.4: ***Staphylococci aureus*** **that was positive for** ***mec*** **A gene**

4.5 Antimicrobial susceptibility profiles of the isolated foodborne bacterial pathogens

4.5.1 Antimicrobial susceptibility patterns of the isolated E. coli

Figure 4.5 shows that 100 % of the *E. coli* isolates were sensitive to kanamycin and ceftazidime but showed highest resistance to Ampicilin (64%) and Sulphamethoxazole (60%). 8% were resistant to chloramphenicol and 4% to Kanamycin.

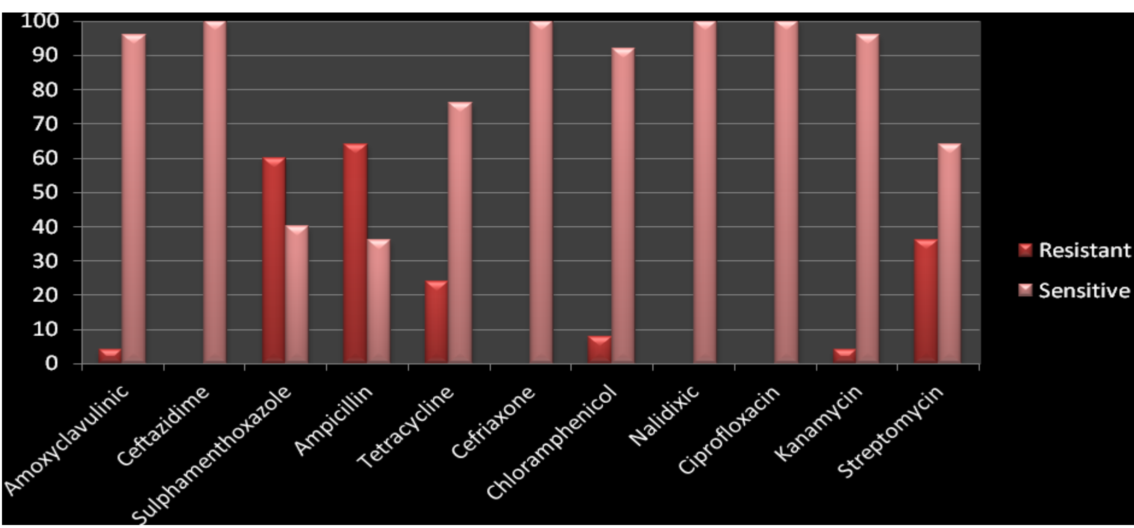


Figure 4.5: Antimicrobial Susceptibility profile of the isolated *E coli*

4.5.2 Antimicrobial susceptibility profile of the isolated Staphylococci aureus

(100%) of the isolated *Staphylococci aureus* were sensitive to Vancomycin and Gentamycin but showed highest resistance to cefuroxime(16.1%) and erythromycin (16%) then followed by ceftriaxone(14.3%), oxacillin,(12.5%) and others as is shown in Figure 4.6 below.

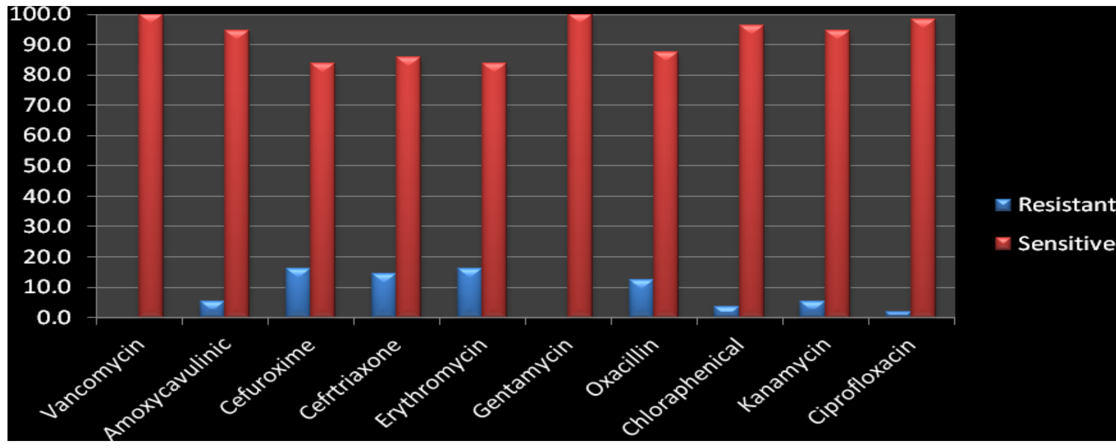


Figure 4.6: Antimicrobial Susceptibility profile of the isolated *Staphylococci aureus*

CHAPTER FIVE

DISCUSSION

5.1 Foodborne bacterial pathogens isolated from money and phones of food handlers

The money sampled from food handlers contaminated with foodborne pathogens (table 4.1), the findings in this study agrees with the results of Kuria *et al*. (2009) which also yielded *E.coli* and *Staphylococci aureus* from food vendors coins in Nairobi. Barua *et al*., (2019) isolated *Staphylococci aureus*, *E.coli* and Salmonella from the Bangladeshi notes all of which are foodborne bacterial pathogens and agrees with this study. The Iranian paper currency Rashed, et *al*., (2006) were also contaminated with *E.coli* and coagulase positive staphylococci among other. 20 shilling coin and 100 shilling note were the most contaminated (figure 4.1) probably because they were the most availed currency by the sampled participants and probably the currency that circulated most in the population. The denomination of paper currencies have a direct correlation with the degree of contamination as lower denomination notes had the most contaminants (Hassan *et al.,* 2011; Pradeep *et al.,* 2012; Moosavy *et al.,* 2013). Thus, it can be inferred that lower denomination notes harbor the highest load of microbes, probably because they are exchanged more frequently from one hand to another during market transactions than the higher denomination notes.

Food handler's phones were contaminated with *E.coli* and *Staphylococci aureus* foodborne pathogens. This study's frequency of bacterial contamination was lower (figure 4.2) compared to a similar study done by Ilusanya, et al., (2012) on food vendors in Ago-Iwoye Town, Ogun State, Nigeria where Staphylococcus aureus (50%), and Escherichia coli (26%), were isolated among others. The population type of foodborne bacteria pathogen found on money in this study was similar to what was found on the cell phones (figure 4.1 and figure 4.2), there is a possibility of cross contamination from handling the phones and money together with the same hands at work because of mobile

banking in the food industry or when carrying the two items together in the purse/bag. It is also possible that the same population of microorganisms is passed on as money circulates.

5.2. *Escherichia coli* pathotypes from money and phones from food handlers

The *E. coli* pathotypes isolated from money and phones of food handlers were Enteropathogenic *Escherichia coli* (EPEC), Enterohaemorrhagic *Escherichia coli* (EHEC), Enteroaggreative *Escherichia coli* (EAEC) and Enterotoxigenic *Escherichia coli* (ETEC) this study findings is similar to Sang, (2016) EIEC,EAEC,EPEC and ETEC were isolated from food handlers stool samples. This finding is significant because they were isolated from food handlers at work and ETEC is a common cause of cause of diarrhea in developing countries (Barati, 2012). A crosectional study done in Nairobi yielded EPEC, ETEC, EIEC, EaggEC and STEC (Odwar *et al.,* 2014) from raw chicken sold in nairobi . In 2008 Schilling, 2008 established that EAEC (8.6%), (ETEC 7.9%) and EPEC (7.4%), were the most frequently identified bacterial pathogenic agents isolated however in this study though done in the same country EHEC was the most isolated E. *coli* pathotypes and this difference could have been due to difference in the population under study. The Maasai community is famous for consumption of raw meat and milk whereas ETEC strains have been recovered from a variety of animals, and cattle are considered the major reservoir for ETEC strains (Sang *et al*., 2012). Recent evidence has indicated that small domestic ruminants are also relevant ETEC reservoirs (Amézquita-López *et al*., 2012; La Ragione *et al*., 2009).

5.3 Positive *Staphylococci aureus* for mecA gene

Seven isolates of *Staphylococcus aureus* were resistant to oxacillin and were tested for *mec*A gene. A study done on Zambian currency isolated 6 MRSA from resturants and hotels Neel, (2012). Presence of MRS is a serious health risk because of how difficult and expensive it is to treat diseases caused by the MRSA strains,they are resistant to ß-lactums resulting in resistance to virtually all available ß-lactums drugs.Their presence

in our currency and from currencies in other countries (Alemu, 2014; Neel, 2013) around the world should be of great concern .The presence of MRSA in food industry has been reported from major food animals mainly pigs cattle and chicken Lee, (2003)and this is a potential risk because transmission to human through food sources is a possibility.

5.4 Susceptibility patterns of the food borne bacterial isolates from money and phones of food handlers

Antimicrobial susceptibility test was carried out on potential foodborne bacterial pathogen. The choice of drugs was based on the commonly used antibiotics for both gram positive and gram negative bacteria. The drugs included chloramphenical 30µg, ampicillin 10µg, co-trimoxazole-25µg, cefuroxime-30µg, gentamycin-10µg, cefotaxime-30µg, amoxicillin-clavulanate-20µg, erythromycin 15 µg, oxacillin 1µg, vancomycin 30 µg, chloramphenicol 30µg and ceftriaxone 30µg, based on the CLSI, (2008).12 % MRSA were resistant to oxacillin, the highest resistance was observed in cefuroxime and erythromycin (21%). All isolates were sensitive to vancomycin and gentamycin (figure 4.6).

E. coli isolates showed resistance to ampicillin, sulphamethoxazole, streptomycin, tetracyclin, chloramphenicol and amoxyclavilinic but highest resistance was seen with ampicillin (16%) and sulphamethoxazole (15%). All isolates were sensitive to ceftazidime, ceftriaxone, nalidixic acid and ciprofloxacin, (figure 4.5).

Multiple antimicrobial resistances among strains of pathogenic *E. coli* have also been reported in Kenya (Kariuki *et al*., 2007; Moyo *et al*., 2011; Sang *et al*., 1997) hence concurs with the results of the current study. This is most likely to have been attributed to the increase in the widespread use of antimicrobial agents which diminishes the efficacy of affordable and available drugs (Sosa *et al*., 2010). The emergence and spread of antimicrobial resistance in bacteria of medical importance imposes serious constraints on the options available for treatment of many infections.

Bacterial infections are often treated empirically with broad-spectrum antibiotics and The resistance rate of drugs in this study may be associated with resistant strains in circulation that have contaminated currencies due to inappropriate use of antibiotic purchased irregularly . There is also purchase of antibiotics over the counter from unqualified drug sellers with several drug alternatives from the prescribed drugs (Kuria, 2009).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study established that the foodborne bacterial pathogens were present on money handled by food handlers (18.8%) which were *E.coli* (0.4%) and 18.4% *Staphylococci aureus*. Their cell phones (21.3%) were also contaminated with foodborne bacterial pathogens which included *Staphylococci aureus* (14.3. %) and *E.coli* (7%).

The study also concludes that virulent strains of *E. coli* were present on the money and phones of food hanlers. The pathotypes characterized in this study yielded; EPEC, ETEC, EHEC, EAEC and EIEC. The food handlers were harboring pathogenic *E. coli* which could lead to outbreaks of food borne diarrhoea infections in the community. Seven isolates of *Staphylococcus aureus* were positive for *mec*A gene, the presence of MRSA is a serious health risk because of how difficult and expensive it is to treat diseases caused by the strains.

The study concludes that the monies and phones of food handlers had antibiotic resistant strains of the *E. coli*; ampicillin (16%) and sulphamethoxazole (15%) and 7(12.5%) isolates of MRSA which were all resistant to oxacillin, the highest antimicrobial resistance was observed in cefuroxime and erythromycin (21%). Therefore money and cell phones are possible vectors of food borne antibiotic resistant pathogens and can act as sources of outbreaks if cross contamination is not addressed.

6.2 Recommendations

It was recommended:

i. Food handlers should be sensitized on the risks involved in handling food after touching money and mobile phones. They should also be encouraged to practice hand hygine

before handling food at any stage and the general public should also be informed of the danger of handling food with the money and mobile phones.

ii. Mobile banking should be embraced as an alternative way of money exchange, the use of card and online money transfers should be encouraged as opposed to the conventional way of handling money. Banks should devise a way of regularly decontaminating currency in circulation

iii. Mobile phone manufacturers should provide plastic covers as accessories that can be washed or sanitized frequently to minimize contamination by pathogenic microorganisms.

iv. Further studies should be done to assess the risk factors associated with occurrence of bacterial pathogens on money and phones.Other groups of microorganisms should also be investigated.

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APPENDICES

Appendix I: Ethical Review Committee clearance

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **March 1, 2012**

TO: **GERTRUDE GATI KISAŃG (PRINCIPAL INVESTIGATOR)**

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

Forwarded 5/3/2012

Dear Madam,

RE: SSC PROTOCOL No. 2127 –2ND REVISION (*RE-SUBMISSION*): ENTEROPATHOGENIC BACTERIA ON MONEY AND CELLPHONES AMONG FOOD HANDLERS FROM SELECTED FOOD OUTLETS WITHIN NAIROBI, KENYA

Reference is made to your letter dated February 20, 2012. We acknowledge receipt of the revised proposal on February 22, 2012.

This is to inform you that the Committee determines that the issues raised at the 197th meeting of 24th January 2012 are adequately addressed. Consequently, the study is granted approval for implementation effective this **1st day of March 2012** for a period of one year.

Please note that authorization to conduct this study will automatically expire on **February 28, 2013**. If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to the ERC Secretariat by **January 17, 2013**. The regulations require continuing review even though the research activity may not have begun until sometime after the ERC approval.

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

Work on this project may begin.

Sincerely,

CHRISTINE WASUNNA,
Ag. SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

In Search of Better Health

Appendix II: Scientific Sterring Committee clearance form

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

ESACIPAC/SSC/9989 **8th December, 2011**

Getrude Kisan'g

Thro'
Director, CMR forwarded 9/12/2011
NAIROBI

REF: SSC No. 2127 (Revised) – Enteropathogenic bacteria on money and cell phones among food handlers from selected food outlets within Nairobi, Kenya.

Thank you for your letter dated 6th December, 2011 responding to the comments raised by the KEMRI SSC.

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

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

For: **Sammy Njenga, PhD**
SECRETARY, SSC

In Search of Better Health

Appendix III: Informed consent form for participants

Title of the Research Study:

Presence of diarrhoeal disease causing bacteria on money and cellphones of food handlers within Nairobi, Kenya.

Principal investigator: Gertrude Gati Kisan'g

Co investigators and Institution affiliation:

1. Dr. Joseph O. Oundo - Centre for Diseases prevention and Control(CDC)
2. Professor Zipporah Ng'ang'a- Jomo Kenyatta University of Agriculture and Technology(JKUAT)

Study location: KEMRI-Centre of Microbiology (CMR)

Purpose of the study:

The purpose of this study is to identify whether there are bacteria on money and cellphones that are likely to cause diarrhoea when ingested in contaminated food. This will provide information that will assist food establishments and other sectors to know how to handle these very important items as they can be a source of transmission of disease causing bacteria.

Description of the study:

The money and cellphone availed by the willing consenting participants will be wiped on the spot using a wet cotton wool that has been dipped in water containing sugar that will allow the bacteria to remain alive during transportation to the laboratory. The availed money and cellphone will then be cleaned using a clean dry cotton wool returned to the owner before the next participant is attended to. The cotton wool swabs will then be labelled with numbers that will not reveal the identity of the participant and

transported to the laboratory for study. On reaching the laboratory these swabs will be transferred in nutritive media that will allow growth of bacteria if any, but not all bacteria are harmful only those that are likely to cause diarrhoea will be further investigated.

Potential Harm, Injuries, Discomforts or Inconvenience, Risks.

There are no known or anticipated harm/risks to the study participants.

Potential benefits:

There are no direct benefits to the study participants but the outcome is expected to enlighten the food industry and the public on possible dangers involved in handling food with money and cellphones.

Alternative:

A recruited participant is free choosing not to participate in the study and one's refusal will not lead to any penalty or loss of benefit.

Confidentiality and dissemination of study results

The identity of the study participant will be kept confidential and all the samples will be coded with numbers from the field to the laboratory. No information that reveals the identity of the participants will be released or published without consent. At the end of the study there will be no way to link the names of the participants with the data.

Reimbursement:

The recruited participant will not be reimbursed for their transport and time; the sampling will take place at their work stations and with permission from their supervisors.

Contacts:

For any questions and or concerns about the study the contact person is Mrs Gertrude Gati of P.O. Box 3351 – 80100, Mombasa. Email address gatitanui@gmail.com.

For any questions pertaining to rights as a research participant ,the contact person is: The secretary ,KEMRI Ethics review committee, P.O Box 54840-00200,Nairobi;Tel numbers :020-2722541,0722205901,0733400003;email address :

erc-secretariat@kemri.org

CONSENT FORM

STUDY NO: ________________________

I.. (Full name of the person from whom swabs from phone and/ or money is being sought), being 18 years or older and having full capacity to consent.

I have been informed about the study which aims at finding whether cellphones and money harbour bacteria that can cause diarrhoea. I have been informed of the nature, implications, duration, purpose, voluntary nature and inconveniences that may reasonably be expected by: - Gertrude Gati.

I am also aware that all data collected will be coded in order to protect my identity, only the research staff will have access to the data and at the end of the study there will be no way to link my name with the data.

I understand that I may at any time during the study withdraw my consent and withdrawal from the study without any loss or penalty. My refusal to participate will involve no penalty or loss of benefit to which I am otherwise entitled. This research has no monetary or other benefits to the participant other than to give valuable information

to all stakeholders on the risks of handling the said items when processing or handling food.

I have been given the opportunity to ask questions concerning the study and they have been answered to my satisfaction. If I have further questions I may contact Mrs Gertrude Gati of P.O. Box 3351 – 80100, Mombasa.

Yes I wish to participate in this study

Signature ....Date...

Name...

No I do not wish to participate in this study

Signature ....Date..

Name...

Appendix IV: Reference primers and controls

Category	Reference strain	Target gene(s)
ETEC	ATCC 35401	*eltB*, *estA*
EHEC	ATCC 43890	*vt1*, *eaeA*
EHEC	ATCC 43889	*vt2*, *eaeA*
EPEC	ATCC 43887	*eaeA*, *bfpA*
EIEC	ATCC 43893	*Ial*
EAEC	97R[a]	pCVD432-harboring strain
***E. coli* (negative control)**	ATCC 11775	No virulence gene

Appendix V: Target primers sequence

Primer	Target gene	GenBank/EMBL accession no. or reference	Primer sequence	Amplicon size (bp)
LT	*eltB*	S60731	5′-TCTCTATGTGCATACGGAGC-3′ 5′-CCATACTGATTGCCGCAAT-3′	322
ST	*estA*	M34916	5′-GCTAAACCAGTA$^{G}_{A}$GGTCTTCAAAA-3′ 5′-CCCGGTACA$^{G}_{A}$GCAGGATTACAACA-3′	147
VT1	*vt1*	AF461172	5′-GAAGAGTCCGTGGGATTACG-3′ 5′-AGCGATGCAGCTATTAATAA-3′	130
VT2	*vt2*	AY143337	5′-ACCGTTTTTCAGATTTT$^{G}_{A}$CACATA-3′ 5′-TACACAGGAGCAGTTTCAGACAGT-3′	298
eae	*eaeA*	AE005595	5′-CACACGAATAAACTGACTAAAATG-3′ 5′-AAAAACGCTGACCCGCACCTAAAT-3′	376
SHIG	*Ial*	12	5′-CTGGTAGGTATGGTGAGG-3′	320
BfpA	*BfpA*	U27184	5′-CCAGGCCAACAATTATTTCC-3′ 5′-TTCTTGGTGCTTGCGTGTCTTTT-3′	367
			5′-TTTTGTTTGTTGTATCTTTGTAA-3′	
EA	Pcvd	X81423	5′-CTGGCGAAAGACTGTATCAT-3′ 5′-CAATGTATAGAAATCCGCTGTT-3′	630
eae	*bfpB*	U27184	5′-GATAAAACTGATACTGGGCAGC-3′ 5′-AGTGACTGTTCGGGAAGCAC-3′	811

Appendix VI: Publication

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Molecular Characterization and Antibiotic Profile of Diarrhogenic *E.Coli* Isolated from Money and Cellphones of Food Handlers in Nairobi, Kenya

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Abstract

Escherichia coli is nonpathogenic facultative flora of the human intestine. However, some strains have transformed to become pathogenic hence diarrheagenic *E. coli*. There is inadequate data on the role money and cellphones play in the transmission of *E. coli* and their antibiotic profiles in Kenya. This study aimed at characterizing diarrhogenic *E. coli* and antibiotics profiles determination. The study participants were enrolled from selected food handling establishments in Kenya. Swabs from money and cell-phones were collected and cultured in appropriate media for the isolation, identification of *E. coli* and antimicrobial susceptibility testing. The *E. coli* pathogens showed some degree of resistant against ampicillin, Sulphamenthoxazole, streptomycin and tetracyclin. Resistance may be associated with decreased potency that can be due to drug degradation/adulteration, or presence of a lower concentration of active ingredients. Six strains of *E. coli* had a combination of genes coding for; EPEC, EHEC and EIEC. Pathogenic *E. coli* are a major causes of diarrheal diseases hence significant in this study since they were isolated from food handlers. Money and cell phones are possible vectors of pathogenic *E. coli* which are antibiotic resistant and can act as sources of outbreaks if proper hand hygiene is not observed.

Keywords: *Molecular characterization, antibiotic profile, resistance, diarrhogenic E. coli, genes*

1. Introduction

Money in form of coins and paper currency is used repeatedly in exchange for goods and services (Oyero and Emikpe, 2007). Due to this, the circulation of money currency from one individual to another potentially spreads microorganisms. It is a very good vector for transmission of diseases (Wamae, 2009). If these currencies are contaminated by pathogenic bacteria, the rate of infection and death rate from these infectious agents will continue to rise (Pope *et al*., 2002). Among the pathogens disseminated in money and cellphones are enteric pathogens such as enterotoxigenic *Escherichia coli, Shigella spp., Salmonella spp.*, and so forth. These are the ones most frequently encountered and are responsible for a variety of diseases like diarrhea, dysentery, and enteric fever. To further compound this problem, enteric bacterial pathogens have been widely reported to demonstrate resistance to several antibiotics (Poonia *et al.,* 2014 & Rai, 2012; Verma *et al.,* 2011). The determination of the efficiency of antimicrobial agents against specific pathogens, either human or animal source, is essential for proper therapy.