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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.

Escherichia coli (*E. coli*) is the most common nonpathogenic facultative flora of the human intestine. However, some strains of *E. coli* have transformed to become pathogenic in humans. This strains of *E.coli* that cause enteric infections are designated diarrheagenic *E. coli*. (Vidal *et al.,* 2005). Identification of Enteropathogenic EPEC is based on demonstration of the eae gene, which is located in the LEE (locus of enterocyte effacement) pathogenicity island, and the bundle forming pilus (bfpA) gene, located on a plasmid called the EPEC adherence factor (EAF) plasmid (Kumar *et al* 2006). Shiga toxin-producing *E. coli* (STEC) is characterized by the production of Shiga-like toxins 1 and 2 (Stx1 and Stx2) and in some strains the presence of the LEE locus related to the attaching and effacement lesion (Müller *et al*., 2006). Strains that cause gastroenteritis in humans is grouped into six categories as Enteroaggregative (EAEC), Enterohemorrhagic (EHEC), Enteroinvasive (EIEC), Enteropathogenic (EPEC), Enterotoxigenic (ETEC), and diffuse adherent (DAEC) *Eschirichia coli.* These are serotyped on the basis of their somatic, flagella, capsular and surface antigen profiles (Hien *et al*., 2008). Each of the six categories has a different pathogenesis and comprises a different set of O: H serotypes. Enteroaggregative *E. coli* (EAEC) has been associated with persistent diarrhea (>14 days), especially in developing countries (Sang *et al*., 2011). EAEC was first recognized as causing diarrhea in infants and HIV patients and in some adults in both developed and developing countries, possession of fimbrial structures have been associated with adhesion to HEp-2 cells and human erythrocytes. The fimbriae are related to the bacterial capacity for adherence to the intestinal surface. The identification of EAEC is mainly by PCR, HEp-2cell culture, and DNA probes (Oundo *et al.*, 2009).

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Serological tests are used to differentiate diarrheagenic *E. coli* from other *E. coli* but direct identification include the use of DNA probes and PCR techniques (Qadri *et al*., 2005; Ramlal *et al*., 2017). There is inadequate data on the role money and cellphones play in the transmission of foodborne pathogens in Kenya. Among the food borne pathogens are *E. coli* which are normal flora in the gut of warm-blooded animals. Some of the *E. coli* are associated with diarrhea and can be determined by serological or molecular probes.

2. Research Problem

Food and waterborne diarrhoeal diseases is the leading cause of mortality of approximately 2.2 million people annually in less developed countries. Developing countries are thought to be more affected by foodborne illness than the developed countries. Foodborne disease surveillance in Kenya is carried out mainly by Ministry of Health but statistics on foodborne diseases are not well analyzed and documented since all diarrheal diseases are lumped together making it difficult to isolate the burden of foodborne diseases from other diseases. 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 amenities to inadequate knowledge on food safety among others. This makes it difficult to establish what factors contribute most to outbreaks of foodborne diseases. There is lack of data on the role money and cellphones play in the transmission of foodborne bacteria especially diarrhogenic *E. coli* and their antibiotics susceptibility profiles.

3. Objective of the Study

The study aimed at determining diarrhogenic *E. coli* using molecular techniques and determining antibiotics susceptibilities.

4. Research Hypothesis

Diarrhogenic *E. coli* cannot be characterized using molecular techniques and their antibiotics profiles cannot be determined.

5. Justification

Money is the main means of exchange for goods and services in Kenya and in other parts of the world. There has been a proliferation of cellphones with reported ownership levels of up to 60 mobile subscriptions per 100 people by 2009. It has been shown from previous studies conducted elsewhere that cell-phones and money are possible vectors of disease with pathogenic bacteria being isolated from the two systems. Money and cellphone are handled in close contact if not together with the introduction of the mobile money transfer technology in the country. There is paucity of data on the role that money and cellphones play in the transmission of bacterial pathogens such as pathogenic/diarrhogenic *E. coli* among food handlers in Kenya. It is, therefore, important to establish a baseline data on the role of cellphones and money as avenues for transmission of diarrhogenic *E. coli* and their drug susceptibility profiles.

6. Research Methodology

6.1 Research Design

The study adopted cross-sectional design in which systematic random sampling was employed in collection of samples from food handlers in selected food outlets under the Kenya Medical Research Institute (KEMRI) Hospitality Industry Support Program (HISP).

This study was carried out in selected food outlets in Nairobi County. Nairobi has a population of 3,138,369 and it is the capital city of Kenya. This city has all categories of hotels and eateries from the deluxe five star establishments to food kiosks as well as back street food vendors. This study focused on food handlers who were randomly sampled from selected food outlets.

The study population consisted of waiters, cooks and food handlers in processing firms recruited in the HISP Program. A sample of 395 participants was determined using the formula by Nagin *et al.*, (Kuria *et al.*, 2009).

$$n=Z^2pq/d^2$$

The total population size was determined using a list of the food handlers provided by the HISP Program. The first participant was determined by random selection; and there after systematic sampling method was employed where every 2nd participant was sampled until the sample size was achieved.

6.2 Laboratory Procedures

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6.2.1 Antibiotic Susceptibility Testing

The E. coli were isolated, identified using appropriate techniques. Antimicrobial susceptibility testing was determined by Disk diffusion technique on Mueller Hinton media (OXOID) and interpreted based on CLSI, (2008).

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 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, *E. coli* 25922 and *C. jejuni* 29428 was used for quality control.

6.3 PCR Amplification

6.3.1 DNA Extraction from E. coli

Extraction was done by simple boiling technique modified after Strommenger *et al.,* (2003). The test organism 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.

6.3.2 Multiplex PCR for E. coli

Pure Taq Ready-To- Go PCR beads (Amersham biosciences Buckinghamshire, UK) with a total reaction volume of 26.4µl, was used in the PCR run; template DNA was 2.0µl; with 24.4 µl of the master mix prepared by adding 2.4 µl of the forward and reverse primer with 22 µl of sterile distilled water. The PCR conditions were set as follows: initial denaturation step of 5 minute at 94^{o}C followed by a further 1 minute of denaturation at 94^{o}C; annealing step at 61^{o} C for 30 seconds, and extension at 72^{o}C for 1 minutes -seconds for 35 cycles, finally 72 for 10 min and cooling at 4^{o}C. The PCR products 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. Target genes with their virulence factors and target primer sequence are shown in Appendix 5 and 6, respectively.

6.4 Data Storage and Analysis

The data was organized and managed using computer software EPI INFO version 3.5.3 (CDC, Atlanta, USA). 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 20.0 (CDC, Atlanta, USA). Descriptive statistics was done to obtain simple frequencies, means and median. Data was presented using tables and words. The $p< 0.05$ was considered statistically significant.

6.5 Ethical Issues

The study was approved by Board of post graduate studies of Jomo Kenyatta University of Agriculture and Technology (JKUAT), and the National Ethical Review committee (ERC). Permission was granted by the hotel management. All the information was treated with confidentiality.

7. Results

7.1 Antimicrobial susceptibility patterns of the isolated E. coli

Figure 1 shows that most of the isolated *E. coli* were sensitive to the panel of antibiotics tested. The *E. coli* pathogens showed some degree of resistant against ampicillin, Sulphamenthoxazole, streptomycin and tetracyclin. Very few were resistant to chloramphenicol, Kanamycin and amoxcyclavulanic acid.

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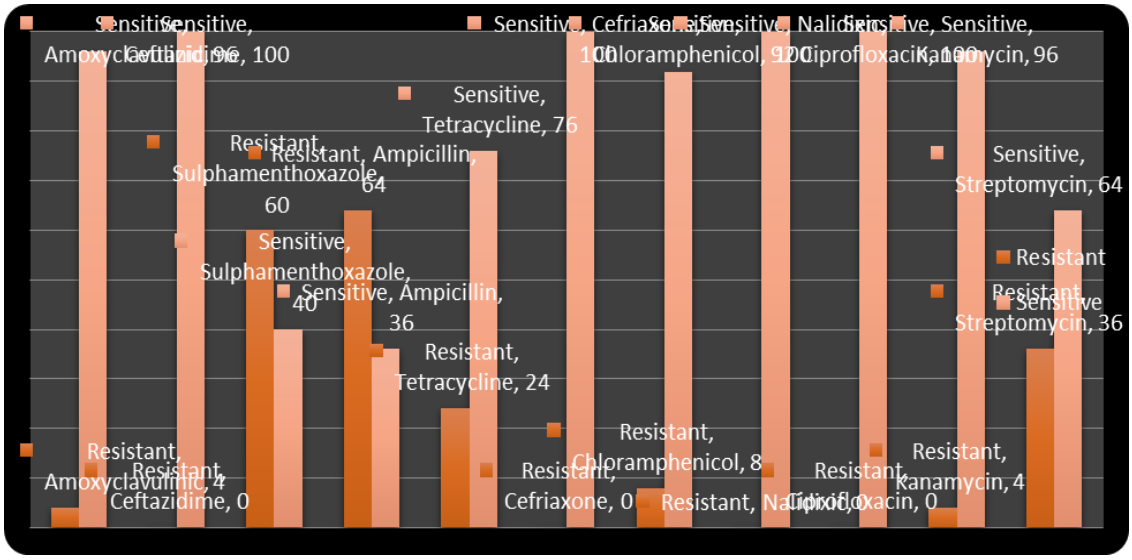

Figure 1 Antimicrobial Susceptibility Profile of the Isolated E. coli

7.2 Identification of Escherichia Coli Pathotypes based on Virulence Factors

Specific PCR primers of ETEC, EPEC, EHEC and EIEC were used. The primer used were LT which targets the *eltB* gene for ETEC and EHEC, vt_1 and eaeA for EPEC, st_1 and st_2 for EIEC and finally ST gene for ETEC. Table 1 shows the strains, genes and their molecular weights in base pairs.

Table 1 Strains, genes and base pairs of E. coli pathotypes

Strain	Genes - bp		
EPEC	vt_2 - 130,	eaeA -376	EA - 630
EHEC	eltB(LT) - 322		
EIEC	stx_2 - 130		
ETEC	*eltB*		

In this study all the *E. coli* isolated were characterized using molecular techniques denoted as polymerace chain reaction (PCR) to check for their virulence factors. Figure 2 shows single primer PCR showing *E. coli* with eltB (LT) coding for ETEC pathotypes.

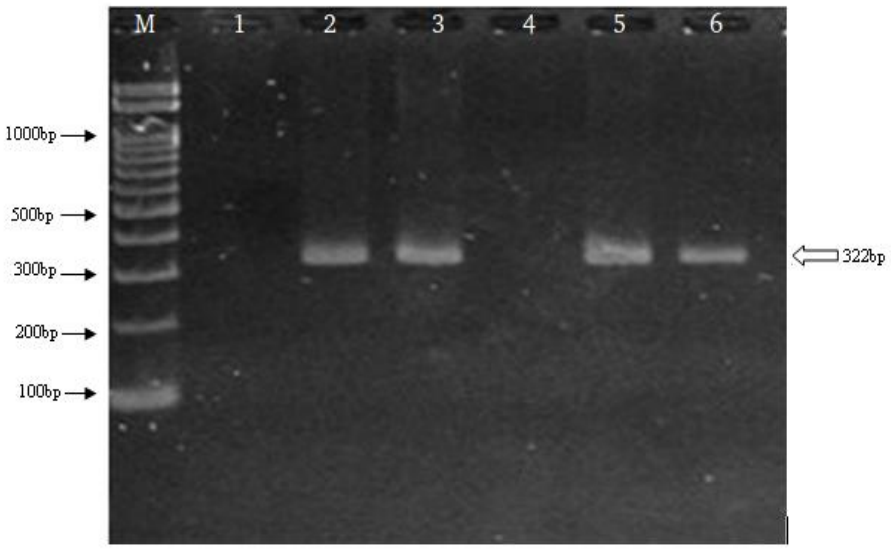

Figure 2 Specific PCR primers of ETEC (Enterotoxigenic E. coli)

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Legend: {Lane M is a Molecular marker, each band equivalent to 100 bp (base pairs) DNA molecular size standard. Lane 1 and 6 are quality control samples where**,** 1 represents negative control sample and 6 (*Escherichia coli* ATCC25922) representing a known positive control sample which was run alongside the tests. Lane 2, 3, 4 and 5 represents ETEC isolates from swabs cultures showing band formation at 322 base pairs upon PCR}.

Table 2 is a summary of the *E. coli* pathotypes based on their virulence factors according to multiplex PCR. Six strains of *E. coli* in this study had a combination of genes coding for the following pothotypes; EPEC, EHEC and EIEC. Most isolates were positive for genes coding for one pathotype such as ETEC, EHEC among others.

Table 2 Multiplex PCR Showing Identified Escherichia Coli Pathotypes

Lane Number	Base pairs	Pathotypes
N	-	Negative control
M	100bp	Molecular marker
1	811	EPEC
2	298	EIEC
3	-	Negative
4	298, 322, 630	EPEC, EIEC, ETEC
5	298, 518	EPEC, EIEC
6	130, 298, 518	EPEC, EIEC
7	322, 298	EHEC, EIEC
8	322	EIEC
P	322	Positive control
M	100pb	Molecular marker

Key: EPEC-Enteropathogenic *E. coli*, ETEC-Enterotoxigenic *E. coli*, EIEC- Enteroinvasive *E. coli*, EHEC- Enterohemaragic *E. coli*

Figure 3 shows a multiplex PCR showing isolates with different genes, some have one, others have two while others have three genes coding for three different virulence factors. The pathotypes of *E. coli* according to the multiplex PCR are summarized in Table 1 and are well indicated in Figure 3.

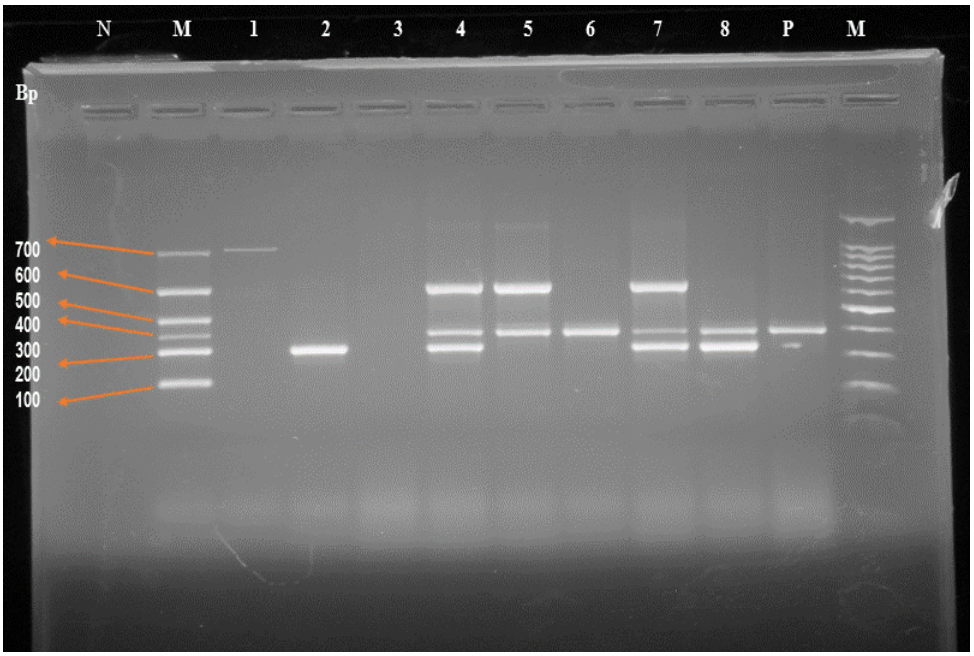

Figure 3: Multiplex PCR showing different virulence gene of *E. coil* isolates

Legend: {Lane M is a molecular ladder of 100 bp; N is a negative control while lane P is a positive control of *E. coli* (ATCC 25922) with st_2 virulence markers of 298 base pairs. Lane 1, 2, 4, 5, 6, 7 and 8 are the *E. coli* with virulence factors}.

8. Discussion

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8.1 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test was carried out on all the E. coli isolated. The choice of drugs was based on the commonly used antibiotics for 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 and ceftriaxone 30µg, based on the CLSI, (2008). Majority of the isolates were sensitive to the panel of antibiotics tested except some *E. coli* which was resistant to ampicillin and Sulphamenthoxazole.

A study on commensal gut flora from children in Sudan found that 39% of children had strains resistant to six antimicrobial agents and over 70% of the children had strains resistant to at least 4 of 6 antimicrobial agents commonly prescribed in the country (Miles *et al*., 2006) however most of the isolates in the current study were sensitive to the panel of antibiotics tested. The difference could be attributed to the fact that Sudan isolates were clinical while the isolates from the current study could be termed as environmental. Multiple antimicrobial resistances among strains of pathogenic *E. coli* have also been reported in Kenya (Kariuki *et al*., 2007; Moyo *et al*., 2011) 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 (Asafo-Adjei, 2017). The emergence and spread of antimicrobial resistance in bacteria of medical importance imposes serious constraints on the options available for treatment of many infections.

Resistance of some particular drugs may be associated with several factors favouring the development of bacterial resistance to antibiotics in the developing countries. They includes:-Less potent activity, as a result of decreased potency that can be due to degradation or adulteration of the drug, or presence of a lower concentration of active substances. Some drugs produced in industrialized countries have been found to have expired and are being distributed in developing countries (Rossit *et al.*, 2007). Sometimes the antibiotics provided may be poorly transported and stored, leading to drug inactivation. Most Hospitals in Kenya do not have clinical microbiology laboratories that perform routine analyses for microbiological diagnosis. Even if some services are in place, international guidelines and quality control for susceptibility testing are often not available or require methods that are not affordable (Sang *et al.*, 2013). There is no information about either the etiology of the infectious diseases or antimicrobial susceptibility which are both essential for clinical practice. Bacterial infections are often treated empirically with broad-spectrum antibiotics over-the-counter availability. In most part of the country, antibiotics can be purchased without prescription in pharmacies, general stores and from street markets. Since many drugs are expensive, some patients purchase incomplete regimens whenever possible and discontinue treatment when the symptoms disappear (Laxminarayan *et al.*, 2013). There is also unnecessary prescription of antibiotics, mainly in cases of acute diarrhea and respiratory infections. There is also purchase of antibiotics over the counter from unqualified drug sellers with several drug alternatives from the prescribed drugs (Kuria, 2009). None of the bacterial pathogens isolated showed resistance to Nalidixic acid, Ciprofloxacin, Kanamycin and Ofloxacin.

8.2 Characterized Escherichia Coli Pathotypes

The *E. coli* is part of the gut flora, (Feng *et al.*, 2002) and fecal oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination (Thompson, 2007). These bacteria are normal floral and are not associated with any diseases although some contain virulence factors. The only way to detect this virulence factors is through molecular techniques. In this study, 6.8% of *E. coli* that were isolated were characterized using molecular techniques denoted as polymerase chain reaction (PCR) to check for toxigenic strains. Six isolates were positive for EPEC, EHEC and EIEC. The presence of these pathogenic strains is significant since they were isolated from food handlers in the hotel industry and are a major cause of diarrheal diseases. The most prevalence pathotypes among the characterized *E. coli* in this study were EPEC. However the ETEC prevalence in this study was lower than reports from Maasai land; where ETEC prevalence of up to 24.1% have been reported (Sang *et al*., 2012). The Maasai community is pastoralists 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 (Ferens *et al*., 2011). Recent evidence has indicated that small domestic ruminants are also relevant ETEC reservoirs (Amézquita-López *et al*., 2012). The current study isolated *E. coli* from phones and money of food handlers while the other studies isolated *E. coli* from clinical and veterinary samples hence the difference in ETEC prevalence.

In Kenya Sang reported EAEC (8.9%), as the most frequent followed by ETEC (1.2%) and EIEC (0.6%) while among the Maasai community ETEC (29.8%) was the most prevalent followed by STEC (24%), EAEC (14.2%) then EPEC (3.5%) (Sang *et al.*, 2011). From an urban community in Nairobi, Makobe *et al*., (2012) identified EPEC (19.3%) as the most prevalent followed by ETEC (7.25%) and EAEC (3.86%). A study observed EAEC as the most frequent *E. coli* pathotype (36.6%) in diarrhoeagenic *Escherichia coli* isolates from children < 5 years from Kenya and Japan (Bii *et al*., 2005). In Tanzania country, EAEC (64.1%) was the most detected followed by EPEC (20.3%) then ETEC (15.6%) (Moyo *et al*., 2011). The differences in prevalence of *E. coli* pathotypes in different geographical areas and samples could be explained by the different social behavioural, life styles and cultural practises among the communities.

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Multiple combination of EPEC, EIEC, ETEC and EPEC, EIEC were observed in some isolates in this study. The varying combination of multiple virulence genes harboured by the ETEC/ EAEC is a rare pathotypes that harbours the phage-mediated Shiga toxin determinant with an Enteroaggregative *gene while* the ETEC/ETEC isolates showed that the strains were a mixture of several different *E. coli* pathogroup-associated properties. A study done in Kenya in 2008 established that EAEC (8.6%), (ETEC 7.9%) and EPEC (7.4%), were the most frequently identified bacterial pathogenic agents isolated (Iijima *et al.*, 2017) however in this study though done in the same country EPEC was the most isolated *E. coli* pathotypes and this difference could have been due to difference in geographical location, samples among other traits.

9. Conclusions and Recommendations

9.1 Conclusions

Most *E. coli* were sensitive to the panel of antibiotics tested although there is a great concern on some pathotypes since they showed some degree of resistant against ampicillin, Sulphamenthoxazole, streptomycin, tetracyclin and chloramphenicol, Kanamycin and amoxcyclavulanic acid. Therefore money and cell phones are possible vectors of food borne antibiotic resistant pathogen and can act as sources of outbreaks if proper hand hygiene is not observed. Six strains of *E. coli* in this study had a combination of genes coding for the following pothotypes; EPEC, EHEC and EIEC. Most isolates were positive for genes coding for one patotype such as ETEC, EHEC among others. Therefore the food handlers were harboring pathogenic *E. coli* which could lead to outbreaks of food borne diarrhea infections in the community.

9.2 Recommendations

Food handlers and the general public should be sensitized on the risks involved in handling food after touching money and mobile phones. They should also be encouraged to practice hand hygiene before handling food at any stage because they contain pathogenic *E. coli* which are drug resistant.

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