Journal of Bacteriology Research Vol. 4(4), pp. 46-54, October 2012
Available online at http://www.academicjournals.org/JBR
DOI: 10.5897/JBR12.008
ISSN 2006- 9871 ©2012 Academic Journals

Full Length Research Paper

Characterization of antibiotic resistance in environmental enteric pathogens from Kibera slum in Nairobi-Kenya

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Accepted 3 August, 2012

Kibera slum is characterized by poor sanitation hence the frequent outbreaks of diarrheal diseases. Emergence of antibiotic resistance by diarrhoea-causing bacteria is an inevitable challenge in the area. Diarrhea-causing bacteria were isolated from water, soil, vegetables, meat and dry foods samples and their antibiotic susceptibility was characterized. 237 samples were aseptically collected and analyzed. Morphological and biochemical characterization was done using Bergey's manual of determinative bacteriology as a reference. 174 *Escherichia coli*, 8 *Salmonella* and 6 *Shigella* isolates were identified. Drug susceptibility of the isolates was done using disk diffusion method where 9 antibiotics from 5 classes of antibiotics were used. The frequencies of resistant isolates to antibiotics were as follows: ampicillin (56.79%), trimethoprim+ sulphamethoxazole (29.63%), augmentin (27.16%), tetracycline (18.52%), streptomycin (13.57%), chloramphenicol (7.41%), nalidixic acid (4.94%), gentamycin (2.47%) and ciprofloxacin (0%). Polymerase chain reaction was done to amplify the antibiotic determinants *tet-A, sul 1* and *dfrb1.* Gel electrophoresis revealed presence of *tet-A* gene and *sul 1* genes but absence of *dfrb1* in 15 isolates that were resistant to tetracycline and SXT. Plasmid extraction and profiling was done and plasmid sizes compared to plasmids of *E. coli* V517 and 39R861. Large and small plasmids were present in the isolates with *tet-A* and *sul 1* genes. Plasmid of 63 bp was present in all isolates. Conjugation experiments confirmed 100% transfer of 63 bp plasmid and 90% complete phenotype in all cases. This study showed presence of contamination of the area by antibiotic resistant diarrhea-causing pathogens, hence proper hygiene procedures and adherence to correct drug prescriptions need to be reinforced.

Key words: Characterization, antibiotic resistance, susceptibility, polymerase chain reaction, transconjugants, phenotype, plasmids.

INTRODUCTION

Worldwide, an estimated one billion people live in informal urban settlements or "slums" (UN-Habitat, 2006). These settlements pose grave threats to the health of their inhabitants, stemming from poor-quality housing, lack of infrastructure and minimal access to refuse collection, health care or other essential services).

A study conducted in Kibera sub location in Nairobi showed that coliform levels for all the water samples tested was above the recommended limit. The mean fecal coliform count in outhouse water was 93/100 ml and 103.4/100 ml for in-house water, thus the water was potentially highly contaminated with enteric pathogens (Chemuliti et al., 2010). According to the Kenyan Ministry of Health Report (2003), diarrhea is the leading cause of death in the Kibera slum followed by malaria and respiratory diseases.

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Table 1. Antimicrobial families, genetic markers and primer sequences for resistance genes tested.

Antimicrobial family	Genetic marker	Primer sequence (Forward and Reverse)	Amplicon size (bp)	Genbank accession no	Reference
Tetracycline	*Tet(A)*	GTGAAACCCAACATACCCC (F) GAAGGCAAGCAGGATGTAG(R)	888	X00006	J. Harel
Trimethoprim	*DhfrIb*	AGTATCATTGATAGCTGCG (F) GTAGTGCGCGAAGCGAAC (R)	517	DQ388123.1	J. Harel
Sulfonamides	*sul1*	TTCGGCATTCTGAATCTCAC(F) ATGATCTAACCCTCGGTCTC(R)	822	X12869	R. C. Levesque

According to Murray et al. (1999), the pathogenic enteric bacteria include organisms such as *Salmonella, Shigella, Vibrio* and *Escherichia coli.* Until the 1940s only *Salmonella* and *Shigella* were considered as gastrointestinal pathogens of medical importance in the family *Enterobacteriaceae.* It is now a well-established fact that *E. coli* is a significant cause of diarrheal illnesses both in infants and adults in many parts of the world (Kinge et al., 2010; Schierack et al., 2009; Kreig and Holt, 1984). Data on clinical isolates is plenty while less attention has been given to environmental isolation of these enteric pathogens (Sharma et al., 2010). Samples from the environment such as water, soil, meat, vegetables and dry foods are probable reservoirs of these pathogens. Studying antimicrobial resistance in humans and animals is important in order to detect changes in patterns of resistance, implement control measures on the use of antimicrobial agents, and to prevent the spread of multidrug-resistant strains of bacteria (Duijkeren et al., 2003).

The progressive increase in antimicrobial resistance among enteric pathogens particularly *Shigella, Vibrio cholerae,* Enteropathogenic *E. coli, Salmonella typhi* and *Salmonella enteriditis* species are becoming a critical concern worldwide, particularly in the developing world where there are high rates of diarrheal diseases which are associated with mortality (Sang et al., 2011; Fricke et al., 2008; Kumar et al., 2008). Surveillance data for antibiotic resistance are necessary to define or update guidelines for empirical treatment, as well as a guide for appropriate drug supplies.

MATERIALS AND METHODS

Characterization and identification of bacteria

A total of two hundred and twenty samples comprising of meat, water, vegetables, soil and dry food were aseptically collected from Gatwekera and transported to the laboratory in a cool box for analysis within 1 h of collection between 10th October 2010 and 1st February 2011. Meat was collected from 15 retail butcheries, vegetables were from 14 open air markets, soil and dry foods were also sampled from these open air markets. The samples were enriched in selenite-F broth overnight, before they were incubated on MacConkey and XLD agar for morphological characterization and subsequently biochemical characterization of the individual colonies. Biochemical tests were done by the use of TSI and API 20 E kit. Identification was done with reference to Bergey's manual of determinative bacteriology.

Antibiotic susceptibility testing

Eighty isolates were selected randomly from all sample types and subjected to antibiotic susceptibility testing, using the method described by Bauer et al. (1966). Cultures were tested for sensitivity to different antimicrobials selected from five classes of commonly used antibiotics namely, amynoglycosides, tetracycline, beta-lactams, sulphonamides, quinolones and chloramphenicol. Quality control was provided for by *E. coli* ATCC 25922. The following antibiotic disks were used: ampicillin (10 µg), augmentin (30 µg), streptomycin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), tetracycline (30 µg), trimethoprim + sulfamethoxazole (25 µg) and gentamicin (10 µg).

PCR amplification of antibiotic resistance genes

The polymerase chain reaction for genes encoding resistance to Sulfonamides, Tetracycline and Trimethoprim was done according to Sambrook and Russel (2007) with some modifications. PCR amplification was performed with 2 µl of the DNA template. The PCR conditions were 30 cycles of amplification at a denaturation temperature of 95°C for 1 min, at an annealing temperature of 55°C for 1 min and an extension temperature of 72°C for 3 min. This step was followed by a final extension at 72°C for 10 min. PCR products were resolved on 1% agarose gels, stained with ethidium bromide, visualized under UV light and photographed. The primer sets for the genes for antibiotic resistance that were targeted are shown in Table 1.

Plasmid extraction

Plasmid extraction and profiling for the antibiotic resistant strains were done by the alkaline lysis technique (Birnboim and Doly, 1979). Plasmid sizes were determined by comparison with plasmids of known molecular sizes from *E. coli* strain V517 which bears plasmids of molecular weight 35.8, 4.8, 3.7, 2.6, 2.0, 1.8, 1.4 MDa and strain 39R861 which has plasmids of molecular weight 98, 42, 24, and 4.6 MDa on 1% horizontal agarose gels.

Table 2. A summary of the biochemical reactions carried out on the four different isolates that were identified in this study.

Biochemical reaction (test)	Isolate identity			
	E. coli	***Salmonella typhymurium***	***Salmonella typhi***	***Shigella flexneri***
TSI	A/Ag	K/A	K/A	K/A
H_2S (TSI)	_	+	+	_
Motility	+	+	+	_
Indole production	+	_	_	_
Urease activity	_	_	_	_
Cirate utilization	_	+	_	_
ONPG	+	_	_	_
LDC	+	+	+	_
ODC	_	+	_	_
Mannitol	+	+	+	+
Sorbitol	+	+	+	_
Rhamnose	+	+	_	_
Melibiose	+	+	+	_
Arabinose	+	+	_	_

TSI, Triple sugar iron; A/Ag, acid slant and butt + gas; K/A, alkaline slant, acid butt, no gas; H_2S, hydrogen sulfide gas; LDC, lysine decarboxylase; ONPG, ortho-nitrophenyl-beta-D galactopyranoside; ODC, ornithine decarboxylase.

Conjugation experiments

Attempts to transfer antibiotic resistance determinants from the resistant bacteria to susceptible recipients were done (Finlay and Falkow, 1988). In each case, appropriate antibiotics were used to select for transconjugants on Mueller Hinton agar. The mating assays were carried out using the nalidixic acid-resistant *E. coli* K12 strain as the recipient. In order to confirm the transfer of the resistance phenotype from donor to recipient, antibiotic susceptibility of the transconjugants was performed using the same panel of antibiotic disks used for the donor and similarly, plasmid extraction and profiling of transconjugants was done to ascertain transfer of R-plasmids.

Data management and analysis

Data was recorded in lab workbooks and later entered in excel worksheets and then analyzed using SPSS package. The output of the analysis was presented in form of frequency tables, bar charts and gel photographs.

RESULTS

Characterization and identification of isolates

After morphological and biochemical characterization of the isolates, 174 *E. coli*, 8 *Salmonella* and 6 *Shigella* were identified. The biochemical reactions that led to identification of the isolates under this study was summarized and presented in Table 2.

Sample source contamination

The highest frequency of contamination was seen in dry foods (93.75%), meat (92.68%), soil (88.90%) while the least contaminated sources were water (76.10%) and vegetables (60.71%) as shown in Table 3.

Antibiotic susceptibility outcomes

The highest frequencies of resistant isolates to various drugs were as follows: ampicillin (56.8%), sulphamethoxazole-trimethoprim (29.63%), augmentin (27.60%) and tetracycline (18.52%). It was also noted that the frequency of resistance to specific antibiotics was not dependent on the sample type from which the isolate was sourced. There was no trend of variation in the number of resistant isolates from certain sample types from one antibiotic to another. This is shown in Table 4.

Out of the 80 isolates that were screened for antibiotic susceptibility, 18.75% showed multiple antibiotic resistance, they resisted 3 or more antibiotics while 81.25% were not multiple antibiotic resistant. The resistance frequencies versus number of drugs to which they were resistant was as follows: 0 drugs (17.50%), 1 drug (42.50%), 2 drugs (21.25%), 3 drugs (8.75%), 4 drugs (3.75%), 5 drugs (2.50%), 6 drugs (2.50%), 7 drugs (1.25%), 8 drugs (0.00%) and 9 drugs (0.00%).

Molecular characterization of the antibiotic resistance

DNA from twenty four isolates that were resistant or gave intermediate outcomes to tetracycline and SXT was subjected to PCR with primers *sul* 1 and *tet A.* The 24 isolates were grouped into two groups of 12 each and DNA from each group was taken through two monoplex

Table 3. The frequency of sample source contamination for the five sources from which the samples were collected.

Source	Samples collected	Contaminated samples	Frequency of contamination (%)
Dry foods	32	30	93.75
Meat	41	38	92.68
Soil	45	40	88.90
Vegetables	56	34	60.71
Water	46	35	76.10
Total	220	180	256.30

Table 4. Frequencies of the resistant isolates obtained from the various environmental samples collected from Gatwekera village of Kibera slums to nine different antibiotics.

Drug	% of resistant isolates (N=80)	Source	*E. coli*	*Salmonella* spp	*Shigella* spp
CIP	0.00% (n=0)	Soil	0	*Salmonella* spp (0)	*Shigella* spp (0)
		Meat	0	*Salmonella* spp (0)	*Shigella* spp (0)
		Water	1	*Salmonella* spp (0)	*Shigella* spp (0)
		Vegetables	0	*Salmonella* spp (0)	*Shigella* spp (0)
		Fish	0	*Salmonella* spp (0)	*Shigella* spp (0)
AMP	56.25% (n=45)	Soil	7	*Salmonella typhymurium*(2)	*Shigella* spp (0)
		Meat	5	*Salmonella typhi* (1)	*Shigella* spp (0)
		Water	11	*Salmonella* spp (0)	*Shigella flexneri* (1)
		Vegetables	11	*Salmonella typhymurium*(2)	*Shigella* spp (0)
		Fish	5	*Salmonella* spp (0)	*Shigella* spp(0)
SXT	30.00% (n=24)	Soil	6	*Salmonella* spp (0)	*Shigella* spp(0)
		Meat	4	*Salmonella* spp (0)	*Shigella* spp(0)
		Water	8	*Salmonella typhi* (1)	*Shigella* spp(0)
		Vegetables	5	*Salmonella* spp (0)	*Shigella* spp(0)
		Fish	0	*Salmonella* spp (0)	*Shigella* spp (0)
S	15.00%(n=12)	Soil	0	*Salmonella* spp (0)	*Shigella* spp(0)
		Meat	2	*Salmonella typhymurium* (1)	*Shigella* spp(0)
		Water	2	*Salmonella typhymurium* (1)	*Shigella* spp(0)
		Vegetables	3	*Salmonella typhymurium* (2)	*Shigella* spp(0)
		Fish	1	*Salmonella* spp(0)	*Shigella* spp(0)
AMC	27.50% (n=22)	Soil	4	*Salmonella typhymurium* (1)	*Shigella spp*(0)
		Meat	3	*Salmonella* spp(0)	*Shigella spp*(0)
		Water	6	*Salmonella typhymurium* (1)	*Shigella spp*(0)
		Vegetables	5	*Salmonella typhymurium* (1)	*Shigella* spp(1)
		Fish	1	*Salmonella* spp(0)	*Shigella spp*(0)
TET	18.75%(=15)	Soil	2	*Salmonella* spp(0)	*Shigella* spp(1)
		Meat	3	*Salmonella typhymurium* (1)	*Shigella* spp(0)
		Water	4	*Salmonella typhymurium* (1)	*Shigella spp*(0)
		Vegetables	1	*Salmonella spp*(0)	*Shigella* spp(0)
		Fish	1	*Salmonella typhymurium* (1)	*Shigella* spp(0)
NA	3.75% (n=3)	Soil	0	*Salmonella* spp(0)	*Shigella* spp(0)

Table 4. Continued

		Meat	1	*Salmonella* spp(0)	*Shigella* spp(0)
		Water	0	*Salmonella* spp(0)	*Shigella* spp(0)
		Vegetables	0	*Salmonella* spp(0)	*Shigella* spp(1)
		Fish	1	*Salmonella* spp(0)	*Shigella* spp(0)
C	7.50%(n=6)	Soil	1	*Salmonella* spp(0)	*Shigella* spp(0)
		Meat	1	*Salmonella* spp(0)	*Shigella* spp(0)
		Water	3	*Salmonella* spp(0)	*Shigella* spp(0)
		Vegetables	1	*Salmonella* spp(0)	*Shigella* spp(0)
		Fish	0	*Salmonella* spp(0)	*Shigella* spp(0)
CN	2.50%(n=2)	Soil	0	*Salmonella* spp(0)	*Shigella* spp(0)
		Meat	1	*Salmonella* spp(0)	*Shigella* spp(0)
		Water	0	*Salmonella* spp(0)	*Shigella* spp(0)
		Vegetables	0	*Salmonella typhymurium* (1)	*Shigella* spp(0)
		Fish	0	*Salmonella* spp(0)	*Shigella* spp(0)

AMC, Amoxicillin; C, Chloramphenicol; Te, Tetracycline; Amp, Ampicillin; SXT, Sulphamethoxazole+Trimethoprim, S, Streptomycin; NA, Nalidixic acid; CIP, Ciprofloxacin; CN, Gentamycin.

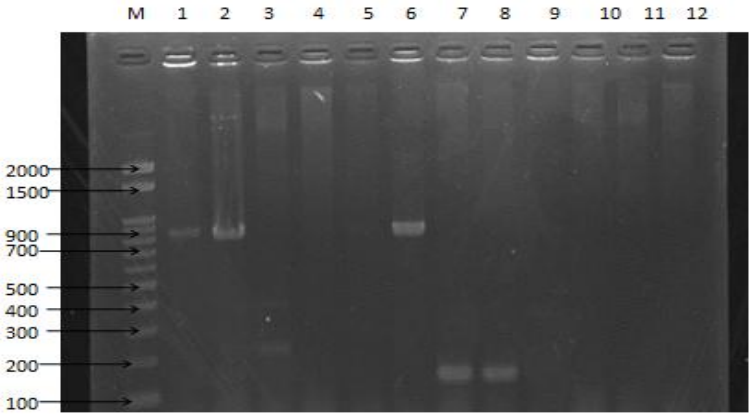

Plate 1. *Sul 1* genes from 12 isolates which were resistant to sulphamethoxazole + trimethopim. Lanes 7 and 4 are for *Salmonella typhimurium* while lanes 2, 3, 5, 6, 8, 9, 10, 11 and 12 are *E. coli*. M is the molecular marker, Hyper ladder (band sizes are in base pairs).

PCRs using the two antibiotic determinants.

In Plate 1, DNA from 12 isolates was amplified for the gene *sul 1.*Only lanes 1, 2 and 6 were positive for the gene encoding sulphonamides resistance (*sul 1)* while lanes 3, 4, 5, 7, 8, 9, 10, 11 and 12 were negative for the gene.

In Plate 2, DNA from 11 isolates was amplified for the gene *Tet A.* Lanes 2, 3 and 4 were positive for the gene encoding tetracycline resistance; *tet A* while lanes 1, 5, 6, 7, 8, 9, 10, 11 and 12 were negative for the gene.

In Plate 3, DNA from 12 isolates was amplified for the gene *Tet A.* Lanes 5, 6, 7, 8, 9, 10, 11, and 12 were positive for the gene encoding tetracycline resistance; *tet A* while lanes 1, 2, 3 and 4 were negative for the gene.

In Plate 4, DNA from 12 isolates was amplified for the gene *sul 1.*Only lanes 9, 10, 11 and 12 were negative for the gene encoding sulphonamides resistance (*sul 1)* while lanes 1, 2, 3, 4, 5, 6, 7 and 8 were positive for the gene.

Fifteen isolates that previously were positive for genetic determinants *sul* 1 and *tet A* or one of the two exhibited the presence of at least one plasmid. All of the fifteen isolates had the plasmid of size 43 bp. This is shown in Plate 5.

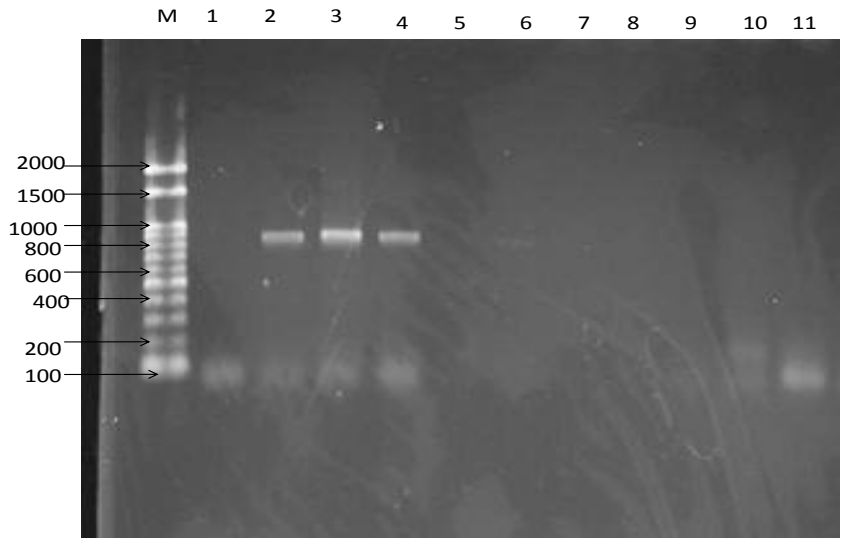

Plate 2. *Tet A* genes for 11 isolates which were resistant to tetracycline. Lanes 7 and 4 are for *Salmonella typhymurium*. 1, 2, 3, 5, 6, 8, 9, 10, 11 while 12 are for *E. coli*. M is the molecular marker, Hyper ladder (band sizes are in base pairs).

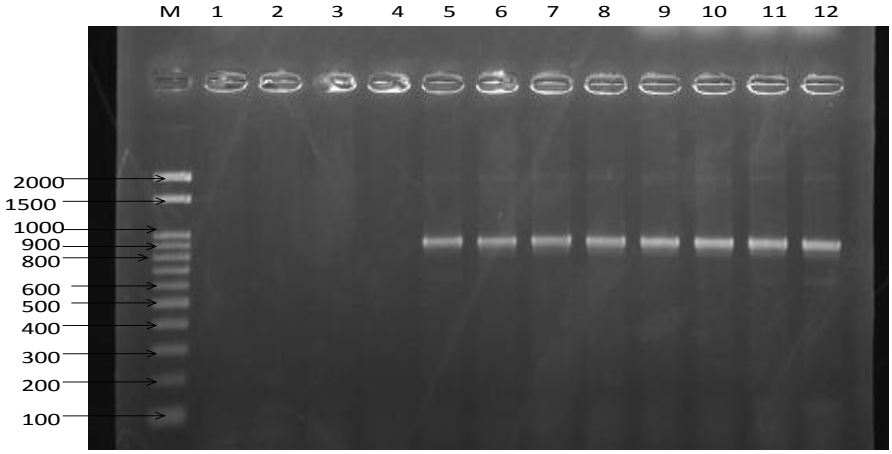

Plate 3. The antibiotic resistance determinant *tet A* genes for 12 isolates that were resistant to tetracycline. Lanes 3, 5 and 11 are for *Salmonella typhimurium* while lanes 1, 2, 4, 6, 7, 8, 9, 10 and 12 are for *E. coli*. M is the molecular marker, Hyper ladder (band sizes are in base pairs).

Conjugation outcomes

Conjugation experiments were done with 15 isolates that had plasmids that were resistant to at least ampicillin and 10 of them had successful transconjugants. Resistance to tetracycline and SXT was transferred in all cases. In 90% of all the successful transconjugants, the donor phenotype and recipient phenotype remained the same. Upon plasmid gel profiling of the transconjugants as shown in Plate 6, it was noted that there was plasmid transfer to all the transconjugants except one which did not have any plasmid at all after the mating experiments.

DISCUSSION

The prevalence of the three microoorganisms identified varied as follows; *E. coli* (91.58%), *Salmonella* (4.21%) and *Shigella* (3.16%). *E. coli* therefore had the highest prevalence in the studied samples followed by *Salmonella* then *Shigella*. Data obtained indicated that foods (meat, vegetables and other dry foods), are potential reservoirs for many pathogenic organisms which were shown to be resistant to many antimicrobials, suggesting a potential public health hazard. These results emphasize the need to implement proactive measures,

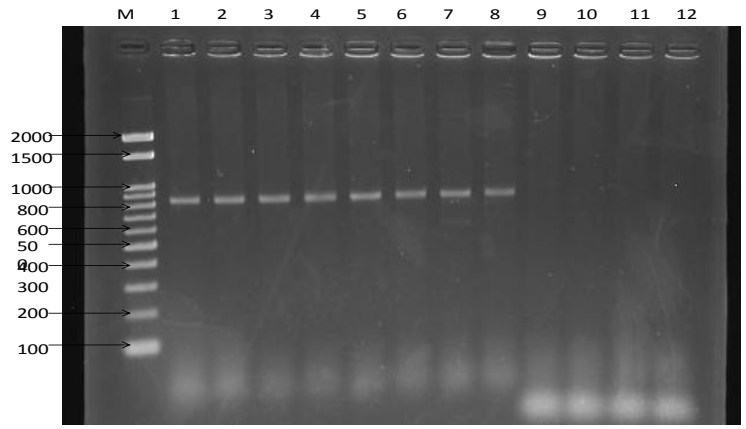

Plate 4. The resistance determinant *sul 1* genes for 12 isolates that were resistant to SXT. Lanes 3, 5 and 11 represent *Salmonella typhimurium..*

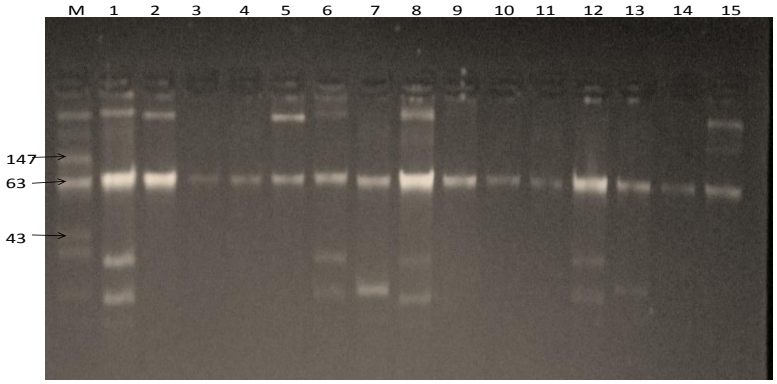

Plate 5. Plasmid DNA obtained from 15 isolates that were confirmed to have both *tet A* and *Sul 1* genes from PCR. M is the plasmid size marker *E. coli* strain 39R861 with band sizes 98, 42, 24 and 4.6 MDa. Lanes 5, 6, 9, 10 and 13 are for *Salmonella* ser Typhimurium while 1, 2, 3, 4, 7, 8, 11, 12, 14 and 15 are for *E. coli* isolates (band sizes are in base pairs).

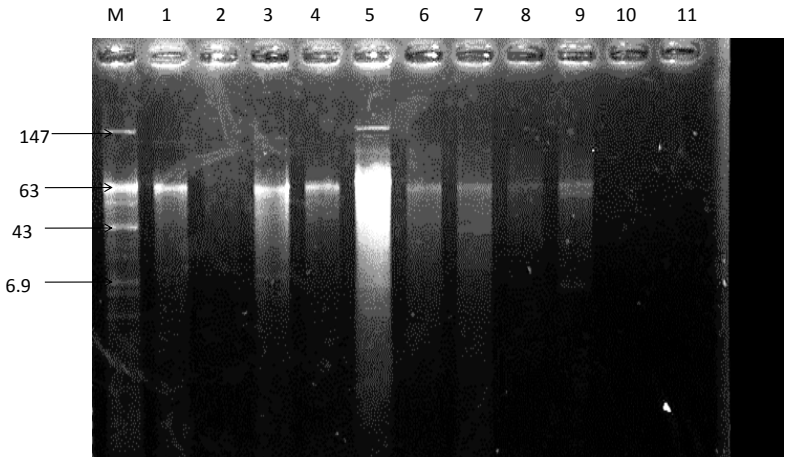

Plate 6. Plasmid DNA extracted from 10 successful transconjugants. M is the molecular marker *E. coli* R39, 7, 3 and 1 are *Salmonella* ser Typhymurium while 2, 4, 5,6,8,9 and 10 are *E. coli* (band sizes are in base pairs).

and more emphasis to be placed on the application of hygienic practices to reduce the risk of infection. Epidemiological reports suggest that meat products are a major agent for the spread of diarrheal illnesses (FAO/WHO, 2002). The isolates were tested for their susceptibility to nine antibiotics that are routinely prescribed for treatment and management of diarrheal infections that is SXT, ampicillin, tetracycline, chloramphenicol, gentamycin, nalidixic acid, ciprofloxacin, streptomycin and augmentin (USPHS/IDSA, 2003). 60% of the isolates were resistant to at least one antimicrobial while the rest (40%) were resistant to two or more antimicrobials (multiple antibiotic resistant). Drug susceptibility tests revealed that the isolates resistance to ampicillin (56.79%), sulfamethoxazole + trimethoprim (29.63%), augmentin (27.60%) and tetracycline (18.52%) were the highest just as was also concluded by Varga et al. (2008).

It also revealed that the most effective drugs to which the microorganisms were most sensitive were ciprofloxacin, gentamycin, chloramphenicol and nalidixic acid. These findings are in agreement with Mengo et al. (2010) where they concluded that the antibiotics that form the mainstay of therapy for typhoid patients in developing countries are ampicilin, cotrimoxazole and chloramphenicol but due to the increasing resistance to anti-bacterials used traditionally for therapy, the use of fluoroquinolones, such as ciprofloxacin and ofloxacin, for the treatment of typhoid has become more common. Gentamycin and ciprofloxacin were the most effective antibiotics in our study. 96.30% of the isolates tested were sensitive to gentamycin while 98.6% of all the isolates tested were sensitive to ciprofloxacin. Gentamycin is rarely abused because of its mode of administration which is mostly intramuscular.

This explains the near zero resistance to this antibiotic, as it cannot be easily abused. Gentamycin was introduced in 1969 as a broad spectrum amynoglycoside which was effective *in vitro* against most gram negative bacilli (Sierbert et al., 1979). Musoke and Revathi (2000), and Kariuki et al. (1997) also isolated gentamycin resistant enteric bacteria. Ciprofloxacin is one of the most expensive antibiotics usually prescribed for typhoid treatment. For all episodes of diarrhea with blood in the stool, the WHO currently recommends treatment with ciprofloxacin or one of the three second-line antibiotics, pivmecillinam, azithromycin and ceftriaxone (WHO, 2007). This has resulted in a cure rate of 99% (Traa et al., 2011). The frequency of resistant isolates varied widely among the different samples for different antibiotics. For instance meat had the highest number of resistant isolates against nalidixic acid, water had the highest number of resistant isolates against chloramphenicol while vegetables had the highest number of resistant isolates against ampicillin. The nature of sample thus did not affect the frequency of resistance.

The gel profiling of the PCR products revealed that the genes for resistance to sulphonamides and tetracycline are available in most of the phenotypically resistant isolates. The antibiotic resistance in those isolates which seem not to possess plasmids was associated with chromosome and/or transposons instead of being plasmid-mediated. This implies that there is no consistent relationship between antibiotic resistance pattern and the number of plasmid bands present. Such findings were also reported by Yah and Eghafona (2008). Sulphamethoxazole in this case was used in combination with trimethoprim but the gene for resistance to trimethoprim was absent in the resistant isolates. This phenomenon indicated the existence of a plasmid carrying multiple resistance to antibiotics. Plasmids were detected virtually in all the MDR isolates. Hence, plasmid mediated resistance is evident in our study. These findings are in agreement with Leversteine et al. (2002b). Plasmids are widespread among enteric bacteria of meat production animals and these emergent plasmids have flexibility in their acquisition of MDR-encoding modules, necessitating further study to understand the evolutionary mechanisms involved in their dissemination and stability in bacterial populations (Fernandez et al., 2011).

Conjugation studies showed the presence of self-transmissible plasmids that were responsible for the transfer of resistance phenotype to the recipient (*E. coli* K12). Resistance to sulphamethoxazole and tetracycline among other antibiotics was shown to be transferred to the recipient through conjugation. This shows that besides being chromosomally encoded, sulphamethoxazole and tetracycline resistance is also plasmid mediated. The acquisition of a new gene may occur by genetic transformation or through mobilization by conjugative transfer. The latter may occur at high frequency and efficiency, and several resistance genes can be acquired simultaneously (Carattoli, 2003). Leverstein et al. (2002a) reported the possibility of transfer of a complete resistance phenotype as is evidenced in 90% of isolates in this study. The high percentage of *E. coli* and *Salmonella* isolates that were MAR to tetracycline, ampicillin, and sulphamethoxazole + trimethoprim, suggested that there has been a misuse of these drugs, which has resulted in these environmental samples that is water, vegetables, meat, soil and dry foods posing a potential threat to humans in the area.

Of interest, ampicillin or augmentin, are not appropriate empiric therapy for common enteric bacteria such as *Shigella* spp. and *Salmonella* spp. because of the high frequency of resistance. The high antibiotic resistance also indicates a negative impact on therapy with these classes of antibiotics, which with time may be rendered completely unhelpful. According to the results of the conjugation experiments, resistance can be fully transmitted from one organism to the other, hence enteric pathogens can transfer resistance to susceptible organisms that share the same environment. Plasmid analysis of the transconjugants showed that the antibiotic

sensitive *E. coli K12* isolates could acquire the R-plasmid from other enteric bacteria such as *E. coli* for a suitable adaptation for survival in the changing antibiotic environment.

Conclusion

The high percentage of *E. coli* and *Salmonella* isolates that were MAR to tetracycline, ampicillin, and sulphamethoxazole + trimethoprim suggested that there has been a misuse of these drugs, which has resulted in these environmental samples that is water, vegetables, meat, soil and dry foods posing a potential threat to humans in the area. According to the results of the conjugation experiments, resistance can be fully transmitted from one organism to the other, hence enteric pathogens can easily transfer resistance to susceptible organisms that share the same environment. Plasmid analysis of the transconjugants showed that the antibiotic sensitive *E. coli K12* isolates could acquire the R-plasmid from other enteric bacteria such as *E. coli* for a suitable adaptation for survival in the changing antibiotic environment.

AKNOWLEDGEMENTS

I greatly appreciate the contribution made by the Kenya Medical Research Institute in funding this project and the staff of the bacteriology lab, for assisting during the benchwork which has led to the success of this work.

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