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Occurrence of Pathogenic Bacteria Harbouring Antibiotic Resistant Genes in River Njoro in Nakuru County, Kenya

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Abstract Njoro River drains an agricultural catchment whose main livelihood activities are livestock rearing and employment in light industries. The limited quantities of piped water supplies coupled with inadequate sanitary facilities experienced in the area can contribute to the spread of antibiotic resistance from antibiotic use in agriculture and human. The objective of the current study was to isolate common waterborne enteric pathogens and test them for antibiotic resistance on some commonly used antibiotics. The organisms that were found to be resistant were also tested for the presence resistant genes. This was done by filtering known quantities of water through membrane filters and plating them on selective and differential media and these were tested for sensitivity to antibiotics. Isolates that showed antibiotic resistance were tested for the presence of tetracycline (Tet A), Sulfamethoxazole (Sul2) genes class 1 intergrase gene and SXT element resistance genes using PCR with appropriate primers. Pathogens including *E. coli* strains, *Salmonella* spp, *Vibrio cholera* and *V. parahaemolyticus* were recorded in this study. Sulfamethoxazole (Sul2) genes were detected in *Klebsiella pneumonia*, *Klebsiella oxytoca* isolates and Entero- aggregative strains of *E. coli*. Tetracycline (tet A) genes were detected in ETEC and EAEC pathogenic strains of *E.coli*. Class 1 integrase was detected in an EAEC strain. The SXT (int) element was not detected in any of the isolates tested.

Keywords: *antibiotics, pathogens, resistance, resistance genes*

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1. Introduction

Water borne pathogens in aquatic environments are mainly derived from disposal of untreated or inadequately treated sewage into a water body, although several human bacteria pathogens are indigenous to estuarine and sea waters (e.g. halophilic *Vibrios*). Infection may be through consumption of contaminated water or direct contact through bathing or from other water recreational activities. The most common water borne pathogen is *Salmonella typhi* which usually causes diarrheal illness commonly referred to as typhoid and paratyphoid fever [1]. *Vibrio cholera* is also important water borne pathogen. It causes cholera which is a disease characterised by acute and very intense diarrhoea which can exceed one litre per hour [2]. *Shigella* is Gram negative non spore forming straight rod like members of the family *Enterobacteriaceae*. There are various species of *shigella* and these include *Shigella dysenteriae* which is an intestinal pathogen that causes a severe form of dysentery. It also produces shiga toxin which is an enterotoxin and is more prevalent in developing countries. Other species include *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei* which cause a mild form of the dysentery [3]. Entero toxigenic *E. coli* (ETEC) serotypes cause infantile gastro enteritis. It is a major cause of diarrhoea in developing countries where there is poor sanitation and inadequate cleaning water [4]. There are various organisms that are being classified as emerging waterborne pathogens. These include the *Mycobacterium avium* complex (MAC).There are two classes of *Mycobacterium avium* complex and they include; *Mycobacterium avium* and *Mycobacterium intracellulare*. The importance of MAC organism was recognised with the discovery of disseminated infections on immuno-compromised patients particularly those with HIV and AIDS. Members of MAC are considered opportunistic human pathogens [5]. Other emerging pathogens include *Helicobacter pylori*, which is a major etiological agent for gastritis and has been implicated in the pathogenesis of duodenal and peptic ulcers and gastric carcinomas; however most individuals infected by *H. Pylori* remain asymptomatic [5]. *Aeromonas hydrophila*has gained public health concern as a pathogen with high incidence of resistance. It is a potential agent for gastroenteritis, septicaemia, wound infection and meningitis [6].

Many organisms harbour antibiotic-resistance genes, eventually inserted into genetic mobile platforms (plasmids, transposons, integrons) able to spread among water and soil bacterial communities [7]. Integrons, in particular, with multiple-resistance gene cassettes, are highly efficient molecular tools used by bacteria for the acquisition and expression of antimicrobial-resistance genes [8]. Chromosomal and plasmid-borne integrons have been identified as one of the crucial factors for the development of multidrug resistance in *Enterobacteriaceae*, as well as in many other bacteria species, by lateral gene transfer of gene cassettes [9]. Notably, class 1 integrons have been found in many multi drug resistant organisms and many Gram-negative species. These genetic elements often contain a 59 integrase gene and a 39 *sul1* gene (encoding sulfamethoxazole resistance) and resistance gene cassettes separated by 59-bp stretches which are involved in the incorporation of additional cassettes within an integron [10]. While these integrons have been plasmid borne in many instances [11], they have become chromosomally integrated in *S. Typhimurium* DT104. Chromosomal integration has been speculated to allow resistance genes to persist even in the absence of antibiotic selection [12]. Tetracycline resistance can be mediated by efflux, ribosomal protection, or chemical modification, but the first two mechanisms are the most clinically significant [13]. A variety of resistance determinants may encode these mechanisms. Depending on the species, the *tet* to *tet* (E) determinants are generally responsible for tetracycline resistance in *Enterobacteriaceae* [14]. Tet (A) determinants encodes efflux pumps.

The effluents containing wastes from human and animals treated with antibiotics that can lead to development of antibiotic resistance in microorganisms including pathogens which find way into River Njoro from agricultural activities and hospitals in the area. Since a good number of people in the area use the river directly for domestic use, agricultural and watering of livestock, continuous use of this untreated river water may have led to increased exposure to drug resistant bacteria posing a great health concern. Incidentally diarrhoea has been reported as number 3 disease burden in Nakuru County in Kenya with the most common causative agents of diarrhoea being waterborne pathogens including; *E. coli, Salmonella typhi, Shigella and Vibrio cholera*. Thus this study proposed to isolate water borne pathogens and test for the presence of anti biotic resistance contributed by use of River Njoro.

2. Materials and Methods

This study was based on River Njoro in Nakuru County in the Rift Valley Region of Kenya. River Njoro descends from the forested Eastern Mau Escarpment (3000 m above sea level) to the valley floor, emptying into Lake Nakuru **Error! Reference source not found.** The River Njoro spans a distance of about 60 km from its origin in the native forests of the Eastern Mau Escarpment (elevation of 2700-3000 meters (m)) to its terminus at Lake Nakuru in the Rift Valley floor. [15]. Njoro River drains an agricultural catchment whose main livelihood activities activities are livestock rearing and employment in light industries (Ref). Limited quantities of piped water supplies and inadequate sanitary facilities are experienced in the area. A good number of inhabitants depend on direct river use for domestic and livestock watering. The river receives runoff inputs from many non point sources in the area in addition to treated sewage effluents from a few settlements.In-stream activities such as bathing, water fetching, laundry cleaning and cattle watering occur [16]. Study sites were chosen from points and nonpoint sources of pollution from agricultural, industrial and settlements in the river catchment sites (Figure 1). These sites included the following; Sigotik which is assumed as unpolluted upstream site, Turkana, a cattle watering point- to capture discharges from Njokerio area, Njoro Canning Factory, to capture effluents from the canning factory and effluents from the University. Njoro Bridge, to capture effluents from Kenya Orchards and Vegetable canning factory. Kiptanui_Daneside and KALRO farms effluents were captured at Njoro Bridge. Kerma, Kenyatta and Ngata captured discharges from Njoro and Kenyatta agricultural areas. Mogoon the furthest point downstream to Captured discharges from Rift Valley Institute of Science and Technology and nearby farms.

2.1. Determination of Antibiotic Resistant Pathogens in Water Samples

To determine pathogenic bacteria in water samples susceptible to antibiotics, membrane filtration procedure was used to filter 1 ml water samples or dilution of it (Ref). To isolate the pathogens, the filters were placed on TCBS (indicate manufacturer), *Salmonella/Shigella* Agar (Manufacturer) and Chromacult agar (MercK, Germany) to isolate *Vibrio* spp, *Salmonella* spp., *Shigella* spp. and *E. coli* respectively. Sensitivity testing of antibiotics stated above was done using CLSI disk susceptibility testing method (Ref). In this method, a bacteria inoculum approximately 1-2 × 10^8 CFU/ml (0.5 McFarland) was applied to the surface of a large (150 mm diameter) Mueller- Hinton agar plate. Paper antibiotic discs were prepared using Whatman filter papers and placed on the inoculated agar surface. Antibiotic disk cartridges were of the following concentrations in µg, for Ampicillin (AM-10), Chloramphenicol (C-30), Streptomycin (S-10), Tetracycline (Te-30). The plates were incubated for 16-24 hours at 35℃ prior to recording of results. The zones of growth inhibition around each of the antibiotic discs were measured to the nearest millimetre. The diameter of the zone was related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameter of each drug was thereafter interpreted.

2.2. Molecular Characterization of Antibiotic Resistant Bacteria

2.2.1. Bacteria DNA Extraction

Bacteria culture resistant to antibiotics were grown overnight in rich broth. DNA was extracted from the colonies that had been grown overnight in Nutrient Agar media. Bacterial cells were emulsified in 250 µllysis buffer (0.1 M NaCl, 50 mM disodium EDTA, 0.1 M Tris-HCl, pH 8) containing 0.5% (w/v) sodium dodecyl

sulfate 0.5% (w/v), proteinase K (0.5 mg/ml), and RNase (0.8 mg/ml). After emulsification, the tube was placed in a water bath at 60℃ for 5 minutes. Two hundred micro-litres of the supernatant was placed in a new Eppendorf tube and overlaid with 30 µl of 6 M NaCl and in 70% ethanol at room temperature. The tubeswerekept at room temperature for five minutes, centrifuged at 1000 X g for three minutes. The supernatant was discarded and crude DNA rinsed with 70% ethanol and dried out by leaving the eppendorf tubes open for a few minutes in the clean bench. Finally the DNA was suspended in 200 µl of sterile water. The DNA was detected by evaluating its concentration (an OD of 1 at 260 nm = 50 µg/ml of DNA) and its purity (OD at 260 nm/OD at 280 nm = 1.7-2.0). Estimation of DNA contents was done by running the DNA sample on an agarose gel (2%) alongside known amounts of DNA of the same or a similar size. The amount of sample DNA loaded was estimated by comparison of the band intensity with the standards using a scanner or imaging system. The standards used were of roughly the same size as the fragment of interest to ensure reliable estimation of the DNA quantity, since large fragments inter-chelate more dye than small fragments and give greater band intensity.

2.2.2. Amplification of Antibiotic Resistant Gene Sequences

Tetracycline family of antibiotics has a wide use in medical and veterinary fields. They are widely used as growth promoters in farm animals and as prophylaxis for plant and aquaculture diseases making them more likely to be widespread in the environment compared to other antibiotics. Tetracycline resistance genes were determined by first extracting DNA from environmental samples. The DNA 16 SrDNA gene was amplified using tet primers (Table 1) described in [17]. PCR solution contained 1.5 mM of each primer, PCR buffer II (Applied Biosystems), 1.5 mM$MgCl_2$, each of the four dNTPs at a concentration of 200 mM and 1 Taq Gold DNA polymerase (Applied Biosystems). All primer pairs tested resulted in PCR products of the predicted size only, demonstrating their high specificity. PCR products (10 µl) were separated by electrophoresis on a 2.0 % agarose gel and visualized under UV light after staining in a 1.0 g ethidium bromide per µ1 solution.

2.3. Statistical Analysis

Data obtained was represented as tables or graphs in Ms. Excel^{TM}. Statistical analysis was carried out on appropriate programs in SPSS^{R} software version 19. Significant level was set at $\alpha = 0.05$. The mean values of physico-chemical characteristics, water quality, and total numbers of bacteria in samples from points and nonpoint sources of pollution from agricultural, industrial and settlements in river catchment sites were compared by ANOVA. The means were separated using a *post- hoc* test. Tukey's test was done to determine the means that were different. The bacterial species from different sites was compared by descriptive statistics. Correlation analysis was conducted to establish relationships between variables.

3. Results

3.1. Determination of Antibiotic Resistant Pathogens in Water Samples

In this study, the pathogens of interest were those that cause diarrhoea. Selective media in this case was used. For *Salmonell*a, Hichrome Salmonella agar was used. In this media, two types of colonies were observed, blue colonies were presumptively *E.coli.* The results for the first month of analysis are tabulated in Table. Purple colonies which were presumptively *Salmonella* species were isolated. In TCBS media, two colony types were observed, dark green colonies which were presumptive for *Vibrio parahymolyticus* and yellow colonies which were presumptive for *Vibrio cholera.* In chromocult media *E. coli* had blue colonies.

Table 1. List of Primers used for PCR

Genes	Primer	Sequence (5'→3')	Amplicon Size (bp)	PCR Conditions			References
				Melting	Annealing	Extension	
Class 1 intergrase	InDS	F: CGGAATGGCCGAGATC R: CAAGGTTCTGGACCAGTTGCG	380	95℃, 1m	58℃, I m	72℃, I m	Dalsgaard *et al.* (2000)
SXT element	Int	F: GCTGGATAGGTTAAGGGCGG R: TCTATGGGCACTGTCCACATTG	592	95℃, 1m	54℃, I m	72℃, I m	Hochhut *et al.* (2001)
Sulfamethoxazole resistance	Sul2	F: AGGGGGCAGATGTGATCGAC R: TGTGCGGATGAAGTCAGCTCC	625	95℃, 1m	54℃, I m	72℃, I m	Hochhut *et al.* (2001)
Tetracycline resistance	TetA-2000	F: GTAATTCTGAGCACTGTCGC R: CTGCCTGGACAACATTGCTT	950	98℃, 1m	58℃, 1m	72℃, 1m	Kobayashi *et al.* (2007).

Table 2. Pathogenic Bacteria Sampling Time 1 and Tukey's Test Showing Temporal Variations between Means of Pathogens from Site to Site

SITE		*Vibrio parahymoliticus*	*Vibrio Cholera*	*E. coli*	*Salmonella spp*
SIGOTIK	Mean	ND	ND	3.13×10^4A	4.03×10^4A
	SD	ND		1.52×10^3	4.16×10^4
TURKANA	Mean	2.16×10^4A	ND	6.40×10^4B	4.20×10^4A
	SD	1.87×10^4		3.41×10^3	721×10^3
CANNING	Mean	$2.06\times10^4{}_B$	ND	5.40×10^4AB	4.00×10^4B
	SD	1.78×10^3		3.98×10^3	1.00×10^3
NGATA	Mean	3.66×10^4A	3.33×10^4B	8.66×10^4C	4.13×10^4A
	SD	5.50×10^3	2.08×10^3	5.22×10^3	8.08×10^3
MOGOON	Mean	1.03×10^4B	ND	2.07×10^4A	3.86×10^4B
	SD	1.78×10^3		2.28×10^3	4.16×10^3

Mean values with different letters in the same column are significantly different at P<0.05)

Table 3. Pathogen Bacteria Sampling Time 2 and Tukey's Test Showing Temporal Variations between Means of Pathogens from Site to Site

SITE		*E. Coli*	*Salmonela*	*Vibrio Cholera*	*Vibrio parahymoliticus*
SIGOTIK	Mean	3.43×10^{4}A	3.06×10^{4}A	2.06×10^{3}A	2.13×10^{3}A
	SD	2.88×10^{3}	1.15×10^{3}	1.78×10^{3}	1.84×10^{3}
TURKANA	Mean	4.03×10^{4}B	4.33×10^{4}B	4.00×10^{3}B	3.20×10^{3}B
	SD	8.73×10^{3}	2.13×10^{3}	8.80×10^{2}	1.0×10^{2}
CANNING	Mean	4.73×10^{4B}	3.46×10^{4}A	3.63×10^{3}C	2.16×10^{3}A
	SD	1.10×10^{3}	5.03×10^{3}	6.6×10^{2}	1.87×10^{3}
NGATA	Mean	4.06×10^{4}B	3.33×10^{4}A	ND	ND
	SD	3.51×10^{3}	4.16×10^{3}	ND	ND
MOGOON	Mean	3.70×10^{4}A	3.30×10^{4}A	ND	ND
	SD	5.00×10^{3}	2.64×10^{3}	ND	ND

Mean values with different letters in the same column are significantly different at P<0.05

Table 4. Trend in Distribution of Pathogenic Bacteria in Water during the study period.

Sampling time	Parameter	CFUs per 100 Ml	
	Pathogen	Site with the highest count	Site with the lowest count
November 2014	*Proteus vulgaris* (colourless)	Ngata : 5.66×10^{4}	Turkana :4.0×10^{4}
	E. coli(blue)	Mogoon :2.0×10^{4}	Sigotik :3.1×10^{4}
	*Salmonell*a (Purple colony)	Turkana :4.2×10^{4}	Mogoon 3.8×10^{4}
	Vibrio cholera (yellow)	Ngata :3.33×10^{4}	
	Vibrio Parahymolyticus (green)	Ngata :3.66×10^{4}	Mogoon: 1.0×10^{4}
January 2015	*Proteus vulgaris* (colourless)	Njoro canning:7.5×10^{4}	Ngata : 5.6×10^{4}
	E. coli (blue)	Njoro Canning :4.7×10^{4}	Sigotik :3.4×10^{4}
	Salmonella (Purple colony)	Turkana :4.3×10^{4}	Sigotik :3.06×10^{4}
	Vibrio cholera(yellow)	Turkana :4.0×10^{3}	Ngata and Mogoon: 0
	Vibrio Parahymolyticus(green)	Njoro canning :2.1×10^{3}	Mogoon: 1.1×10^{3}

It is important to note that for the first month, *Vibrio* species were only found in Ngata and Mogoon whereas there were no *Vibrio* species isolated in other sites as shown in Table 2.

In the second month, *Vibrio* species were in more sites than in the rainy month i.e. Turkana Mogoon and Njoro canning where as there no *Vibrio* species were isolated in Ngata and Sigotik as shown in Table 3.

A statistical analysis using ANOVA to find the variation in the number of pathogenic bacteria in the first sampling time indicated a significant difference in number of *Vibrio* species, F, = 769, $p < 0.05$. However there was no significance difference in the number of other pathogenic bacteria. The same was observed in the Second sampling where there was no significance difference in the number of pathogens from site to site except for *Vibrio* species.

A Pearson's correlation was run to determine the relationship between pathogenic bacteria and other pathogens, coliform bacteria, HPC and antibiotic resistant organisms. *Salmonella* had a positive correlations with streptomycin resistant organisms ($r=0.819$ $p<0.01$). There was also a positive correlation between *Salmonella* species and total coliforms ($r=0.529$ $p< 0.05$), faecal coliforms ($r=0.670$ $p< 0.05$), HPC ($r= 0.805$ $p< 0.01$). There was a positive correlation between *E. coli* and *Vibrio* species ($r= 0.781$ $p< 0.01$).

3.2. Antibiotic Susceptibility Testing of the Pathogenic Bacteria

A total of seventy four isolates were tested for resistance to the four antibiotics using CLSI disk diffusion methods and photographs of some of the results for *E. coli* and *Salmonella* responses to various antibiotics are shown in Plate 1and 2. The organisms were deemed resistant, intermediate or susceptible based on disk zone diffusion diameter chart. The percentage of the resistant strains towards each antibiotic was then calculated and represented in Figure.

Plate 1. A plate showing *E. coli* susceptibility testing for four antibiotics based on the zones of inhibition it is resistant to the four antibiotics i.e. tetracycline, ampicillin, streptomycin and chloramphenicol

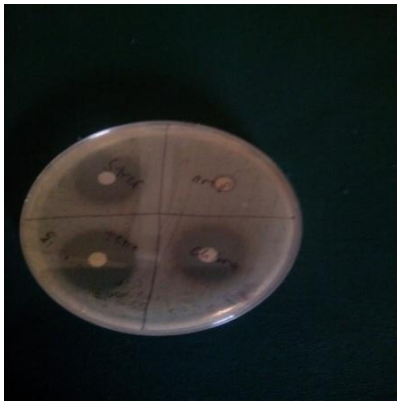
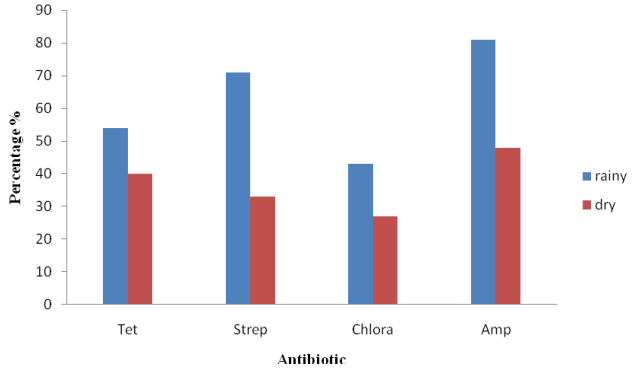


Plate 2. A plate showing *Salmonella* resistant to Ampicillin and Susceptible to tetracycline, streptomycin and chloramphenicol

Generally during the rainy month there were more resistant strains isolated as compared to the dry month. From these observations, 54% of the strains were resistant to tetracycline in the rainy month whereas 40% of the strains were resistant to tetracycline in the dry month. During the rainy month 71% of the strains were resistant to streptomycin where as only 33% of the strains showed resistance to streptomycin in the dry month. 43% of the pathogens were resistant to chloramphenicol in the rainy month compared to 27%. Finally, 81% of the pathogens were resistant to ampicillin during the rainy month and 48% of the pathogens were resistant to ampicillin during the dry month. Ampicillin showed to have more resistance as compared to all the other antibiotics.



Key: Tet-tetracycline, Strep- Streptomycin, Chlora- Chloramphenicol, Amp -Ampicillin

Figure 1. Percentage of Antibiotic Resistant Organism for Each Antibiotic during Dry and Rainy Months

Moreover, it was noted that there were some pathogens which had resistance to more than two antibiotics i.e. multidrug resistant strains. 32% of all the pathogens tested were resistant to more than two antibiotics and out of this 27% of the strains were resistant to all the four antibiotics.

3.3. Molecular Characterization of Antibiotic Resistant Bacteria

In this study, the presence of tetracycline (Tet A), sulfamethoxazole (Sul2), Class 1 intergrase (InDs) resistant genes and SXT (Int) element which confer resistance to antibiotics were determined. Molecular Marker used 1 kb plus DNA marker (100-3000) bp ideal for determination of size of PCR products.

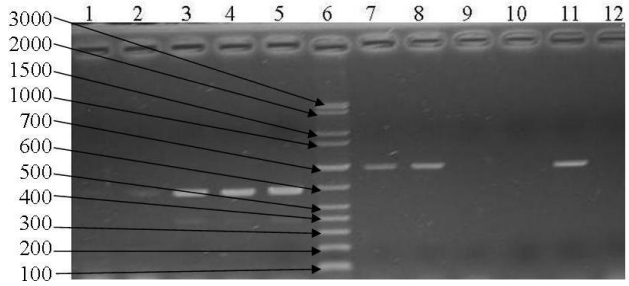
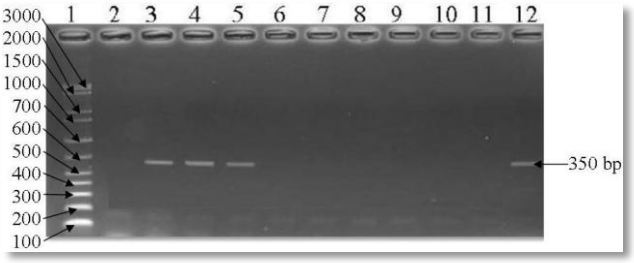


Figure 2. Ethidium Bromide-Stained Agarose gel (2%) Containing PCR Products by Sulfamethoxazole Resistant Genes, Sul2 625 bp Primers and Template DNA from the Indicated Bacteria Strains and Reaction of Tet A (950bp)

Table 5. Bacteria Isolates on the Wells 1 To 12 in Figure

WELL	SAMPLE CODE	Isolate
1	41(Mogoon)	*Klebsiellaoxytoca*
2	42(Turkana)	*Klebsiellaoxytoca*
3	46(Mogoon)	EAEC
4	47(Sigotik)	EAEC
5	+ control	*E. coli* KDH 521 (MDR from CMR-KEMRI)
6	Gel pilot 1kb plus Ladder,	
7	42(Turkana)	*Klebsiellaoxytoca*
8	46(Mogoon)	EAEC
9	47(Sigotik)	EAEC
10	+ control	*E. coli* KDH 521 (MDR from CMR-KEMRI)
11	+ve control	
12	-ve control	

From the plate above, it was evident thatTet A genes (950 bp) were present in EAEC isolates (Well 3 and 4) for EAEC strains isolated in water samples from Mogoon and Sigotik respectively. Sul 2 genes (625bp) were also present as seen in *Klebsiellaoxytoca* and EAEC (well 7 and 8) isolated from Turkana and Mogoon respectively.



Gelpilot 1kb plus Ladder,

Figure 3. Ethidium bromide-stained agarose gel (2%) containing PCR products by InDS, TetA-2000 (bacteria isolate) primers and template DNA from the indicated bacteria strains Showing Tet A resistance genes

Table 6. Bacteria isolates on well 1 to 12 of figure 12

WELL	SAMPLE CODE	Isolate
1	41(Mogoon)	*Klebsiella oxytoca*
2	42(Turkana)	*Klebsiella oxytoca*
3	46(Mogoon)	EAEC
4	47(Sigotik)	EAEC
5	48(Turkana)	ETEC
6	49(Canning)	EAEC
7	50(Ngata)	*Klebsiella pneumonia*
8	2a(Turkana)	*Aeromonas hydrophila*
9	13a(Turkana)	*Pseudomonas aeruginosa*
10	-ve control	*E.coli*
11	-ve control	*E.coli*
12	+ve control	*E. coli* KDH 521 (MDR from CMR-KEMRI)

There was evidence of **InDS** (350 bp) genesfor enteroagressive *E.coli* strains in well 3and 4 isolated from Mogoon and Sigotik respectively and also in an entroagressive strains isolated in Turkana while it was negative in the other bacterial isolates. Interestingly, the same EATEC strains from Mogoon and Turkana had also shown resistance to sulfamethoxazole (Figure 2).

4. Discussion

4.1. Pathogenic Bacteria in Water

In this study, pathogenic bacteria that cause dysentery and diarrheal infections were isolated. These are *E.coli*, *Salmonella* and *Shigella*spp. which were isolated in all the sites five sites. In the rainy month Ngata had the highest number of pathogens isolated whereas Mogoon had the lowest number of pathogens isolated. In the dry month however Turkana and Njoro canning had the highest number of pathogens isolated where asSigotik had the lowest number of pathogens isolated. On the other hand *Vibrio* species were not isolated in all the sitesduring the dry month in November. During the rainy month, only Ngata and Mogoon had *Vibrio* species isolated. In January the dry month, Turkana, Njoro canning and Mogoon had *Vibrio* species isolated. Faecal contamination of the river from both animal and human sources may have led to the hyper-eutrophication of water [16]. This factor combined with the increasingly brackish, slow moving tributaries and increased anthropogenic activities might have adversely affected the ecological balance in River Njoro [18]. The combination of the above could in essence have created the ecological niche necessary for the proliferation of these pathogens.

The widespread occurrence of drug resistant microorganisms especially pathogens in our environment has necessitated the need for regular monitoring of antibiotics susceptibility trends to provide the basis for developing rational drug prescription programs and making policy decisions that govern the use of antibiotics [19]. Microorganisms undergo selection pressures in the presence of toxic compounds and develop resistance. The most common resistance is to metal and antibiotics, which can be a result of bio-essentiality or of abuse of the metal and/or antibiotics. The inferences drawn from chart can be resistant, intermediate or susceptible. Resistant indicates that clinical efficacy has not been reliable in treatment studies, Intermediate implies clinical applicability in body sites where the drug is physiologically concentrated or when a high dosage of the drug can be used and Susceptible implies that an infection due to the organism may be treated with the concentration of antimicrobial agent used, unless otherwise contraindicated. Susceptibility testing in this study showed that most of these organisms were resistant to more than two antibiotics. Generally ampicillin had the highest resistance whereas chloramphenicol was the most susceptible drug in this study. Worth noting was the fact that the pathogens isolated during the rainy month were more resistant than those isolated during the dry months. This could be due to emptying of resistant enteric pathogens into the river water by the rain water and agricultural runoffs. Multidrug resistance was also observed in this study whereby 32% of the isolates were resistant to more than two drugs and 27% of the isolates were resistant to all the four test antibiotics i.e. chloramphenicol, tetracycline, ampicillin and streptomycin.

This study targeted four genes that confer antibiotic resistant genes, Tet A genes, Sulfamethoxazole resistant genes, Class 1 integron and SXT element. Class 1 intergrase genes, Tet Aand Sulfamethoxazole resistant genes were isolated but no detectable SXT element genes were detected. From the results, it was evident that the Tet gene was present in most of these bacteria isolated in the study. This is a cause for alarm since Tetracycline continues to be used for treatment in a variety of Gram-positive and Gram-negative, intracellular bacteria and protozoan infections, as well as for non-infectious conditions. Tetracycline is also an important antibiotic for prophylaxis or treatment, alone or in combination with other antibiotics, for *Bacillus anthracis*, *Francisellatularensis* and/or *Yersinia pestis*; all listed as potential weapons in biological terrorism [20].

This study targeted four genes that confer antibiotic resistant genes,Tet A genes, Sulfamethoxazole resistant genes, Class 1 integron and SXT element. Class 1 intergrase genes were isolated but no detectable SXT element genes were detected. This study established EAEC, ETEC and other disease causing organisms such as *Klebsiella oxytoca* and *Klebsiella pneumonia* among others were harbouring these antibiotic resistant genes. Also it was noted that most of the pathogens isolated were resistant to tetracycline, ampicillin, streptomycin, and chlolamphenicol. The use of these drugs should be closely monitored.

Sulfamethoxazole resistance gene (Sul2) was determined. Trimethoprim-sulfamethoxazole (TMP-SMX) is the most efficacious agent for the prevention of *Pneumocystis carinii* infections in human immunodeficiency virus (HIV)-infected individuals. It is, however, also a potent broad-spectrum antibacterial agent [21], and long-term use may result in the selection of resistant bacteria. Hence, numerous HIV infected patients worldwide have received and will continue to receive TMP-SMX prophylaxis for long periods of time, increasing the potential for development of resistant bacteria in these individuals, which can then be spread to the community at large. Sul 2 primers were used to target this gene. The gene was evidently present in the isolated pathogenic bacteria.

5. Conclusion and Reccomendation

There was a very high number of antibiotic resistant pathogenic bacteria isolated in this study. These pathogens were *Salmonella typhi*, *Proteus vulgaris*, *E. coli* and *Vibri*o species.Tetracycline resistant genes, class 1 intergrase, and sulfamethoxazole resistant genes were isolated from R. Njoro. River Njoro water, forms a destination for tetracycline and sulfamethoxazole resistant bacteria and might serve as an environmental reservoir for antibiotic-resistance that may be transferred to other environmental and pathogenic organisms through horizontal gene transfer.

There should be enforcement of all environmental related laws to protect and conserve the environment surrounding rivers in the country. Of importance are NEMA and USEPA regulations regarding environmental protection. The KEBs, and WHO guidelines on drinking water quality is important regarding public health. Government should adopt alternative safer water sources for communities still in use of river water.

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