DISTRIBUTION AND DIVERSITY OF ANTIBIOTIC RESISTANT BACTERIA IN NJORO RIVER IN NAKURU COUNTY

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ABSTRACT
Many in-stream activities occur in River Njoro whereby communities fetch water for domestic use, watering of animals and Laundry. Furthermore communities use the river for irrigation, washing of motor vehicles and for sewage disposal. Of major concern is that many pharmaceuticals used in the farms and hospitals as well as antibiotic resistant microbes find their way to the river in runoff and sewage. Presence of antibiotic resistance in river water exposes human and animals to contamination during these in-stream activities. Thus the current study determined the levels of bacteria resistant to various types of antibiotics both medical and veterinary used for disease treatment in the catchment. The levels of residual antibiotics present in river water and sediments were investigated as well as various physical chemical conditions and indicators of faecal pollution in the river. The total numbers of bacteria resistant to five antibiotics studied varied in sediments collected in different sites (ANOVA, $P < 0.05$). Turkana site showed highest resistance to ampicillin, tetracycline and streptomycin while Njoro canning factory showed highest numbers for gentamycin and chloramphenical. Indicators of faecal pollution were found in all sites even in Sigotik (the most upstream site) recording an *E. coli* density of 4.13×104±15.28 per 100 ml of water. Physical-chemical measurements were able to show site differences. A typical example is BOD whereby Njoro Canning factory had the highest value of 6.99±0.20 mg L^{-1}whereas Sigotik the furthest point upstream had BOD of 1.28±0.13 mg L^{-1}. Positive *Shigella* spp., *E.coli* spp., *Salmonella* spp., *Vibrio cholera* and *V. parahaemolyticus* were recorded in Turkana and Ngata sites. There is cause for alarm due to the high antibiotic resistant bacteria in River Njoro. We recommend proper treatment of the river water before use or alternative safer water sources for consumption.
Keywords: River Njoro, Antibiotic resistant bacteria, Physico-chemical parameters, Microbiological indicators

INTRODUCTION
Bacteria resistant to antibiotics pose a serious threat to human life. Resistance to antibiotics such as β-lactams, macrolides, fluoroquinolones (quinolones) and tetracycline have been reported (Jury *et al.*, 2012). Nalidixic acid (NA) is a broad spectrum, first generation synthetic quinolones antibiotics that was discovered in 1962 and is effective against Gram negative bacteria thus used for the treatment of urinary tract infections (UTI). Chloramphenicol (CHL) is a broad spectrum antibiotic discovered in 1949 and is routinely used as treatment of eye infections and serious infections caused by anaerobes. Tetracycline (TC) is another example of broad spectrum antibiotic, discovered in 1945 and is used against a diverse numbers of infections including UTI, skin infections (acne), sexually transmitted diseases as in gonorrhoea and Chlamydia, as well as eye infections. The dependable and simple use of antimicrobial substances led to the propagation of antibiotic resistant strains and this narrowed the option for alternative treatment (Jury *et al*., 2012).

The widespread emergence of antibiotic resistance particularly multidrug resistance (MDR), among bacterial pathogens has become one of the most serious challenges to clinical therapy (Levy *et al*., 2004). Multi drug resistant bacteria can be defined as bacterial species resistant to more than one class of antimicrobial agents (Siegel *et al*., 2008). Infections caused by MDR bacteria are difficult to treat. Another pathogenic group of big concern is extended spectrum beta lactamase (ESBL) isolates including members from *Enterobacteriaceae* and *E. coli* (Dahbi *et al*., 2013). The MDR bacteria are increasing public health problems and few therapeutic options are available to treat them. Their increasing incidence in the environment can lead to the proliferation of health problems in immuno-compromised patients which might be very difficult to treat with existing antibiotics (Reinthaler *et al*., 2014).

Antibiotics are released to the aquatic environment in different pathways. After the administration to humans, they are excreted as metabolites but also a considerable amount is eliminated in unchanged form as parent compounds via urine and faeces into the sewage. Hospitals are also one of the most important contributors of the occurrence of the antibiotics into the aquatic environment (Lindberg *et al*., 2004). Use of antibiotics in veterinary medicine for the treatment of bacterial infections of animals as well as prophylactic agents is another source of contamination. The animal excreta are the major

source of contamination as most of these substances end up in manure. The manure and slurry (urine and faeces) are either stored or directly applied to the farms (Babic *et al*., 2006).

Faecal antibiotic-resistant bacteria, secreted in human or animal intestines under antibiotic treatment (Salyers *et al.,* 2004) may enter water mainly from treated effluents of wastewater treatment plants (WWTP) (Reinthaler *et al.,* 2003), field runoffs (Peak *et al.,* 2007) and direct discharge of untreated wastewater. These faecal bacteria might then be able to transmit antibiotic resistance to autochthonous bacteria through lateral transfer when the resistance genes are carried by conjugative plasmids and transposons (Van Elsas *et al.,* 2003).

Mechanisms for horizontal transfer of antibiotic resistance genes have been reported in the environment. These include conjugation, transduction and transformation. Emerging trends in the increase of resistant microbes in water is a challenge in disease control. For instance, diarrhoea has been reported as number three disease burden in Nakuru County. The most common causative agents of diarrhoea are *E.coli, Salmonella typhi, Shigella and Vibrio cholera*. These bacteria are mostly water-borne pathogens. A substantial component of the population in Kenya depends on river water for domestic use and for watering animals. This river water is contaminated with effluents from sewage, agricultural runoff and effluents from hospitals. Continuous use of this untreated river water has led to increased exposure to drug resistant bacteria posing a great health concern. As the medics try to counteract this menace of drug resistance by changing the drugs that are not effective, the problem has worsened due to emergence of multidrug resistant bacteria. Despite the seriousness of this issue, information regarding the antibiotic resistance from Kenya surface waters is not readily available hence the proposal to carry out this study.

This study investigated the distribution and diversity of antibiotic resistant bacteria in polluted River Njoro based on the following objectives: (1) to measure concentrations of microbiological water quality indicators from selected sampling sites on river Njoro in Nakuru County, (2) to determine concentrations of residual antibiotics in river water and sediments from selected sites in river Njoro using high performance liquid chromatography (HPLC) methods and (3) to isolate and characterize bacteria resistant to selected antibiotics in water and sediments from selected sampling sites in River Njoro. It was expected that results would be useful for future management of wastes and protection of the River Njoro.

MATERIALS AND METHODS

Study Site

Njoro River descends from the forested Eastern Mau Escarpment (3000 m above sea level) to the valley floor emptying into Lake Nakuru (Figure 1).

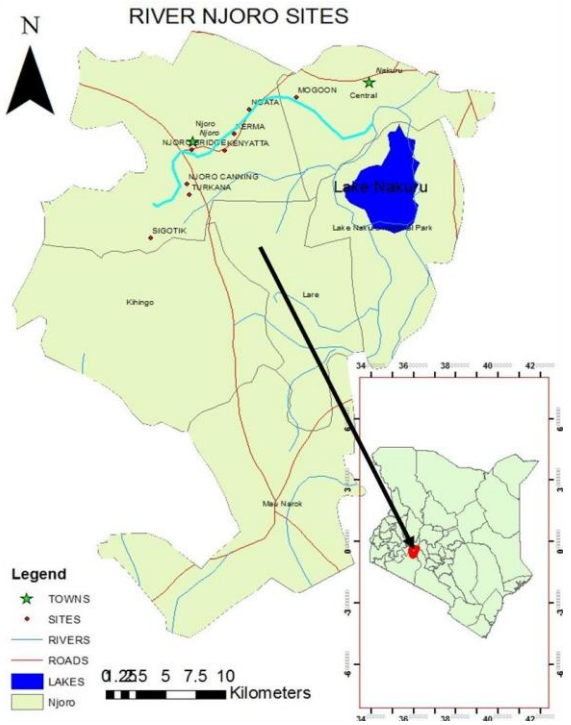

Figure 1: Geographical Location of River Njoro (Source: Koech, 2014)

The 280 km^{2}watershed consists of mixed small-scale and large commercial agriculture; mostly rain fed, and extensively grazed livestock rearing. Study sites were chosen from points and nonpoint sources of pollution from agricultural, industrial and settlements in river catchment sites. Suggested Sampling sites on Njoro River were chosen from: Sigotik which is assumed as unpolluted upstream site, Turkana cattle watering point to capture discharges from Njokerio, Njoro canning to capture effluents from the canning factory and effluents from the University, Njoro Bridge to capture effluents from Kenya Orchards canning factory, Kiptanui, Daneside and KARI farms, Kerma Watering point, Ngata to capture discharges from Njoro and Kenyatta and Mogoon to capture discharges from Technology and nearby farms. For comparison, samples were obtained from sites with little or no anthropogenic activities.

Sample Collection and Processing

Three replicates of water and sediment samples were collected at the sampling sites during both the dry season in January and wet season in November. About 10 cm sediment core was sampled using a 5 cm diameter polyvinyl chloride core at the sampling site. Water samples were collected into 500 ml sterile water sampling bottles filled to 100 ml level, placed in a cool box and taken to the laboratory for analysis within six and not more than 24 hours post-sampling.

Determination of Physical-chemical Parameters

The physical-chemical parameters measured on site on every sampling occasion were: temperature, pH, electrical conductivity (EC), total dissolved solutes (TDS), dissolved oxygen (DO) and saturation. Temperature was measured using one Wagtech International portable meter while the pH and the DO were measured using pH meter and DO meter respectively on-site. Biochemical oxygen demand was determined by incubating in the dark a sealed sample of water for five days and measuring the loss of oxygen from the beginning to the end as described by Raud *et al*. (2012).

Determination of Antibiotic Residues in Water

Residues of antibiotics commonly used in agriculture and medicine that find their way to water and sediment samples through sewage and waste water disposal were obtained by solvent extraction. Detection and quantification was done using HPLC. A reverse phase C18 column (150×4 nm) was used with methanol as the elution solvent. The flow rate was 0.5 ml/min and detection was accomplished using UV detector set at 288- 254 nm. Antibiotic standards including tetracycline, chloramphenicol, streptomycin, ampicillin and gentamycin were used as positive controls in HPLC measurements. Standards were used to ascertain accuracy.

Microbiological Water Quality Indicators

Microbiological quality assessment of water samples was carried out as described in APHA (2005). Samples (100 ml of water) or dilutions of it were filtered through Millipore membranes (45 mm diameter and 0.45 µm pore size) and membranes transferred to appropriate media for incubation. Thus, membranes for total coliforms and *E. coli* were grown on Chromacult agar (Merck) at 37^{o}C for 24 hours. The number of colonies of each type was counted and total number multiplied by dilution factor to give the number per 100 ml. Total coliforms were obtained by counting all the blue and pink colony-forming units (CFUs) and expressed per 100 ml of water sample.

Pollution with easily degradable organic matter was analysed by determining densities of heterotrophic plate counts (HPC) by the pour plate method. This was done by pour plating 1 ml of undiluted or diluted water samples with plate count agar (Merck). The total number of CFUs was counted in the dilution containing 30 to 300 CFUs per plate. Mean number of colonies counted from replicate samples were multiplied by the dilution factor to obtain number of HPCs per ml.

Isolation and Identification of Antibiotic Resistant Bacteria

Total number of antibiotic resistant bacteria

To test for total bacteria resistant to antibiotics proportion in water or sediment resistant to specific antibiotics the procedure described by McArthur and Tuckfield (2000) was used. Ten serial dilutions of sediment or water were made by suspending one gram of the first two cm sediment layer in 9 ml of 1% peptone water and vortexed gently. A 100 µl spread plated on nutrient agar containing 100 µg ml^{-1}cycloheximide and 100µg ml^{-1}of antibiotics including: tetracycline, streptomycin, chloramphenicol and ampicillin. Each sample was plated in triplicates on each antibiotic agar separately. Control plate contained only cycloheximide to control fungal growth. The proportion of total bacteria resistant to a specific antibiotic was calculated following incubation at 20^{o} C for six days in dilution containing 30 – 300 colonies per plate.

Identification of antibiotic resistant bacteria

Pure cultures of well isolated antibiotic resistant strains that looked different based on colony morphology were made from each plate and streak plated on nutrient agar amended with 100 µg –ml^{-1}of each antibiotic. Re-streaking was repeated until pure cultures were obtained. Pure cultures were stored at 4^{o}C on agar slants. For long term storage the isolates were preserved in sterile 20% glycerol in deionised water and kept at -70^{0}C.

Morphological, Cultural Identification and *biochemical characterisation*

The pure cultures were streaked on nutrient agar plates and single colonies examined for colonial characteristics (size appearance, colour, margins, elevation, texture etc.). A loopful of 24 hr old culture were gram stained and observed for cell shapes and gram reaction under oil immersion objective (X10) magnification of a bright field microscope. Gram negative isolates were confirmed by string formation visible with naked eyes on cells mixed with a drop of 3% Sodium hydroxide on a glass slide. Results for each isolate were tabulated. Standard biochemical

tests were done on each isolate as per Bergys Manual of Systematic Bacteriology (Holt *et al.*, 1994) and results recorded.

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 100 ml water samples or dilution of it. To isolate the pathogens, the filters were placed on Thio citrate bile salt (TCBS), Salmonella/Shigella agar and chromacult agar to isolate *Vibrio* and *Salmonella* spp., *Shigella* spp. and *E. coli* respectively. Sensitivity testing of antibiotics stated above was done using Clinical and Laboratory Standards Institute disk susceptibility testing method.

Statistical Analysis

Data obtained was represented as Tables or graphs in Microsoft 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 physical-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 analysis of variance (ANOVA). The bacterial species for different sites was compared by descriptive statistics.

RESULTS AND DISCUSSION

Physico-chemical characteristics influence the growth and diversity of microbial populations in aquatic environment. According to water quality guidelines for drinking water, the results indicated that the various water sources were of poor microbiological quality.

PH

This is an important factor that determines the suitability of water for various purposes, including toxicity to animals and plants. In the present study, pH was found generally alkaline in all the eight sites throughout the study. This might be due to increasing draining of domestic effluent water to the river and microbial activities. In this study the pH was ranging between 8.87±0.14 to 7.16±0.60. pH values in all the sites showed the same seasonal trend in all the sampling sites with Njoro canning generally having the lowest PH. Neutral pH is suitable for growth of bacteria such as *Caulobacter* spp, *Gallionella* spp, and *Pseudomonas* spp, which predominate in streams with low nutrient composition. However with increased pH levels there is a tendency of bacteria to die (Mwachiro, 1993).

Temperature

In polluted water, temperature can have profound effects on DO and BOD. The fluctuation in river water temperature usually depends on the season, geographic location, sampling time and temperature of effluents entering the stream (Ahipathy and Puttaiah, 2006). In this study the temperature values varied between 18.70±0.10^{o}C to a low of 14.17±0.06^{o}C. Mogoon had the highest temperature recorded throughout the sampling periods. This could greatly be contributed to the time of the day the temperature was being taken. Sigotik generally had relatively low temperature conditions observed, this could constitute an advantage for the maintenance of the quality of water due to lower microbial activity.

Conductivity

This is a measure of the ability of an aqueous solution to carry an electric current. This ability depends on the presence of ions; on their total concentration, mobility, and valence; and on the temperature of measurement. In this study the values of EC varied between of 350.67±0.58 µS cm^{-1} to a low of 126.10±0.26 µS cm^{-1}. Njoro Canning had higher values of Electrical conductance. Increasing levels of conductivity and cations are the products of decomposition and mineralization of organic materials (Abida, 2008). In all the lower values of conductivity were observed in rainy season due to dilution with rain water and highest in dry seasons owing to evaporation and reduced discharge of sewage water to the river.

Dissolved Oxygen Content (DOC)

In this study, the levels of dissolved oxygen were ranging between 8.56±0.45 mg L-1 and 6.2±0.45 mg L-1. Oxygen is the single most important gas for most aquatic organisms; free oxygen or dissolved oxygen (DO) is needed for respiration. Dissolved oxygen levels below 1 mg L-1 will not support fish; levels of 5 to 6 ppm are usually required for most of the fish population. The average value of DO levels (6.5mg/l) indicates the average quality of river water (APHA, 2005). The DO values were found highest during rainy seasons and minimum during dry seasons. This might be due to natural turbulence and high algal productivity caused by bacterial decomposition of organic matter. The low dissolved oxygen at Njoro Canning was possibly due to the higher water temperature. This is because the solubility of oxygen decreases with increasing temperature. The low dissolved oxygen can as well be attributed to the sluggish flow of the water which may caution increasing accumulation of organic load and human activities with the river system.

Biological Oxygen Demand (BOD)

This is a measure of the oxygen in the water that is required by the aerobic organisms. The biodegradation of organic materials exerts oxygen tension in the water and increases the biochemical oxygen demand (Abida, 2008). The BOD in this study was ranging between 6.99±0.20 mg L^{-1} to a low of 1.28±0.13. Generally throughout the study Njoro canning had the highest BOD values and the lowest BOD values were observed at Sigotik. Rivers with low BOD have low nutrient levels; therefore, much of the oxygen remains in the water. Unpolluted, natural waters will have a BOD of 5 mg/l or less because BOD directly affects the amount of dissolved oxygen in rivers and streams. The greater the BOD, the more rapidly oxygen is depleted in the stream. This means less oxygen is available to higher forms of aquatic life. Sources of BOD include leaves and woody debris, dead plants and animals, animal manure, effluents from pulp and paper mills, wastewater treatment plants, feedlots and food-processing plants, failing septic systems and urban storm water runoff (USEPA, 1997).

Microbiological Water Quality Indicators

According to water quality guidelines for drinking water, the results indicated that the various water sources were of poor microbiological quality. The lowest level of faecal coliforms recorded in both seasons was 3.13 x 10^4 cfu·ml^{-1}. However, according to Department of Water Affairs and Forestry (DWAF) (1996) the maximum limit for no risk of faecal coliforms is 0 cfu·100 ml^{-1}. The lowest total coliform recorded throughout the sampling seasons was 6.20 x 10^4 cfu·ml^{-1} .The counts exceeded the 5 cfu·100 ml^{-1}, which is the maximum recommended limit for no risk (DWAF, 1996; Water Research Commission [WRC], 1998). Both total and faecal coliforms in this investigation exhibits more counts during the dry season than in the rainy season. This might be due to discharging of domestic wastes containing faecal matters to the river body and open defecation along the sides of river bank during the dry season. The low counts during rainy season might be due to cold climatic condition, which is not supportive for bacterial duplication in a greater extent or due to dilution effects due to increased water volume in the river. So in all the stations Total and Faecal coliforms counts of river water are beyond the permissible limit and were not suitable for drinking purpose without pre-treatment. The maximum allowable limit for no risk in terms of heterotrophic bacterial count is 1.0 x 10^2 cfu·ml^{-1} (WRC, 1998). However in this study, the lowest HPC were observed at Sigotik which recorded a count of 4.33 x 104 cfu·ml^{-1} which was way above the recommended amount to render the water safe for drinking.

Pharmaceuticals in the Environment

Distribution of the antibiotics in water detected along river Njoro is presented in Figure 2. In this study, out of the four test antibiotics two of them were detected in significant amounts. These are ampicillin and chloramphenical. Ampicillin was detected in the range of 0.04- 0.06 mg/ L and chloramphenical was detected in the range of 0.01-0.10 mg/L. In the United States, for example, the expected environmental concentration (more commonly termed the predicted environmental concentration; PEC) is used differently to trigger ecological effects testing for human drugs versus those for livestock. A PEC for human pharmaceuticals of >0.1 μg/L necessitates aquatic ecotoxicity testing, whereas a lower concentration results in a categorical exclusion from testing. In the case of veterinary drugs, only aquaculture-related medicines are subject to aquatic testing if the water PEC is >1 μg/. Therefore the concentrations observed along River Njoro were higher than the recommended amounts of antibiotics in aquatic environment raising a great concern. A final concern regards the utilization of prophylactic antibiotics in aquaculture. The heavy use of these compounds, several of which are non-biodegradable increases antibiotic selective pressure in water, facilitating the transfer of antibiotic resistance determinants between aquatic bacteria, including fish and human pathogens and allows the presence of residual antibiotics in commercialized fish and shellfish products (Alonso *et al.*, 2001).

Antibiotic-resistant organisms from humans and animals are released into the sewage by contaminated sites (including urine), faeces, eventually corpses and manure. There were high numbers of antibiotic resistant organisms in all the study sites along River Njoro even in the reference point (Sigotik) (Table 3). The antibiotic resistant isolates were in the range of 3.50×10^4 – 8.2×10^4 for tetracycline and for Streptomycin they ranged from 3.6×10^4 – 1.30×10^5, for chloramphenical the resistant isolates were in the range of 3.10×10^4 – 6.83×10^4 and finally for Ampicillin the range of resistant isolates were ranging between 3.0×104 to 1.79×10^5. Generally there were more resistant strains in the dry season as opposed to the rainy season and this could be attributed to low temperatures which do not support proliferation of bacteria. Ampicillin and streptomycin had the highest number resistant of strains.

Table 1: Turkey's test showing temporal variations between means of physico-chemical parameters

Site	Time (months)	N	Water quality parameter					
			DO (mgl^{-1})	pH	BOD ($mg\ l^{-1}$)	Temp (°C)	% Sat	Cond (μscm^{-2})
Sigotik	November 2014[1]		7.79a	8.59a	1.28a	14.97a	103.63a	106.17a
	January 2015[2]	3	8.05a	8.76a	4.02a	12.26b	98.73b	156.3b
	February[2]	3	7.7a	8.27b	5.21a	14.16c	98.66bc	206.8c
	March[1]	3	6.41b	7.16c	1.3a	15.86d	100.36d	260d
	April[1]	3	7.53a	7.35cd	0.98a	15.46e	99.46bce	99.86e
Turkana	November 2014[1]	3	7.55a	8.17a	2.1a	16.2a	100.93a	145.33a
	January 2015[2]	3	7.01a	8.77a	3.89a	16.43a	93.2b	243b
	February[2]	3	7.48a	8.5a	6.21a	16.16a	100.23a	319c
	March[1]	3	7.17a	8.08a	1.58a	16.63a	99.1a	300d
	April[1]		7.38a	7.64b	1.86a	16.46a	97.7ab	148.3a
Canning	November 2014[1]	3	7.36a	8.14a	6.99a	16.1a	98.1a	160.03a
	January 2015[2]	3	6.38b	8.26a	5.8a	17.3a	86.36b	261.33b
	February[2]	3	4.71c	8.13a	0.13a	15.53a	58.36c	365c
	March[1]	3	6.27b	8.1a	5.74b	16.96a	97.53a	400d
	April[1]	3	7.55a	7.55b	6.58a	16.4a	99.00a	150.53a
Njoro	November 2014[1]	3	7.45a	8.17a	1.95a	15.87a	98.37a	150.37a
Bridge	January 2015[2]	3	4.57a	8.23a	2.59a	17.1a	88.5b	279.00b
	February[2]	3	7.78a	8a	4.35a	15.46b	101.46a	347.66c
	March[1]	3	6.68a	7.36a	2.73a	16.3a	95.46c	350.00d
	April[1]	3	7.36a	7.53a	3.02a	16.76a	96.8a	152.23e
Kenyatta	November 2014[1]	3	7.33a	8.35a	2.2a	16.63a	97.9a	154.7a
	January 2015[2]	3	7.06a	8.45a	4.57abc	17.9a	272.66b	96.4b
	February[2]	3	8.03b	8.12b	4.33abc	18.1a	110.76c	350.66c
	March[1]	3	7.58ac	8.00c	1.92b	18.66b	105.66d	400.00d
	April[1]	3	7.26a	7.7d	1.69c	17.6a	97.43ab	152.4e
Kerma	November 2014[1]	3	7.35a	8.25a	1.81a	17.5a	99.53a	154.93a
	January 2015[2]	3	6.83a	8.62b	3.64a	19.7b	96.06a	269ab
	February[2]	3	8.04b	8.04c	4.67a	18.27c	110.8b	332.66c
	March[1]	3	8.09b	8.22ac	2.52a	18.73d	111.7b	300d
	April[1]	3	7.35a	7.6d	2.29a	17.7e	98.3a	151.4e
Ngata	November 2014[1]	3	7.59a	8.15a	1.66a	16.67a	100.4abc	153.4a
	January 2015[2]	3	6.72a	8.53b	3.45a	20.00b	94.73ac	267.33b
	February[2]	3	7.29a	8.1a	3.97a	17.30ab	97.83ac	343c
	March[1]		7.55a	8.2a	0.87a	18.00ab	106.13b	320d
	April[1]	3	7.4a	7.55c	1.09a	17.93ab	98.96c	150.9e
Mogoon	November 2014[1]	3	7.76ab	8.63a	1.76a	17.07a	101.57ab	150.67a
	January 2015[2]	3	7.2ab	8.58a	3.28a	19.83a	98.96a	259.00b
	February[2]	3	7.91a	8.19b	4.58b	17.9a	105.36b	342.66c
	March[1]	3	6.9b	8.35c	0.7c	18.7a	99.66ab	320.00d
	April[1]	3	7.54ab	7.64d	1.22a	18.23a	99.23a	151.80e

Median values with different letters in the same column are significantly different at $P < 0.05$.Superscript 1 and 2 indicates rainy and dry month respectively.

Although only ampicillin and chloramphenical was found in the water, resistance to these antibiotics could have been acquired through other means besides selective pressure by the antibiotics. Indeed, faecal antibiotic-resistant bacteria selected in human or animal intestines under antibiotic treatment (Salyers *et al.*, 2004) may enter the water environment mainly from treated effluents of wastewater treatment plants (WWTP) (Reinthaler *et al.*, 2003; Webster *et al.*, 2004) field runoffs (Peak *et al.*, 2007) and direct discharge of untreated wastewater. These faecal bacteria might then be able to transmit antibiotic resistance to autochthonous bacteria through lateral transfer when the resistance genes are carried by transferable and/or mobile genetic elements, principally conjugative plasmids and transposons (Van Elsas and Bailey, 2002; Schlüter *et al.*, 2007). In addition, some authors have

reported indirect evidence of the transfer of antibiotic-resistance genes in aquatic habitats (Goni-Urriza *et al.*, 2000; Séveno *et al.*, 2002; Tennstedt *et al.*, 2003). This circulation of resistance genes constitutes a latent hazard for human health. Turkana and Njoro canning had the highest antibiotic resistant bacteria. This is due the high rate of pollution in these sites as evidenced by physico-chemical parameters and microbiological quality indicators.

Table 2: Turkey's test showing temporal variations between means of microbiological indicators

Site	Time (months)	N	*E. coli*	Total coliforms
Sigotik	November 2014[1]	3	4.13×10^4a	7.83×10^4a
	January 2015[2]	3	3.13×10^4a	7.20×10^4ab
	February[2]	3	3.16×10^4a	6.20×10^4b
	March[1]	3	4.03×10^4a	7.63×10^5ab
	April[1]	3	3.16×10^4a	6.26×10^4b
Turkana	November 2014[1]	3	4.46×10^4a	1.09×10^4a
	January 2015[2]	3	3.60×10^4a	8.36×10^4a
	February[2]	3	4.83×10^4a	1.14×10^4a
	March[1]	3	5.36×10^4a	1.37×10^5a
	April[1]	3	6.16×10^4a	9.43×105a
Canning	November 2014[1]	3	4.90×10^4a	1.06×10^4a
	January 2015[2]	3	3.40×10^4a	1.11×10^4a
	February[2]	3	8.06×10^4a	2.74×10^4a
	March[1]	3	1.09×10^4b	3.5×10^5b
	April[1]	3	3.33×10^4a	7.50×10^5a
Njoro Bridge	February[2]	3	3.66×10^4a	1.20×10^4a
	March[1]	3	4.90×10^4b	1.20×10^5a
	April[1]	3	3.36×10^4c	1.05×10^4a
Kenyatta	February[2]	3	3.43×10^4a	1.12×10^5a
	March[1]	3	4.30×10^4a	7.73×10^4ab
	April[1]	3	4.36×10^4a	1.32×10^4ac
Kerma	February[2]	3	3.33×10^4a	1.06×10^4a
	March[1]	3	3.96×10^4a	107×10^5b
	April[1]	3	3.56×10^4b	1.28×10^5c
Ngata	November 2014[1]	3	5.93×10^4ac	996×10^4a
	January 2015[2]	3	3.13×10^4b	7.70×10^4a
	February[2]	3	4.73×10^4c	1.40×10^4b
	March[1]		4.13×10^4bc	9.70×10^4a
	April[1]	3	4.10×10^4bc	1.91×10^5c
Mogoon	November 2014[1]	3	5.93×10^4a	2.11×10^3a
	January 2015[2]	3	3.33×10^4a	6.86×10^4a
	February[2]	3	3.86×10^4a	7.13×10^4a
	March[1]	3	5.66×10^4a	1.14×10^5a
	April[1]	3	3.63×10^4b	1.03×10^4b

Median values with different letters in the same column are significantly difference at $P < 0.05$. Pearson's correlations showed that there was a positive correlation between the *E. coli* (faecal coliform) and total coliforms ($r = 0.565$, $P < 0.05$).

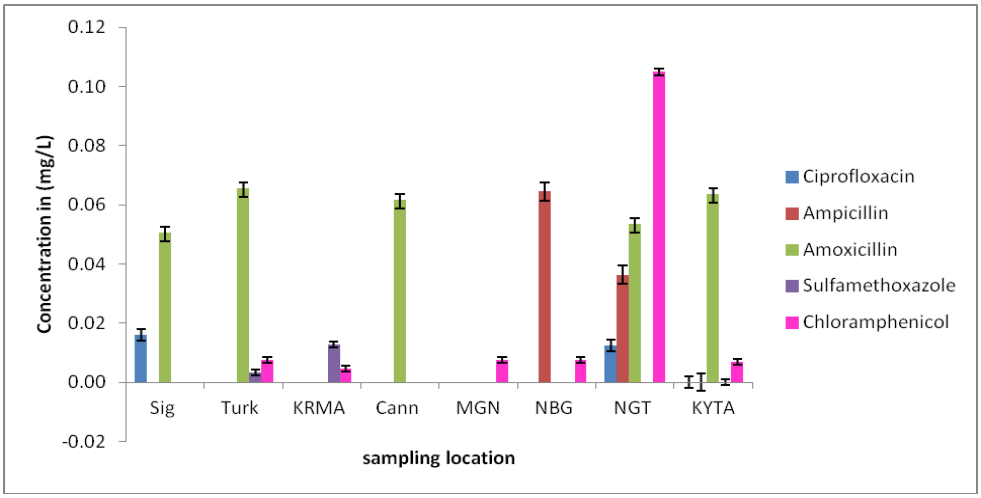

Figure 2: Concentration of antibiotics in water from sites on Njoro River. Note: Sig-Sigotik, Turk-Turkana, KRMA-Kerma, Cann-Canning, MGN-Mogoon, NBG-Njoro Bridge, NGT-Ngata, KYTA-Kenyatta.

Table 3: Range of Total Antibiotic Resistant Bacteria

Season	Parameter	CFUs per gm^{-1} Wet Sediment	
	Antibiotic	Site with highest count	Site with lowest count
Season1	Tetracycline	Turkana $5.56\times10^4\pm3.8\times10^3$	Sigotik: $3.5\times10^4\pm3.4\times10^2$
	Streptomycin	Turkana $1.3\times10^5\pm1.4\times10^3$	Sigotik: $6.23\times10^4\pm3.0\times10^4$
	Chloramphenical	Turkana : $6.6\times10^4\pm5.4\times10^2$	Sigotik: $3.1\times10^4\pm1.0\times10^2$
	Ampicillin	Njoro canning :$1.79\times10^4\pm1.1\times10^3$	Mogoon: $4.9\times10^4\pm8.1\times10^3$
	Control	Njoro canning : $2.7\times10^5\pm4.9\times10^3$	Ngata: $5.5\times10^5\pm1.8\times10^3$
Season2	Tetracycline	Njoro canning $8.2\times10^4\pm5.0\times10^2$	Ngata: $3.53\times10^4\pm4.1\times10^2$
	Streptomycin	Njoro canning $5.66\times10^4\pm8.1\times10^2$	Sigotik: $3.66\times10^4\pm7.6\times10^3$
	Chloramphenical	Turkana : $6.83\times10^4\pm3.1\times10^3$	Mogoon:$4.3\times10^4\pm1.5\times10^2$
	Ampicillin	Turkana: $7.06\times10^4\pm3.23\times10^3$.	Sigotik: $3.0\times10^4\pm1.7\times10^3$
	Control	Turkana : $5.1\times10^5\pm1.3\times10^3$	Ngata: $1.2\times10^5\pm2.4\times10^3$

The antibiotic resistant organisms were identified using biochemical tests and the following are bacteria isolates identified. *E. coli* was identified and further tests were performed to determine its pathotype. Most of the *E. coli* isolated were non-pathogenic while a few strains were entero-aggregative *E. coli* (EAEC), entero -pathogenic *E. coli* (EPEC) and entero- toxigenic *E. coli* (ETEC). *Klebsiella* species were also isolated and these were *K. oxytoca* and *K. pneumonia*. The *Enterobac*ter species isolated were *E. aerogenes* and *E. cloacae* and *E. amnigenus*. Two *pseudomonas* species were also isolated and these were *P. Aeroginosa* and *P. putida*. *Aeromonas* species isolated were *A. hydrophila* and *A. sobria*. *Yersinia enterocolitica* and *Citrobacter frendii* were also isolated.

In this study we were able to isolate pathogenic bacteria that cause dysentery and diarrheal infections. These are *E.coli*, S*almonella* and *Shigella* which were isolated in all the five sites. In the rainy season Ngata had the highest number of pathogens isolated whereas Mogoon had the lowest number of pathogens isolated. In the dry season, however, Turkana and Njoro canning had the highest number of pathogens isolated where as Sigotik had the lowest number of pathogens isolated. On the other hand *Vibrio* species were not isolated in all the sites. During the rainy season, only Ngata and Mogoon had *Vibrio* species isolated. During the dry season however, Turkana, Njoro canning and Mogoon had *Vibrio* species isolated. Table 3 gives the site to site variation of the number of pathogens isolated.

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 prescription programs making policy decisions (Omigie *et al.*, 2006). 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. Susceptibility testing in this study showed that most of these organisms were resistant to more than two antibiotics. Seventy four isolates were tested for resistance to the four antibiotics using CLSI disk diffusion methods. The percentage of the resistant strains was then calculated and 54% of the strains were resistant to tetracycline in the rainy season whereas 40% of the strains were resistant to tetracycline in the dry season. During the rainy season, 71% of the strains were resistant to streptomycin whereas only 33% of the strains showed resistance to streptomycin in the dry season. About 43% of the pathogens were resistant to chloramphenical in the rainy season compared to 27%. Finally, 81% of the pathogens were resistant to ampicillin during the rainy season and 48% of the pathogens were resistant to ampicillin during the dry season. Generally we came to the conclusion that during the rainy season there were more resistant strains isolated as compared to the dry season. Generally, ampicillin had the highest resistance whereas chloramphenical was the most susceptible drug in this study. Worth noting is the fact that the pathogens isolated during the rainy season were more resistant than those isolated during the dry season. 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.

CONCLUSION

This study confirmed the role of River Njoro as a reservoir of antibiotic resistance bacteria which can disseminate antibiotic resistance genes to other human pathogens and so constitute a problem for human health. Therefore, it will be vital for public health workers to create awareness for the need to observe good health practices such as boiling drinking water or seeking alternative sources of drinking water in the study area.

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REFERENCES

Abida, B. 2008. Study on the quality of water in some streams of Cauvery River. *Journal of chemistry*, *5*(2), 377-384

Ahipathy, M.V. and Puttaiah, E.T. 2006. Ecological characteristics of VrishabhavathyRiver in Bangalore (India). *Environmental geology*, *4 9*(8), 1217-1222.

Alonso, A., Sanchez, P. and Martinez, J.L. 2001. Environmental selection of antibiotic resistance genes. *Environmental Microbiology*, *3* (1), 1-9.

APHA, A. 2005. WEF, 2005. *Standard methods for the examination of water and wastewater*, *21*, 258-259.

Babic, M., Hujer, A.M. and Bonomo, R.A. 2006. What's new in antibiotic resistance? Focus on beta-lactamases. *Drug Resistance Updates*, *9* (3), 142-156.

Boxall, A., Rudd, M.A., Brooks, B.W., Caldwell, D.J., Choi, K., Hickmann, S. And Van Der Kraak, G. 2012. Pharmaceuticals and personal care products in the environment: what are the big questions? *Environmental Health Perspectives*, *120* (9), 1221-1229.

Dahbi, G., Mora, A., López, C., Alonso, M.P., Mamani, R., Marzoa, J. and Blanco, J. 2013. Emergence of new variants of ST131 clonal group among extraintestinal pathogenic *Escherichia coli* producing extended-spectrum β-lactamases. *International Journal of Antimicrobial Agents*, *42*(4), 347-351.

Department of Water Affairs and Forestry (DWAF). 1996. South African Water Quality Guideline for Domestic Use (2nd edn.), Pretoria

Ellis, R.H., Hong, T.D. and Roberts, E.H. 1991. Effect of storage temperature and moisture on the germination of papaya seeds. *Seed Science Research*,*1* (01), 69-72.

Goñi-Urriza, M., Capdepuy, M., Arpin, C., Raymond, N., Caumette, P. and Quentin, C. 2000. Impact of an Urban Effluent on Antibiotic Resistance of Riverine Enterobacteriaceae and Aeromonas spp. *Applied and Environmental Microbiology*, *66* (1), 125-132.

Holt, J.G., Krieg, N.R., Sneath, P.H., Staley, J.T. and Williams, S.T. 1994. Bergy's Manual of determinate bacteriology.

Idia, U. P., Omigie, O., Tatfeng, Y., Omorogbe, F.I., Aisabokhale, F. and Ugbodagah, O.P. 2006. Antimicrobial susceptibility and plasmid profiles of *Escherichia coli* isolates obtained from different human clinical specimens in Lagos–Nigeria. *J Am Sci*, *2*, 70-76.

Jury, L.A., Jump, R.L., Olds, D.M., Seifi, N., Kypriotakis, G., Peron, E. P. and Donskey, C.J. 2012. Effective antimicrobial stewardship in a long-term care facility through an infectious disease consultation service: keeping a LID on antibiotic use. *Infection Control and Hospital Epidemiology*, *33*(12), 1185-1192.

Levy, S.B. and Marshall, B. 2004. Antibacterial resistance worldwide: causes, challenges and responses. *Nature medicine*, 10, S122-S129.

Lindberg, R., Jarnheimer, P.A., Olsen, B., Johansson, M. and Tysklind, M. 2004.Determination of antibiotic substances in hospital sewage water using solid phase extraction and liquid chromatography/mass spectrometry and group analogue internal standards. *Chemosphere*, *57*(10), 1479-1488.

Lippmann, M.O.R.T.O.N. (1998). The 1997 USEPA standards for particulate matter and ozone. *Issues in environmental science and technology*, *10*, 75-100.

McArther, J.V. and Tuckfield, R.C. 2000. Spatial patterns in antibiotic resistance among stream bacteria: effects of industrial pollution. *Applied and Environmental Microbiology*, 66 (9), 3722-3726.

Mokaya, S.K., Mathooko, J.M. and Leichtfried, M. 2004. Influence of anthropogenic activities on water quality of a tropical stream ecosystem. *African Journal of Ecology*, *42*(4), 281-288.

Mwachiro, E.C., Makilla, D., Bett, D.K. and Ndeje, G.K. 2012. A Comparative Study of Cage and Earthern Pond culture of Oreochromis Jipe, in Lake Jipe, Taita Taveta District Kenya.

Ochieng, O.B., Chenje, M.E., and Mulwa, F.B. 2015. Distribution and reproductive patterns of the Epinephelus genus groupers off Kenyan South coast marine waters. *Journal of Fisheries and Aquatic Science*, *10* (3), 159.

Okuda, T., Yamashita, N., Tanaka, H., Matsukawa, H. and Tanabe, K. 2009. Development of extraction method of pharmaceuticals and their occurrences found in Japanese wastewater treatment plants. *Environment international*, *35* (5), 815-820.

Omigie, O., Tatfeng, Y., Omorogbe, F.I., Aisabokhale, F. and Ugbodagah, O.P. 2006. Antimicrobial susceptibility and plasmid profiles of *Escherichia coli* isolates obtained from different human clinical specimens in Lagos–Nigeria. *J Am Sci*, *2*, 70-76.

Peak, N., Knapp, C.W., Yang, R.K., Hanfelt, M.M., Smith, M. S., Aga, D. S. and Graham, D.W. 2007. Abundance of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different antibiotic use strategies. *Environmental Microbiology*, *9* (1), 143-151.

Raud, M., Tutt, M., Jõgi, E. and Kikas, T. 2012. BOD biosensors for pulp and paper industry wastewater analysis. *Environmental Science and Pollution Research*, *19* (7), 3039-3045.

Reinthaler, F.F Galler, H., Feierl, G., Petternel, C., Haas, D., Grisold, A.J. andZarfel, G. 2014. KPC-2 and OXA-48 carbapenemase-harbouring *Enterobacteriaceae* detected in an Austrian wastewater treatment plant. *Clinical Microbiology and Infection*, *20*(2), 132-134.

Rowe-Magnus, D.A. and Mazel, D. 2002.The role ofintegrons in antibiotic resistance gene capture. *International Journal of Medical Microbiology*, *292* (2), 115-125.

Salyers, A.A., Gupta, A. and Wang, Y. 2004. Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends in microbiology*, *12*(9), 412-416.

Schlüter, A., Szczepanowski, R., Pühler, A. and Top, E.M. 2007. Genomics of IncP-1 antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool. *FEMS microbiology reviews*, *31* (4), 449-477.

Séveno, N.A., Kallifidas, D., Smalla, K., van Elsas, J.D., Collard, J.M., Karagouni, A.D. and Wellington, E.M. 2002. Occurrence and reservoirs of antibiotic resistance genes in the environment. *Reviews in medical microbiology*, *13* (1), 15-27.

Siegel, R.E. 2008. Emerging gram-negative antibiotic resistance: daunting challenges, declining sensitivities, and dire consequences. *Respiratory Care*, *53* (4), 471-479.

Smith, A.J., Balaam, J.L., and Ward, A. 2007.The development of a rapid screening technique to measure antibiotic activity in effluents and surface water samples. *Marine Pollution Bulletin*, *54* (12), 1940-1946.

Suvarna, A.C., and Somashekar, R.K. 1997. Evaluation of water-quality index of river cauvery and its tributaries. *Current Science*, *72* (9), 640-646.

Tennstedt, T., Szczepanowski, R., Braun, S., Pühler, A. and Schlüter, A. 2003. Occurrence of integron-associated resistance gene cassettes located on antibiotic resistance plasmids isolated from a wastewater treatment plant. *FEMS Microbiology Ecology, 45(3)*, 239-252.

USEPA. 1997. The 1997 US EPA standards for particulate matter and ozone. *Issues in environmental science and technology*, *10*, 75-100.

Van Elsas, J.D. and Bailey, M.J. 2002. The ecology of transfer of mobile genetic elements. *FEMS microbiology ecology*, *42* (2), 187-197.

Van Elsas, J.D., Turner, S., and Bailey, M.J. 2003. Horizontal gene transfer in the phytosphere. *New Phytologist*, *157*(3), 525-537.

Water Research Commission (WRC). 1998. Quality of Domestic Water Supplies. Vol. 1- Assessment

Guide. WRC Report No TT101/ 98. Pretoria, South Africa.

Webster, L.F., Thompson, B.C., Fulton, M.H., Chestnut, D.E., Van Dolah, R.F., Leight, A. K. and Scott, G.I. 2004. Identification of sources of Escherichia coli in South Carolina estuaries using antibiotic resistance analysis. *Journal of Experimental Marine Biology and Ecology*, *298* (2), 179-195.

Yillia, P.T., Kreuzinger, N. and Mathooko, J.M. 2008. The effect of in-stream activities on the Njoro River, Kenya. Part II: Microbial water quality. *Physics and Chemistry of the Earth, Parts A/B/C*, *33* (8) 729-737.