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DISTRIBUTION AND DIVERSITY OF ANTIBIOTIC RESISTANT BACTERIA IN SELECTED AGRO-INDUSTRIAL POLLUTION IN NJORO RIVER, NAKURU, KENYA

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ABSTRACT
Many in-stream activities occur in River Njoro. Consequently, many pharmaceuticals used in farms and hospitals and antibiotic-resistant microbes end up in the River through runoff and sewage. Presence of antibiotic resistance exposes humans and animals to contamination during in-stream activities. This study determined bacteria resistant to both medical and veterinary antibiotics in the catchment. Residual antibiotics and physical chemical conditions and indicators of feacal pollution were investigated. The bacteria resistant to five antibiotics studied varied significantly (P<0.05). Turkana site had highest resistors to ampicillin, tetracycline and streptomycin, while Njoro Canning Factory had highest resistors to gentamycin and Chloramphenical. Indicators of faecal pollution were found in all sites including Sigotik with 413.33±15.28 *E.coli* per 100 ml of water. Physical chemical measurements showed site differences. The Njoro Canning Factory BOD was 6.99±0.20 mg L^{-1}, whereas Sigotik BOD was 1.28±0.13 mg L^{-1}. Presumptive positive *Salmonella*, *V. cholera* and *V. parahaemolyticus* species were found in Turkana and Ngata sites. There is cause for alarm due to the high numbers of antibiotic resistant bacteria in River Njoro. Proper treatment of the River water before use is recommended, or alternative safe water sources for these communities should be found.
Keywords: Physiochemical parameters, Microbiological indicators

INTRODUCTION
Drug resistant bacteria are resistant to varieties of classes of antibiotics like β- lactams, macrolides, fluoroquinolones (quinolones) and tetracycline (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 UTI. Chloramphenicol (CHL) 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 these 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 in 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. For example, methicillin resistant *Staphylococcus aureus* (MRSA) that causes skin and wound infections is resistant to most antibiotics including β-lactams (ampicillin, methicillin, oxacillin, cephalosporin, carbapenems etc.). Methicillin resistant *Staphylococcus aureus* is difficult to treat with conventional antibiotics for *Staphylococci* (Goldstein et al., 2012). Another example is vancomycin resistant *Enterococci* (VRE) that causes urinary tract infections (UTI), bacteraemia, and meningitis. Vancomycin resistant *Enterococci* isolates are resistant to vancomycin, the drug of choice for the treatment of Gram positive infections. Another pathogenic group of big concern is extended spectrum beta lactamase (ESBL) isolates including members from *Enterobacteriaceae*, and *E. coli* (Dahbet al., 2013).MDR bacteria are an increasing public health problem and few therapeutic options are available to treat these infections. The increasing incidence of MDR presence 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 (Reinthaleret 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. Many researchers have shown the incomplete removal of pharmaceuticals during wastewater treatment processes. 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 the most of these substances end up in manure. The manure and slurry (urine and faeces) are either stored or directly applied to the farms. Drugs may persist in solid environmental matrices for a long time. The persistence depends on their photo stability, binding and adsorption capacity, degradation rate and leaching into the water. Strongly absorbing pharmaceuticals tend to accumulate in soils or sediment and by contrast, highly mobile pharmaceuticals have a potential to resist degradation and tend to leach into the groundwater and to be transported with the groundwater, drainage water and surface water run-off to surface waters (Babicet al., 2006).

Faecal antibiotic-resistant bacteria, secreted in human or animal intestines under antibiotic treatment (Salyerset al., 2004), may enter the water environment mainly from treated effluents of wastewater treatment plants (WWTP) (Reinthaleret 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 and Bailey, 2003).This circulation of resistance genes constitutes a latent hazard for human health. 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 (Rowe-Magnus and Mazel, 2002).

Mechanisms for horizontal transfer of antibiotic resistance genes have been reported in the environment. These include conjugation, transduction and transformation. There is need to investigate various environments to establish for the presence of these genes and come up with mitigation measures to control

and manage such pollution in the environment. Emerging trends in the increase of resistant microbes in water is a challenge in disease control. 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. Data obtained will be useful for future management of wastes and protection of our surface waters.

The aim of the study is to investigate the distribution and diversity of antibiotic resistant bacteria in agro-industrial polluted river Njoro, (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 HPLC methods. (3)To isolate and characterize bacteria resistant to selected antibiotics in water and sediments from selected sampling sites in 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). The 280 km^2watershed consists of mixed small-scale and large commercial agriculture; mostly rain fed, and extensively grazed livestock rearing. The rapidly growing Egerton University community and neighbouring town of Njoro, with associated commercial and agro-industrial areas, are situated along the river's middle section. Dense urban slums of Nakuru Municipality, fourth largest city in Kenya, and peri-urban settlements border the lowest reaches. The watershed population, estimated at 300,000, has grown and continues to grow rapidly. Annual runoff for the wet upper portion (116 Km^2) has varied greatly around its mean of 147 mm/year with a recent low of 25 mm in 1984 (Chemilil, 1995). Faecal pollution point sources, in the middle and lower reaches, include poorly treated sewage discharges, and wastewater from a cannery, small dairies, and several small slaughterhouses. Numerous non-point sources include a wide variety of direct in-river and diffuse land-based livestock and human sources throughout the watershed and the possibility of wildlife sources in the less inhabited extreme upper reaches. Lack of improved water supplies in the Njoro watershed means the majority of residents and their animals have no option but to use river water for daily needs (SUMAWA, 2005). Smallholder cattle, many dairy, make the daily round-trip journey to water at over 38 main public and many other small watering points along the river and stream network, shared with households and water vendors fetching domestic supplies. Cattle and water transporting donkeys are frequently observed defecating in the river and grazing in riparian areas along its length. Growth in smallholder households in the middle and upper Njoro watershed has brought a rapid livestock expansion in the last 10–15 years, raising environmental concerns (SUMAWA, 2005).

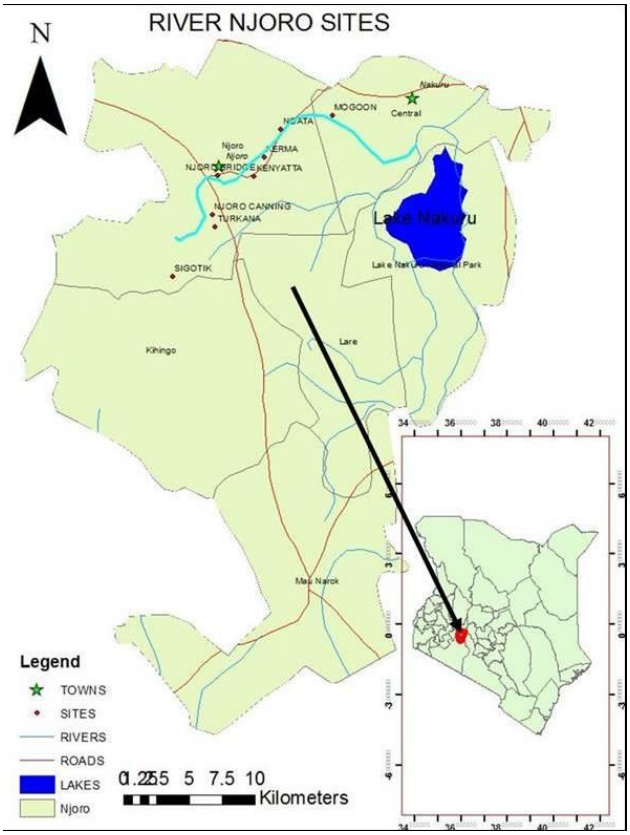

Figure 1: Geographical Location of River Njoro(**Source:** Koech, 2014)

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 area, 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 areas, Mogoon to Capture discharges from Technology and nearby farms. For comparison purposes 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 and wet seasons. About 10cm sediment core was sampled using a 5cm diameter PVC core at the sampling site. 100 ml of water samples was collected using 500ml sterile water sampling bottles, placed in a cool box and taken to the laboratory for analysis within 6 and not more than 24 hours of sampling.

Determination of Physiochemical Parameters

The following physio-chemical parameters were measured on site on every sampling occasion; temperature, pH, electrical conductivity (EC), total dissolved solutes (TDS), dissolved oxygen (DO) and saturation were measured using portable meters. The temperature and PH was measured using one Wagtech International portable meter PH and the DO of the sampled water was measured by use of PH meter and DO meter respectively and was done 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 was obtained by solvent extraction. Detection and quantification was done using high performance liquid chromatography. A reverse phase C18 column (150×4nm) was used with methanol as the elution solvent. The flow rate was set at 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 was used as positive controls in HPLC measurements.

Microbiological Water Quality Indicators

Microbiological quality assessment of water samples was carried out as described in APHA (2005). Samples (100ml 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 and incubation conditions for faecal pollution indicators. Thus, membranes for total coliforms and *E. coli* were grown on Chromacult agar (Merck) at 37^{o}Cfor 24 hours. *E.coli* CFUs appeared blue in this medium while other coliforms appear pink. The number of colonies of each type was counted and total number multiplied by dilution factor to give the number per 100ml. Total coliforms were obtained by counting all the blue and pink 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 300CFUs 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 I gm of the first 2 cm sediment layer in 9ml of 1% peptone water, vortexed gently and 100 µl spread plated on nutrient agar containing 100µg 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 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^{o}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 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% KOH 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 TCBS, Salmonella/Shigella Agar and chromacult agar 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.

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 physio- 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 bacterial species for different sites was compared by descriptive statistics.

RESULTS AND DISCUSSION

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

PH 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 of water may not be as important in pure water because of the wide range of temperature tolerance in aquatic life, but in polluted water, temperature can have profound effects on dissolved oxygen (DO) and biological oxygen demand (BOD).The fluctuation in river water temperature usually depends on the season, geographic location, sampling time and temperature of effluents entering the stream (Ahipathy, 2006). In this present 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 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 the present 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 is one of the most important factors in stream health. Its deficiency directly affects the ecosystem of a river due to bioaccumulation and biomagnifications. The oxygen content in water

samples depends on a number of physical, chemical, biological and microbiological processes. DO values also show lateral, spatial and seasonal changes depending on industrial, human and thermalactivity. 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 (O2) or DO is needed for respiration. DO levels below 1 ppm 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). DO values were found highest during rainy seasons and minimum during dry seasons, which might be due to natural turbulence and higher algal productivity produces O_2 by photosynthesis in rainy period and active utilization in bacterial decomposition of organic matter. The low dissolved oxygen at Njoro Canning was possibly due to the higher water temperature. The solubility of oxygen decreases with increasing temperature (Ellis, 1989). 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 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). 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. 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).

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 3.13×10^4 cfu·ml^{-1}. However, according to DWAF (1998) 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 6.20×10^4 cfu·ml^{-1} .The counts exceeded the 5 cfu·100 ml^{-1}, which is the maximum recommended limit for no risk (DWAF, 1996: 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 countsof river water are beyond the permissible limit and was not suitable for drinking purpose without pretreatment. The maximum allowable limit for no risk in terms of heterotrophic bacterial count is 1.0×10^2 cfu·ml^{-1} (DWAF, 1996, 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 are introduced in the environment from human and veterinary applications at volumes comparable with total pesticide loadings (Brain et al, 2006). Antibiotic resistance is not the only possible adverse effect of antibiotic release in water environments, and ecotoxicity tests are starting to be introduced to document these effects (Yamashita et al, 2006). Distribution of the antibiotics in water detected along river Njoro are 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 U.S., 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/, (VICH, 2004). 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).

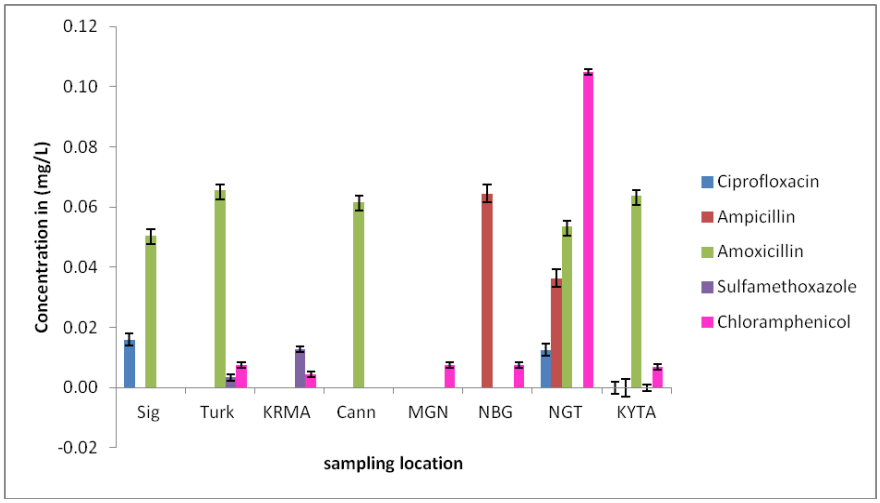

Figure 2: A graph of concentration of antibiotics from sites on Njoro River
Note: Sig-Sigotik, Turk-Turkana, KRMA-Kerma, Cann-Canning, MGN-Mogoon, NBG-Njoro Bridge, NGT-Ngata, KYTA-Kenyatta.

Antibiotic-resistant organisms from humans and animals are released into the sewage by contaminated sites (including urine), faeces, eventually corpses and manure. In particular, wastewater from hospitals and intensive farming facilities (under concentrated animal feeding operations) is probably a major source of pathogenic and antibiotic-resistant organisms and antibiotic-resistance genes that are released into the environment). There was a high number of antibiotic resistant organisms in all the study sites along River Njoro even in the reference point (Sigotik)(Table 1) 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, this could be attributed to low temperatures which do not support proliferation of bacteria. Ampicillin and streptomycin had the highest number resistant of strains. Although only ampicillin and chloramphenical was found 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; Ferreira da Silva et al., 2007), field runoffs (Peak et al., 2007; Stine 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 number of antibiotic resistant bacteria. This is due the high rate of pollution in these sites as evidenced by physiochemical parameters and microbiological quality indicators.

Table 1: Total Antibiotic Resistant Bacteria

Season	Parameter	CFUs per gm^{-1}Wet Sediment	
	Antibiotic	Site with highest count	Site with lowest count
Season 1	Tetracycline	Turkana 55666±384230	Sigotik : 35000±34641
	Streptomycin	Turkana 130000.±1473906	Sigotik : 62333±3089
	Chloramphenical	Turkana : 66000±54562	Sigotik : 31000±1000
	Ampicilin	Njoro canning :179333±1149840	Mogoon ;49333±8144
	Control	Njoro canning : 2716666±490747	Ngata ; 550000±185202
Season 2	Tetracycline	Njorocanning 82000.00±500.00	Ngata : 35330±416
	Streptomycin	Njorocanning 56661±814.	Sigotik : 36660±763
	Chloramphenical	Turkana : 68330±3165.	Mogoon : 41330±1550
	Ampicilin	Turkana :70660.±3239.	Sigotik : 30000±1734
	Control	Turkana : 510000±1307	Ngata : 121000±2424

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 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 are *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. The table below gives the site to site variation of the number of pathogens isolated.

Table 2: Pathogenic Bacteria Isolated

Season	Parameter	CFUs per 100 Ml	
	Pathogen	Site with the highest count	Site with the lowest count
Season 1	Proteus vulgaris (colourless)	Ngata : 56600±251	Turkana :40000±173
	E. coli(blue)	Mogoon :20700±2287	Sigotik :31300±15
	*Salmonell*a (Purple colony)	Turkana :42000±72	Mogoon :38600±41
	Vibrio cholera (yellow)	Ngata :33300±21	
	Vibrio Parahymolyticus (green)	Ngata :36600±55	Mogoon: 10300±178
Season 2	Proteus vulgaris (colourles)	Njoro canning:75000±13228	Ngata : 56600±251
	E. coli(blue)	Njoro Canning :47333±11015	Sigotik :34333±2886
	Salmonella (Purple colony)	Turkana :43333±21385	Sigotik :30666±1154
	Vibrio cholera(yellow)	Turkana :4000±888	Ngata and Mogoon: 0

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 (Hideomi et al., 1977). 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 CLSK disk diffusion methods. Thepercentage 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 season71% of the strains were resistant to streptomycin where as only 33% of the strains showed resistance to streptomycin in the dry season. 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 was 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 bacteriawhich 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, boil drinking water and seek alternative sources of drinking water in the study area.

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