

Original Research Article

Antimicrobial susceptibility patterns of Enterobacteriaceae isolated from domesticated animals and the environment in Lake Victoria, Kenya



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ARTICLE INFO

Article history:

Received 11 December 2012

Accepted 11 October 2013

Available online 21 October 2013

Keywords:

Enterobacteriaceae

Antimicrobial resistance

Reservoirs

Aquatic environment

ABSTRACT

Faecal coliform levels in Lake Victoria waters progressively reduced away (0–150 m) from the lake shores. *Enterobacter*, *Escherichia coli*, *Klebsiella*, *Proteus* and *Citrobacter* were recovered at high frequencies from water and fish than domesticated animals. Goats, chicken, donkey and cattle are important reservoirs of *E. coli* susceptibility to antimicrobials varied, based on the bacterial species, with about 53.8% of the isolates showing resistance to at least one class of antibiotics. The study provides a picture of resistance factors readily retained by the Enterobacteriaceae within the basin and implies that the lake may be an important reservoir of antimicrobial resistance genes.

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1. Introduction

The Lake Victoria catchment is estimated to have over 30 million people within the three countries Kenya, Uganda, and Tanzania (Okedi, 2005). Communities in the Lake Victoria basin interact with the lake ecosystem on a daily basis through fishing as well as collecting water for domestic and commercial purposes, thus the lake has a major influence on water borne and related communicable diseases in this region (Tanzam et al., 2005).

The Enterobacteriaceae family consists of a wide range of gram-negative bacilli of clinical significance. These bacteria are causative agents of many food-borne infections in humans. The Enterobacteriaceae inhabit a wide variety of niches that include the human gastrointestinal

tract, the gastrointestinal tract of animals, and various environmental locations.

High frequencies of antimicrobial resistance among members of the Enterobacteriaceae family isolated from clinical cases has been reported within the Lake Victoria region of Kenya (Shapiro et al., 2001; Brooks et al., 2003, 2006; Onyango et al., 2008). No attempts have been made to demonstrate the distribution, occurrence, and possible reservoirs of enterobacterial species within the Lake Victoria basin however, thus negligible information exist about their antimicrobial resistance patterns.

Studies within the Lake Victoria region have shown that more than 80% of *Shigella* spp. and *Salmonella* spp. isolated from patients attending hospital were resistant to ampicillin, tetracycline and trimethoprim-sulfamethoxazole. Resistance of bacterial isolates to more than one antimicrobial agent was also commonly reported in these studies (Brooks et al., 2003; Shapiro et al., 2001).

The aim of this study therefore was identifying and comparing patterns of antimicrobial agent resistance; and

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determining the distribution and occurrence of members of Enterobacteriaceae in livestock, in fish and in the environment of the Lake Victoria basin. Understanding the level of resistance to antimicrobial agents among faecal bacteria provides a good indicator for resistance problems to be expected with pathogens within the basin. Monitoring the prevalence of resistance in such indicator bacteria from different populations, animals, fish, and the environment makes it feasible to compare the prevalence of resistance and also to postulate resistance patterns expected within the environment.

2. Materials and methods

This study took place within the Lake Victoria basin of Nyanza province in western Kenya, targeting both rural and urban communities. Generally fishing, cattle rearing, and subsistence farming are the principal occupation for the rural communities within the study areas (Fig. 1). The samples were collected from five fish landing beaches along Lake Victoria (Sirongo, Dunga, Homa Bay, Mbita town, and Luanda Konyango). These sites were chosen based on fish production and proximity to an urban town and human activities.

The study was based on a repeated cross-sectional study design, and took a prospective approach. The samples were collected between January and December 2010.

Freshly deposited faeces from domesticated animals (within a radius of 500 m from the fish landing site) were picked using a sterilized spoon and placed in a sterile container (Greiner Bio-One). Water samples were collected by submerging pre-sterilized pipette to depth of 30 cm below the surface and extracting a 100 ml sample which was dispensed in sterilized 250 ml Pyrex glass bottles, three water samples were collected from each sampling site (at the shores 0 m, 100 m and 150 m). 500 g of fish samples were purchased from fishermen at landing sites for freshly landed samples and sundried fish products were sourced from markets. Soil samples were collected aseptically using a sterilized spoon from six points and pooled together to form a representative sample for that site. All the samples were transported on ice in insulated containers to Maseno University biomedical laboratory for analyses.

3. Microbial isolation

Upon arrival at the laboratory in Maseno, fish samples were processed according to FAO (1992). 25 g of fish was cut and homogenized aseptically in 225 ml buffered peptone water (Himedia Lab. Pvt. Mumbai, India) followed by direct plating onto selective media XLD (Himedia Lab. Pvt. Mumbai, India) and MacConkey agar (Himedia Lab. Pvt. Mumbai, India) and by overnight pre-enrichment of homogenate. This was then followed by enrichment by

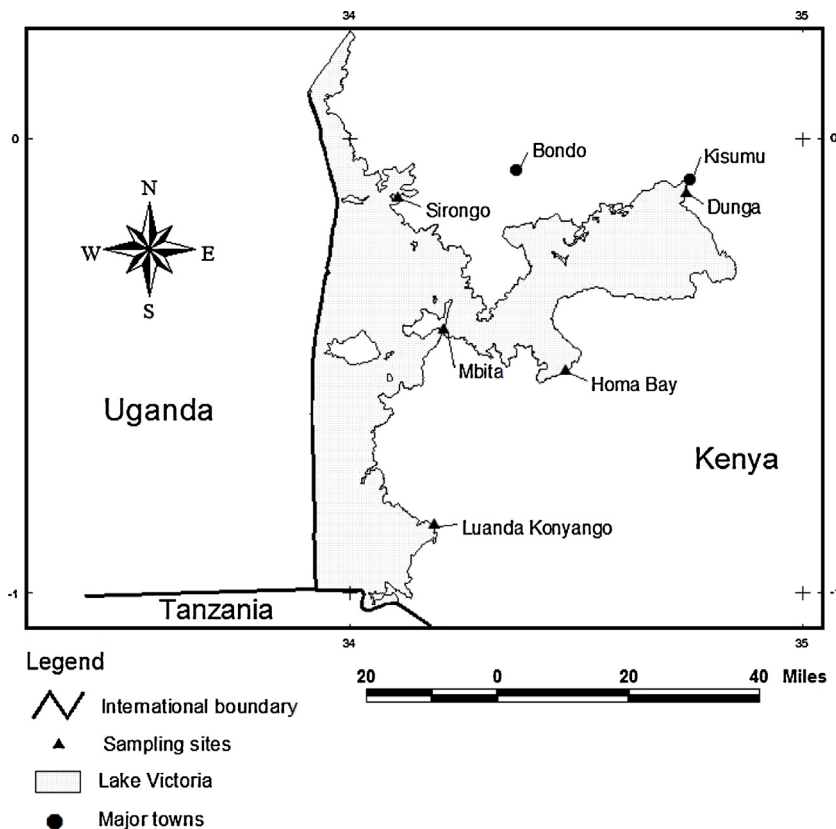


Fig. 1. Map of Lake Victoria (Kenya) showing the study sites.

adding 1 ml sample into 10 ml selenite F (HIMedia Lab. Pvt. Mumbai, India) and tetrathionate broths (HIMedia Lab. Pvt. Mumbai, India) incubated at 37 °C for 18 h before plating on selective media.

Soil samples were processed by transferring 2 spoonfuls of the pooled soil sample into a pre-sterilized, Whirl Pak bag. 100 ml of sterile phosphate-buffered water was then added and mixed for 2 min and then mixture was then filtered through a pre-sterilized 28 µm-pore-size nylon filter as described by van Elsas and Smalla (1997). The filtrate was then used to recover the Enterobacteriaceae by direct plating on selective media and overnight enrichment in selenite F and tetrathionate broth followed by plating on MacConkey and XLD agar (HIMedia Lab. Pvt. Mumbai, India).

Water was processed by taking 10 ml of sample and adding it to 90 ml of buffered peptone water followed by plating on MacConkey and XLD agar and enrichment by 1 ml of pre-enriched sample in 10 ml selenite F and tetrathionate broths incubated at 37 °C for 18 h, followed by plating on MacConkey and XLD agar. To enumerate total coliform counts as described by Anazoo and Ibe (2005). Serial dilutions of 10⁻¹–10⁻⁵ were prepared from which 1 ml was transferred to sterile petri dishes and MacConkey agar added and incubated at 37 °C for 48 h. After which plates with red colonies were enumerated and reported as colony forming units per ml (cfu/ml)

Faecal samples were processed by direct plating on selective media and by enrichment in 10 ml selenite F for 18 h at 37 °C followed by plating on selective media as described by Kariuki et al. (2002).

All incubations were at 37 °C for 18 h. Characteristic colonies were based on morphological characteristic and subjected to biochemical test TSI, LIA, citrate agar and indole all from HIMedia Lab. Pvt. Mumbai, India. The isolates were further confirmed to genera or species level using API 20 E (BioMerieux, France). All Enterobacteriaceae isolates were stored at –20 °C in tryptic soya broth plus 15% glycerol.

Susceptibility tests with six antibiotics namely ampicillin (10 µg), tetracycline (30 µg), cefuroxime (30 µg), nalidixic acid (30 µg), chloramphenicol (30 µg) and gentamicin (10 µg) (Oxoid Inc, UK) were performed using the standard Kirby-Bauer disc diffusion method on Mueller Hinton (HIMedia Lab. Pvt. Mumbai, India). The plates were then incubated at 37 °C for 18–20 h. The diameters (in millimetres) of clear zones of growth inhibition around the antimicrobial agent disks, including the 6 mm disc diameter was measured by using precision callipers (CLSI, 2002). A standard reference strain of *Escherichia coli* (ATCC 25922) was used as a control. The breakpoints used to categorize isolates as resistant to each antimicrobial agent were those recommended by CLSI (2002).

Data were entered in Ms Excel Windows XP professional 2003 and analyzed by Minitab 13.1 Windows 95. Anova and Mann–Whitney test were used to determine significant differences in zones of inhibition between species and sample site. Data for the antimicrobial agent resistance of each bacterial isolate were reported as the diameter of the zone of inhibition (in mm).

4. Results

Total coliforms were enumerated from 45 water samples. Significant differences among the sampling points ($p < 0.05$) – total coliform counts reduced away (0–150 m) from the lake shores, the mean log₁₀ observed were 3.77 cfu/ml at the shores, 3.00 cfu/ml 100 m away and (2.64 cfu/ml) from 150 m (Table 1).

One hundred and sixty isolates from the Enterobacteriaceae family were retrieved for antimicrobial agent resistance profiles from 207 samples (water 45, cow 33, goat 16, chicken 23, donkey 10, fish 70, and soil 10). Overall, *E. coli* was an important isolate recovered among all the types of samples collected. Cattle, donkey, chicken, and goats, recorded relatively higher frequencies of occurrence, with goats the highest levels of *E. coli* recovery at 95%, followed by chicken, donkey and cattle at 88.5%, 85.7%, and 75%, respectively. Fish had the lowest rates with 29.8% for Nile perch, 15.4% *Rastrineobola argentea* fresh and 11.8% *R. argentea* dried (Fig. 2). Other Enterobacteriaceae recovered and included are shown in Table 2. *Enterobacter*, *Citrobacter*, *Klebseilla* and *Proteus* were largely recovered from fish, water, and chicken samples as compared to cattle, goat and donkey (Fig. 3), whereas *Salmonella* spp. were recovered from soil, donkey, chicken, fish, and goat.

The antimicrobial susceptibility profiles for the Enterobacteriaceae isolated in this study show that 53.8% isolates were resistant to at least one class of antibiotics. Resistance to ampicillin occurred most frequently 29.4%, followed by tetracycline 8.1%, and multiple resistances of tetracycline and ampicillin 7.5% (Table 3). Resistance to ampicillin was common among *Citrobacter* spp. 90% ($n = 10$) and *Enterobacter* spp. 80.7% ($n = 31$) whereas resistance to tetracycline was also common among *Proteus* spp. 81.8% ($n = 11$). *E. coli* isolates showed equal resistance of about 36.8% ($n = 68$) among the two antibiotics ampicillin and tetracycline. Among the *E. coli*, 67.7% ($n = 68$) were sensitive to the six antibiotic tested (ampicillin, tetracycline, cefuroxime, nalidixic acid, chloramphenicol, and gentamicin) whereas only 19.4% ($n = 31$), 30% ($n = 10$), and 1% ($n = 10$) of *Enterobacter* spp., *Klebseilla* spp., and *Citrobacter* spp., respectively, were sensitive to all antibiotics tested. All the *Proteus* spp., *Providencia* spp., and *Serratia* spp. tested were resistant to at least one of the antibiotics (Fig. 4).

The study compared antimicrobial agents disc diffusion zone sizes obtained from *E. coli* and those from other Enterobacteriaceae. There were significant differences among *Enterobacter* spp. (ampicillin ($p < 0.0001$), nalidixic acid ($p < 0.0002$), gentamicin ($p < 0.0005$), and cefuroxime

Table 1

Shows means of total coliform loads (cfu/ml) for the three sampling points monitored within the five study sites.

Sampling point (n)	Mean (log ₁₀)/ml	Min (log ₁₀)/ml	Max (log ₁₀)/ml
0 m (15)	3.77 ± 0.27	3.4	4.3
100 m (15)	3.00 ± 0.5	2	3.78
150 m (15)	2.64 ± 0.45	2	3.48

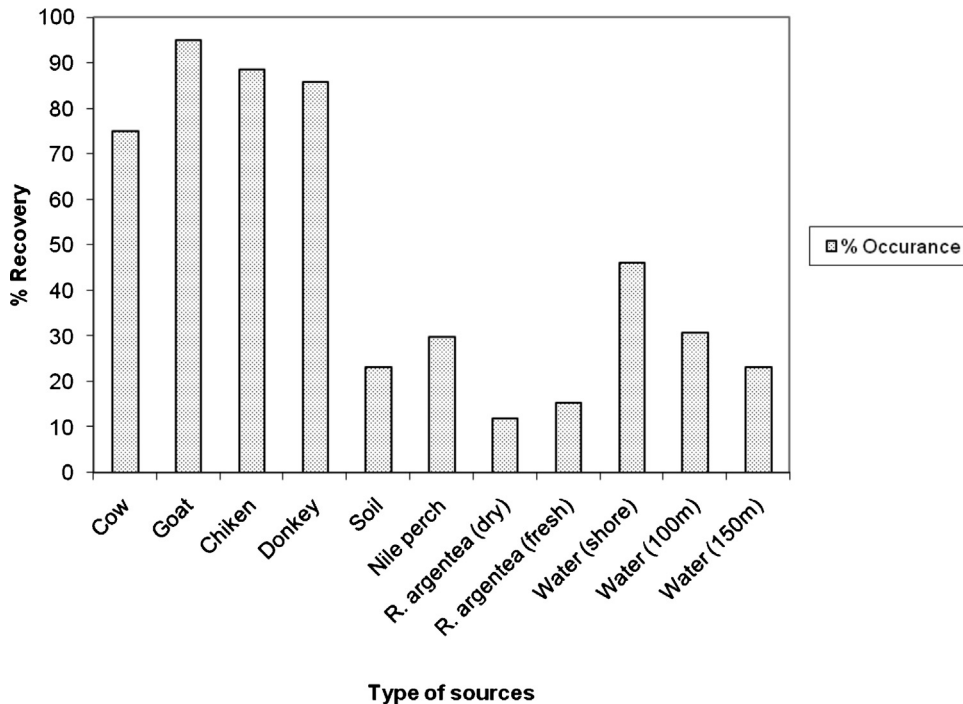


Fig. 2. Percentage occurrence of *E. coli* among the samples collected for analysis from the basin.

($p < 0.026$); *Citrobacter* spp. (ampicillin ($p < 0.0001$) and chloramphenicol ($p < 0.005$); *Proteus* spp. (tetracycline ($p < 0.02$), nalidixic acid ($p < 0.002$), chloramphenicol ($p < 0.0004$), gentamicin ($p < 0.001$), and *Klebsella* spp. (ampicillin ($p < 0.0001$)).

Table 2
Species of Enterobacteriaceae recovered from sources sampled from the Lake Victoria basin.

Enterobacteriaceae family	Number of individuals
<i>Escherichia coli</i>	68
<i>Escherichia hermannii</i>	1
<i>Proteus</i> spp.	
<i>P. vulgaris</i>	9
<i>P. mirabilis</i>	2
<i>Enterobacter</i> spp.	
<i>E. cloacae</i>	21
<i>E. sakazakii</i>	10
<i>Klebsella</i> spp.	
<i>K. pneumonia</i>	3
<i>K. oxyalocan</i>	7
<i>Salmonella</i> spp.	
<i>S. arizonae</i>	5
<i>Salmonella</i> other species	14
<i>Serratia</i> spp.	
<i>S. liquefacans</i>	2
<i>S. fricaria</i>	2
<i>Citrobacter</i> spp.	
<i>C. freundii</i>	4
<i>C. youngae</i>	2
<i>C. breckii</i>	2
<i>C. koseri</i>	2
<i>Providencia struattii</i>	3
<i>Morganella morganii</i>	2
<i>Pantoea</i> spp.	1
Total	160

Significant differences ($p < 0.05$) were observed among tetracycline and ampicillin with soil and fish isolates giving the smallest inhibition zones respectively.

Salmonella arizonae showed 40% ($n = 5$) resistance to ampicillin, whereas other *Salmonella* spp. isolated were sensitive to all the six antimicrobials tested. While comparing the disc diffusion zones among *Salmonella* spp. and *S. arizonae*, significant differences ($p < 0.05$) were observed suggesting *S. arizonae* had smaller inhibition zones meaning less sensitive to the antimicrobials tested. When compared to *E. coli* isolates, *Salmonella* spp. recovered from soil samples were all sensitive to tetracycline whereas all the *E. coli* recovered from soil were resistant.

5. Discussion

The study demonstrates that the lake water has high levels of total coliforms ranging from \log_2 cfu/ml to $\log_{4.3}$ cfu/ml. The progressive variation in the levels of total coliform from the shore line at 0–150 m demonstrates that contamination could be linked to continuous loading here. Human activity could be important in this respect, since the shores are inhabited and the lake used for cleaning household utensils, clothing, bathing, and watering domesticated animals. All these activities are responsible to increase the levels of coliform loads in the water. The reduction of coliform levels away from the shores could be indicative of the coliforms being killed by ultraviolet light (Whitman et al., 2004) or more diluted by the open lake waters.

This study demonstrates that cattle, chicken, goats, and donkeys are important reservoirs for *E. coli* within the Lake

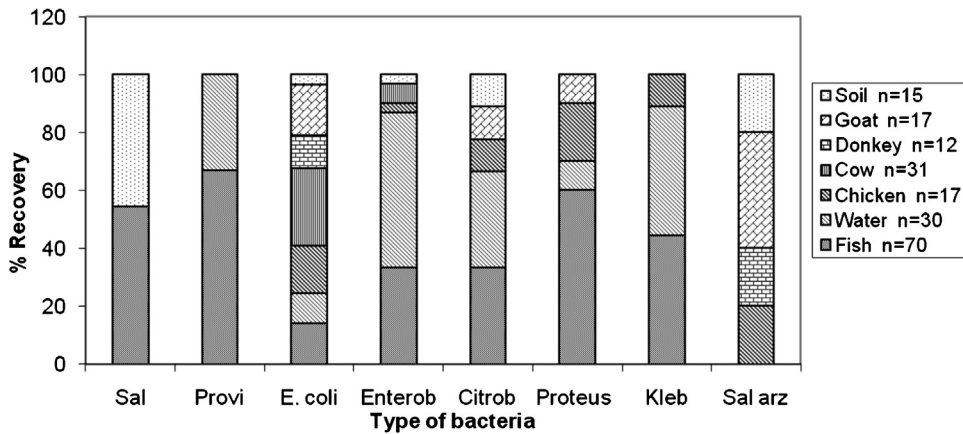


Fig. 3. The percentage recovery of some common Enterobacteriaceae by sources Enterob = *Enterobacter*; Citrob = *Citrobacter*; Kleb = *Klebsella*; Sal arz = *Salmonella arizonae*, Sal = *Salmonella*; Provi = *Providencia*.

Victoria basin, compared to *E. coli* from fish which was low. The harbouring of *E. coli* by these hosts is influenced by the frequency with which a host individual is exposed to *E. coli*; the probability that an exposure event will result in

the establishment of a population; and the mean length of time the *E. coli* population can persist in the host, or alternatively the rate at which the host loses its population of *E. coli* (Gordon and Cowling, 2003).

Table 3
The different patterns of antimicrobial resistance shown by the isolates.

Type of resistance	Frequency	Genera/species with resistance
tet	13 (8.1%)	<i>E. coli</i> (6), <i>Proteus</i> (5), <i>Providencia</i> (2)
amp	47 (29.4%)	<i>E. coli</i> (6), <i>Klebsella</i> (5), <i>Enterobacter</i> (25), <i>Citrobacter</i> (9), <i>Serratia</i> (3), <i>E. humananii</i> (1), <i>S. arizonae</i> (2) <i>Pantoea</i> spp. (1)
cxm	1 (0.6%)	<i>Proteus</i> (1)
tet + amp	12 (7.5%)	<i>E. coli</i> (9), <i>Klebsella</i> (1), <i>Proteus</i> (1), <i>Citrobacter</i> (1)
amp + na	1 (0.6%)	<i>Enterobacter</i> (1)
tet + c	2 (1.3%)	<i>Proteus</i> (1), <i>Providencia</i> (1)
amp + c	1 (0.6%)	<i>Citrobacter</i> (1)
amp + cxm	1 (0.6%)	<i>Proteus</i> (1)
tet + amp + c	3 (1.9%)	<i>E. coli</i> (1), <i>Morganella</i> (1), <i>Klebsella</i> (1)
amp + na + c	1 (0.6%)	<i>Serratia</i> (1)
tet + amp + na + c	1 (0.6%)	<i>Proteus</i> (1)
tet + amp + c + cxm	1 (0.6%)	<i>Proteus</i> (1)

tet = tetracycline; amp = ampicillin; c = chloramphenicol; cxm = cefuroxime; na = nalidixic acid.

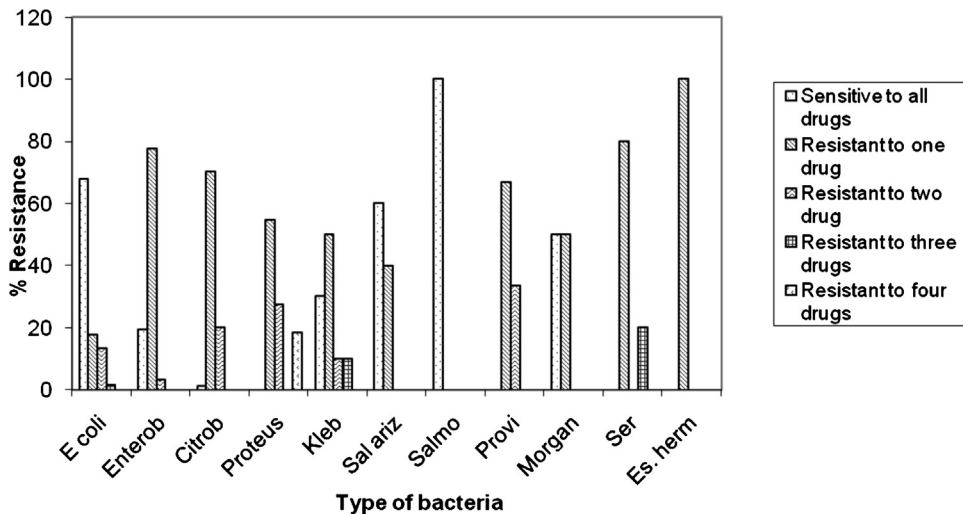


Fig. 4. Percentage of isolate exhibiting antimicrobial agent resistance by genera Enterob = *Enterobacter*; Citrob = *Citrobacter*; Kleb = *Klebsella*; Sal arz = *Salmonella arizonae*, Salmo = *Salmonella*; Provi = *Providencia*; Morgan = *Morganella*; Ser = *Serratia*; Es. hum = *Escherichia humananii*.

The study found that *Enterobacter*, *Citrobacter*, *Klebsiella*, and *Proteus* were largely recovered from fish, and water samples as compared to domesticated animals; this demonstrates that they survive better than *E. coli* in the environment.

The study further shows that within the Lake Victoria basin, soil and livestock may form important reservoirs, for *Salmonella* spp. and *S. arizonae*, respectively. Reptiles have been reported to be important reservoirs for *S. arizonae* and recovery from domesticated animals and human has also been reported (Mahajan et al., 2003; Bauwens et al., 2006; Di Bella et al., 2011).

We postulate that the process of drying fish (*R. argentea*) could be responsible for contamination of the fish with *Salmonella* spp. Generally, *R. argentea* is dried on top of fishing nets spread directly on the ground at fish landing sites (Abila and Jensen, 1997). In all the freshly caught *R. argentea* sampled no recovery of *Salmonella* was made, whereas recovery was made from four samples of dried *R. argentea*. Secondly, all the *Salmonella* spp. recovered from both soil and fish were sensitive to all the six antibiotics tested, compared to the *S. arizonae* recovered from domesticated animals that were resistant to ampicillin.

The antimicrobial susceptibility profiles for the Enterobacteriaceae isolated in this study show that over half of the environmental isolates were resistant to at least one class of antibiotics. Brooks et al. (2003) reported higher resistance levels of 90% and 80% for tetracycline and ampicillin respectively for *Salmonella* spp. and *Shigella* spp. among clinical isolates within the Lake Victoria basin. This finding, therefore, may imply that the use of antimicrobials agents in clinical situations may be an important factor for selecting for resistance within the basin.

The occurrence of resistance to various antimicrobials tested demonstrates that there is a relatively high maintenance of resistance among environmental Enterobacteriaceae. Mutation (Martinez and Baquero, 2000) or by acquisition of resistance genes such as plasmids (Davies, 1994) are two main mechanisms through which bacteria may develop antibiotic resistance. Stabilization of such resistance among bacteria has been suggested to be due to compensatory mutations that restore fitness without loss of resistance. Analysis of disc diffusion zone sizes of *E. coli* and those of other Enterobacteriaceae demonstrated that non *E. coli* Enterobacteriaceae have smaller mean inhibition zones as compared to *E. coli*, therefore, show less susceptibility. This finding may imply that other factors outside the scope of this study may play an important role in the selection and maintenance of resistance genes among the Enterobacteriaceae family in the basin. The exposure to environmental pollutants and changes in nutrient composition has been found to lead to selective pressures favouring certain bacteria or genotypes within water bodies (Lin et al., 2004). In this study, non *E. coli* Enterobacteriaceae were recovered mainly from water and fish sources and therefore these factors could have played an important role in selection for resistance against antimicrobials. However, other studies have suggested that resistance to ampicillin and tetracycline are almost ubiquitous in Enterobacteriaceae, with the exception for the *Salmonellae* (Livermore, 1995, 1996).

This study further shows that resistance to tetracycline and ampicillin among Enterobacteriaceae in the Lake Victoria basin may increase risk of becoming resistant to additional antimicrobial agents. It is possible that such resistance may be conserved in the bacterial populations over time regardless of selection pressure, resulting in an overall increase in resistance over time, or be a result of independent, simultaneous development of resistance to different agents or a result of co-selection of resistance determinants (Sayah et al., 2005).

A recent study by Sifuna et al. (2008) has however shown that *E. coli* isolated from sundried fish sold in markets within the Lake Victoria region showed multiple antibiotic resistances, which was also not transferable by conjugation. Therefore, we postulate that Lake Victoria may be an important reservoir for antimicrobial resistant genes. Poor water resource management practices, such as release of industrial waste and use of agrichemicals within the catchments have been documented (World Agroforestry Centre, 2006), and may serve to sustain the resistance genes among the Enterobacteriaceae within the Lake Victoria basin, whereas human, fish, and livestock watered from the lake may serve to transfer or disperse the genes. Ash et al. (2002) has also reported a similar situation for several rivers in the United States of America as being reservoirs for antibiotic resistant microbes.

Conflict of interest

The author(s) declare that they have no competing interests.

Financial disclosure

This study was funded by the National Council for Science and Technology – Research Number NCST/5/003/PG/75.

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