World Academy of Science, Engineering and Technology
International Journal of Biomedical and Biological Engineering
Vol:5, No:3, 2011

Prevalence and Antimicrobial Susceptibility Patterns of Enteric Bacteria Isolated from Water and Fish in Lake Victoria Basin of Western Kenya

Jackson H. O. Onyuka, Rose Kakai, David M. Onyango, Peter F. Arama, John Gichuki, and Ayub V. O. Ofulla

***Abstract*—**A cross sectional study design and standard microbiological procedures were used to determine the prevalence and antimicrobial susceptibility patterns of *Escherichia coli*, *Salmonella enterica* serovar *typhimurium* and *Vibrio cholerae* O1 isolated from water and two fish species *Rastrineobola argentea* and *Oreochromis niloticus* collected from fish landing beaches and markets in the Lake Victoria Basin of western Kenya. Out of 162 samples analyzed, 133 (82.1%) were contaminated, with *S. typhimurium* as the most prevalent (49.6%), followed by *E. coli* (46.6%), and lastly *V. cholerae* (2.8%). All the bacteria isolates were sensitive to ciprofloxacin. *E. coli* isolates were resistant to ampicillin, tetracycline, cotrimoxazole, chloramphenical and gentamicin while *S. typhimurium* isolates exhibited resistance to ampicillin, tetracycline, and cotrimoxazole. The *V. cholerae* O1 isolates were resistant to tetracycline and ampicillin. The high prevalence of drug resistant enteric bacteria in water and fish from the study region needs public health intervention from the local government.

***Keywords*—**Aquatic environments, Antimicrobial resistance, Enteric bacteria, Lake Victoria Basin

I. Introduction

ESCHERICHIA *coli* and *Salmonella enterica* serovar *typhimurium* are among the most common causes of gastroenteritis in humans [1]. *Escherichia coli* and other groups of coliforms may be present where there has been faecal contamination originating from warm-blooded animals [2]. *E. coli* is recognized as a good indicator of faecal contamination. It is identified as the only species in the coliform group found exclusively in the intestinal tract of human and other warm-blooded animals and subsequently excreted in large numbers in feces, approximately 10^9 per gram [3]. Salmonellosis is a food and water-borne bacterial infection of man and animals. *Salmonella* causes a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia [4]. It is estimated that food-borne *Salmonella* infections are responsible for 1.3 million illnesses annually worldwide, resulting in 16,000 hospitalizations and 600 deaths [5]. *Vibrio cholerae* O1 is responsible for the life threatening secretory diarrhea, mostly associated with epidemic outbreaks when sanitary conditions are not optimum [6], and the Asiatic cholera outbreaks have been linked to consumption of unsafe food and water such as drinking lake and river water, food sold by the roadside and feasting at funeral gathering [7]-[8].

The emergence of bacteria resistant to antimicrobials is common in areas where antimicrobials are used [9]. Water and food contaminated with antibiotic-resistant bacteria is a major threat to public health, as the antibiotic resistance determinants can be transferred to bacteria of human clinical significance [10]. The prevalence of antimicrobial resistance has increased during the recent decades [11] partly due to selection pressure caused by the indiscriminate use and misuse of antimicrobials including antibiotics given to veterinary animals [12]-[13].

The emergence of antimicrobial resistance to members of the *Enterobacteriaceae* family is posing major problem in the management of bacterial infections [14] and the occurrence of antimicrobial resistant bacteria is also increasing in aquatic environments [15]-[16]. Fish living in natural environment are known to harbor pathogenic *Enterobacteriaceae* [17]. Invasion of fish muscles due to the breakage of immunological barriers of fish by pathogens is likely to occur when the fish are reared in aquaculture ponds contaminated with faecal coliforms, *E. coli* and *Salmonella*, of greater than 10^3, 10^4, and 10^5 per 100ml in pond water, respectively [18]. Drug resistant bacteria may naturally occur in aquatic environments and make their way to humans and spread drug resistant genes leading to persistence of ill health in the human population [19]. *In vitro* transfer of plasmids carrying resistance determinants has also been recorded from fish pathogens to human pathogens including *V. cholera*e [20]. In

J. H. O. Onyuka is with Maseno University, Department of Biomedical Science and Technology,Box 333-40105 Maseno, Kenya. (Tel. +254 57 31620 Ext. 3150; fax: 254-057-351153; e-mail: lonyuka@yahoo.com).

R. Kakai, is with Maseno University, Department of Biomedical Science and Technology Box 333-40105 Maseno, Kenya. (Tel. +254 57 31620 Ext. 3150; fax: 254-057-351153; e-mail: kakairm@yahoo.com).

D. M. Onyango is with Maseno University, Department of Zoology, Box 333-40105, Maseno, Kenya. (phone: +254-573-1620 Ext 3438; fax: 254-057-35221; e-mail: donyango@maseno.ac.ke).

P. F. Arama is with Maseno University, Department of Department of Botany and Horticulture, Box 333-40105, Maseno, Kenya. (phone: +254-573-1620; fax: 254-057-351153; e-mail: aramapk@yahoo.com).

J. Gichuki is with Kenya Marine and Fisheries Research Institute, Kenya, Box 1881, Kisumu, Kenya. (phone: +254-057-530045/2021461; e-mail: gichukij@yahoomail.com).

A.V.O. Ofulla is with Maseno University, Department of Biomedical Science and Technology Box 333-40105, Maseno, Kenya. (Tel. +254 57 31620 Ext. 3150; fax: 254-057-351153; e-mail: ofulavo@gmail.com).

fact, fish has been found to be a source of *Salmonella* infection [21], and *Salmonella* has been isolated from aquaculture fish and shrimps [22] indicating that bacteria present in such ecosystem can be transmitted to humans.

Fish such as *Rastrineobola argentea* and *Oreochromis niloticus* are foods that are consumed by many people living around Lake Victoria due to their nutritive value and high protein content [23]-[24]. Fish from Lake Victoria represents 85% of Kenya's fish supply and constitutes 25% of total catch from Africa's inland fisheries [25]. People in the Lake Victoria region also rely on lake water for drinking and daily use. Therefore contamination of fish and water sources with antimicrobial resistant bacteria can pose serious public health risk to people living in the Lake Victoria region and beyond. This study was therefore designed to determine the prevalence and antimicrobial susceptibility patterns of enteric bacteria isolated from water and fish in Lake Victoria basin, western Kenya.

II. Materials and Methods

A. Study Sites

The water and fish (*Rastrineobola argentea* and *Oreochromis*) samples for this study were collected from three fish landing beaches namely Dunga, Luanda Rombo and Sirongo and from three markets: Kisumu Municipality, Luanda and Bondo within L. Victoria basin of western Kenya (Figure 1). Sirongo beach is in Bondo county, Central Sakwa location, Uyawi sub location which has an area of 34.5 km^2 and a population density of 234. Luanda Rombo beach is in Suba county, Mbita division, Wanyama sub location with an area of 7.0 km^2 and a population density of 317. Dunga beach is in Kisumu county, Winam division, West Kolwa location, Nyalenda B sub-location with an area of 4.7 km^2 and a population density of 6,886 [26]. Kisumu municipality market is in Kisumu city, Winam Division; Luanda market is along Kisumu-Busia road while Bondo market is in Bondo Township, Bondo county, Nyanza province, Kenya. The choice of the two fish types' *Rastrineobola argentea* and *Oreochromis niloticus* was based on the fact that they are the most commonly consumed fish species in the Lake Victoria basin region [23].

B. Sample Collection

Using a cross-sectional study design, water (250ml per sample in sterile bottle) and one kilogram of fresh fish samples (500-600 pieces of *Rastrineobola argentea*) and whole fresh *Oreochromis niloticus*, respectively, were randomly collected from the fish landing beaches and from the markets and placed in sterile plastic bags. Within each fish landing beach, or from each market, the samples (whether water or fish species) were taken from three different locations, and at each location, samples were taken from three different points, meaning that nine (9) samples (whether water or fish) were taken from each fish landing beach or market.

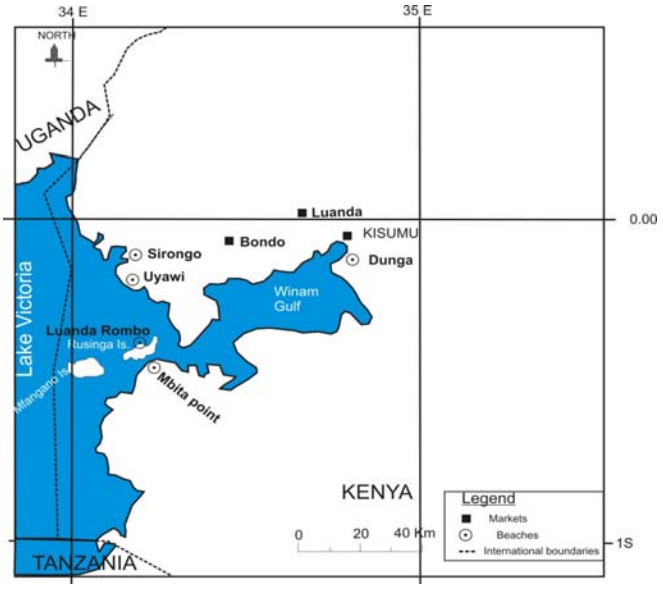

Fig. 1 A map of Winam Gulf of Lake Victoria showing the sampling sites

The water samples from the beaches were collected from different points, separated by at least 10-15 meters along the lake shorelines, and from 10-20 liter plastic containers (or buckets) used for storing water for washing fish by different fresh fish traders in the markets. The fresh fish samples from the fish landing beaches were collected (bought) from fishermen from different landed fishing boats, and those from the markets also collected (bought) from the fish traders from where the respective water samples were collected. All the collected water and fish samples (in clearly labeled containers or plastic bags, respectively) were then transported in cooler boxes with ice packs within four (4) hours of collection to the Maseno University School of Public Health and Community Development, Department of Biomedical Science and Technology laboratory for microbial analysis.

C. Sample Processing and Bacterial Culture

The water and fresh fish samples from the field were unpacked and coded for laboratory analysis. 100mls aliquots from each of the 250ml water samples, and weighed ten grams of fish samples (either 10-15 whole pieces of *Rastrineobola argentea*, or cut muscles with skin from lateral line of *Oreochromis niloticus*) were used for analysis. The fish samples were macerated for 3 min in 90 ml saline (0.85% NaCl) in a blender (Sanyo^{TM}) and topped up with saline to make up 100ml of fish slurry. The 100ml water samples and the resultant 100ml slurry stocks were respectively transferred into sterile labeled 250ml flasks in readiness for bacteriological analysis.

Determination of total viable non specific bacterial counts was done by the aerobic plate count method using plate count agar according to AOAC method 966.23 [27]. All the experiments were done in triplicate. Briefly, using a micropipette and sterile tips, aliquots of 1 ml of the various sample preparations (i.e. from the 100ml water samples or fish

slurry) were aseptically inoculated into sterile test tubes containing 9mls of plate count agar, vortexed, and then poured or plated into sterile Petri dishes and allowed to set for about 15 minutes. The loaded Petri dishes were then inverted and incubated at 37^0C (Gallenkamp, Germany) for 24 hrs. By using Quebec colony counter, the number of colonies per plate was counted, and the means from triplicate experiments for each sample determined and recorded.

Escherichia coli analysis of the various sample preparations (i.e. from the previous 100ml water samples or fish slurry) was done using most probable number (MPN) procedure or the multiple tube fermentation test which detects the coliform bacteria as indicator for faecal contamination [28] followed by biochemical assay for species identification. Multiple tube techniques utilize selective and differential liquid media into which multiple aliquots of serial dilutions of samples are inoculated. The technique involves three successive steps, namely, presumptive test, confirmed test and complete test [29].

In the presumptive test, 10mls of phenol red lactose broth (HiMedia Lab. Pvt. Mumbai, India) was added into each of 3 sets of 25ml tubes (with inverted Durham's tubes' inserts). Each set contained three tubes (i.e., there were 9 tubes in total). The loaded tubes were sterilized by autoclaving. The tubes were allowed to cool and then inoculated with a ten-fold difference in water and fish samples inoculums volumes, i.e., 0.1ml, 1ml, and 10ml per tube and incubated at 37^0C (Gallenkemp, Germany). After 48 hours, the tubes were examined for acid and gas production. Change of phenol red lactose broth to yellow indicated acid formation [28]. Each set was scored for the number of positive tubes and the score of all the three sets recorded and used with the standard Most Probable Number (MPN) table to determine the number of coliforms in the water or fish samples.

The presumptive test was then followed by the confirmative test, the complete test and IMViC tests. The confirmative test was performed by streaking a positive presumptive tube onto eosin methylene blue agar (EMB agar) (HiMedia Lab. Pvt. Mumbai, India). This agar contains lactose and the dyes eosin Y and methylene blue. When *E. coli* grows on EMB, it ferments so much acid that the two dyes precipitate out in the colony producing a metallic green sheen appearance. A positive confirmative test is then the presence of green sheen colonies on EMB streaked from a positive presumptive test.

The complete test was performed by inoculating a tube of phenol red lactose broth with green sheen colonies from positive confirmative tests. Simultaneously, a loop of colony was streaked onto a slant of nutrient agar. Both tubes were incubated at 37^0C for 48hrs. The culture on the nutrient agar was analyzed by Gram staining.

The presumptive positive samples from the above tests were subjected to further biochemical assays. The biochemical tests performed included Indole production, Methyl red, Vogues-Proskauer test, Citrate test, Oxidase production and catalase production. These biochemical tests were performed as per standard microbiological methods [30]. Analytical grade chemicals and reagents obtained from HiMedia were used in all the tests. An *E. coli* ATCC 25922 culture obtained from Kenya Medical Research Institute (KEMRI) microbiology laboratory was used as a control for the biochemical tests.

The isolation of *Salmonella* spp. from the various samples' (water and fish) was done by resuscitation method of Harrigan [31]. Aseptically, 2ml of the water sample or fish slurry (from the 100ml stocks) was enriched in selenite F media (HiMedia Lab. Pvt. Mumbai, India) and incubated at 37^0C for 24hrs. For identification of *Salmonella*, positive selenite F cultures were touched at the top and streaked on xylose lysine desoxycholate agar (HiMedia Lab. Pvt. Mumbai, India) and incubated at 37^0C for 24 hrs. Suspected colonies were identified based on characteristic colony morphology, Gram staining, and motility tests [28]. The xylose lysine desoxycholate agar colonies were streaked onto nutrient agar and incubated at 37^0C for 2 hours and the resultant colonies again streaked on triple sugar iron (TSI) agar and incubated at 37^0C for 24 hours. Slants with H_2S production, i.e. blackening at the butt, were taken as positive for *Salmonella*. Positive isolates for *Salmonella* were streaked on nutrient agar and incubated at 37^0C for 24 hours. Further biochemical screening for *Salmonella* was done using the indole, methyl red, Voges-Proskauer and citrate (IMViC) reaction and confirmed by slide agglutination test following Kauffmann-White scheme serotyping [32].

The isolates were serotyped by slide agglutination with homologous *Salmonella* O and H group antisera (Bio-Rad, Marne-la-Coquette, France) [33]-[34]). Briefly, using a clean microscope slide, a drop of antiserum was placed on the slide at one end and a drop of sterile normal saline (0.85% NaCl) placed at the opposite end of the same slide, 3 to 4 colonies were suspended in 0.3ml sterile saline and cells suspension was made. Onto the drops of serum and normal saline, one loopful of the cell suspension was placed, and then mixed well. The slide was shaken gently for one minute. Normal saline served as the control. Agglutination observed within one minute was regarded as positive for *Salmonella* and for the same isolate monovalent was used using the same procedure.

Further, H antigen was used for tube agglutination using cultures grown for 8hrs at 37^0C in heart infusion broth which were diluted with an equal volume of saline containing 1% formaline. Antigen suspension of 0.5ml was added to 0.05ml of each specific H serum. The tubes were then shaken well for 2min, allowed to stand in a water bath at 50-52^{0}C for 1hr, then observed for agglutination. Agglutination of the suspension in tubes was positive result and a confirmation for *Salmonella enteric serovar typhimurium.*

The isolation of *Vibrio cholera* from the various samples' (water and fish) was done by enriching 2mls of water sample or fish slurry (from the 100ml stocks) using 3 tubes with alkaline peptone water (pH 8.6) and incubated at 37^0C for 24hrs. Top surface of alkaline peptone water culture was touched with a loop and inoculated on Thiosulphate Citrate

Bile Salt (TCBS) agar (Fluka, Sigma-Aldrich, Switzerland) plates which is a differential and selective media for *Vibrio* species and then incubated at 37^0C for 24hrs. From TCBS agar plates, sucrose-fermenting yellow, shiny colonies measuring 2 to 4 mm in diameter suspected for *V. cholerae* were subcultured in brain heart infusion agar and incubated at 37^0C for 24hrs and then subjected to biochemical tests i.e. indole, methyl red, Voges Proskauer and citrate, triple sugar iron agar, oxidase, catalase and string tests [28].

For identification of the serotypes, slide agglutination serology test was done using polyvalent O1 and O139 antisera (Denka Seiken Co. Ltd, Tokyo, Japan) for confirmation of *V. cholerea*. A drop of O1 and O139 antisera was placed on two different slides at one end and a drop of sterile normal saline (0.85% NaCl) placed at the opposite end of each slide. Using an inoculation loop, 2 to 3 discrete colonies were picked from TSI and emulsified in 0.3 ml sterile saline and mixed thoroughly to make cell suspension. The slide was then tilted back and forth to observe agglutination within 30 seconds to 1 minute. If clumping appeared on cell suspension which had antisera, then this was regarded as positive to Ogawa antiserum and negative to Inaba antiserum as a conformation test of *V. cholerae* O1 [35].

All the confirmed isolates of *E. coli, S.enterica serovar typhimurium*, and *V. cholerae* O1 were streaked on tryptic soy agar plates and incubated at 37^0C for 24hrs in readiness for antimicrobial susceptibility testing.

D.Antimicrobial Susceptibility Testing

The identified isolates of *E. coli, S. enterica serovar typhimurium*, and *V. cholerae* O1 were tested by the standard disc diffusion method on Mueller-Hinton agar (HiMedia Lab. Pvt. Mumbai, India) which relies on the zone size [36] and results interpreted as described by Clinical and Laboratory Standards Institute [37]. From the tryptic soy agar plates, discrete colonies were picked using sterile wire loop and transferred to a tube containing 5 ml of sterile saline, vortexed and adjusted to 0.5 using McFarland. Sterile cotton swab was then dipped, rotated and pressed firmly on the tube walls above the culture to remove excess inoculums from the swab. This was then evenly swabbed on the dried surface of Mueller-Hinton agar plates ensuring even distribution of the bacterium. The antimicrobial agents and their concentrations used were as follows: ampicillin 10mcg, chloramphenical 10mcg, erythromycin 15mcg, gentamicin 10mcg, norfloxacin 10mcg, ciprofloxacin 5mcg, tetracycline 30mcg, methicilin 5mcg, and co-trimoxazole 25mcg, (HiMedia Lab. Pvt., Mumbai, India). The antimicrobial loaded discs were placed on the bacteria loan using sterile forceps and incubated at 37^0 C for 18 to 24 hrs. Isolates were classified as sensitive or resistant using the criteria described by the disc manufactures (HiMedia Lab. Pvt. Mumbai, India). *Escherichia coli* ATCC 25922 from KEMRI microbiology laboratory was used as the quality control strain.

E. Data Analysis

Data entry and analysis was done using Excel windows 2003. ANOVA was used for determining the differences in prevalence of *E. coli, S. typhimurium* and *V. cholerae* between the various beaches and markets. Descriptive statistics (percentages) was used to establish the differences of the antimicrobial resistance patterns between water, *R. argentae* and *O. niloticus.*

III. RESULTS

Water samples from Dunga beach were the most contaminated with total viable counts of 2.5 CFU/ml, followed by water sampls from Kisumu market 2.45 CFU/ml and then Luanda market 2.3 CFU/ml, Figure 2. Water samples from Sirongo beach were the least contaminated with 2.28 CFU/ml. Fish samples from Kisumu market showed the highest total viable count with 2.34 CFU/g for *R. argentea* and 2.29 CFU/g for *O. niloticus* followed by Dunga beach 2.30 CFU/g for *R. argentea* and 2.26 CFU/g for *O. niloticus*. Sirongo beach recorded the lowest viable colony counts for both *R. argentea* and *O. niloticus*, 2.12 CFU/g and 2.15 CFU/g, respectively, Figure 2.

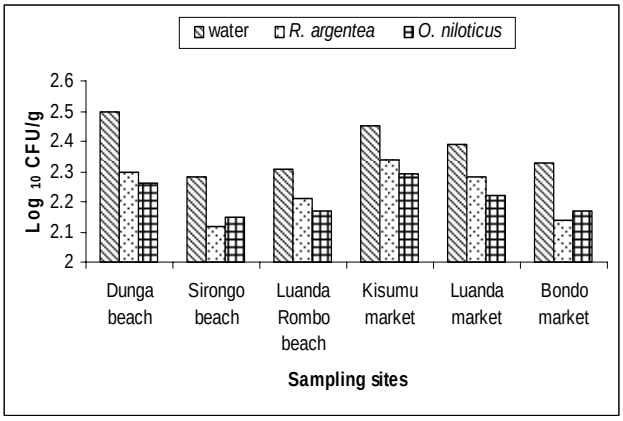

Fig. 2 The total coliform count from water, *R. argentea* and *O. niloticus* from various sampling sites

Out of 162 samples analysed, 133 (82.1%) were contaminated with various bacteria species (Table 1). *Salmonella typhimurium* was the most prevalent with 49.6% among the isolates followed by *E. coli* 46.6% and the least prevalent was *V. cholerae* 3.8%. Dunga had the highest number of bacteria isolated 33.8% followed by Kisumu market with 19.9% and the least was Sirongo beach with 11.3%. Out of 45 isolates from Dunga, *Salmonella* isolates were 48% while 44.4% were *E. coli* and *V. cholerae* were 6.7%. From Kisumu market, out of the 21 isolates, 52.4% were *Salmonella*, 42.9% were *E. coli* and 4.8% were *V. cholerae*. On the other hand, Sirongo beach yielded the lowest number of microbes with a total of 11.3% isolates, out of these 53.3% were *E. coli*, and 46.7% were *Salmonella*. The differences in prevalence between the bacteria species (*S. enterica serovar typhimurium*, *E. coli* and *V. cholerae*), and between the various beaches and markets was significant by two way ANOVA, $P < 0.01$.

Antimicrobial susceptibility results for *E. coli*, *S. enteric serovar typhimurium* and *V. cholerae* are shown in Table 2. In water, over 60% of *S. enteric serovar typhimurium* isolates were resistant to cotrimoxazole, ampicillin and tetracycline. Over 70% of *E. coli* were resistant to cotrimoxazole, tetracycline and ampicillin and for *V. cholerae*, resistance was over 66% to ampicillin, tetracycline and cotrimoxazole. All the water isolates were sensitive to norfloxacin and ciprofloxacin. However, in *R. argentea* all the isolates were 100% sensitive to ciprofloxacin. Over 68% of *E. coli* isolates were resistant to tetracycline, ampicillin and cotrimoxazole. Of the 23 *S. enteric serovar typhimurium* isolates, 73% were resistant to cotrimoxazole, tetracycline and ampicillin while in *V. cholerae* isolates, 100% were resistant to ampicillin and tetracycline.

TABLE I
NUMBER OF SAMPLES CONTAMINATED WITH ENTERIC BACTERIA (*S. TYPHIMURIUM*, *E. COLI* AND *V. CHOLERAE* O1) FROM WATER AND FISH SAMPLES COLLECTED FROM DIFFERENT BEACHES AND MARKETS IN THE LAKE VICTORIA BASIN OF WESTERN KENYA

Sampling location and type (number*)	No. of samples positive for bacteria species			Total
	S. typhymurium	*E. coli*	*V. cholerae*	
Dunga beach				
Water	5	8	2	
R. argentea	8	5	1	
O. niloticus	9	7	0	
Total	22	20	3	45
Sirongo beach				
Water	2	4	0	
R. argentea	3	2	0	
O. niloticus	2	2	0	
Total	7	8	0	15
Luanda Rombo beach				
Water	2	5	1	
R. argentea	3	3	0	
O. niloticus	3	2	0	
Total	8	10	1	19
Kisumu Fish market				
Water	3	3	0	
R. argentea	4	4	0	
O. niloticus	4	2	1	
Total	11	9	1	21
Luanda Fish market				
Water	3	3	0	
R. argentea	2	3	0	
O. niloticus	4	2	0	
Total	9	8	0	17
Bondo Fish market				
Water	4	2	0	
R. argentea	3	2	0	
O. niloticus	2	3	0	
Total	9	7	0	16
Grand Total	66 (49.6%)	62 (46.6%)	5 (3.8%)	133

* the water, *R. argentea* and *O. niloticus* samples were nine (9) from each of the beaches or markets

Out of 19 *E. coli* isolated from *O. niloticus*, over 50% were resistant to ampicillin and cotrimoxazole and over 85% of *S. enterica serovar typhimurium* isolates were resistant to tetracycline and cotrimoxazole, and 100% of *V. cholerae* isolates were resistant to cotrimoxazole and tetracycline. All the isolates from water, *R. argentae* and *O. niloticus* were sensitive to ciprofloxacin.

TABLE II
ANTIMICROBIAL RESISTANCE PATTERENS OF E. COLI, S. TYPHIMURIUM AND V. CHOLERAE O1 ISOLATES FROM WATER AND FISH (R. ARGENTEA AND O. NILOTICUS) SAMPLES

Antibiotic	Number (%) of isolates resistant to antibiotic			Total
	Water			
	E. coli n = 25	*S. typhimurium* n = 19	*V. cholerae* n = 3	47
Ampicillin	16 (64)	13 (68.4)	2 (66.7)	
Chloramphenicol	7 (28)	5 (26.3)	0	
Erythromycin	4 (16)	2 (10.5)	0	
Gentamicin	8 (32)	3 (15.8)	0	
Norfloxacin	0	0	0	
Ciprofloxacin	0	0	0	
Methicillin	4 (16)	3 (15.8)	0	
Tetracycline	19 (76)	12 (63.2)	2 (66.7)	
Co-trimoxazole	20 (80)	14 (73.7)	2 (66.7)	
	R. argentea			
	E. coli n =19	*S. typhimurium* n =23	*V. cholerae* n =1	43
Ampicillin	14 (73.7)	14 (60.9)	1 (100)	
Chloramphenicol	5 (26.3)	8 (34.8)	0	
Erythromycin	0	3 (13.0)	0	
Gentamicin	4 (21.1)	3 (13.0)	0	
Norfloxacin	1 (5.3)	1 (4.3)	0	
Ciprofloxacin	0	0	0	
Methicillin	2 (10.5)	5 (21.7)	0	
Tetracycline	14 (73.7)	16 (69.6)	1 (100)	
Co-trimoxazole	12 (63.2)	17 (73.9)	0	
	O. niloticus			
	E. coli n =18	*S. typhimurium* n =24	*V. cholerae* n =1	43
Ampicillin	13 (72.2)	10 (41.7)	0	
Chloramphenicol	7 (38.9)	9 (37.5)	0	
Erythromycin	2 (11.1)	1 (4.2)	0	
Gentamicin	3 (16.7)	1 (4.2)	0	
Norfloxacin	2 (11.1)	0	0	
Ciprofloxacin	0	0	0	
Methicillin	2 (11.1)	3 (12.5)	0	
Tetracycline	2 (11.1)	17 (70.8)	1 (100)	
Co-trimoxazole	12 (66.7)	16 (66.7)	1 (100)	

n = number of isolates from water or fish, % = number of antibiotic resistant isolates/total number of isolates X 100.

IV. DISCUSSION

Total coliform counts results from this study has shown that water samples from Dunga beach and Kisumu market were more contaminated and the water samples from Sirongo beach were the least contaminated. Similarly, fish samples from Kisumu market and Dunga beach were the most contaminated, and fish samples from Sirongo beach were the least contaminated, suggesting that contaminated water may contribute to the microbial load in the fish. The prevalence data from the most probable number (MPN) method and subsequent bacteria species (*E. coli*, *S. typhimurium* and *V.*

cholera) identification and isolation from water and fish also showed that Dunga beach and Kisumu market samples had the highest number of bacteria isolated and Sirongo beach had the lowest number of bacteria isolated. Dunga beach is at the shores of Lake Victoria in Kisumu town within Winam gulf and the lake water at the beach could be more polluted compared to lake water at Sirongo which is in Bondo county very far from an urban setting and less populated. The lake generally receives large quantity of treated and untreated waste water discharged from human and industrial sources [16]. Further, rivers and rainfall could introduce enteric pathogens from distant sources into shore water [38] hence contributing to lake water pollution. Discharge of untreated municipal effluent into rivers and the lakes, compounded by lack of awareness of good hygiene practices, can also directly contribute to the degradation of river and lake water quality for habitats and domestic use [39].

The reason why fish from Kisumu market were more contaminated was not determined by this study but it could be attributed to the poor sanitary conditions of storage and handling of fresh fish in the market or unhygienic fish transportation methods from the beaches to the market. Kisumu municipality authorities therefore needs to enforce higher standards of hygiene in the market and Kenya Fisheries department should also set up high standards for transporting fresh fish from the beaches to the markets.

Results from this study showed that *Salmonella* was the most prevalent pathogen which could explain the high prevalence of diarrhoea in Kisumu region [40]. *V. cholerae* though not very prevalent was also isolated from water and fish. *V. cholerae* is not only able to survive but also able to grow in fresh water habitats [41]. *V. cholerae* found in the waters of cholera endemic areas exists in biofilm-like aggregates in which cells are in conditional viable state [42]. They are metabolically impended cells which can regain its metabolic activity under specific *in vitro* conditions. Such vibrio cells might play a critical role in the transmission of pathogens [43].

The results from this study showing that fish collected from the beaches were contaminated with enteric bacteria may be due to use of contaminated water collected directly from the lake by local artisanal fish processors as a result of lack of piped water. This could further be enhanced by transportation of fish in dirty fishing boats and dirty packaging baskets by the fisher folks. The fish can also be contaminated through zooplankton, phytoplankton including algae as results from previous studies have shown that zooplanktons, algae and phytoplanktons are normally together in the harvested fish in the nets hence possibility of fish contamination [44]-[45].

Results from this study have also shown that the enteric bacteria isolated from water and fish were resistant to some antibiotics. All the bacteria isolated from water were sensitive to norfloxacin and ciprofloxacin; and all the bacteria isolates from *R. argentea* were 100% sensitive to ciprofloxacin. In this study therefore, a multidrug resistance pattern was observed for *E. coli,* and *Salmonella typhimurium* with cotrimoxazole, tetracycline and ampicillin. However, the bacteria species were susceptible to the antibiotics ciprofloxacin and norfloxacin. Resistance to cotrimoxazole, tetracycline and ampicillin might be related to their overuse as opposed to norfloxacin and ciprofloxacin which are not used for treating enteric infections. The emergence and dissemination of antimicrobial resistance among *E. coli, Salmonella enteric serovar typhimurium* and *Vibrio cholerae* O1 strains is an increasing global health problem that is complicating the therapeutic management of severe salmonellosis and diarrhogenic diseases [46].

The findings from this study are consistent with those reported previously by Onyango [16]-[47] of high resistance pattern of *Salmonella* spp isolated from the fish landing beaches to antibiotics frequently used for treating salmonellosis and diarrhoeagenic diseases in the local community. The high prevalence of resistance to tetracycline, ampicillin and co-trimoxazole in *E. coli* in the region has also been reported by Sifuna [48], in which *E. coli* demonstrated resistance mostly to ampicillin, and tetracycline. Similar results were reported by Sack [49] and Shapiro [7] which attributed resistance to use of tetracycline for mass prophylaxis during cholera outbreaks. The resistant pattern reported in this study can also be linked to use of these drugs in veterinary practise and leading to resistance in human as also previously reported by Laxminarayan [50]. Due to widespread acquisition of resistance, it is important that the susceptibility tests are routinely done to guide antibiotic treatment and policy [51]-[52]-[53]. Antimicrobial resistance genes can be readily transmitted between commensal *Enterobacteriaceae* and enteropathogens *in vivo* and *in vitro* [10] hence these organisms may cause hard to treat persistent diseases to human if interventions and proper policies are not instituted in timely manner.

The finding from this study implies that a high percentage of cases of diarrhea in western Kenya may be caused by antimicrobial-resistant bacteria to the cheaper and commonly used antibiotics, thus illustrating the effect of longstanding, unregulated antimicrobial use. A survey of enteric pathogens isolated from patients attending selected district hospitals in western Kenya revealed high prevalence of resistance to commonly used antibiotics including tetracycline, cotrimoxazole and ampicillin [54]. This suggests that the strains circulating in the human population are also found within the aquatic and fish environment. Most enteric pathogens easily share genes for antimicrobial resistance, and the continuous selective pressure applied by the over the counter availability of these agents, as well as the prescription of these agents at most clinic visits, has potentially lethal consequences for a region plagued by epidemics of cholera. Judicious use of antimicrobial therapy requires the education of health workers and patients, adequate laboratory diagnostic capabilities, and government regulation [53].

None of the *E. coli, Salmonella*, and *V. cholerae* were resistant to ciprofloxacin. Several studies have shown that ciprofloxacin offers advantages in the treatment of salmonellosis, reaching high concentrations in serum and faeces [55]-[56]. Widespread use of ciprofloxacin for treating

all *E. coli*, *Salmonella*, and *V. cholerae* infections should be discouraged to avoid selection of resistant strains. This study provide valuable information to the ministry of medical services and public health, fisheries and other agencies in making policy decisions aimed at reducing microbial contamination of fish and water, and the indiscriminate use of antibiotics. There is need for research on antibiotic susceptibility surveillance in the aquatic environments where fresh fish and water are obtained for human consumption.

ACKNOWLEDGMENT

The authors acknowledge Maseno University, School of Public Health and Community Development, Department of Biomedical Science and Technology for allowing the research to be undertaken in their premises, and the School of Graduate Studies for administrative support. This research was supported by funds from Commission of Higher Education (CHE), Kenya, held by Prof. Ayub V. O. Ofulla, and by International Society of Infectious Diseases (ISID)-USA small grant awarded to Jackson H. O. Onyuka.

REFERENCES

[1] B. A. McCormick, S. P. Colgan, C. Delp-Archer, S. I. Miller, and J. L. Madara,. (1993) *Salmonella typhimurium* attachment to human intestinal epithelial monolayers: Transcelluar signaling to subepithelial neutrophile. *Journal of Cell Biology*. 123:895-907, 1993.

[2] K. K. Chao, C. C. Chao, and W. L. Chao. Suitability of the traditional microbial indicators and their enumerating methods in the assessment of fecal pollution of subtropical freshwater environments. *Journal of Microbiology Immunological Infection.* vol 36, pp 288-293, 2003.

[3] E. E. Geldreich E. E. Bacterial populations and indicator concepts in feces, sewage, storm water and solid wastes. In G. Berg (ed). *Indicators of viruses in water and food*. Ann Arbor Science Publishers, Inc., Orlando, Fla. 1983, pp 51-97, 183.

[4] S. S. Gaikwad, and Y.Y. Parekh. Salmonellosis in the systemic form due to multiple drug resistanct Salmonella typhimurium. *Journal of Postgraduate Medicine*, vol. 30, pp. 159-162, 1984.

[5] Center for disease Control and Prevention. Division of epidemiology and surveillance capacity development, Annual report. 2006.

[6] World Health Organization. *Annon*. "Cholera" (2010). http://www.who.int/topics/cholere/en/index.html.

[7] R. L, Shapiro, P. Waiyaki, B. L. Nahlen, L. Slutsker, and R. O. Muga. Transmission of *Vibrio cholerae* O1 in rural western Kenya associated with drinking water from Lake Victoria: an environmental reservoir for cholera. *Am. J. of Tropical Medicine and Hygiene.* Vol. 60, 271-276, 1999.

[8] J. C. Acosta, C. M. Galinndo, J. Kimario, K. Senkoro, and G. Urassa. Cholera outbreak in southern Tanzania: risk factors and patterns of transmission. *Emergence of Infectious Diseases.* Vol. 7(3), pp. 583-587, 2001.

[9] E. C. Ibezim. Microbial resistance to antibiotics. *African journal of Biotechnology* Vol. 4 (13) pp. 1606-1611, 2005.

[10] D. P. Blake, K. Hillman, D. R. Fenlon, and J. Low. (2003). Transfer of antibiotic resistance between commensal and pathogens members of the *Enterobacteriaceae* under ileal conditions. Journal of Applied Microbiology, vol. 95, pp. 428–436, 2003.

[11] E. J. Threlfall, L. R. Ward, J. A. Frost., and G. A. Willshaw. The emergence and spread of antibiotic resistance in food-borne bacteria. *International Journal of Food Microbiology*. 2000 62:1-5.training programme.

[12] Joint Expert Technical advisory Committee on Antibiotic Resistance. The use of antibiotic on food producing animals. In: antibiotic resistant bacteria in animals and humans. 1999.

[13] R. J. Bywater. (2004). Veterinary use of antimicrobials and emergence of resistance in zoonotic and sentinel bacteria in the EU. Journal of Veterinary Medicine. vol. 51, pp. 361-363, 2004.

[14] P. K. Ashok. Bacterial resistance of antimicrobial agents and microbiological quality among *E. coli* isolated from dry fishes in southeast coast of India, 2008.

[15] T. W. Schwatz, Kohenen, and B. Jansen. Detection of antibiotic resistant bacteria and their resistant genes in wastewater, surface water and drinking water biofilms. *FEMS Microbiol Ecol*, vol. 43, pp. 325-335, 2003.

[16] M. D. Onyango, S. Wandili, R. Kakai, and E. N. Waindi. (2009). Isolation of *Salmonella* and *Shigella* from fish harvested from Winam gulf of Lake Victoria. *J. of Inf. in Dev. Count.* Vol. 3(2), pp. 99-104, Sep. 2009.

[17] T. V. R. Pillay. Fish and Public Health and disease. In Aquauclture, Principles and practices, Pillay, TVR (Ed.). Fishing News Book, Famham, UK., ISBN: 0-85238-168-9 1990 pp: 174-215.

[18] M. C. Guzman, M. A. Biston, L. M. Tamagninii, and R. D. Gonzalez. (2004). Recovery of *Escherichia coli* in fresh water, *Jenynsia mulidenttata* and *Bryconamericus inhering*. *Water Research*. Vol. 38, pp. 2368-2374, 2004.

[19] P. N. Acha and B. Szyfres. Zoonoses and communicable diseases common to man and animals. Vol. 1 Bacterioses and Mycoses 3rd ed. Scientic and Technical Publication No. 580. Pan American Health Organization, Regional Office of the WHO, Washington, USA, 384, 2003.

[20] R. Hayashi, K. Horada, S. Mutsuhashi, and M. Inoue. Conjugation of drug resistance plasmid from *V. anguillarum* to *V. parahaemolyticus*. *Microbial Immunology*. Vol. 26, pp. 479-487, 1982.

[21] M. Masette. A comparative study of storage tissue of warm and cold water fish in view of the current market demands. A PhD thesis, United Nations University, UNU- Fisheries training programme 1999.

[22] L. E. Wyatt., R. Nickelson, and C. Vanderzant. (1979). Occurrence and control of *Salmonella* freshwater catfish. *Journal of Food Science*. Vol. 44 pp. 1069-1073, 1979.

[23] R. O. Abila. Economic analysis of the domestic and export markets of Kenya's Nile perch and its products. In: Proceedings of FAO expert consultation on fish technology in Africa (Kisumu, Kenya). Report no. 574 FAO (Rome). pp. 254-260, 1998.

[24] S. A. Abdullahi, D. S. Abolude and R. A. Ega. (2001). Nutrient quality of four oven dried freshwater catfish species in Northern Nigeria. *Journal of Tropical Biosciences.* Vol. 1(1) pp. 70-76, 2001.

[25] N. K. Gitonga. (2006). Approaches to achieving safety of fish and fishery products in East Africa. Fisheries department of Kenya Lake Victoria Environment Management Programme. 2006.

[26] Kenya National Bureau of Statistics. The 2009 Kenya population and housing census. Volume 1A, August, 2010.

[27] AOAC. Official Methods of Analysis of AOAC International 16th edition. Methods 950.46, Washington D. C, 1995.

[28] APHA/AWWA/WEF. (1998). Standards Methods for the examination of water and wastewater. 20th edition. American Public Health Association/American Water works Association/Water Environment Federation,Washington, DC, USA,ISBN:0-87553. pp 235-7, 1998.

[29] S. Tharannum, S. N. J. Sarah, M. Chandini, J. Vanitha, T. S. Manjula, and S. C. Shyam. Molecular confirmation of the presence of coliforms in drinking water using polymerase chain reaction *Kathmandu Uni. J. of Sc. Eng. and Tech*. Vol. 5, pp. 130-136, 2009.

[30] J. Cappuccino and N. Sherman: Microbiology- A laboratory manual (8th ed). Benjamin-cummings Publisher Co Inc., New York, pp. 137-149, 2007.

[31] W. F. Harrigan. *Laboratory methods of food microbiology*, 3rd edition (pp 165-183). Sandiego: Academic Press Ltd 1998.

[32] F. Kauffmann. The Bacteriology of enterobacteriaceae. Munksgaard, Copenhagen, Denmark, 1966.

[33] M. Y. Popoff, J. Bockemühl, and F. W. Brenner. Supplement 1998 (no. 42) to the Kauffmann-White scheme. *Res. Microbiology*. Vol.151, pp. 63–65, 2000.

[34] M. Y. Popoof, and L. Le Minor. Antigenic formula of *Salmonella* serovars. 8th edition. World Health Organization Collaborating Center for Reference and Research on *Salmonella*. Pasteur institute, Paris, France. 2003.

[35] National Centre for Infectious Diseases. Laboratory Methods for the Diagnosis of Epidemic Dysentery and Cholera. Center for Diseases Control and Prevention, Atlanta, Georgia, USA, pp. 49-50, 1999.
[36] A. W. Bauer, and J. C. Kirby. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. Vol. 45 pp. 493-496, 1999.
[37] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests. Approved standards M2-M9. Wayne, PA. CLSI. 2007.
[38] J. Baudart, K. Lemarchand, A. Brisabois, and P. Lebaron. (2000). Diversity of Salmonella strains isolated from the aquatic environment as determined by serotyping and amplification of the ribosomeal DNA spacer regions. *Applied environmental microbiology*. Vol. 66, pp.1544-1552 2000.
[39] S. G. Robin, A. S. Posa, V. Joeli, R. Alan, C. Clire, P. Craiy and L. Alena. (2004). Royal Swedish Academy of Science. *Ambio* 2004. Vol. 33: No. 1-2 pp1-23.
[40] Annual bulletin for Health service Access utilization and coverage, Nyanza, Dept. of HMIS, MoH, 2007.
[41] M. Vital, H. P. Fuchslin, F. Hammes, and T. Egli. (2007). Growth of *Vibrio cholerae* O1 Ogawa eltor in freshwater. *Microbiology*. Vol. 153 pp. 1993-2001, 2007.
[42] M. S. Islam, B. S. Drasar, and R. B. Sack. The aquatic environment as reservoir of Vibrio cholerae: A review *J. of Diarrhoeal Diseases Research*. Vol. 11, pp. 197-206, 1994.
[43] P. Watnick, and R. Kolter. Steps in development of a *Vibrio cholerae* biofilm. Mololecular Microbiology. Vol. 34 pp. 586-595, 1999.
[44] J. Reidl, and K. Klose. *Vibrio cholerae* and cholera: Out of water and into the host. *FEMS Microbiol. Rev*. Vol. 26, pp. 125-139, 2002.
[45] A. Worden, M. Seidel, S. Smriga, A. Wick, and F. Malfatti. Trophic regulation of *Vibrio cholerae* in costal marine waters. *Environmental Microbiology*. Vol. 8, pp. 21-29, 2006.
[46] S. Sirinavin, and P. Garner. Antibiotics for treating salmonella gut infections. Cochrane Database Systems Review. CD001167, 2000.
[47] M. D. Onyango, F. Machoni, R. Kakai, and E. N. Waindi. (2008). Multidrug resistance of *Salmonella* enteric serovars *Typhi* and *Typhimurium* isolated from clinical samples at two rural hospitals in western Kenya. *J. of Infec. in Dev. Count.* Vol. 3(2), pp. 99-104, Jan. 2008.
[48] A. W. Sifuna, E. N. M. Njagi, P. Okemo, A. Munyalo, G. O. Orinda. (2008). Microbiology quality and safety of *Rastrineobola argentea* retailed in Kisumu town markets, Kenya. *East Africa Medical Journal*. Vol. 85(10), pp. 509-13, Oct. 2008.
[49] D. A. Sack. Antimicrobial resistance in shigellosis, cholera and compylobacteriosis. In Background document for the World Health Organization global strategy for containment of antimicrobial resistance (2001).
[50] R. Laxminarayan. *Antibiotic resistance*: An emerging environmental health threat. Washington, DC: RFF press, 2002.
[51] C. A. Hart, and S. Kariuki. (1998). Antimicrobial resistance in developing countries *BMJ*. Vol. 317, pp. 647-650, 1998.
[52] F. Gallarda, J. Ruiz, F. Marco, K. J. Towner, and J. Vila. Increase in incidence of resistance to ampicillin, chloramphenical and trimethoprim in clinical isolates of *Salmonella* serotype *typhimurium* with investigation of molecular epidemiology and mechanisms of resistance. *J. Med. Microbiol*. Vol. 48 (4):, pp. 367-74, 1999.
[53] Kariuki, M. Gichia, R. Kakai, D. Kusenererwa, W. Macharia, T. Menge, S. Morpeth, G. Mwabu, L. Ndegwa, B. OlackB, J. Orwa, J. Pandit, G. Revathi, C. Winters, H. Gelband and R. Laxminarayan. Situation analysis: Antibiotic Use and Resistance in Kenya. Global Antibiotic Resistance Partnership – Kenya country report, 2011.
Available at: www.resistancestrategies.org
[54] R. Kakai. Antibiotic resistance in Western Kenya. Global Antibiotic Resistance Partnership (GARP) inaugural meeting. Fairview Hotel Nairobi, Kenya. 6th – 7th Aug 2009.
Available at: http://www.docstoc.com
[55] G. Eduardo, S. Carlos, E. Juan, C. Carlose, M. Rose, and R. Rose. Ciprofloaxacin in the treatment of cholera: A randomized double blind control clinic trial of a single dose in Peruvian adults.1995.
[56] E. J. Threlfall, J. A. Skinner, and L. R. Ward. (2001). Detection of decreased in vitro susceptibility to ciprofloxacin in *Salmonella enterica* serotypes Typhi and Paratyphi *A. J Antimicrob Chemother* Vol. 48(5), pp 740-1, Nov, 2001.