International Journal for Biotechnology and Molecular Biology Research Vol. 2(9), pp. 150-162, 7 September, 2011
Available online http://www.academicjournals.org/IJBMBR
ISSN 2141-2154 ©2011 Academic Journals

Full Length Research paper

Bacteriological quality and diarrhoeagenic pathogens on River Njoro and Nakuru Municipal water, Kenya

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Accepted 18 August, 2011

Waterborne diarrhoeal pathogens are considered a re-emerging threat and are responsible for considerable morbidity and mortality, especially in developing countries. The objective of this study was to determine bacteriological quality, biochemical oxygen demand and presence of diarrhoeagenic pathogens in water samples collected from River Njoro and Nakuru Municipal water. A total of 432 samples were tested, 216 from River Njoro and 216 from Nakuru Municipal water. Bacteria indicator numbers (arithmetic mean MPN/ml) varied from 24.4 (source) to >2700.0 (midstream) for total coliforms and 3.6 (source) to 1880.0 (midsream) for faecal coliforms in River Njoro. There was a consistent increase in bacteria loading as the river flowed from the source (Nessuit) to downstream sites. The biochemical oxygen demand (BOD) ranged from 2.0 mg/L at the source of the river to 44.0 mg/L at Njoro bridge. The frequency of enteropathogenic bacteria isolated in 216 samples collected from River Njoro were; *Aeromonas hydrophila* 52%, *Hafnia alvei* 29.2%, *Salmonella typhimurium* 18%, *Salmonella typhi* 17%,Enteroaggregative *Escherichia coli* 9.2%, Necrotoxigenic *E. coli* 7.4% and Enteropathogenic *E. coli* 3.2%. Only *H. alvei* 4.6% and *A. hydrophila* 6.5% were isolated from Nakuru Municipality water and no enteropathogens were detected at Nakuru town centre, Kiti and Milimani estates. River Njoro has been found to be heavily contaminated with indicator bacteria, organic material and diarrhoegenic pathogens. This suggests need to educate people regarding good health practices, proper waste disposal, boiling drinking water and seek alternative sources of drinking water in the study area.

Key words: Biochemical oxygen demand, coliforms, diarrhoea.

INTRODUCTION

Waterborne bacterial pathogens are considered re-emerging threat causing acute and chronic diarrhoea among the infants, elderly and immunosuppressed individuals. In developing countries most of the rural communities are poverty-stricken, lack access to potable water supplies and rely mainly on polluted river, stream, well and pond water sources for their daily water needs (WHO, 2003; Nevondo and Cloete, 1999). In areas where potable water supplies have been provided, these supplies are unreliable and insufficient; forcing residents to revert to traditional contaminated river sources (WWAP, 2006).

Kenya is a water-scarce country and water-borne diseases have proven to be a prime threat to human health in the country as in other parts of the world (WWAP, 2006). Consequently, a significant proportion of residents in rural communities in Kenya are exposed to water-borne disease and their complications (Shapiro et al., 2001). These diseases cause crippling, devastating and debilitating effects on rural residents and further exacerbate the already strained health burden and facilities in the country (Kariuki et al., 1994; Kiruki et al., 2006).

High prevalence of undiagnosed diarrhoea at Njoro Health Centre and Nakuru Provincial General Hospital (NPGH) has been closely linked to consumption of pathogen-polluted waters (NPGH, 2009). In 2001 to

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2004, 5883 diarrhoea cases were reported at Nakuru provincial general hospital (NPGH) and pathogens isolated include; *Entamoeba histolytica* (10%), *Giardia lamblia* (2%), *Escherichia coli* (1%), *Salmonella typhi* (27%) and other parasites (12%). Of the cases reported, 48% of them were not diagnosed and were treated empirically (NPGH, 2009). It has been alleged that the reported cases of water related diseases at Njoro Health Centre account for over 50% of all illness (NPGH, 2009).

River Njoro (60 km) drains a small predominantly rural catchment (280 km^2) in southwestern Kenya, approximately 160 km northwest of Nairobi (Figure 1). It is a high altitude stream with its source at the eastern segment of Mau Hills (2700 m a. s. l) (Mathooko, 2001). It is the major source of water for domestic purposes (drinking, washing, cooking and bathing), industrial and agricultural use for the surrounding community including Nakuru town, Njoro town, Kenya Agricultural Research Institute (KARI) and Njoro Canning factory. Previous studies on River Njoro have reported the deteriorating state of affairs with regard to changes in land use within the catchment area. This is believed to have seriously affected water quality, especially the middle and lower reaches, where the stream is seriously polluted (Kundu et al., 2004; Yillia et al., 2008; Yillia et al., 2009). Despite the poor water quality status, many residents depend on the stream for their daily water needs as water supply in the riparian settlements is acutely inadequate (Yillia et al., 2008). There is no information on the prevalence of diarrhoegenic pathogens in River Njoro and as a consequence, the ecology, epidemiology and identity of these bacteria remains poorly understood.

Faecal coliforms and *E. coli* are of great importance among bacterial indicators used in water quality definition and health risks (Giannoulis et al., 2005). The large numbers of *E. coli* present in human gut and the fact that they are not generally present in other environments support their continued use as the most sensitive indicator of faecal pollution available (Edberg et al., 2000). Baudizsova (1997) found that the other thermo-tolerant and total coliforms were capable of growth in non-faecal polluted river water while *E. coli* was not and supports recommendation for *E. coli* to be used as the sole indicator bacteria for faecal contamination (Tallon et al ., 2005).

BOD was used to infer the general quality of the water and its degree of pollution by biodegradable organic matter including domestic sewage. Most pristine rivers have a BOD_5 of less than 1 mg/L. Moderately polluted rivers may have BOD in the range 2 to 8 mg/L (Clair et al., 2003). Excessive BOD can result in death of fish and other oxygen-sensitive aquatic species. In humans excessive BOD can lead to increased incidence of gastrointestinal diseases including diarrhoea, caused by exposure to pathogenic bacteria in water (WWAP, 2006).

This study assessed the microbial quality of River Njoro and Nakuru Municipal water. Moreover, potential enteropathogens were isolated and further characterized using microbiological, serological and biochemical techniques. Direct detection of pathogens gave the best evidence of microbial contamination.

MATERIALS AND METHODS

Sample collection and processing

The sample size was calculated by the formula of Lwanga and Lememshows (1991) using prevalence rate of 16%. Prevalence was estimated by counting each water sample testing positive once, regardless of the number of isolates (Pedersen et al., 2006). The high sample size (216) was used in order to increase the power and reliability of the study and its findings.

$n = Z^2.p.q/d^2$

n = the desired sample size, p = prevalence of condition under study, q = 1–p, Z = standard normal deviation = 1.96 (from the tailed normal table).

$1.96^2 (0.16X0.84)/0.05^2$

$= \frac{3.84 \times 0.1344}{0.0025} = 206$ water samples.

The sites sampled on river Njoro were Nessuit, Beeston, Njokerio, Njoro canning, Njoro bridge and Ngata point (Figure 1). Likewise, Nakuru Municipal water from Milimani, Kiti, Ronda, Shabab, Nakuru town centre and Bondeni sites were similarly analyzed. The sampling sites at River Njoro were selected to encompass potential sources of contamination included: pristine watersheds, farming areas, sewage polluted and industrial discharge points. The study was performed in Biochemistry Laboratory, Egerton University, Kenya, between January 2007 and March 2009. Water samples for bacteriological examination were collected aseptically using sterile water-sampling bottles containing 1% Tryptone water. Samples were collected to the volume of 100 ml in sterile glass bottles. All samples were immediately sealed, labeled, placed in dark containers and processed within 4 h of collection to ensure sample integrity. Water samples were pre-enriched in buffered peptone water (BPW) and incubated at 37°C for 3 h then filtered through 0.22 µm cellulose nitrate membrane (Whatman GmbH, Germany). A total of 432 samples were collected from the 12 different sites of River Njoro and Nakuru Municipality during the dry and rainy seasons.

Biochemical oxygen demand (BOD)

The biochemical oxygen demand (BOD) was used as an indication of the extent of organic pollution in water sources. Five-day BOD ($BOD_{5)}$) levels in water samples were determined using standard laboratory procedures (APHA, 2008). BOD_5 levels in water samples from River Njoro were determined using Lovibond OxiDirect BOD meter (Dortmund, Germany) and by ensuring inhibition of nitrification during incubation. The BOD_5 range of 0 to 200 mg/L was selected. Water samples were prepared by checking pH to conform to the required pH range (pH 6.5 to 7.5).

Enumeration of coliforms

Coliforms were estimated using a three-tube most probable number method (MPN) according to American Public Health Association (APHA) Standard Methods (2008). Depending on the water type, river water or municipal water, dilutions of 10^1 to 10^{-4} (10.0, 1.0, 0.1,

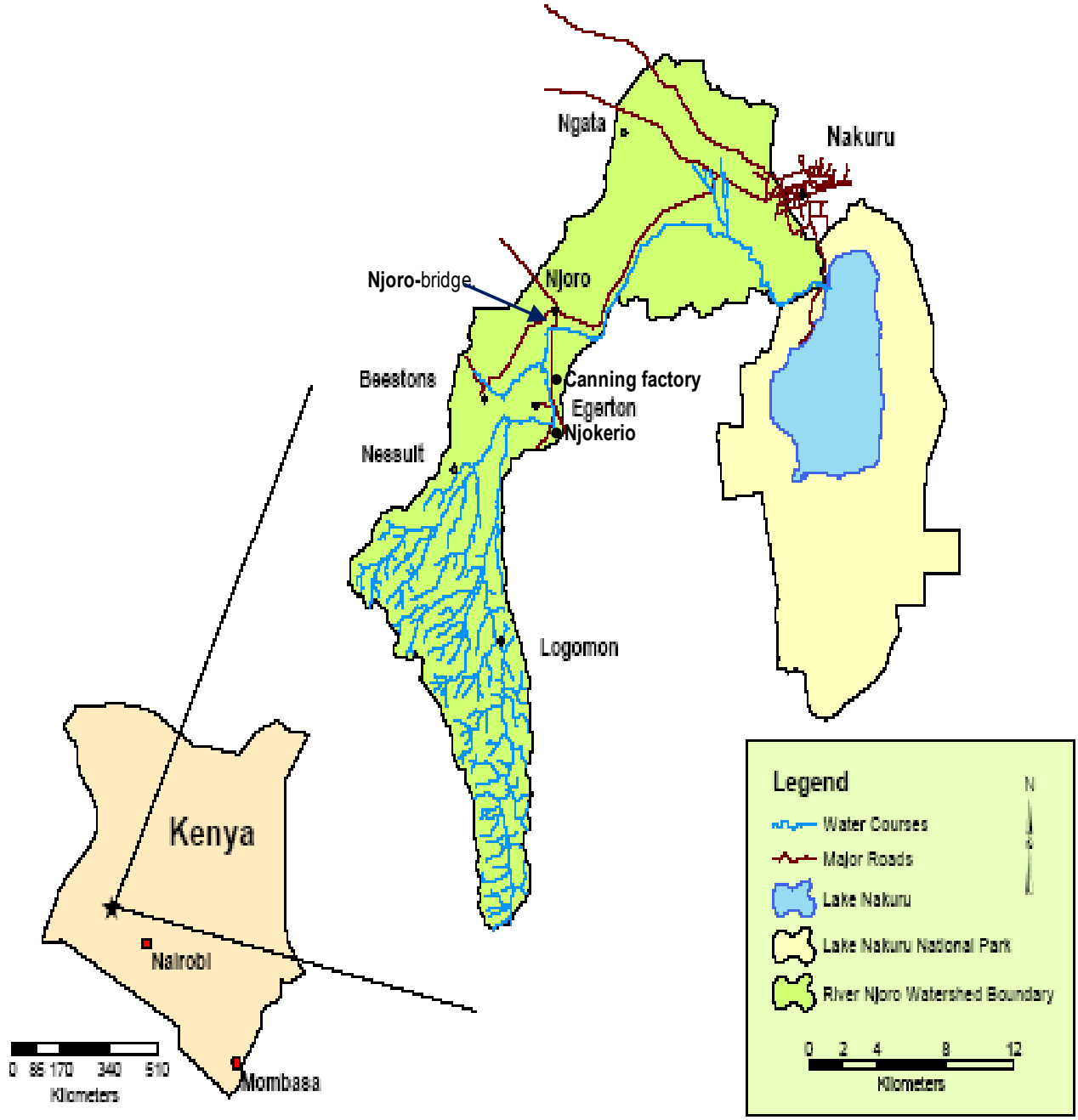

Figure 1. Sampling sites at River Njoro and Nakuru Municipal water.

0.01, 0.001, 0.0001 ml) were prepared in 1 ml of 0.1% buffered peptone water (BPW) (Oxoid Ltd, UK) and 1 ml of each dilution inoculated into six tubes. Three tubes from each dilution were incubated at 37°C and three at 44.5°C. Tubes showing acid and gas productions after incubation at 37°C were scored as positive for total coliforms. Tubes showing acid and gas at 44.5°C after 24 h were confirmed as faecal coliforms by plating on MacConkey and Eosin Methylene Blue and examining for typical colonies. Counts per 1 ml were calculated from MPN tables as described in the United States Department of Agriculture, Food Safety and Inspection Services (USDA-FSIS, 2008).

Identification of enteropathogenic bacteria

Isolation of *E. coli*

Membrane filters (Whatman GmbH, Germany) were then aseptically removed from the membrane holder, inverted and re-cultured on nutrient agar, then subcultured on MacConkey agar and Eosin Methylene Blue (EMB) agar and incubated for 24 h at 37°C. All morphologically distinct colonies that were dark pink (that is, strong lactose fermenters) on MacConkey agar and Blue-black colonies having a greenish metallic sheen on EMB agar were

streaked onto blood agar plates and incubated for 18 to 24 h at 37°C. Isolated colonies were confirmed as *E. coli*-positive using three biochemical tests: indole (+), oxidase (-) and potassium hydroxide (+) (Cheesbrough, 2004) and API-20E (BioMerieux, Durham, North Carolina, USA). Each isolate identified as *E. coli*-positive was enriched in Buffered peptone water (BPW) or Tryptic Soy Broth (TSB) and stored at -80°C. Positive (established strains from American Type Culture Collection or other known sources) and negative (*E. coli* K-12) controls were included for each assay.

Isolation of *H. alvei*

Membrane filters (Whatman GmbH, Germany) were then aseptically removed from the membrane holder, inverted and re-cultured on nutrient agar, then subcultured on Hektoen enteric agar and incubated for 24 h at 37°C. All morphologically distinct colonies that were blue-black (non-lactose fermenters) were streaked into blood agar plates and incubated for 18 to 24 h at 37°C. Isolated colonies were confirmed as *H. alvei*–positive using API-20E (BioMerieux, Durham, North Carolina, USA). Each isolate identified as *H. alvei*-positive was enriched in Buffered peptone water (BPW) or Tryptic soy broth (TSB) and stored at -80°C.

Isolation of *Salmonella* species

Water samples were pre-enriched in Selenite F broth and incubated at 37°C for 3 h then filtered through 0.22 µm cellulose nitrate membrane (Whatman GmbH, Germany). Membrane filters were then aseptically removed from the membrane holder, inverted and re-cultured on nutrient agar, then subcultured on Xylose Lysine Deoxycholate (XLD) agar and incubated for 24 h at 37°C. All morphologically distinct colonies with pink black-centred colonies were inoculated onto Triple sugar agar slant and incubated for 18 to 24 h at 37°C to identify reactions typical of *S. typhi*. Most *Salmonella* species produce a K/AG H_2S^+ reaction (Alkaline/Acid and gas) on Tripple Sugar Iron Agar (TSI). However, *S. typhi* can be biochemically differentiated from other *Salmonellae* species by being citrate negative, not producing gas and forming only small amounts of H_2S on TSI (MacFaddin, 2000). *S. typhimurium* caused cracks on the TSI agar due to gas production and large amount of H_2S produced. Each culture was then streaked into blood agar plates and incubated for 18 to 24 h at 37°C. All presumptive positive isolates were also tested with API-20E (BioMerieux, Durham, North Carolina, USA). Each isolate identified as *S. typhi*-positive was enriched in Tryptic Soy Broth (TSB) and stored at -80°C.

Isolation of *A. hydrophila*

Membrane filters (Whatman GmbH, Germany) were then aseptically removed from the membrane holder, inverted and cultured on Aeromonas Medium Base (RYAN) Agar (Oxoid, UK), then subcultured on Bile Aesculin Agar (Oxoid, UK-Basingstoke) and incubated for 24 h at 37°C. Aeromonas agar was impregnated with ampicillin (5.0 µg/ml) as an additional selective agent to screen for and isolate *Aeromonas* species. Isolated colonies were confirmed as *A. hydrophila*–positive using API-20E (BioMerieux, Durham, North Carolina, USA). Reference strains used as positive controls were *A. hydrophila* 7966 ATCC and *E. coli* 25922 ATCC. Sterile deionized water was used as a negative control.

Biochemical characterization of isolated bacteria

API-20E test strip (BioMerieux, Inc., USA) was used to identify the enteric Gram-negative rods (Murray et al., 1999).

Serological testing of pathogens

Serological identification was carried out on *E. coli* and *S. typhi* to identify pathogenic strains. Isolated cultures were inoculated on Nutrient agar plates overnight and then typed against their respective antisera as described by Bopp et al. (2003) for 'O' and 'H' agglutination reactions. Selected strains were subjected to slide agglutination with polyvalent antisera (Behring, Marburgy, Germany) against somatic antigens of NTEC (O2, O4, O4:H, O4:H111 O11, O12, O25); EAEC (O55, O111, O125) and EPEC (O128:H12, O142, O119:H6) serotypes.

Statistical analysis

The results on most probable number (MPN) values were analysed using paired t-test to compare total or faecal coliforms in the wet and dry season respectively. Paired t-test was also used to compare River Njoro BOD_5 levels during the dry and wet season. ANOVA (analysis of variance) and Post Hoc tests (Duncan, version 17.0) were used to compare total coliforms, faecal coliforms and BOD levels along different sites of River Njoro. $P<0.05$ value was considered to indicate statistically significant differences. The SPSS PC 17.0 software package (SPSS Inc., Chicago, III., USA) was used for data analysis.

RESULTS

Water quality and microbiological analysis

It is evident from the results that Nakuru Municipal water from Town centre, Kiti and Milimani estates were satisfactory for drinking purposes since the water samples had mean total coliform count below 0.03 MPN/ml (Table 1). However, the Nakuru Municipal water from Ronda and Bondeni estates were contaminated with coliforms counts of >3.0 MPN/ml. The arithmetic mean numbers of indicator bacteria at six sites on River Njoro are shown in Table 2. There was a consistent increase in bacterial loading as River Njoro flowed downstream from its source (Nessuit). The seasonal variation in bacterial indicators during the dry (December to April) and wet (May to October) seasons shows that significantly higher faecal coliform counts ($P \leq 0.05$) were recorded at Njoro canning, Njoro bridge and Ngata sites during the wet season compared with the dry season but there were no significant difference in total coliforms ($P \geq 0.05$) between the dry and wet season along the six sites. River Njoro water was grossly polluted with the detected total coliforms being as high as 1388.0 MPN/ml during the dry season and >2700.0 MPN/ml during the rainy season at Njoro bridge.

At both upstream (Nessuit point) and downstream (Njoro bridge) the coliform counts were increasing. Data for the period, January 2007 to March 2009 indicates that the total coliforms level at Nessuit were in the range of 3 to 10 times higher than the maximum permissible level of 3 MPN/ml specified for drinking purposes. The scenario

Table 1. Bacteriological assessment of water quality from Nakuru Municipal water.

Water source	Seasons					
	Total coliforms (MPN ml^{-1})		P value	Faecal coliforms (MPN ml^{-1})		P value
	Wet	Dry		Wet	Dry	
Shabab						
Mean± SD	1.63±0.61	2.50± 0.31	0.006	0.10 ±0.12	0.091 ±0.12	**0.940**
Range	0.93-2.40	2.10-2.90		0.03- 0.30	0.03- 0.30	
Bondeni						
Mean± SD	7.30±1.07	4.72±0.96	0.044	2.00±0.58	0.09 ±0.12	**0.001**
Range	5.70-8.70	4.00-6.40		1.20-2.60	0.03-0.30	
Town centre	<0.03	<0.03		<0.03	<0.03	
Kiti	<0.03	<0.03		<0.03	<0.03	
Milimani	<0.03	<0.03		<0.03	<0.03	
Ronda						
Mean± SD	9.60±1.87	4.52 ±1.10	0.010	7.36±2.00	4.14 ±1.33	**0.023**
Range	7.40- 12.00	3.70-6.40		4.50- 9.40	3.00- 6.40	

Results are expressed as mean ± SD for five replicates. P values in bold represent significant difference in the total and faecal coliforms between the wet and dry season (Student's t-test).

Table 2. Bacteriological assessment of water quality from River Njoro.

Water source	Seasons					
	Total coliforms (MPNml^{-1}		P value	Faecal coliforms (MPN ml^{-1})		P value
	Wet	Dry		Wet	Dry	
Nessuit						
Mean± SD	35.00 ±7.54	30.00 ±5.87	0.110	3.66 ±0.78	4.54±2.78	**0.586**
Range	27.00-43.00	25.00-40.00		2.90-4.60	2.30- 9.40	
Beeston						
Mean±SD	47.60± 6.67	24.40± 3.90	0.084	4.42± 1.02	4.32±3.16	**0.960**
Range	29.00-64.00	17.00- 38.00		3.60-6.10	0.72-9.40	
Njokerio						
Mean±SD	148.00±35.64	110.00±18.61	0.163	101.60±12.84	101.80±12.70	**0.970**
Range	110.00-200.00	93.00-140.00		90.00-120.00	90.00-120.00	
Njoro canning						
Mean±SD	1780.00^{a}±507.00	1066.00^{a}±542.00	0.145	1440.00^{a}±798.70	414.00^{a}±24.80	**0.045**
Range	1100.00-2400.00	640.00-2000.00		400.00-2300.00	380.00-440.00	
Njoro Bridge						
Mean±SD	2700.00^{a}±1177.00	1388.00^{a}±536.00	0.083	1880.00^{b}±486.80	520.00^{a}±165.40	**0.002**
Range	1500.00-4600.00	900.00-2100.00		1100.00-2400.00	380.00-750.00	

Table 2. Contd.

Ngata						
Mean±SD:	2060.00[a]±1250.00	984.00[a]±372.70	0.064	1146.00[a]±282.10	466.00[a]±101.40	**0.013**
Range:	900.00-4100.00	640.00-1600.00		900.00-1600.00	380.00- 640.00	

Results are expressed as mean + SD for five replicates. P values in bold represent significant difference in the total and faecal coliforms between the wet and dry season (Student's t-test). abP<0.05 represent significant difference in total or faecal coliforms in River Njoro along the sampled sites (ANOVA, Post Hoc tests).

Table 3. Summary of biochemical oxygen demand of River Njoro water samples (2007-2009).

Section of the River	Wet season (BOD_5)(mg/L)	Dry season (BOD_5)(mg/L)	P values
Nessuit			
Mean±SD	3.00±0.35	2.00±0.23	**0.005**
Range	2.50-3.40	1.60-2.20	
Beeston			
Mean±SD	7.56[c]±2.14	4.28±1.29	**0.035**
Rang	5.80-10.00	3.00-6.00	
Njokerio			
Mean±SD	22.40[d]±2.61	19.14[c]±1.23	**0.015**
Range	19.80-26.00	7.30-20.60	
Njoro canning			
Mean±SD	31.10[e]±2.40	38.10[d]±1.90	**0.004**
Range	28.00-34.00	36.20-40.80	
Njoro Bridge			
Mean±S	36.20[f]±3.00	44.00[e]±5.70	
Range	32.00 -40.00	36.20-50.20	**0.012**
Ngata			
Mean±SD	43.60[g]±2.80	36.10[d]±1.50	**0.007**
Range	40.00-47.00	34.00-38.00	

Results are expressed as mean +SD for five replicates. P values in bold represent significant difference in the BOD5 between the wet and dry season (Student's t-test).cdefgP<0.05 represent significant differences in the BOD5 during the wet and dry season along the sampled sites (ANOVA, Post Hoc tests).

worsens as the river transverses through the Njokerio and Njoro canning factory. By the time the river gets to Njoro bridge, the total coliform count increases to over 900 times higher than the maximum permissible level of 3 MPN/ml.

Biochemical oxygen demand (BOD)

The BOD_5 levels in River Njoro during January 2007 to March 2009 are shown in Table 3. Mean BOD_5 levels ranged between 2.0 and 44.0 mg/L. In general, BOD_5 levels increased as the river flowed downstream with Njoro bridge recording the highest value at 44.0 mg/L and the lowest value was recorded at Nessuit (2.0 mg/L).

While the river is almost fit for drinking upstream (Nessuit), the scenario is really bad as it flows through populated Njokerio, Egerton and Njoro-bridge. The BOD_5 levels during wet season were higher than during the dry season at Njokerio point. However, the trend changed drastically as the river flowed past Njoro canning and Njoro bridge where the BOD_5 levels during dry season (38.1 and 44.0 mg/L) were higher than the wet season ((31.1 and 36.2 mg/L) respectively. Extreme values were

Table 4. Number of pathogenic *E. coli* and *S. typhi* serotypes.

*Isolate number	Serotype/strain	Number (Total)
E/RNJ/0908	O128:H12 (EPEC)	2
E/RNJ/0912	O142:NM (EPEC)	3
E/RNJ/0925	O119:H6 (EPEC)	2
E/RNJ/0806	O55 (EAEC)	8
E/RNJ/0907	O111(EAEC)	2
E/RNJ/0811	O125 (EAEC)	10
E/RNJ/0918	O4:H (NTEC)	8
E/RNJ/0919	O11 (NTEC)	1
E/RNJ/0923	O12 (NTEC)	3
E/RNJ/0935	O25 (NTEC)	3
Total		(43)
S/RNJ/0801-0936	'O':9,12,Vi: H (*S.typhi*)	(37)

EPEC (Enteropathogenic *E. coli*, NTEC (Necrotoxigenic *E. coli*), EAEC (Enteroaggregative *E. coli*). *- Similar serotypes though isolated from different sites and at different times.

observed at Ngata (43.6 mg/L) and Njoro bridge (44.0 mg/L). The least polluted was upstream (Nessuit) where BOD_5 values were found to be ≤ 3.0 mg/L. For unpolluted waters, BOD_5 values should range from 1 to 3 mg/L. The maximum permissible level of BOD_5 for bathing and drinking is 3 mg/L.

Presumptive Identification of diarrhoegenic pathogens from River Njoro and Nakuru Municipal water

A total of 432 water samples from River Njoro and Nakuru Municipal water were tested for enteropathogenic bacteria (Table 5). The combination of culturing bacteria on selective media, biochemical tests and serotyping revealed a variety of different bacterial species. They occurred in different water samples at varying frequencies. Bacteria isolates cultured on MacConkey, Eosin Methylene Blue, Hektoen enteric, S-S agar, XLD, Aesculin Bile agar and Aeromonas agars gave characteristic growth of *E. coli*, *H. alvei*, *S. typhi*, *S. typhimurium* (Figure 2A) and *Aeromonas hydrophila* (Figure 2B).

Serological and biochemical characterization of isolated bacteria

E. coli were detected in 100 of 216 (46%) water samples collected from River Njoro and 43% of these were pathogenic *E. coli*. All the presumptive *E. coli* isolates were subjected to serogrouping. A number of serogroups were identified in water samples that have been associated with NTEC (O2, O4, O11, O12, O25); EAEC (O55, O111, O125) and EPEC (O128, O142, O119:H6) serotypes. The total number of serotyped pathogenic *E. coli* and *S. typhi* are indicated in Table 4. Biochemical identification was confirmed for *E. coli*, *H. alvei, S. typhi* and *A. hydrophila* using API 20E strips (Biomerieux, USA) as shown in Figures 3 to 6. All the *E. coli* isolates were oxidase, tryptophan deaminase, H_2S, arginine dihydrolase negative and did not utilize citrate (Figure 3). *E. coli* fermented most of the common sugars including; glucose, mannose, sorbitol, rhamnose, sucrose, melibiose and arabinose (yellow) but were unable to ferment inositol and amygdalin (blue). All the isolates also elaborated the enzymes O-nitrophenyl-β-D-galactosidase(ONPG), tryptophanase (indole) and did not produce acetoin (VP) and gelatinase (GEL). These biochemical characteristics are typical for most *E. coli* strains.

H. alvei strains were oxidase and indole negative, lysine and ornithine decarboxylase positive, O-nitrophenyl-β-D-galactopyranoside positive and produced acetoin (VP) (Figure 4). Several reactions including ability to produce acetoin (VP), utilize citrate, ferment inositol and failure to hydrolyze tryptophan suggested that these isolates were *H. alvei* and not *E. coli* strains. Moreover, they could not ferment common sugars like sorbitol, sucrose and melibiose which are usually fermented by *E. coli*.

API 20E is designed to identify members of the family Enterobacteriaceae but a number of tests that it contains are also useful for the biochemical characterization and identification of other Gram-negative bacteria including *Aeromonads*. Therefore, API 20E strip was used in this study to biochemically characterize *A. hydrophila* isolates. *A. hydrophila* isolates were oxidase, indole, lysine decarboxylase, arginine dihydrolase and O-nitrophenyl-β-D-galactopyranoside positive, hydrolyzed esculin and produced acetoin (VP) (Figures 5 and 6).

Table 5. Number (percentage) of enteropathogenic bacteria at different sites along River Njoro and Nakuru Municipal water.

Sample site	Samples tested	EAEC	EPEC	NTEC	*H. alvei*	*S. typhi*	*S. typhimurium*	*A. hydrophila*
Shabab	36	0 (0)	0 (0)	0 (0)	2 (5)	0 (0)	0 (0)	2 (5)
Bondeni	36	0 (0)	0 (0)	0 (0)	8 (22)	0 (0)	0 (0)	7 (19)
Nakuru Town	36	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Kiti	36	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Milimani	36	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ronda	36	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (14)
Sub-total	216	0 (0)	0 (0)	0 (0)	10 (4.6)	0 (0)	0 (0)	14 (6.5)
Nessuit	36	0 (0)	0 (0)	0 (0)	8 (22)	0 (0)	3 (8.3)	3 (8.3)
Beestons	36	5 (14)	0(0)	0(0)	16 (44)	0 (0)	0 (0)	2 (5)
Njokerio	36	2 (5)	5 (14)	2 (5)	0 (0)	7 (19)	8 (22)	12 (33)
Njoro canning	36	5 (14)	1 (2.8)	1 (2.8)	3(8.3)	15 (42)	9 (25)	32 (88.9)
Njoro Bridge	36	8 (22)	1 (2.8)	12 (33)	1(2.8)	10(27)	14 (39)	36 (100)
Ngata	36	0 (0)	0 (0)	1 (2.8)	34 (94)	5 (14)	5 (14)	27 (75)
Sub-total	216	20 (9.2)	7(3.2)	16(7.4)	62(29.2)	37 (17)	39 (18)	112 (52)
Grand total	432	20 (4.6)	7 (1.6)	16 (3.7)	72 (17)	37 (8.5)	39 (9)	126 (29.2)

EPEC (Enteropathogenic *E. coli*, NTEC (Necrotoxigenic *E. coli*), EAEC (Enteroaggregative *E. coli*).

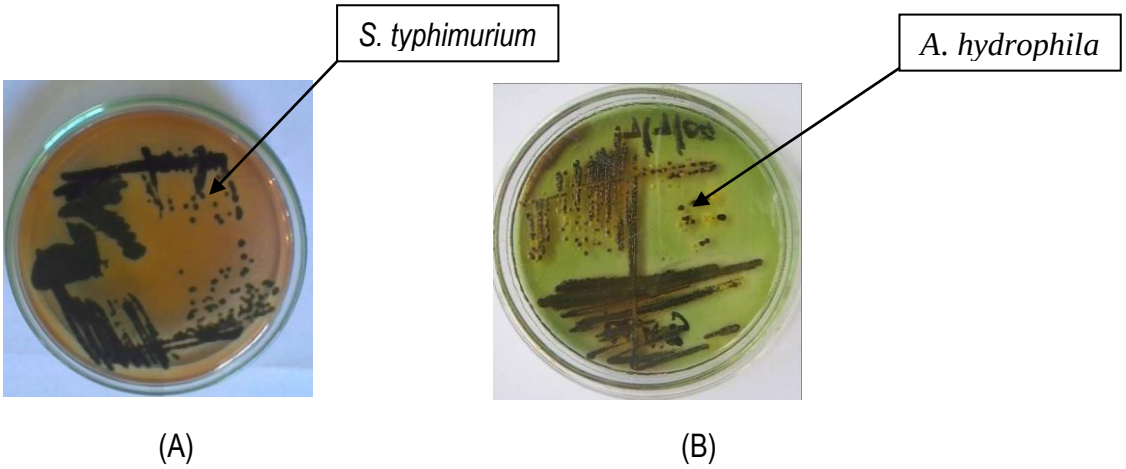

Figure 2. (A) S-S agar cultures showing black *S. typhimurium* colonies and (B) Aeromonas agar showing dark-brown colonies of *A. hydrophila*, isolated from Njoro bridge at River Njoro.

They fermented various sugars, including glucose, mannose, sucrose, amygdalin and arabinose. These biochemical characteristics were observed with ATCC 7966 *A. hydrophila* and are typical for most *A. hydrophila* strains.

S. typhi isolates were lysine decarboxylase and H_2S positive. Among the common sugars, only D-glucose, D-manose, melibiose and sorbitol were fermented. Several reactions were negative including ONPG, ADH, CIT, UREA, TDA, IND, VP, GEL and oxidase. *S. typhi* did not ferment inositol, rhamnose, sucrose, amygdalin and arabinose.

Overall, 73% water samples were positive for one or more enteropathogens. The enteropathogenic bacteria isolated from River Njoro were; *A. hydrophila* 52%, *H. alvei* 29.2%, *S. typhimurium* 18%, *S. typhi* 17%, Enteroaggregative *E. coli* 9.2%, Necrotoxigenic *E. coli* 7.4% and Enteropathogenic *E. coli* 3.2% (Table 5). No *E. coli* O157:H7 was isolated from both River Njoro and Nakuru Municipal water.

The only pathogens isolated from Nakuru Municipal water were *H. alvei* 4.6% and *A. hydrophila* 6.5% (Table 5). No pathogenic *E. coli*, *S. typhi* and *S. typhimurium* were isolated from Nakuru Municipal water. Water from Nakuru town centre, Kiti, Milimani estates was free from pathogenic contamination. The isolated enteropathogens

Figure 3. API 20E strips after inoculation with *E. coli.*

Figure 4. API 20E strips after inoculation with *H. alvei.*

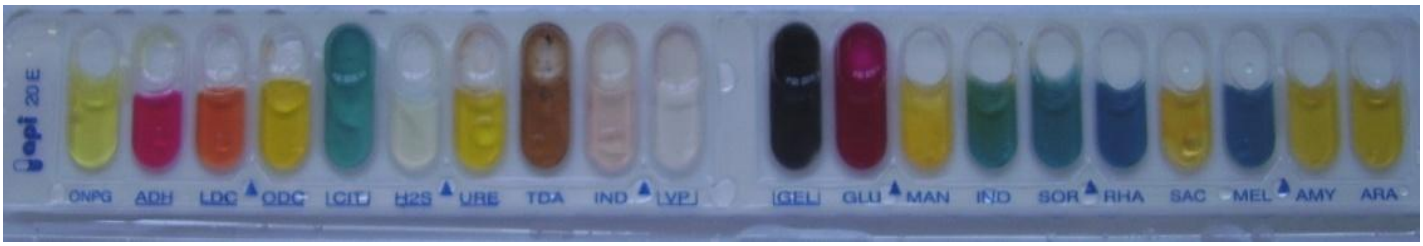

Figure 5. API 20E strips after inoculation with *A. hydrophila.*

cause numerous diarrhoea-associated diseases in the area including typhoid fever caused by *S. typhi*, gastroenteritis caused by *H. alvei* and *A. hydrophila* and diarrhoea caused by *S. typhimurium* and pathogenic *E. coli* (EPEC, EAEC and NTEC). Consequently, the potential health risk posed by the consumption of water from river sources by rural residents and consumers in the Njoro District must not be underestimated.

DISCUSSION AND CONCLUSIONS

Nakuru Municipal water from Town centre, Kiti and count Milimani estates were satisfactory for drinking purposes since the water samples had total and faecal coliforms below 0.03 MPN/ml (Table 1). However, the total coliform count from water collected from Bondeni and Ronda estates in Nakuru Municipality exceeded the set standards (3 MPN/ml) of treated water suitable for drinking. The samples collected during dry season had the lowest total coliform counts (4.72 and 4.52 MPN/ml) while the one sampled after the rains had the highest total coliforms (7.3 and 9.6 MPN/ml) at Bondeni and Ronda respectively. This could be due to leakages through cracks in the distributing pipes. The proximity of latrines and sewage lines also enhances the chances of cross contamination. Much more disturbing was the isolation of enteropathogens like *H. alvei* and *A. hydrophila* from Bondeni, Shabab and Ronda estates.

The fact that tap water is the most popular source of

Figure 6. API 20E strips after inoculation with A. *hydrophila* (ATCC 7966).

drinking water in these estates is a risk to human health.

This study indicated that River Njoro is extremely polluted with faecal material and that the quality substantially deteriorates as it flows from the source through the densely populated area of Njokerio, Njoro canning factory, Njoro bridge, Njoro secondary school and Njoro market to Lake Nakuru. Ideally, the water would be expected to be pristine in quality at its source but average bacterial numbers were moderately high for total coliforms (35 MPN/ml) and faecal coliforms (4.54 MPN/ml) in wet and dry seasons respectively (Table 2). This could be due to faecal contamination by birds and wild animals found in the Mau forest, catchment area. At Njokerio and Njoro bridge (KARI) the level of contamination is much higher because of the animal faeces from the main abattoir and agricultural fertilizers from the nearby farms. Moreover, the catchment area for the River Njoro has in the recent past undergone high levels of deforestation and human settlement, resulting in the decline of rainfall during the wet season.

Both total and faecal pollution in Njoro were significantly higher in the rainy season than in the dry season, presumably due to higher run-offs of animal wastes from pasture and rangelands in the nearby farms and slaughterhouses or discharge of improperly treated sewage to River Njoro during operational malfunctioning or during heavy storms. This appears to be the situation in several tropical areas where it has been shown to be associated with higher rates of diarrhoea (Musa et al., 1999). Although the river could have self-cleansing capacity, the capacity could be strained by persistent pollution overloads. This is attributed to its being flanked by expanding human habitats and vibrant socio-economic activities. Many rivers are reportedly prone to high bacterial levels due to heightened ecological activities and may therefore be unsuitable for human consumption (Lacorchak et al., 1998; Levy et al., 2011).

BOD_5 levels increased as the river flowed downstream with Njoro bridge recording the highest value at 44.0 mg/L and the lowest value was recorded at Nessuit (2.0 mg/L). This is in agreement with data obtained from River Odzi, a clean, non-urban African river in the Eastern Highlands of Zimbabwe (Jonnalagadda and Mhere, 2001). When polluted with agricultural run-off and effluents from fisheries, BOD_5 levels in River Odzi increased tremendously but normally they were much lower in the dry season and relatively higher in the wet season.

The BOD_5 levels recorded upstream (Nessuit) was much lower than that reported by Yillia et al. (2009), however, the BOD_5 levels midstream (Njoro bridge) was more than eight times higher than that reported by the same authors. The higher BOD_5 levels at Njoro bridge could have been exacerbated by the influx and settlement of Internally displaced individuals (IDPs) at Njoro Town (due to post-election violence of 2007), resulting in dumping of garbage, human and non-human waste materials into the river. The practice of direct dumping of solid wastes and discharge of sewage effluents into streams has been identified as contributing to enhance BOD_5 levels in streams (Sangodoyin and Sanyalou, 1997). The general increase in pollution levels at the middle reaches during dry weather has been further attributed to the increase in in-stream activities of people and livestock as water sources become increasingly scarce at this time of the year and the impact of the intermittent input of wastewater from damaged or poorly managed sewage outfalls becomes significant with decrease in stream flow (Mokaya et al. 2004; Yillia et al. 2008; Jenkins, 2008; Yillia et al., 2009). These results are in marked contrast to those obtained from highly polluted rivers in Ghana, for example 80.0 mg/L for River Karle and 153.0 mg/L for Agbobloshie River in Accra (Biney, 1986). However, River Subin, in Kumasi, Ghana had similar BOD_5 values at the source (12.0 mg/L) but became more heavily loaded with organic matter downstream (217 to 419 mg/L) (Obiri-Danso et al., 2005).

Pathogens isolated from Nakuru Municipal water were *H. alvei* (4.6%) and *A. hydrophila* (6.5%). It is also encouraging to note that water from Nakuru town centre, Kiti and Milimani estates were free from pathogenic contamination. The enteropathogenic bacteria isolated from the various water sources of River Njoro were; *A. hydrophila* (52%) *H. alvei* (29.0%), *S. typhimurium (*18%), *S. typhi (*17%), EAEC (9.2%), NTEC (7.4%) and EPEC (3.2%). These findings provide evidence for significant presence of EAEC, NTEC, EPEC, *S. typhi*, *S. typhimurium* and *A. hydrophila* in River Njoro. The occurrence of these pathogens poses a real health risk through consumption or direct contact with untreated

water from River Njoro and this probably contribute to the observed high levels of diarrhoea reported in Njoro Health Centre. It is likely that other pathogens (example, *Proteus mirabilis*, *Vibrio cholerae* e.t.c.) are also present in River Njoro. These enteropathogens cause numerous diarrhoea-associated diseases in the area. Such diseases include typhoid fever caused by *S. typhi*, gastroenteritis caused mainly by *H. alvei* and *A. hydrophila* and diarrhoea caused by *S. typhimurium* and pathogenic *E. coli* (EPEC, EAEC and NTEC). Consequently, the potential health risk posed by the consumption of water from river sources by rural residents and consumers in the Njoro District must not be underestimated.

In addition to diarrhoea, isolated enteropathogens are incriminated in other diseases. For example *E. coli* may cause neonatal meningitis, cystitis and pyelonephritis whereas *Aeromonas* species may cause septicaemia in immunocompromised patients. Apart from descriptions of a diarrhoeagenic potential *H. alvei* (Ratnam, 1991; Reina et al., 1993), several cases have been reported in which it has been associated with extra-intestinal human infections such as septicaemia, liver abscess, peritonitis and pneumonia (Gunthard and Pennekamp, 1996). Typically, extra-intestinal infections are nosocomial and develop in patients with underlying diseases or predisposing factors.

The level of contamination of Nakuru Municipal water was low and in some Nakuru estates like Nakuru town centre, Kiti and Milimani there were no pathogens present. Therefore, the present system of water treatment using chlorine appears to be functioning well. The major source of water for Nakuru Municipality are the Turasha treatment works at Gilgil, Mereroni treatment works at Nakuru town and a number of boreholes including Kabatini, Baharini and Banita boreholes. The water quality and monitoring of microbial, physical and chemical contaminants is effectively carried out by the Department of Water Quality and Pollution Control in the Ministry of environment and natural resources, Kenya. The same department also monitors the BOD, chemical oxygen demand (COD) and other chemicals in the effluent discharged into the water bodies by various industries and institutions in the area. As the population grows, more faecal discharge is shed into River Njoro and chances of microbial contaminations increases so the situation requires continued monitoring of aquatic ecosystems. Basic education on disease causation directly relating to human faecal contamination of water collection points must be considered in the short term. The detection of EAEC, NTEC and EPEC strains means that these water sources present a health hazard to the users. Among the water isolates, EAEC (9.2%), EPEC (3.2%) and NTEC (7.4%) were frequently encountered. However, the percentage occurrence was low compared to those isolated from Rivers in South Africa (EPEC (34.1%), NTEC (35.6%) and EAEC (14.1%) (Obi et al., 2004). EAEC is an emerging diarrhoegenic pathogen associated with traveller's diarrhoea and has been identified in patients with persistent diarrhoea infected with HIV (Nataro, 2005; Samie et al., 2007). EPEC has been shown to be a major cause of diarrhoea in young children (Kuhnert et al., 2000), while NTEC is responsible for diarrhoea in cattle (Debroy and Maddox, 2001). NTEC is also isolated from animals and humans and can belong to the same serogroups and produce or carry genes coding for fimbrial and fimbrial adhesions (Mainil and Daube, 2005). The isolation of NTEC from River Njoro could confirm the faecal contamination of this river by grazing cattle and raises the question of possible zoonotic infections.

Currently, efforts are being made to characterize the virulent genes in *E. coli* using polymerase chain reaction (PCR) to detect virulence gene markers namely; bundle forming-pillus (Bfp-326bp) genes coding for EPEC, cytotoxic necrotizing factor (cnf_1-278bp) gene coding for NTEC and enteroaggregative gene (eaeC-630bp) and *Shigella* enterotoxin 1(set1A-309bp) coding for EAEC.

Many pathogens that are missed by routine cultures, serological assays and biochemical tests can be detected by PCR. PCR is fast, sensitive and capable of copying a single DNA sequence of viable and non-viable cell over a billion times within 3 to 5 h (McDaniel et al., 1995; Clark, 2009). PCR can also be used to detect previously unknown organisms directly in environment or clinical specimens by using broad range primers.

According to the results it can be concluded that the microbial quality of the River Njoro water sources was poor and unacceptable for human consumption due consistent increase in total and faecal coliforms and pathogenic loading downstream and it poses risks to health for people abstracting the water downstream and Lake Nakuru ecosystem. Generally, the BOD_5 levels were moderately high, indicating organic contamination, nutrient enrichment and gradual deterioration of the water quality in River Njoro.

This indicates the potential risk of infection for consumers and calls for prompt intervention to mitigate the socio-economic and health impact of water-borne diseases in these rural communities. This study and other studies on domestic consumption of water in rural communities in the developing world (Mavura et al., 2006; Nevondo and Cloete, 1999; Acho-chi, 2001; Lehloesa and Muyima, 2000) showed the challenges of health and water resources in Kenya and other developing countries. The provision of potable water for rural communities is important in order to satisfy basic needs and is easily seen as crucial for assessing social development in developing countries (Acho-chi, 2001). The Kenyan government is thus alerted about the urgent need to address water supply problems in rural communities, where a substantial proportion of the population resides. It was concluded that Nakuru Municipal water from Town centre, Kiti and Milimani

estates were satisfactory for drinking purposes. 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. The medical personnel should be encouraged to investigate and ascertain the prevalence of the earlier mentioned diarrhoegenic pathogens in both clinical and water samples.

ACKNOWLEDGEMENTS

We thank the department of Biochemistry and Molecular Biology, Egerton University and Deutscher Akademischer Austausch Dienst (DAAD) for material and financial support.

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