



Original Paper

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## Microbial quality and antibiotic resistant bacterial pathogens isolated from groundwater used by residents of Ongata Rongai, Kajiado North County, Kenya

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### ABSTRACT

Groundwater represents an important source of drinking water and its quality is currently threatened by a combination of over-abstraction and microbiological and chemical contamination. Nairobi, Kenya and its environs is plagued with un-planned dwelling residential settlements, with limited potable water sources which are often contaminated by bacterial pathogens. This coupled with increased incidences of antibiotic resistance among the pathogens is alarming. This study used the MPN technique to determine indicator organisms, and the agar diffusion method to determine resistance in isolated pathogenic bacteria. The total coliforms values recorded were as high as  $1.12 \times 10^2 (\pm 8.8 \times 10^1)$  MPN/100 ml, fecal coliform loads were  $8.63 (\pm 7.01)$  MPN/100 ml for the shopping centre. Total bacterial counts values recorded were as high as  $5.64 \times 10^4 (\pm 1.77 \times 10^4)$  CFU/ml at the shopping centre. Numerous organisms that are potential enteric pathogens such as *Vibrio* sp., *Salmonella* sp., *Shigella* sp., *P. aeruginosa* and *E. coli* were isolated from the water samples. There was high resistance to many antibiotics particularly sulphamoxazole (98.5%), kanamycin (95.3%) and ampicillin (87.5%). The most active antibiotics were chloramphenicol with resistance level of 45.4% and streptomycin (59.4%). The study indicates that water from the studied boreholes was not suitable for human consumption and therefore calls for urgent intervention. There is a high likelihood that congestion and lack of proper waste and waste water management is responsible for contamination of aquifers in this region.

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**Keywords:** groundwater, contamination, coliforms, potable water, antibiotic resistance.

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### INTRODUCTION

Rapid population growth is evident worldwide and has resulted in an increased demand for essential amenities including

accessibility of potable water. To address this demand, groundwater is increasingly being exploited as one of the sources of water globally (Mumma et al., 2010). Nevertheless,

unregulated urban growth and land development is on the rise with Nairobi and its environs equal affected. And water resources are being degraded due to high demand and untreated waste water entering the environment from industry and domestic sources. However, adaptation and planning of water resources is difficult, as many African countries have no established water quality monitoring programmes (RSC, 2010).

Ongata Rongai is a fast growing urban centre situated in Kajiado County on the outskirts of Nairobi. This town has become an alternative for Nairobi residence resulting into high population densities and overcrowded housing (Kazungu et al., 2011; Omwenga et al., 2009). This population pressure makes groundwater an important source of drinking water despite its quality being threatened by a combination of over-abstraction and microbiological and chemical contamination (Aydin, 2007; Rei et al., 2003; Shittu et al., 2008). This compromises water quality standards as set by the World Health Organization (WHO) which dictates that there should be no tolerable limit for pathogens in the water intended for consumption (WHO, 2010).

Antibiotic resistance has become a major concern in most countries Kenya included. Previous studies have continued to show an increase in drug resistance in waterborne pathogens which cause infectious diarrheal diseases which are a major cause of morbidity and mortality in developing countries (Alikhani et al., 2013). In the last decades, resistance against the commonly used drugs for the treatment of enteric infections including ampicillin, tetracycline, and co-trimoxazole has increased (Alikhani et al., 2013). The rising incidences of resistance

limits the number of effective drugs available and a good example is that currently, Ciprofloxacin is the only antibiotic recommended by WHO for management of bloody diarrhoea due to *Shigella* organisms, now that widespread resistance has developed to other previously effective antibiotics (WHO, 2013). This trend has led the current trend of pathogens not responding to the currently used drugs risking possible uncontrollable outbreaks (CDC, 2013).

There is very little published data on the quality of ground water sources in this area even though the area residents depends solely on groundwater in an area that has a poor sewerage system hence predisposing them to infection (Kazungu et al., 2011). The area is characterized by high numbers of boreholes and septic tank in this has resulted to poor sewage system and less than 100% accessibility of portable water (Chaturvedi, 2008). In this kind of infrastructure, Ongata Rongai residents are at high risk of possible disease outbreak or diarrhoeal disease (Levy et al., 2012). This coupled with the rising antibiotic resistance incidences is alarming and has been linked to unregulated use of including the rise of multidrug resistant bacteria (Olusegun et al., 2009).

Studies on the exploitation as a drinking water source and vulnerability of groundwater to contamination in Kenya are limited even when there is such high need for the resource. The aim of this study was to examine the microbial quality of groundwater in Ongata Rongai and to determine the diversity and antibiotic susceptibility profiles of potential bacterial pathogens isolated from this source.

## MATERIALS AND METHODS

### Sample collection and preparation

Study design: A cross-sectional study was carried out at Ongata Rongai, in Nairobi Kenya. A total of one hundred and forty four (144) groundwater samples were collected from selected sampling sites in Kware, Nkoroi, Stage 1 and Shopping centre. The water samples were collected in sterile glass schott bottles and transported in cool boxes kept below 4 °C and analysed immediately upon arrival in the laboratory.

### Total bacterial counts

Standard spread plate method was used to enumerate total bacterial counts (TC) and standard microbial analytical methods in the isolation and enumeration of faecal coliforms (FC) (Jackson et al., 2011). In addition, Most Probable Number (MPN) per 100 ml determined according to APHA, (2005).

### Detection of pathogenic bacteria

Standard analytical methods were also used in the detection of various pathogenic bacteria species which included *Salmonella* species, *Shigella* species, *E. coli*, *Vibrio* species (Maarita and Okemo, 2009) and *Pseudomonas* species. These bacterial species were isolated using respective selective media such as; SS agar, DCA agar and MacConkey agar which were used in isolation of *Salmonella* and *Shigella*. Sorbitol MacConkey agar was used to isolate *E. coli*, Thiosulphate bile salt sucrose agar was used to isolate *Vibrio* sp and Nutrient agar for *Pseudomonas* (Maarita and Okemo, 2009). Morphological characterization (colony shape, colour and Gram staining) as well as biochemical

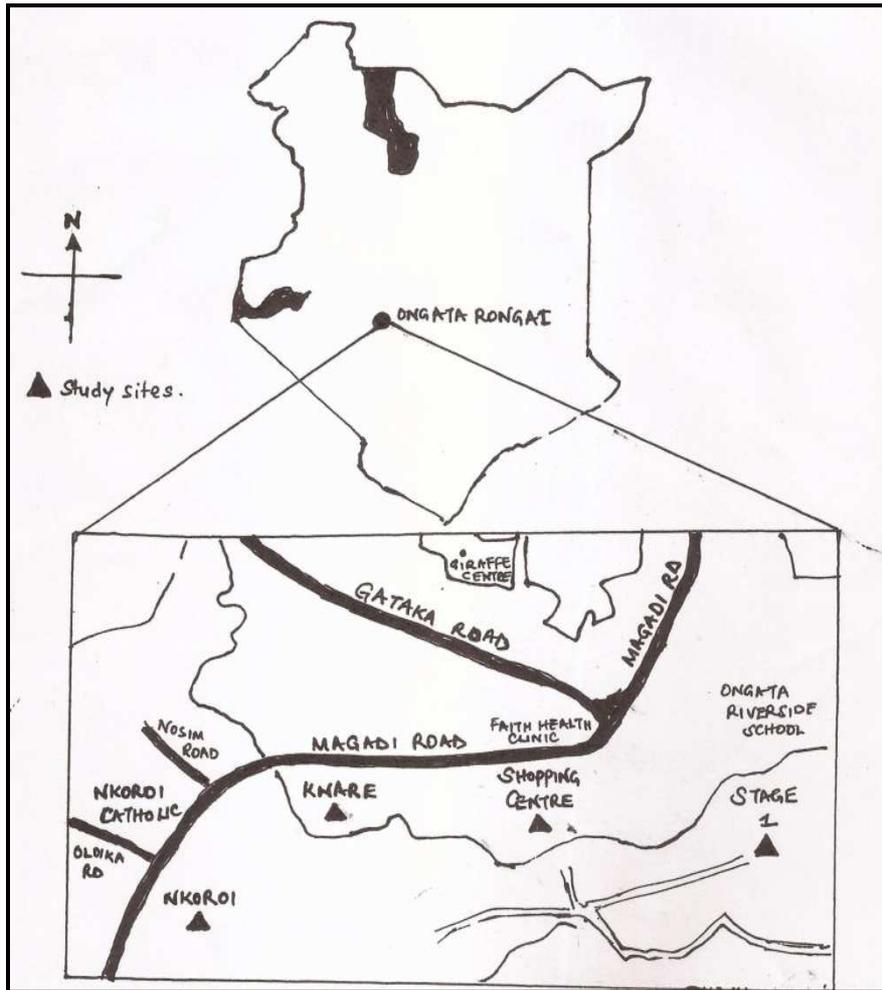
profiling of the isolates were used in the identification of the suspected pathogenic bacteria. These tests include TSI (triple sugar iron), glucose fermentation, cytochrome oxidase test, motility, indole test, citrate utilization and urease (Yah et al., 2007; Samie et al., 2011).

### Antimicrobial susceptibility testing

Kirby Bauer disc diffusion technique (Bauer et al., 1966) was used and 0.5 MacFarland's 10<sup>8</sup> organisms/ml employed in inoculum suspension preparations according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (Olaitan et al., 2011) Antibiotic sensitivity discs included: Ampicillin (25 µg); Cotrimoxazole (25 µg); Streptomycin (10 µg); Chloramphenicol (30 µg); Kanamycin (30 µg); Gentamycin (10 µg); Tetracycline (25 µg) and Sulfamethoxazole (200 µg). The antibiotic sensitivity tests were performed by disc diffusion technique using Commercial antibiotic discs (Hi media, PVT and Difco-Michigan) on Mueller Hinton agar plates (Byarugaba et al., 2011).

### Data analysis

SPSS computer software version 16.0 was used for data entry and statistical analysis. Groups significance tests were performed using one way ANOVA (analysis of variance) at 95% confidence level and P value of <0.05 was considered as significant. The means were separated using Turkey's test. Correlation test was also used to find out whether density of total counts, total coliforms and faecal coliforms were related.



**Figure1:** Map of Ongata Rongai.

## RESULTS

### Total microbial quality of groundwater samples

Generally, the microbial quality of the ground water in this study was found to be contaminated (Table 1). All four sites had high CFU (Coliform Forming Units) counts. The shopping centre recorded the highest ( $5.64 \times 10^{4b} \pm 1.77 \times 10^4$ ) CFU counts and Stage 1 ( $4.32 \times 10^{4a} (\pm 4.42 \times 10^3)$ ) as the least. However, there was no significant difference in the CFU

counts compared across different sampling sites ( $p=0.320$  at  $p>0.05$ ).

Total and faecal coliform counts exceeded the recommend limits of 10 per ml and 0 per 100 ml for coliforms. The highest coliform counts were recorded at the Shopping centre ( $1.12 \times 10^2 \pm 8.8 \times 10^1$ ) even though there was no significant difference ( $P=0.483$  at  $P>0.05$ ) in the total coliform counts among the four sampling sites. Highest faecal coliform counts were recorded at the

shopping center ( $8.63 \times 10^0 \pm 7.01$ ) while the lowest counts ( $4.83 \times 10^{0a} \pm 3.84$ ) were recorded at Nkoroi. However, there was no significant difference in the means of faecal coliform counts at the different sampling sites recorded ( $P=0.520$  at  $P>0.05$ ).

A correlation test revealed that faecal coliform counts showed a positive correlation with total coliforms ( $r=0.67$ ,  $P=0.046$ ) and also showed a positive correlation ( $r=0.55$ ,  $P=0.55$ ) with the Heterotrophic Plate count results from the different sites.

The shopping centre had the most polluted water source both in terms of Total counts ( $5.64 \times 10^{4b} \pm 1.77 \times 10^4$ ) and faecal coliform counts ( $8.63 \times 10^{0a} \pm 7.01$ ). The total counts were significantly different ( $P=0.035$ ). However, there was no significant difference ( $P=0.30$ ) among the other sampling sites. Faecal coliform counts however appear different but there was no significant difference ( $P=0.520$ ) among the four sampling sites.

#### Pathogenic bacteria isolation

Various pathogenic bacteria were detected in the groundwater samples (Table 2). *Vibrio cholera*, *Salmonella* spp, *Shigella* spp, *E. coli* and *Pseudomonas* spp were detected. *Salmonella* spp was detected in all study sited except Kware, *Shigella* spp was at stage 1 and Kware, *E. coli* at Stage 1 and Shopping centre, *Pseudomonas* spp was detected in stage Kware and Nkoroi and while *Vibrio* spp was detected from all the sampled area (Table 2).

#### Antibiotic resistance patterns

Isolated pathogenic bacteria showed high resistance to sulphamethaxazole (98.5%), kanamycin (95.3%) and ampicillin (87.5%) (Table 3). However, all the isolates showed

least resistance to chloramphenicol (45.5%) and streptomycin (59.4%). Resistance to streptomycin and chloramphenicol were not significantly different ( $P=0.43$ ). However, significant differences were noted between the two antibiotics compared to the rest ( $P=0.035$ ).

All (100%) of the *Salmonella* isolates showed resistance to ampicillin and kanamycin (Table 3). However, some of the isolates showed sensitivity to gentamycin (37.5%) and cotrimoxazole (18.8%). *Salmonella* isolates had the least mean resistance ( $67.2\% \pm 42.3$ ), *Shigella* isolates were highly resistant to cotrimoxazole (100%) and sulphamethaxazole (100%). Sensitivity to streptomycin was noted with 50% of the *Shigella* isolates showing sensitivity followed by cotrimoxazole (30%). *Vibrio* isolates showed the least resistance incidences with less than 50% of the isolates showing resistance to streptomycin and chloramphenicol.

*E. coli* isolates were all (100%) resistant to ampicillin, tetracycline, cotrimoxazole, streptomycin and sulphamethaxazole. In addition, half of the isolates (50%) showed resistance to gentamycin and chloramphenicol. *E. coli* had the second highest mean resistance ( $82.8\% \pm 68.2$ ) among the isolates. There was no significant difference ( $P=0.520$ ) in the sensitivity of the isolates except for *Shigella* sp. whose sensitivity was significantly different ( $P=0.01$ ). All the *Pseudomonas* isolates (100%) were resistant to ampicillin, cotrimoxazole, streptomycin, kanamycin and gentamycin. The isolates were least resistant to chloramphenicol (40%). *Pseudomonas* isolates exhibited the highest mean resistance ( $87.5\% \pm 70.2$ ) among all the isolates.

**Table 1:** Mean bacteria densities, total coliforms and fecal coliforms in groundwater samples from Ongata Rongai, Nairobi, Kenya.

Sample (n=48)	Total counts (CFU/ml)	Total coliform MPN/100 ml	Fecal coliform MPN/100 ml
Kware	3.64x10 <sup>4a</sup> (±2.46x10 <sup>3</sup> )	2.63x10 <sup>1a</sup> (±1.58x10 <sup>1</sup> )	7.6x10 <sup>0a</sup> (±6.67)
Nkoroi	3.66x10 <sup>4a</sup> (±2.42x10 <sup>3</sup> )	1.08x10 <sup>1a</sup> (±0.6x10 <sup>1</sup> )	4.83x10 <sup>0a</sup> (±3.84)
Stage 1	4.32x10 <sup>4a</sup> (±4.42x10 <sup>3</sup> )	8.03x10 <sup>1b</sup> (±6.3x10 <sup>1</sup> )	7.33x10 <sup>0a</sup> (±6.34)
Shopping centre	5.64x10 <sup>4b</sup> (±1.77x10 <sup>4</sup> )	1.12x10 <sup>2bc</sup> (±8.8x10 <sup>1</sup> )	8.63x10 <sup>0a</sup> (±7.01)

Means flanked by the same letter (a,b or c) show no significant difference according to Turkey's test at 5% level.

**Table 2:** Presence-absence table of selected pathogenic bacteria.

Location	<i>Salmonella</i>	<i>Shigella</i>	<i>E. coli</i>	<i>Pseudomonas</i>	<i>Vibrio</i>
Kware	A	P	A	P	P
Nkoroi	P	A	A	P	P
Stage 1	P	P	P	A	P
Shopping centre	P	A	P	A	P

A-Absence; P-Presence

**Table 3:** Percentage resistance incidences among Ongata Rongai borehole isolates *Vibrio* spp, *Salmonella* spp, *Shigella* spp, *E. coli* and *Pseudomonas* spp against 8 selected antibiotics.

Antibiotic	<i>Vibrio</i> sp. n=11	<i>Salmonella</i> n=16	<i>Shigella</i> n=10	<i>E. coli</i> n=8	<i>Pseudomonas</i> n=9
AMP(25 mg)	100	100	60	100	100
TE(25 mg)	82	75	100	100	70
COT(30mg)	82	62	50	100	100
S(10 mg)	36	50	40	100	100
K(30 mg)	81	100	90	62.5	100
GEN(10mg)	82	44	80	50	100
SX(200 mg)	100	88	100	100	90
C(30 mg)	45.5	18.75	60	50	40
Mean*	75.9(±54.2)	67.2(±42.3)	72.5(±49.8)	82.8(±68.2)	87.5(±70.2)

Amp- ampicillin, TE –tetracycline, COT-cotrimoxazole, S-streptomycin, K-kanamycin, GEN-gentamicin, SX-sulfamethoxazole and C-chloramphenicol.

## DISCUSSION

Water plays a major role in the spread of communicable diseases (Ahmad et al., 2009), more importantly, contaminated drinking water is a major contributor to the problem of diarrheal disease and in order to address the problem of unsafe drinking water, methods are needed to assess what constitutes good quality versus contaminated drinking water (Levy et al., 2012).

In addition, antibiotic resistance has significantly become a problem and the use of antibiotics, whether for prophylaxis or chemotherapy, does not only impact on pathogenic bacteria but also the commensals. This therefore leads to resistance arising as a consequence of mutations in microbes and selection pressure from antibiotic use that provides a competitive advantage for mutated strains (Laxminarayan et al., 2013).

In this study, the level of contamination and the presence of potentially pathogenic bacteria in groundwater were determined. A total of five (5) different pathogenic bacteria were isolated and their antimicrobial resistance profile determined. From this analysis, the presence of pathogenic bacteria, confirmed the high risk rate the residents of Ongata Rongai are exposed.

From the total bacterial counts, heterotrophic plate count, total coliform load and faecal coliform loads the rates were significantly high for Kware, Nkoroi, Stage 1 and Shopping centre locations. Based on these counts 100% of the water sources that were sampled were found to be contaminated (Kuitcha et al., 2010) and not portable human consumption with its count exceeding that of WHO drinking water standards (Kuitcha et al., 2010; WHO, 2012).

Boreholes due to the way they are constructed are thought to be physically protected from contamination as they are not in direct contact with man and animals. As a result of this, it is assumed that groundwater provides safe drinking water (Xu and Braune,

2010). However, it has been observed elsewhere that some groundwater sources clearly do not provide potable water (Thokozani, 2012), and this study confirms such findings and warns that individual cases must be carefully evaluated.

Presence of septic tanks which are located near the aquifers as well as surface run-off carrying waste and waste waters may find its way into aquifers through seepage. This endangers microbiological quality of groundwater (Samie et al., 2011). Overcrowding and overdependence on groundwater from boreholes in Ongata Rongai exposes this resource to contamination thus endangering the residents. Such overcrowding has been cited as a source of danger in non-planned settlements (Samie et al., 2011).

In this study, the pathogenic species that were most frequently isolated were *Salmonella*, followed by *Vibrio* sp and then *Shigella* sp. This provides a warning to the community that there could be frequent infection among the human population in the area especially with *Salmonella* which was the most prevalent bacterial pathogen. *Salmonella* is a zoonotic bacterium of public health significance, with considerable economic impact (Bottie et al., 2013) and is passed in the faeces and urine and therefore people get infecting as they eat and drink contaminated food or water (Ashbolt, 2004).

Findings from antimicrobial susceptibility test, *Salmonella* isolates were highly resistant to ampicillin and Kanamycin with 100% of the isolates showing resistance (Table 3). Similar findings were obtained by Zelalem et al. (2011) in a separate study. This implies that ampicillin resistance is widespread and could be as a result of plasmid-mediated penicillin resistance (PPNG) (Mehta et al., 2011).

The *Vibrio* sp. isolates showed high resistance to the antibiotics tested against them and with least resistance to Streptomycin whereby 36% of the isolates were resistant

followed by Chloramphenicol (44.5%). However, for Tetracycline and Cotrimoxazole, 82% of the isolates were resistant. The study revealed a high occurrence of tetracycline-resistant *Vibrio* isolates. However, a study carried out by Jackson et al. (2011) in western Kenya revealed that 66.7% of *Vibrio* isolates showed resistance to both Tetracycline and Cotrimoxazole, slightly lower than results obtained from this study.

In Kenya, a study of diarrheal pathogens (Nelson et al., 2009), including *Shigella*, *Campylobacter*, *V. cholerae* and *Salmonella* found that of the cases of diarrhea that were given antibiotic treatment, 51% had pathogens that were not susceptible to the antibiotic given and this may reveal the extent of inappropriate prescribing. It also demonstrated the extent of resistance across species to antimicrobials that are commonly used, and such findings provide additional evidence of emerging danger in Kenya. Subsequently highlighting the need to constantly monitor antibiotic use and resistance is crucial.

### Conclusion

This study shows that Ongata Rongai groundwater sources are contaminated with possibility of waste of human and animal origin. This shows that exploitation of groundwater sources, land development as well as trends in antibiotic resistance in the area should be monitored constantly, and groundwater use regulated to ensure individuals follow the necessary legal requirements. Waste management solutions should be strategized by the area municipality as a long term solution to the problem.

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