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PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSIS IN TREATED, STORED AND DRINKING WATER IN NAKURU NORTH, KENYA.

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ABSTRACT

Nakuru North sub-county is located 160 Km Northwest of Nairobi at an altitude of 1859 m above sea level. It has an area of 593 Km2 with a population density of 25.3 per Km2. People living in the area suffer from enteric diseases probably due to consuming contaminated water. The aim of this study was to determine the physico-chemical characteristics of water that has been treated and stored, isolate microbes from the water and carry out antimicrobial sensitivity test of the isolates. A crossectional study was carried out and a total of 540 samples from water that had been treated through boiling (135), chlorination (135), filtration (135) and solar disinfection (SODIS) and stored by the residents (135). Physico-chemical parameters which included pH, chloride ions concentration and dissolved oxygen were determined and microorganisms isolated and confirmed by biochemical tests. Out of the five hundred and fourty (540) samples examined 35% (189/540) were positive for all the microbial isolates. The prevalence of total coliforms was 51.8 %, E coli (32.3%) and Salmonella (15.9 %). Total coliforms showed the highest mean resistance (26.0 %) followed by Salmonella (16.9 %) while E. coli showed the least (15.5 %). However, there was no significant deference (p=0.98) in resistance among totalcoliforms, E. coli and Salmonellaat 0.05 level of significant. This study established that water that had been treated and stored by the residents in Nakuru North contains high levels of microorganisms and solar disinfection is effective in treating water upon exposure to sunlight for three to five hours per day. In addition, a high percentage of the isolates were resistant to the tested antimicrobials indicating a possibility of antimicrobial miss use and abuse. There is need for proper storage of water after treatment and prudent usage of antimicrobials.

KEYWORDS: Antimicrobial, contamination, Microorganisms, Physico-chemical, susceptibility.

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INTRODUCTION

Nakuru North Sub-County in Nakuru County in the Great Rift Valley. The salient features of the area are volcanic soils, calderas and hot springs. The Sub-County is located 160 km Northwest of Nairobi at an altitude of 1,859 m above sea level, with an area of 593 km^2. It has a population density of 25.3 per km^2. It is specifically located between longitudes 35^o 28' and 35^o 36' East and latitude 0^o 13' and 1^o 10' South. The main livelihoods in the Sub-County are mixed farming, employment and trade [13].

Access to safe drinking water is essential for healthy living. Studies by Kenya Food Security Steering Group (KFSSG) [13] indicate that 60 % of people living in Nakuru North are aware of reduced microbial infection when drinking water is treated. Despite this, cases of enteric diseases are on the increase. This makes it important to determine the physico-chemical properties and analyze the microbial content of water that has been treated and stored in the study area.

One of the methods that is used in water treatment world over is boiling. Boiling kills all microorganisms in water when the temperature reaches 100 degrees Celsius [6]. For this method of water treatment to be effective, it is recommended that water be allowed to boil for up to a minimum of 1 minute [19]. In every 1000 feet above sea level, one minute of boiling should be added to the initial 1 minute [5]. However, the method requires fuel which is expensive to get for most residents of Nakuru North [13].

Chlorination is the most widely used chemical method in Nakuru North sub-county for water disinfection because it is simple in application and it does not require the use of fire wood which has become scarce in the recent past [25]. In this method, gaseous chlorine (Cl_2) or liquid sodium hypochlorite bleach, (NaOCl) is added to, and reacts with water to form hypochlorous acid. The acid forms strong oxidizing agents in water which react with a wide variety of microorganisms [22].

Among the various water filtration methods in use in Nakuru North, the most widely used is ceramic water filtration, which employs the use of candle-filters [15]. Microorganisms are removed from water as it passes through the filter from the top compartment to the lower compartment which stores the water up to when it is used. To enhance the effectiveness of filtration, the filters are impregnated with silver nitrate or colloidal silver which are bacteriostatic [17]. Previous studies have shown that design of the filters, method of filter production and quality vary from one manufacturer to another which may lead to variation in effectiveness of the method in water treatment [11].

Another major problem in Nakuru is the occurrence of microorganisms with antimicrobial resistance. Though there are no documented results of this menace in Nakuru North, people have continuously suffered from drug resistance upon treatment. Studies carried out elsewhere in Kenya indicate thatmore than 50 % of microorganisms isolated from treated water are multi-drug resistant [18]. In a recent study carried out in Nairobi, Kenya, involving isolation of microorganisms from abattoirs sewage, the isolates were most resistant to lincomycin, ampicillin and methicillin and most sensitive to chloramphenical, gentamicin and cotrimoxazole. Such microorganisms could get into water bodies and later be taken home for drinking. The study also demonstrated that the microbes were resistant to antibiotics commonly used as feed additives (tetracycline, streptomycin and sulfonamides) or therapeutics (penicillin and tetracycline) [2].

METHODOLOGY

SAMPLE COLLECTION

Samples were collected from households in Arutani, Bahati, Ndungiri, Ndundori, Kabatini, Lanet, Maili 5, Munanda, Rugongo and Wendo sub-locations in Nakuru North that treated their water through boiling, chlorination and filtration. In high population areas, 13 samples were collected while 14 samples were collected from low population areas. The sample size for both the house holds and water samples was determined using Fisher's formula [10] indicated below:

$$n = \frac{Z^2 pqD}{d^2}$$

where; n = sample size, p = anticipated prevalence which was 4.6 % (0.046) in this study, q = failure which was calculated as 100-4.6 giving 95.4 % (0.954), Z = appropriate value from the normal distribution for the desired confidence level which was 1.96 in this study, d = allowable error (0.05) and D = design effect which was given a value of 2 because replication was carried out.

Based on prevalence of 4.7 % of diarrhoea cases attributed to taking contaminated water in the study area [13], confidence interval of 95 % and maximum allowable error of 5 %, the sample size was determined as indicated below:

$$n = \frac{1.96^2 (0.046 \times 0.954)2}{0.05^2} = 134.9 = 135$$

A total of 135 samples each of boiled, chlorinated and filtered water were collected from ten sampling points. The samples were collected in glass sample bottles which had been sterilized using sodium hypochlorite followed by thorough rinsing using dionized water. The samples were transported to Rift valley regional water testing laboratories in Nakuru town in ice cooler boxes and analyzed within 2 h after collection. The pH of samples was determined at the point of collection using pH meter while chloride ions and dissolved oxygen were determined by titration.

ISOLATION AND ENUMERATION OF ORGANISMS

Briefly, 100ml of water sample was filtered using membrane filters using 0.22 µm cellulose nitrate membranes (Whatman GmbH, Germany). The filters were then aseptically removed from the membrane holder, inverted and cultured on MacConkey agar plates. The plates were incubated at 37 ^{o}C for 24 h for isolation of coliforms. For isolation of *Escherichia coli*, the filters were placed on Eosine methylene blue (EMB) agar for 24 h at 37 ^{o}C. Blue-black colonies having a greenish metallic sheen were confirmed as *E. coli* using biochemical tests. For isolation and enumeration of *Salmonella species,Salmonella-Shigella* agar were inoculated and incubated at 37^{o}C for 24 h. The typical colonies based on morphological characteristics were confirmed using biochemical tests [24].

ANTIMICROBIAL SUSCEPTIBILITY TEST OF ALL THE ISOLATES

The antimicrobial susceptibility testing was carried out by use of Kirby Bauer disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) [7]. Sterile wire loop was used to pick 3 colonies of each microorganism isolated from treated water and emulsification in 3 ml of sterile physiological saline was carried out. Standardization of the suspended colonies was performed by diluting the normal saline suspension until the turbidity matched the 0.5 McFarland Standards. A sterile cotton swab was dipped into the standardized suspension, drained, and used for inoculating 20 ml of Mueller-Hinton agar in a 150 mm disposable plate (STERLIN, UK). The zones of inhibition were measured in millimeters and graded according to sensitive, intermediate or resistant after incubation for 24 h at 37 ^{o}C based on clinical Laboratory Standards Infections (CLSI) guidelines for interpretation. This was followed calculation of % resistance, intermediate or sensitive by getting the number of isolates in each case, dividing it by the total number of isolates then multiplying by 100 [3]. Multiple antibiotic resistance (MAR) was also determined.

The MAR Index of an isolate is defined as a/b, where a represents the number of antibiotics to which the isolate was resistant and b represents the number of antibiotics to which the isolate was subjected [4].

RESULTS

PHYSICO-CHEMICAL ANALYSIS

The pH in the wet season ranged between 8.5-6.0 in boiled water, 8.3-5.5 in chlorinated water and 8.7-5.0 in filtered water. However, during the dry season the pH ranges were 9.0-6.2 in boiled water, 9.1-6.2 in chlorinated water and 8.9-5.2 in filtered water. The Cl^- ions in the wet season varied between 8.2-4.0 in boiled water, 8.5-4.2 in chlorinated water and 7.3-3.9 in filtered water. On the other hand, during the dry season, the Cl^- ranged between 8.5-4.2 in boiled water, 8.2—4.3 in chlorinated water and 8.4-3.5 in filtered water. In addition, the DO in wet season ranged between 5.6-3.0 in boiled water, 8.5-6.2 in chlorinated water and 8.5-5.6 in filtered water. In the dry season, the variations in DO were 5.2-3.5 in boiled water, 7.7-6.3 in chlorinated water and 7.5-5.3 in filtered water (Table 1).

Table 1: Physico-chemical characteristic of treated water in Nakuru North from May 2012 to April 2013 during the wet and dry season

Sampling Site	Boiling						Chlorination						Filtration					
	pH		Cl^-		DO (mg/l)		pH		Cl^-		DO(mg/l)		pH		Cl^-		DO(mg/l)	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Arutani (N=14)	8.1	9.0	8.0	8.5	5.4	5.2	8.3	8.7	8.5	7.5	8.5	7.3	8.2	8.9	7.2	7.5	6.5	6.3
Bahati (N=13)	7.5	8.2	8.2	8.5	4.2	4.2	7.1	8.2	7.2	8.2	7.1	7.0	7.1	7.2	7.4	8.4	7.1	7.0
Ndungiri (N=13)	8.0	7.8	7.1	7.8	3.0	3.5	8.2	9.1	8.1	6.8	7.6	7.6	8.2	7.5	6.1	6.8	5.6	6.6
Ndundori (N=13)	6.0	6.2	4.0	4.2	4.0	4.1	6.0	6.2	4.2	5.2	7.3	7.4	5.9	5.2	3.9	5.2	6.3	6.4
Kabatini (N=14)	7.0	7.2	6.7	5.5	5.1	4.1	6.9	7.4	5.7	4.5	8.4	7.5	7.3	7.2	6.7	4.5	8.5	7.5
Lanet (N=14)	7.5	7.8	7.5	6.5	5.4	5.2	7.2	6.8	8.5	5.5	8.1	7.7	6.5	6.8	7.3	7.5	7.1	6.1
Maili 5 (N=13)	8.5	8.7	6.2	6.3	5.6	5.1	7.5	7.7	6.2	7.3	7.3	6.3	8.7	8.6	6.2	6.3	6.3	5.3
Munanda (N=14)	6.5	6.9	4.1	4.5	3.9	3.9	5.5	6.9	5.1	4.3	7.5	7.1	6.5	6.5	4.5	3.5	7.5	7.1
Rugongo (N=14)	6.0	6.3	4.0	4.4	3.7	3.8	6.0	6.5	4.4	4.4	6.2	7.2	5.0	6.2	4.0	4.5	6.2	6.2
Wendo (N=13)	7.8	7.8	7.1	7.2	5.5	5.2	7.5	7.8	6.1	6.9	7.2	6.9	6.8	6.6	6.7	7.2	7.2	6.2
Mean	**7.2**	**7.5**	**6.2**	**6.3**	**4.5**	**4.4**	**7.0**	**7.5**	**6.4**	**6.1**	**7.5**	**7.2**	**7.0**	**7.7**	**6.0**	**6.1**	**6.8**	**6.5**

A = wet season; B = dry season; DO = Dissolved oxygen

MICROBIAL ANALYSIS

The meantotal coliform count, *E.coli* count and *Salmonella*from boiled water in the wet season were 5.1 x10^4, 3.6 x10^4 and 3.3 x10^4 respectively while in the dry seasons the means were total coliform (5.8 x10^4), *E. coli* (5.3 x10^4) and *Salmonella* (4.8 x10^4).In chlorinated water the means were total coliform (1.8 x10^4), *E.coli* (1.5 x10^4) and *Salmonella* (1.4 x10^4) in the wet season. During the dry season, the means were total coliform (1.3 x10^4), *E. coli* (1.4 x10^4) and *Salmonella* (1.5 x10^4) in the wet season. From filtered water, the means during the wet season were; total coliform (4.7 x10^4), *E.coli* (3.4 x10^4) and *Salmonella* (3.4 x10^4). During the dry season, the means were; total coliform (5.0 x10^4), *E. coli* (5.2 x10^4) and *Salmonella* (4.6 x10^4) (Table 2).

Table 2: Mean colony forming units of microbes from stored water after boiling, chlorination and filtration in Nakuru North from May 2012 to April2013

Sampling site	Boiling						Chlorination						Filtration					
	T. coliform (x10⁴)		*E.coli* (x10⁴)		*Salmonella* (x10⁴)		T.coliform (x10⁴)		*E.coli* (x10⁴)		*Salmonella* (x10⁴)		T.coliform (x10⁴)		*E.coli* (x10⁴)		*Salmonella* (x10⁴)	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Arutani (N=14)	10	09	06	07	05	06	02	01	01	03	02	03	08	07	06	08	04	07
Bahati (N=13)	07	08	05	08	04	07	01	02	01	02	01	02	07	06	06	08	05	08
Ndungiri (N=13)	06	07	06	07	06	08	04	00	03	00	03	00	05	08	05	07	06	05
Ndundori (N=13)	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
Kabatini (N=14)	05	08	04	07	04	05	03	01	03	03	01	03	05	08	03	06	05	05
Lanet (N=14)	09	09	04	09	05	07	05	03	04	02	03	02	08	09	05	07	04	06
Maili 5 (N=13)	08	09	05	08	04	08	02	04	01	03	01	02	07	07	04	08	04	07
Munanda (N=14)	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
Rugongo (N=14)	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
Wendo (N=14)	06	08	06	07	05	07	01	02	02	01	02	03	07	05	05	08	06	08
Mean	**5.1**	**5.8**	**3.6**	**5.3**	**3.3**	**4.8**	**1.8**	**1.3**	**1.5**	**1.4**	**1.4**	**1.5**	**4.7**	**5.0**	**3.4**	**5.2**	**3.4**	**4.6**

p-value = 0.01; A = Wet season; B = Dry season; T. Coliform = total coliform

ANTIMICROBIAL SENSITIVITY TEST

The pure microbial isolates were tested with seven different antibiotics to establish their levels of resistance. The mean resistance in total coliform was 26.0 %, *E. coli* (15.5 %) and *Salmonella* (16.9 %). Total coliform (12 %), *E. coli* (11.2 %) and *Salmonella* (5.2 %) were intermediate resistant to the tested antibiotics. In addition, 60 % of total coliform, 73.3 % of *E. coli* and 78.0 % of *Salmonella* were resistant. The MAR indices were total coliform (0.8), *E. Coli* (0.5), and *Salmonella* (0.3) (Table 3).

Table 3: Antimicrobial susceptibility test (%) results for the seven antimicrobials in Nakuru North from May 2012 to April 2013 following the CLSI (2013) pattern

Antibiotic	Total coliform (N= 98)			*E. coli* (N=61)			*Salmonella* (N=30)			p-value
	R	I	S	R	I	S	R	I	S	
CL30	61.2	0.0	38.8	7.3	20.4	72.3	6.7	11.4	81.9	0.4
NA30	65.8	30.2	4.0	72.5	14.9	12.6	96.2	0.0	3.8	0.1
C30	4.3	1.9	93.8	2.0	4.6	93.4	0.0	1.9	98.1	0.2
CIP5	5.0	23.0	72.0	1.1	30.2	68.7	1.9	19.0	79.1	0.1
CN10	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	100.0	0.5
AML10	30.7	19.3	50.0	21.1	5.6	73.3	11.4	1.9	86.7	0.3
SXT25	15.1	9.6	75.3	4.5	2.8	92.7	1.9	1.9	96.2	0.2
Average	26.0	12	62.0	15.5	11.2	73.3	16.9	5.2	78.0	
MAR	**0.8**			**0.5**			**0.3**			

CL30 = Cephalexin; NA30 = Nalidixic Acid; C30 = Chloramphenicol; CIP5 = Ciprofloxacin; CN10 = Gentamicin; AML10 = Amoxycillin; SXT25 = Sulfa-trimethoprim; R = resistant; I = intermediate; S = sensitive

DISCUSSION

We analyzed drinking water that had been treated and stored for contamination. The presence of coli organism in treated water indicates that the water is feacally contaminated either at the point of use or from the source [9]. These organisms are dangerous to human health when water is used for drinking and have the potential of causing diseases of public health importance. Although the methods used for treating this water could be effective in eliminating microorganism, high levels of microorganisms were isolated (Table 2). This may be attributed to poor storage of the water after treatment. The results obtained for physico-chemical parameters in all the ten sampling points both in the wet and dry seasons correspond to those obtained from untreated water in a previous study by [20]. The values obtained were in agreement with WHO recommended values for drinking water [28]. However, the values of the dissolved oxygen (DO) obtained from boiled water were low in both seasons (Table 1). This could be as a result of boiling which drives away dissolved oxygen in water [26].

Although the microbial levels of water obtained from Arutani, Kabatini, Lanet Bahati, Ndungiri Maili 5 and Wendo sub-locations were higher than World Health Organization recommendations for drinking water, the microbial load of water in Ndundori, Munanda and Rugongo were within the recommendations

both in the wet and dry seasons [28]. The reason for this possibly lies on the location of these regions on hilly areas which are the origins of river Chania and Kandutura which are important water sources in the region. This implies that the treated water was obtained from less potentially contaminated sourced as contamination of river water increases downstream [1]. However, the level of microorganisms obtained in the wet season was lower than those in the dry season (Table 2). This may be associated to dilution effect from incoming flood water flowing into water sources from which the treated water was obtained [27]. In addition, the number of microorganisms obtained from chlorinated water was lower than those obtained from boiled and filtered water. This could be associated with the effect residual chlorine has on microorganisms after chlorination of water [21].

The high level of microorganisms in boiled water agrees with a study carried out elsewhere [23]. This could be attributed to inadequate boiling of water [21]. In addition, the presence of thermo-tolerant organisms such as *E. coli* could have led to these results. The results of isolation of microorganisms from filtered water agree with previous studies by [29]. Poor fabrication of the filter candles by local manufactures could be a contributing factor [16].

In this study, the microbial isolates were most resistant to nalidixic acid, cephalexin and amoxycillin. However, they were most sensitive to gentamicin, chloramphenicol and sulfa-trimethoprim. These results therefore suggest that nalidixic acid, cephalexin and amoxycillin are not effective in controlling the microorganisms. The results agree with a previous study by [12]. People in Nakuru North practice mixed farming and administer antibiotics in boosting their animals' body immunity during fattening before sale [8]. This may explain the reason for antimicrobial resistance observed in this study because antibiotics residues end up in animal products that later get consumed by humans. In addition, some strains of *Escherichia* and *Salmonella* have capsular K and Vi antigens which protect them from access to antimicrobials [2]. Moreover, water sources having antimicrobial deposits, use of antimicrobials as animal feeds addictives and lot of usage of antimicrobial as disinfectants in the environment have greatly contributed to antimicrobial resistance [9]. The MAR indexes of all the isolates were higher than 0.2 which indicated that the microbes originated from a high risk area where antibiotics are often used [14].

CONCLUSION

The high levels of microorganism isolated from treated water in this study indicate that either drinking water is not properly treated or storage of water after treatment is not properly carried out. We recommend health awareness strategy by the public health officers in order to sensitize people in the study area on the need of practicing proper personal hygiene. There is also a need of creating awareness on the efficiency of solar disinfection as a method of water treatment in the area. Reduction of antibacterial resistance is hereby called if the problem is to be curbed.

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