Bacteriological assessment of chlorinated and non-chlorinated water in Morogoro Municipality, Tanzania

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SUMMARY

This cross sectional study was conducted to assess the bacterial contamination in chlorinated and non-chlorinated water in Morogoro Municipality from October 2013 – January 2014. Fifty two samples were collected from selected taps (chlorinated samples) and directly from the water sources (non -chlorinated samples). The total viable count (TVC) was performed on nutrient agar while the total coliform count (TCC) was done by Most Probable Number (MPN) using MacConkey broth. It was found that the TVC ranged between 530 CFU/100 mL and 600 CFU/100 mL during dry season and the same range during rainy season. The TVC ranged between 530 CFU/100 mL and 600 CFU/100 mL during dry season and 600 CFU/100 mL to 650 CFU/100 mL during rainy season. The results for TCC positive chlorinated samples during dry season ranged between 3.6/100 mL and 150/100 mL while during the rainy season it ranged between 15/100 mL and 150/100 mL. The highest TCC were found at Department of Animal Science and Production in both season for chlorinated water and 41% of non- chlorinated water during dry season while all the samples of non-chlorinated water during rainy season had high TCC count. Also the results further showed that TCC for positive non- chlorinated samples during dry season ranged between 210/100 mL and >1100/100 mL while it was >1100/100 mL during rainy season. In chlorinated water, significant difference (p=0.332) was observed when TVC during dry season was compared with that of rainy season. Based on the results of this study, it is concluded that chlorinated and non- chlorinated water show high number of TCC than that recommended by WHO and Tanzania Bureau of Standards (TBS). However, there was low TVC which was within recommended standards. The high TCC observed in treated water in this study may pose a risk of acquiring water-borne diseases to the Morogoro community.

Keywords: Bacteria, coliform, tap water, Morogoro Municipality.

INTRODUCTION

Water is vital to life and an important requirement to all kinds of living organism. Humans use water for drinking and other domestic uses, thus such use of water needs water which is clean and safe. Otherwise water may be the source of different health risks including biological, chemical and physical hazards. For example, cholera outbreak in Dar es Salaam has always been caused by contaminated water (WHO, 2011). Again several other effects like people with mottled teeth in Northern and Central Tanzania is caused by high level of fluorine in water (Yoder *et al.*, 1998). Such water contamination problems may be caused by natural causes or human related activities; the latter has a significant contribution to incidences of waterborne diseases.

In many developing countries, diarrheal diseases remain a major killer in children. Estimates by WHO and UNICEF (2004) indicate that 80% of all

illnesses in developing countries is related to water and sanitation; and that 15% of all child deaths under the age of 5 years in developing countries are caused by diarrheal diseases (WHO and UNICEF, 2004; Thompson and Khan, 2003). In Tanzania for example, water-borne diseases contributed up to 10% of all diseases during 1985 (Jiwa *et al.*, 1991). A number of water-borne diseases including diarrhoea, dysentery, typhoid and cholera have been reported in Tanzania (Temu *et al.*, 2007; Penrose *et al.*, 2010; Mahende *et al.*, 2015; Chuma *et al.*, 2016).

Despite the Tanzania government's efforts to provide safe and adequate potable water to the majority of the population, water-borne diseases are still the problem. Diarrhoeal diseases and all forms of gastroenteritis average at 12% in Morogoro region (NBS, 2005). Interestingly, diarrhoea in children in Morogoro Municipality is reported to be up to 57.2% (Oketcho *et al.*, 2012). The most common problems of water-borne diseases are those

caused by infectious organisms like bacteria, protozoa, virus and helminthes. These microorganisms cause water-borne diarrheal diseases, including salmonellosis, campylobacteriosis, amoebiasis, shigellosis, cholera, or giardiasis are widespread in areas with contaminated water (Thompson and Khan, 2003; WHO and UNICEF, 2004; Grabow, 1996; Chuma *et al.,* 2016). Use of contaminated water, poor sanitation and poor hygiene causes up to 88% of diarrhoeal deaths around the world (Black *et al.,* 2013).

As means of control of the problems caused by contaminated water, authorities responsible with water supply have different means of water treatment. The most common method for water treatment in developing countries is chlorination (WHO, 1997). The method is effective, cheap and user friendly and is recommended by WHO as a solution to water-borne diseases in developing countries. This however disinfects water against bacteria, but chlorine is not effective against viruses, protozoa and helminthes (WHO, 1997). Other water treatment methods like flocculation and biological filtration may be useful but resources and infrastructure limitation in most developing countries has become obstacles to put these in use.

According to the census of 2012, Morogoro Municipality has the human population of 352,904 and demand of clean drinking water is 40,755m^3/day (MORUWASA, 2013). The community depends on piped water from Morogoro Urban Water Supply and Sanitation Authority (MORUWASA) which outsources the water mainly from Mindu dam and rivers like Morogoro river. Therefore 85% of all water provided to the Municipality comes from MORUWASA, 5% have own water sources, and the remaining 10% fetches water direct from streams, rivers and locally dug wells. Chlorination is the only means of water treatment practiced in Morogoro Municipality still there are several water-borne diseases. NBS (2005) and Oketcho *et al.* (2012) found that diarrhea cases are at high rate despite water treatment by chlorination this implies that water treatment methods used may not be effective. Yet there has been no study which tried to assess the effectiveness of water chlorination in Morogoro water treatment plants. This study was conducted to assess the bacterial load and coliform count in chlorinated water supplied in Morogoro Municipality.

MATERIAL AND METHODS

Study area

The study was conducted in Morogoro Municipality which covers 260 km^2 and has a population of 352,904 people (PHC, 2012). The Municipality is located in the Eastern part of Tanzania, 169 kilometres west of Dar es Salaam, the country's largest city and commercial centre, and 223 kilometres east of Dodoma, the country's capital city. The Municipality is supplied with water from different water sources such as Mindu dam, Morogoro river and independent Sokoine University of Agriculture (SUA) water supply. With this study the representative treated tap water from each source was used.

Water sample size and collection

Twenty four chlorinated water samples from the randomly selected taps supplied by three different sources (Mindu dam, Morogoro river and SUA independent source) were collected for analysis. Of these, 12 were collected during dry season and 12 during rainy season. Sample was collected from the representative tap at LITA, and Folkland (Morogoro river supply), Kichangani and Mafiga (Mindu dam source), Collegeof Veterinary Medicine and Biomedical Sciences and Department of Animal Science and Production (DASP) from SUA Independent water supply). Before water sample was collected from the tap, the tap openings were sterilized using flame and then water was allowed to run for 3 minutes. Then 500 mL of water sample was collected into sterile glass bottle and immediately the sample was placed in the cool box with ice packs.

To get the true picture on what is in the water sources, 28 un-chlorinated water samples from the selected sampling points at the three water sources (Mindu dam, Morogoro river and SUA independent source) were collected for analysis. The approach to sampling was done as described by WHO (1984). Briefly, the sterile sample bottle was opened and sunk at approximately six inches below the surface of the water. A total of 500 mL of water sample was collected leaving an air space of 2.5 cm close to the rid and immediately the sample was placed in the cool box with ice packs. Fourteen samples were collected during each of the dry and rainy seasons.

Therefore, a total, 52 water samples were collected for the whole study. Subsequently after the field work of each day, the water samples were being shipped to the University Microbiology laboratory for analysis within 24 hours after sampling.

Laboratory sample analysis

Total viable count (TVC)

TVC of water samples was determined as described by Harrigan and McCance (1976) and TZS (2007) in a such way that normal saline (9 mL) was added in ten test tubes arranged in a single row. Then 1 mL of water sample was added in each test tube above, followed by serial dilution where 1 mL was transferred from one test tube to another and the last 1 mL was discarded. The diluted samples were inoculated in the Petri dishes that contain nutrient agar and incubated at 37°C overnight. The TVC on the plates was performed using a protocol described previously (ISO 721, 2007). Colony forming units (cfu) were counted on at least two critical dilution plates by the aid of colony counter. Two consecutive plates with 15 to 300 colonies were considered for record (ISO 4833:2003(E)). The countable colonies were converted into the mean colony forming units per millilitre (CFU/ml) using a formula: $N = \Sigma C / (n_1 + 0.1n_2)d$ where N = the number of bacteria counted, C = sum of colony counted in two successful dilutions, n_1 = the number of dishes retained in the first dilution, n_2 = the number of dishes retained in the second dilution and d = dilution factor corresponding to the first dilution (ISO 4833:1991(E)).

Total Coliform Count (TCC)

Most Probable Number (MPN) method was adopted for determination of TCC. Nine test tubes with the Durham tubes in inverted position were arranged in three rows. The first row test tubes were added with 10 mL of double strength MacConkey broth media whereas the second and third rows were added with single strength media MacConkey broth. In the test tubes of the first row, 10 mL of the sample was inoculated where as in the second and third row a test tube 1 mL and 0.1 mL of the sample was inoculated respectively. The inoculated tubes were incubated at 37ºC over for 24 hours. Tubes with positive results were indicated by the change in colour of the media from pink to yellow and the formation of gas in the Durham tube. The MPN tables for 3 rows tubes were used to report the result of the MPN of coliform bacteria per millitre of water (WHO, 1984; Harrigan and McCance, 1976).

Data analysis

The data was analysed using Microsoft Excel Spread sheet 2010 to obtain Central tendency and One way Analysis of Variance (ANOVA) was used to compare the results of TCC and TVC in each season. Significance differences were observed at $P < 0.05$.

RESULTS

Results for assessment of bacteria load in chlorinated water by MPN and TVC methods are shown in Table 1. The results showed that TCC for positive chlorinated samples during dry season ranged between 3.6/100 mL and 150/100 mL with mean value of 47.2/100mL while it ranged between 15/100 mL and 150/100 mL with mean value of 40.5/100mL during rainy season. The highest coliform count was found at DASP in both seasons for chlorinated tap water. The results show no statistical significant when TCC during dry season was compared with TCC during rainy season (P=0.731). Also the results for TVC ranged between 530 CFU/100 mL and 600 CFU/100 mL during dry season and have the mean value of 561.6 CFU/100mL and the same range during rainy season with mean value of 572.5 CFU100/mL. Though the results show statistical significant when two seasons were compared (P=0.332).

Table 1. Bacteriological results of TCC and TVC in chlorinated water (tap water) during dry and rainy seasons

Water source	Tap water sampling sites	Dry season		Rainy season	
		TCC/mL	TVC/mL	TCC/mL	TVC/mL
Morogoro river	LITA 1	0.092	5.5	0.23	5.8
	LITA 2	0.036	5.3	0.20	5.6
	Folkland 1	0.15	5.6	0.43	5.9
	Folkland 2	1.5	6.0	0.20	5.4
Mindu dam	Kichangani 1	0.20	5.9	0.21	5.3
	Kichangani 2	0.23	5.3	0.23	6.0
	Mafiga 1	0.43	5.9	0.15	5.8
	Mafiga 2	0.21	5.8	0.93	5.6
SUA independent water supply	FVM 1	0.28	5.7	0.23	6.0
	FVM 2	0.11	5.0	0.20	5.7
	DASP 1	1.5	5.8	0.35	5.6
	DASP 2	0.93	5.6	1.5	6.0

Additionally, assessment of bacteria load in non-chlorinated water is shown in Table 2. The results showed that TCC for positive non-chlorinated samples during dry season ranged between 210/100 mL and >1100/100 mL while it was >1100/100 mL during rainy season. No statistical difference was observed when the TCC results of three sites were compared in each season. Also, it was established that the TVC ranged between 530 CFU/100 mL and 600 CFU/100 mL during dry season and 600 CFU/100 mL to 650 CFU/100 mL during rainy season. The TVC was also statistically insignificant between sites and season.

Table 2. Bacteriological results of TCC and TVC in non-chlorinated water during dry and rainy seasons

Water source	Sampling sites	Dry season		Rainy season	
		TCC/mL	TVC/mL	TCC/mL	TVC/mL
Morogoro river	Point 1	>11	6.3	>11	6.5
	Point 2	11	6.2	>11	6.3
	Point 3	>11	6.1	>11	6.1
	Point 4	2.1	6.0	>11	6.0
Mindu dam	Point 1	11	6.0	>11	6.2
	Point 2	11	5.9	>11	6.3
	Point 3	>11	6.2	>11	6.4
	Point 4	>11	6.3	>11	6.2
SUA independent water supply	At 0700h	>11	6.2	>11	6.2
	At 1000h	11	6.3	>11	6.3
	At 1300h	11	6.1	>11	6.1
	At 1600h	2.1	6.0	>11	6.4

DISCUSSION

The purpose of this study was to assess bacterial contamination in chlorinated tap water during both dry and rainy season. The sampling of water from the selected water taps was done from October 2013 to January 2014. It was found that all the water samples (chlorinated) contained coliform contrary to the standard by WHO (0 count per 100 mL) suggesting that water was likely contaminated with faeces. This predisposes the water consumers to dangers of waterborne pathogens. Also this call for thorough water treatment by the responsible authority and the water consumers should be educated to further treat the water at household level. The study found that during the dry and rainy seasons the TCC was high in chlorinated water which exceeded the standard according to WHO (0 count per 100 mL) and TBS which is 0 to 10 counts per 100 mL. This is an interesting finding since it was expected that chlorinated water would have no or very low counts of coliform bacteria. Similar findings was also noted by Shayo *et al.* (2007) in small community supplies in Kingolwira village in Morogoro, that faecal coliform ranging from 0.93 x 10^3 to 2.1 x 10^2/100 ml. The possible causes of high TCC in chlorinated water can be caused by several factors including presence of new contaminations that were likely due to leakages in the pipes in the pipeline distribution network.

In addition, inefficiency chlorination process may be another possibility for the high coliform count. It is suggested that for chlorination to be effective, water pH should be less than eight, turbidity of less than 5 Nephelometric Turbidity Unit (NTU) and 0.5 mg/L free chlorine residues for 30 minutes as contact time. Nevertheless, detection of bacteria in chlorinated water may be caused due to presence of suspended particles. Studies show that majority of the bacteria in water are attached to particles which act as a shield against chlorine. Microbes entrapped in particles or adsorbed onto surfaces are shielded from disinfection and are not inactivated by the process of chlorination. A study by Wolfe *et al.* (1985) reported presence of coliforms and faecal coliforms in chlorinated water. Again a study by Ridgway and Olson (1982) showed that the majority of viable bacteria in chlorinated water were attached to particles. All these are evidences that not always the chlorinated water is free from microbial contaminations.

Another finding of this study is the observed high TCC in non-chlorinated water. Under general situation contamination may occur due to natural and human related causes. The natural causes include nature of the water catchment area may predispose the water source to contamination. Natural water runoff that may cause floods and erosion enhances contamination from the upland areas. The other natural cause of water contamination is decomposing organic matter and faeces of wild animals. Meanwhile, the human related activities give effluent discharges which find their way to waterbodies and play a big role in water contamination. Such effluents may be municipal and industrial wastes, stormwater runoff and infiltration from waste disposal sites and animal wastes (Chapman, 1996). Mindu dam catchment areas and valleys of Morogoro river have a lot of human activities which are likely to be sources of water contamination which was observed in the current study (Mdegela *et al.,* 2010).

Furthermore, the results of TVC shows the highest count was 650 CFU/100 mL which is within the recommended standard by WHO (TVC should not exceed 1000 CFU/100 ml at 37°C). However, comparison of TCC and TVC for rainy and dry seasons showed no statistical significant (P>0.05) meaning that levels of contamination was almost the same during the two seasons. Although TCC and TVC recorded during the rainy season were slightly high. During the rainy season, there is a lot of surface run off which may carry all the contaminations from uplands to water bodies like dams and rivers. Nevertheless, the dry period is also

associated with high use of water for irrigation and other human related activities which may expose the water bodies to contaminations. Also, during the dry season, there are low levels of water in the water bodies that may lead to concentration effects of the contaminants that even a minor contamination is detectable. All these account for a lack of differences in levels of bacterial contamination noticed during the rainy and dry seasons in the study areas.

Based on the results of this study, it is concluded that chlorinatedand non-chlorinated water show high number of TCC than that recommended by WHO and TBS. However, there was low TVC which was within recommended standards. High TCC poses a risk of acquiring water-borne diseases to the Morogoro community. Since high bacterial contamination was recorded in both chlorinated and non-chlorinated water, the community in Morogoro municipality should retreat the tap water. The water authority, MORUWASA should recheck the chlorination process they do to water and try to be doing routine water quality monitoring to ascertain the quality of water they supply to their clients.

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