Bacteriological Assessment of Drinking Water in Asmara, Eritrea

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Abstract
Asmara city uses drinking water from artificially dammed surface water reservoirs that are generally prone to direct entry of pollutants. The aim of this study was to assess the quality of this water by investigating for the prevalence of coliforms and enteric bacteria which indicate fecal pollution in water. A total of 88 samples were collected from the two main reservoirs, Mainefhi and Toker as well as treatment stations and consumer taps supplied from them. Microbiological analyses were done for the detection of total coliforms, thermotolerant coliforms (TtC) and enteric bacteria. The Most Probable Number (MPN) tests for coliform counts indicated a very high percentage of TtC, with 60% to 100%, for both water reservoirs. Seasonally, there was no significant difference in the findings, with 60% and 67% for the rainy and dry seasons respectively for Mainefhi (p=1.000, Fisher's exact test) and 100% each for both seasons for Toker reservoir. No coliforms and enteric bacteria were detected in treated water from the treatment stations. Conversely, high presence of indicative bacteria and seasonal variation was observed for consumer taps. For those supplied from Mainefhi reservoir, the rainy season finding for TtC of 67% was significantly higher than for the dry season level of 8% (p=0.002, Fisher's exact test), whereas for the Toker reservoir supplied, the differences were not significant, with 28% and 42%, for the rainy and dry seasons, respectively (p=0.712, Fisher's exact test). The findings showed that a notable part of Asmara drinking water is contaminated with fecal coliforms indicating fecal pollution. Therefore, regular and frequent examination as well as appropriate maintenance and follow-up of the water supply system should be practiced to assure it conforms to the national and international drinking water quality standards.

Key words: Asmara drinking water, Mainefhi reservoir, Toker reservoir, Microbiological analysis, Faecal pollution, Coliform bacteria
Introduction:
Water is undoubtedly the most precious natural resource that exists on our planet and life on earth would be non-existent without it. However essential water is to human life, it is still being polluted greatly by human activities. The main sources of water for human use are surface waters and ground waters. Of these, surface water sources are the most polluted.

Water is a scarce resource in Eritrea as a country (Gebremariam, 2005) and Asmara, its capital city, suffers a severe shortage of drinking water supply, particularly in the dry seasons of every year. There are no perennial natural fresh surface water bodies in the country, they are only seasonal, being dry all year along, except for the rainy season (Habtezion, 2011). For this reason artificially dammed water bodies and reservoirs that collect rain water runoffs are the sources of drinking water for Asmara city and many other urban centers in the country. As it is usual for urban centers in developing countries, one would expect the quality of the water from surface water reservoirs to be deteriorated because of the direct entry of pollutant wastes and untreated sewage from catchment areas into such water bodies and inefficient management of the drinking water treatment and distribution systems. Actually, a policy paper of the Eritrean government stated that pollution of the country’s meager water resources is a serious concern (GoE, 1994).

Unfortunately, many pathogens, are transmitted through drinking water supply. Diarrhea, which is caused by poor water quality, sanitation and hygiene is one of the ten top causes of deaths in children under 5 years in Eritrea. A report based on data from National Health Management and Information System (NHMIS) in Eritrea clearly demonstrated that diarrhea was a major public health problem in the country which needed attention (WHO, 2011; Mufunda, et al, 2006).

Ideally, drinking water should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution. Detection of faecal indicator bacteria in drinking water provides a very sensitive method of quality assessment and it is not possible to examine water for every possible pathogen that might be present (WHO, 1996). Actually, coliform bacteria are the commonly used bacterial indicator for assessing the quality of water (Rompré, et al, 2002). Coliform bacteria are abundant in the feces of warm-blooded animals but can also be found in soil, aquatic environments and vegetation. Studies suggest that Escherichia coli (E. coli) is a more reliable indicator of fecal pollution and the occurrence of pathogens in water than other coliforms (Leclerc, et al 2001). Apart from that, many countries still use thermotolerant coliform (TtC) enumeration to provide a legal basis to state water quality. For example, according to Canadian Legislation either E. coli or fecal (TtC) coliforms can be used if experience shows that greater than 90% of the fecal coliforms are E. coli (Health and Welfare Canada, 1992). Enumeration of TtC was taken as the main indicator in this study as 'the count of TtC bacteria is an acceptable alternative' (WHO, 2011) and usually, more than 95% of TtC isolated from water are the gut organism E. coli (UNEP/WHO, 1996).

The present study was undertaken to investigate the bacteriological quality of Asmara water supplied from the two surface water reservoirs, with emphasis on the prevalence of faecal coloforms as pollution indicators as well as other enteric pathogenic bacteria in Asmara drinking water.

Materials and Methods

Sample Collection:
A total of 88 water samples were collected from different parts of Asmara drinking water supply for the two seasons, 52 during the rainy season months of July and August and 36 during the dry season months of February and March. Out of the total sampled, 16 were from the two untreated water reservoirs of Mainefhi and Toker, 12 from treated water at the two treatment stations attached to the reservoirs and 60 from taps of consumers supplied from the two reservoirs. Random sampling was adopted for the study.
The water samples were collected in pre-sterilized screw capped Borosilicate glass bottles of 250 ml volume aseptically, according to APHA and WHO guidelines (APHA, 1998; WHO, 2006). For samples collected from consumer taps, five drops of 10% sodium thiosulphate solution was added to the bottles in order to neutralize the residual free chlorine. The samples were then transported to the laboratory in a cold box with ice packs and preserved in refrigerators until analysis time, which was done within 24 hours of collection.

**Bacteriological Analysis:**
In the laboratory, all samples were subjected to Multiple tube fermentation test for determination of Most Probable Number (MPN) of total coliforms and TtC (faecal coliforms) according to UNEP/WHO Guidelines (1996) and then different biochemical tests were carried out on samples that showed growth of coliforms for the identification of enteric bacterial species based on Barrow and Feltham’s Manual (2003).

**Multiple tube fermentation test:** This test comprised two steps: (a) presumptive test and (b) confirmatory test. The presumptive test was done for total coliforms using 15 test tubes containing 10 ml MacConkey Broth medium and inverted Durham's tubes which were sterilized and inoculated with sample water. Inoculation was done as follows:
(i) 10ml of the original sample added to each of 5 tubes containing double-strength medium.
(ii) 1 ml of the original sample added to each of 5 tubes containing single-strength medium.
(iii) 0.1 ml of the original sample added to each of 5 tubes containing single-strength medium.
All tubes were incubated at 37°C for 48 hours for the observation of acid and gas production. First reading was taken after 24 hours to record positive tubes, and the negative ones were incubated for another 24 hours.

For the confirmatory test, all the tubes positive for presumptive test were subcultured into 10 ml Brilliant green lactose broth (BGLB) with inverted Durham's tubes for confirmation of total coliforms and 10 ml of Tryptone water to determine the presence of TtC after indole production. The BGLB tubes were incubated at 37°C for 48 hours and the Tryptone water at 44°C for 24 hours. The BGLB tubes showing acid and gas and Tryptone tubes positive for indole production were taken as positive for the respective types of coliforms.

From the distribution of the positive tubes, Most Probable Number (MPN) of both Total coliforms and TtC was determined by referring to standard probability table for estimation of Total Coliforms (UNEP/WHO, 1996).

**Identification of enteric bacteria:** This was carried out by different biochemical tests such as Indole, Methyl Red (MR), Voges-Proscauer (VP), Citrate (Simon’s), Triple Iron Sugar (TSI) and Urease tests on the water samples that showed growth of microorganisms.

**Statistics:** SPSS, PASW Statistics 18 was used for analysis. Significance tests were run with Fisher's Exact Test for the differences in the results of seasonal variations, specifically for TtC.

**Results and Discussion:**
Results of the MPN test (Table 1) show that samples from both raw water source reservoirs have very high growth rates for both the rainy and dry seasons, ranging from 60% to 100%, and especially those from Toker reservoir which showed 100% growth consistently. It is common to get high amounts of fecal pollution indicator organisms in untreated waters from open surface reservoirs as they can be contaminated by wastes
of mammals and birds, from manure used in agriculture, storm runoff and from human sewage. Apparently, primary sources of these types of bacteria in water are animal and human wastes. Comparison of these findings with previous similar studies of Asmara water supply was not possible as such published studies could not be found. However, there are similar findings in surface water sources of other urban water supplies of developing countries, where all samples from the surface water sources were found contaminated with both groups of coliforms. For example, Khartoum, Sudan (Ibrahim, et al, 2010), Dhaka, Bangladesh (Ackerjee, et al, 2011), Sragodha city, India (Haydar, et al, 2013), and Hilla city Iraq (Naji, et al, 2011). Regarding seasonal variations in contamination, there was no significant difference in both reservoirs, especially for TtC, with 60% and 67% for the rainy and dry seasons, respectively, for Mainefhi (p=1.000, Fisher's exact test) and 100% each for both seasons for Toker reservoir. Conversely, growth of both Total coliforms and TtC, was not detected in treated water samples taken from both treatment stations of Asmara drinking water supply. This means it conforms to WHO standards (WHO,1997) and hence indicating that the treatment process in the stations was bacteriologically satisfactory.

On the other hand, the samples from taps of consumers demonstrated variable rates of growth of the coliforms for the two seasons. Samples from consumer taps supplied from Mainefhi treatment station showed very high growth of both coliforms during the rainy season, with 72% for total coliforms and 67% for TtC, and low rates during the dry season, with 17% for total coliforms and 8% growth for TtC. The seasonal variation for TtC at consumers supplied from Minefhi was significant with p=0.002, Fisher's exact test. Whereas samples supplied from Toker reservoir indicated percentages slightly lower for the rainy season, with 39% and 28%, than those for the dry season, which were 58% and 42% for total coliforms and TtC, respectively. The seasonal variation for TtC here was not significant with p=0.712, Fisher's exact test. These results are in agreement with those reported by Shar, et al, in Pakistan (2008). This increase of contamination in consumer taps during the rainy season may be due to the rise of ground water levels and leakage into the pipes of the water distribution system of those areas, which may also be rusty and leaking due to old age, as Asmara's water distribution pipes were laid during the colonial times, in the 1920's and again in 1968 to 1972 with the expansion of the city. In general, however, these results of the drinking water from consumer taps indicate high contamination levels. There are similar findings from other cities of developing countries, some examples are, Bahrdar city, Ethiopia with 37% (Tabor, et al, 2011), Nyala city, Darfur-Sudan 45.2% (Abdelrahman, et al, 2011), Lonar city, Maharashtra, India 70% (Borul et al, 2013), and Lahore city, Pakistan 37.2% (Anwar et al, 2010).

The ranked classification of the magnitude of the MPN count of TtC in samples from both the untreated and treated water supply sources is presented in Table (2). From among samples of the untreated reservoir water, only two for the rainy and one for the dry seasons, from Mainefhi reservoir, are in the category conforming to WHO standards. The low risk category includes one sample from Mainefhi for the rainy season and two each from both reservoirs for the dry season, while the rest of the samples are in the intermediate and high risk categories. In contrast, all samples from both the treatment stations as well as, 3 (33%) and 13 (72%) samples for the rainy season collected from Mainefhi and Toker supplied consumer taps, respectively, and 11 (92%) and 7 (58%) samples for the dry season collected from Mainefhi and Toker supplied consumer taps, respectively, fall in the WHO compliance category. As for the rest of the samples from consumer taps, 4 (22%) from the Mainefhi supplied and 3 (17%) from the Toker supplied, both for the rainy season are in the 'low risk' classification, while 7 (39%) from Mainefhi supplied for the rainy and 3 (25%) from Toker supplied for the dry seasons fall in the intermediate and high risk categories, respectively. There are no samples in the 'very high risk' classification for both seasons.
According to Table (3), the untreated water samples for both seasons collected from both reservoirs demonstrated high presence of enteric bacterial species, with 100% of samples from Toker reservoir for both seasons; and 100% and 60% for the dry and rainy seasons, respectively, for Mainefhi reservoir. For samples from consumers taps, the findings show detection rate of 61% for the rainy and 17% for the dry seasons for those supplied from Mainefhi source, indicating the rainy season results as 3.6 times higher than those of the dry season. The results of samples from the Toker source supplied consumer taps, conversely, demonstrate that the rainy season findings are more than two times lower than those of the dry season results. One can observe that the findings in this table also tally with those of Table (1).

Biochemical tests were done for a total of 39 water samples, 24 collected during the rainy and 15 during the dry seasons, that demonstrated growth during the Presumptive test phase of the MPN method. The 14 samples were from the reservoir (total of 16) samples while the 25 were from the consumer tap (total of 60) samples. As shown in Figure (1), a total of 12 types of enteric bacteria, out of which 5 were detected in both seasons. Aeromonas hydrophila was the most identified species, as it was identified in 47% of samples in the dry season and in 13% of samples of the rainy season. Proteus rettgeri (Providencia) was the next as it was identified in 33% samples from the rainy season. Other species following were Citrobacter diversus in 16% and 13% of rainy and dry seasons, respectively, and Escherichia coli in 13% of samples for each season. The remaining eight organisms, Klebsiella pneumoniae sub-spp pneumoniae and Pseudomonas aeruginosa, detected in both seasons, Enterobacter aerogenes, Moraxella, Serratia marcescenes and Yersinia enterocolotica, identified in the rainy season only, and Klebsiella pneumoniae sub-spp rhinoscleromatis and Shigella sonnei detected in the dry season only, were identified each in one sample.

Of particular concern in this study is the high presence of TtC and enteric bacteria, which are indicative of faecal contamination, particularly in consumer taps. Such problems may indicate post-treatment contamination in the water distribution process during its passage in water pipes. The contamination can occur due to defective joints, back siphonage, rusted pipelines crossing over the sewage pipes and low/high pressure in the pipelines. In piped supplies, discontinuity also increases the likelihood of contamination as the risk of back siphonage into the distribution network is increased when pipes are at lower pressure than the surrounding soil. Such phenomena are possible with Asmara city supply lines as most of the pipelines are of metal and very old. Although the current government of Eritrea, after independence in 1991, had started replacement of the old lines with new PVC pipes, the work done so far covered only a small part of the city. In addition, in recent years, with acute shortage of water resources, there are frequent discontinuities of water distribution through the lines to the consumers. The increase in bacterial contamination during the rainy season may also be explained by the fact that with the rising of the ground water levels, water may enter the distribution system due to the leakages of pipes.

The present findings are of notable public health concern as they clearly indicate that a significant percentage of the water supply of Asmara city is contaminated with fecal coliforms. They do not conform to the WHO guideline values, which specify that E. coli or TtC bacteria must not be detectable in any 100 ml sample of all water directly intended for drinking (WHO, 2011). Accordingly, keeping in view with the contamination levels of drinking water seen in the city of Asmara and the grave public health consequences that could emanate, it is of vital importance that water be examined regularly and frequently throughout the year and appropriate maintenance and follow-ups are put into practice. It is also essential that the replacement of the old water lines, that the city administration had started, be facilitated and hastened. Moreover, water quality treatment facilities be strengthened with appropriate and advanced devices and instruments.
Table 1: MPN results of the water samples that are positive for indicator bacteria by season

<table>
<thead>
<tr>
<th>Location</th>
<th>Percent of samples showing indicator bacteria</th>
<th>Number of samples by season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rainy season</td>
<td>Dry season</td>
</tr>
<tr>
<td></td>
<td>T.C.</td>
<td>TtC.</td>
</tr>
<tr>
<td>Mainefhi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source water</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Treatment station</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Consumer taps</td>
<td>72</td>
<td>67</td>
</tr>
<tr>
<td>Toker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source water</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Treatment station</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Consumer taps</td>
<td>39</td>
<td>28</td>
</tr>
<tr>
<td>WHO Guideline values&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Total and Thermotolerant Coliforms must not be detectable in any 100 mL sample</td>
<td></td>
</tr>
</tbody>
</table>

T.C. = Total coliforms; TtC. Thermotolerant coliforms

<sup>a</sup> World Health Organization guideline values for water intended for drinking [4].

Table 2: Magnitude of indicator Thermotolerant coliforms detected by source and location

<table>
<thead>
<tr>
<th>Count in MPN/100 mL</th>
<th>Rainy Season</th>
<th>Dry Season</th>
<th>Remarks&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reservoir</td>
<td>Treatment Station</td>
<td>Consumer</td>
</tr>
<tr>
<td></td>
<td>MN</td>
<td>TK</td>
<td>MN</td>
</tr>
<tr>
<td>&lt; 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>2 - 10</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11 - 100</td>
<td>1</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>101</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>&gt; 1000</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

TOTAL 5 5 3 3 18 18 3 3 3 3 12 12

Trt. St. = Treatment Station; MN = Mainefhi Reservoir; TK = Toker reservoir

<sup>a</sup> The corresponding values indicated in the first column are ranked according to magnitude of risk as specified in WHO [18] literature, especially for 'thermotolerant (faecal) coliforms or E. coli in water supplies'.

<sup>b</sup> When computing MPN values per 100 ml of sample, with the 'five 10-ml, five 1-ml and five 0.1-ml' test portions usage (applied in this study) MPN value given to all negative results is '< 2' instead of '0' [10].
Table 3: Water sources and percent (number) of samples with Enteric bacterial contamination.

<table>
<thead>
<tr>
<th>Location</th>
<th>RAINY SEASON</th>
<th>DRY SEASON</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. samples</td>
<td>of Samples</td>
</tr>
<tr>
<td></td>
<td>per each location</td>
<td>Mainefhi reservoir</td>
</tr>
<tr>
<td>Reservoir</td>
<td>5</td>
<td>60 (3)</td>
</tr>
<tr>
<td>Treatment station</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Consumer taps</td>
<td>18</td>
<td>61 (11)</td>
</tr>
</tbody>
</table>

Figure 1: Type and percent of Enteric bacteria identified in the water samples.

Enteric species

* Kl. Sub-spp. pneumoniae = Klebsiela pneumoniae sub-spp. pneumoniae; * Kl. Sub-spp. rhinoscleromatis = Klebsiela pneumoniae sub-spp. rhinoscleromatis; * P. rettgeri = Proteus rettgeri (Providentia)

Conflict of Interest:
The authors declare that they have no conflict of interest.

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