Original article

Microbiological quality and safety of bottled water brands sold in Ethiopia

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Abstract

Background: Water plays an indispensable role in promoting public health. Even though the quality of piped drinking-water is getting better and better, the majority of the world population tend to consume different bottled water brands due to the assumption that bottled waters are safer and healthier than tap water.

Objectives: The aim of the study was to check whether the bottled water brands sold in Addis Ababa are bacteriologically safe for consumption.

Methods: Samples of 11 domestic and 2 imported bottled water brands (N = 325) were randomly purchased from retail stores and supermarkets in Addis Ababa on five different occasions and analyzed for heterotrophic organisms, thermos-tolerant coliforms, *Intestinal enterococci*, *Aeromonas* species, and *Pseudomonas* species between July 2013 and May 2014.

Results: According to the findings, heterotrophic organisms were detected in all brands, but 66.2% of all samples. The rest 33.8% of the samples (60% imported and 29% of domestic brands, by using Fisher's exact test, $P > 0.05$) were found to be free of heterotrophic organisms. And yet, 83.1% of all the samples assumed to be safe for human consumption (< 100 colony forming units). Similarly, *Pseudomonas* spp., *Aeromonas* spp., and *Intestinal enterococci* were detected from 75.4%, 21.5%, and 3% of samples respectively. But no thermo-tolerant coliforms were detected.

Conclusions: The presence of heterotrophic organisms and *Pseudomonas* species in the majority of the samples implied that some bottling companies might have faulty water treatment or packaging practices. Thus, preventive and corrective actions may need to be taken in order to improve on the purity of the water. [*Ethiop. J. Health Dev.* 2014;28(3):178-184]

Introduction

It has long been known that water plays an important role in the transmission of infectious microorganism to humans (1). The understanding of the public about the dangers of waterborne diseases, in particular during travel has led to an increased demand for bottled drinking water. This escalated demand may be due to their convenient packaging, ease of access, and better taste than tap water (2). However, the main reason rests on the assumption that bottled water is safer and healthier for consumption than piped water (3). Even then, bottled water (especially the so-called natural water) is not sterile and may contain naturally occurring microorganisms as well as those introduced during manufacturing and packaging processes (4).

Potentially pathogenic organisms, even strict pathogens could be found in bottled water products. Most notably, the cholera epidemic of 1974 in Portugal (5), an outbreak of *Pseudomonas aeruginosa* infections in Germany (6), and detection of *Staphyclococus aureus* in water processed and bottled in Zimbabwe (7) can rightly be considered as primary cases in point about the risk of transmission of dangerous pathogenic organisms to consumers through bottled water.

Apart from the above-mentioned examples, water quality indicator organisms were often observed. A microbiological assessment done in Zimbabwe reported that 6.7%, 6.7% and 11.7% of the bottled water samples contained heterotrophic organisms, *Pseudomonas* spp. and coliform bacteria respectively above the limits recommended by the WHO (7). A similar study in Spain (8) found considerable number of heterotrophic organisms in mineral water samples. Even more alarming finding from Trinidad showed that 5.2% of the tested samples contained coliforms and nearly 1.5% had E. coli (9). Another study from Greece again detected species of *Pseudomonas*, *Aeromonas*, and *Intestinal enterococcus* from bottled water samples (10). In addition, a research conducted in Tanzania revealed that heterotrophic bacteria were present in 92%, total coliform from 4.6% and fecal coliform bacteria from 3.6% of bottled water samples analyzed (11). A similar study from Egypt also found out that about 54.8% of analyzed samples were below the acceptable national standards (12).

In the midst of all this, however, it is being witnessed that the market demand and the profit from this business is becoming so overwhelming that the number of bottling companies is increasing steadily all over the world (2, 4) including in developing countries like Ethiopia (13), and this trend is likely to continue. Although, there are no confirmed health outcomes from consumption of bottled water in Ethiopia, it is sensible to be skeptical about the microbiological quality of these products. The studies conducted about the quality of drinking water, especially bottled products, are rather few and limited in scope (14). Hence, the importance of this study can be amplified due to an extensive distribution of bottled waters in the

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country especially in urban centers. Over and above this, bottled water products are being consumed by a wide range of the population including the immune-compromised groups. The present study was conducted to evaluate the bacteriological quality of the most widely distributed bottled water brands in Addis Ababa, Ethiopia, by giving special attention to indicator organisms, particularly thermo-tolerant coliforms and *Intestinal enterococci*, as well as organisms that show the general cleanliness of the production process such as heterotrophic organisms, *Pseudomonas* and *Aeromonas* species.

Methods

***Study Design*:**
A cross-sectional study was conducted within a time frame of about 11 months (between July 2013 and May 2014).

Study Area:
Samples of different bottled water brands were collected on five different occasions in Addis Ababa and tested for their microbial quality.

***Sample Collection and Handling*:**
Five bottles of water from 13 widely available and sold bottled water brands were collected (A, B, C, D, E, F, G, H, I, J, K, L, and M) randomly from retail stores and supermarkets at five different occasions and analyzed within 24hours for their microbiological quality in the Public Health Microbiology Laboratory of the Ethiopian Public Health Institute. From 13 brands included in the study, 11 of them were produced and bottled in Ethiopia and the other two were imported brands, specifically from Europe. To neutralize the bottle/volume effect, 500mL bottles were purchased. For the sake of comparison, 25 tap water samples were collected from different locations in Addis Ababa in accordance with the ISO Guidelines (15) and a similar laboratory procedure was followed for bottled water samples.

Sampling:
Each time five bottles (of the same batch of production) of water from each brand were drawn from the refrigerator and made ready for analysis as a single sample. Each bottle was thoroughly mixed by inverting up and down to have an even distribution of microorganisms, and then equal volume (20mL) of water from each bottle of water was taken and mixed together in a sterile bottle to have a composite sample of 100mL. Such a composite sample was then marked for identification, and run as a single composite sample. As a result, a total 65 composite samples (325 bottles from 13 brands) were incorporated in the study.

***Laboratory Procedures and Analysis*:**
Laboratory tests were done for heterotrophic bacteria, thermos-tolerant coliforms, *Intestinal enterococci*, *Pseudomonas* spp., and *Aeromonas* spp. for all bottled and tap water samples. These organisms were chosen for this assessment as they are being used as indicators of water quality deterioration and contamination from domestic wastes. Specifically, the presence of thermo-tolerant coliforms and *Intestinal enterococci* has been used as indicators of fecal contamination. They may even indicate the presence of microbial pathogens (16).

With the exception of heterotrophic organisms – which were enumerated through pour plate techniques from 1mL of water sample – we used membrane filtration (MF) procedures to detect and count thermo-tolerant coliforms, *Intestinal enterococci*, *Aeromonas* spp., and *Pseudomonas* spp. from 100mL of water samples. This method is being considered as the most accurate, less labor-intensive, and widely used method for quantitative studies in bottled water microbiology (17). We did this by pumping 100mL composite sample of each brand through membrane filters, and then by putting under appropriate media and temperature. Filtration of samples was performed by nitrocellulose membranes (0.45µm pore size, 47mm diameter, Pall–Gelman Laboratory) (18-20).

***Heterotrophic bacteria*:** According to WHO, there is no single universally dominant method for enumeration of heterotrophic bacteria (1). For this particular study, however, pour plate technique using nutrient agar at incubation temperature of 35-37°C for 48hours was used. Pour plates were prepared by mixing 1mL of water sample with nutrient agar (at a temperature of 45.0±1.0°C), in Petri dishes. Following incubation under the conditions specified, the number of colonies that grew on the medium were counted (17-18).

***Thermo-tolerant coliforms and intestinal enterococci*:** The investigation of thermo-tolerant coliforms was done on 100mL samples of water. The procedure used for this specific assessment was membrane filtration followed by incubation of the membranes on selective medium (Tryptone Bile X-Glucuronide) at 44–45°C and then enumeration of colonies after 24hours. Similarly, *Intestinal enterococci* were isolated by plating the membranes on Slanetz and Bartley medium and incubating at 37°C for 48 hours. The isolated organisms were confirmed by transferring the membranes onto Bile-Aesculin-Azide agar, and preheated at 44°C (1, 17, and 19).

***Pseudomonas* and *Aeromonas* species:** For *Pseudomonas* species, 100mL composite samples from each brand were filtered through membrane filters and then placed on a solid medium containing magnesium chloride and potassium sulfate to enhance pigment production. The medium was made selective by the addition of cetyl trimethyl-ammonium bromide and nalidixic acid. After 48 hours of incubation at 37°C, *Pseudomonas* spp. showed their characteristic blue-green colored colonies when available. In the case of *Aeromonas* species, the medium used was *Aeromonas* agar and incubated at 37°C for 24 hours. The growth of these microbes was heralded by their distinctive yellowish green colonies (20).

***Statistical Analysis*:**
The results obtained from the laboratory analysis were evaluated and summarized by a combination of descriptive and inferential statistical methods. Frequency distribution tables were prepared when appropriate and one-sample *t*-test was performed to check whether the mean heterotrophic plate counts (HPC) observed form each brand were significantly different from the reference value of 100 colony forming units (cfu) per mL of drinking water set by the Ethiopian Standards and the WHO. Moreover, Fisher's exact tests were performed using MINITAB®15 to determine whether statistically significant differences existed between the microbial counts of domestic and imported brands. The microbiological results obtained from this assessment were also compared with the Ethiopian Standards, the WHO guidelines and findings of other studies (1). For all statistical tests, *P*-value of < 0.05 was considered statistically significant.

Results

In this study 65 composite samples (N = 325 bottles) of 11 domestic and 2 imported bottled water brands were analyzed between July 2013 and May 2014.

***Heterotrophic Organisms*:**
The outcomes of the investigation showed that heterotrophic organisms were detected from all the sampled brands, but only from 66.2% of the samples. In comparison, 60% of samples of imported and 29% of domestic brands were free of heterotrophic organisms. Likewise, 83.1% of samples (90% of imported and 81.8% of domestic brands) showed less than 100 colony forming units (cfu) per mL (Table 1 and 2). As for the density of colonies grown from the analyzed samples, great differences between brands and also between samples of the same brand were observed. The colony forming units counted from samples ranged from zero to too many to count (TMTC) per mL (Table 3). Tap water samples, on the other hand, contained lower than 10cfu per ml for all samples (Table 1).

***Thermo-tolerant Coliforms and Intestinal Entrococci*:**
It can be reported auspiciously that colonies of thermo-tolerant coliforms were not observed from all 65 samples of all domestic and imported brands. The tap water samples were also found to be free of these organisms. But in this assessment, *Intestinal enterococci* were detected in one of the thirteen brands (Brand F), but only from 3% of all samples (Table 1).

Table 1: **The number (%) of samples from each brand which different microbes detected**

Brand Code	**Microorganisms detected in bottled water samples from each brand, number (%)**				
	Heterotrophic bacteria (>100cfu per ml)	**Thermo-tolerant coliforms per 100 ml**	**Enterococci per 100 ml**	***Pseudomonas* species per 100 ml**	***Aeromonas* species per 100 ml**
A	0 (0)	0 (0)	0 (0)	4 (80)	1 (20)
B	1 (20)	0 (0)	0 (0)	4 (80)	2 (40)
C	1 (20)	0 (0)	0 (0)	4 (80)	2 (40)
D	1 (20)	0 (0)	0 (0)	3 (60)	1 (20)
E	2 (40)	0 (0)	0 (0)	4 (80)	2 (40)
F	3 (60)	0 (0)	2 (40)	4 (80)	2 (40)
G	1 (20)	0 (0)	0 (0)	4 (80)	0 (0)
H	0 (0)	0 (0)	0 (0)	3 (60)	0 (0)
I	0 (0)	0 (0)	0 (0)	4 (80)	1 (20)
J	1 (20)	0 (0)	0 (0)	4 (80)	0 (0)
K	0 (0)	0 (0)	0 (0)	4 (80)	1 (20)
L	1 (20)	0 (0)	0 (0)	3 (60)	1 (20)
M	0 (0)	0 (0)	0 (0)	4 (80)	0 (0)
Tap	0 (0)	0 (0)	0 (0)	2 (40)	1 (20)

***Pseudomonas and Aeromonas Species*:**
Pseudomonas species were the most abundant microorganisms detected in this assessment. This study showed that all the sampled brands contained *Pseudomonas* species. It was found that 75.4% of the bottled water and 40% of tap water samples were contaminated with *Pseudomonas* species. In comparison 76.4% of domestic and 70% of imported bottled water brands contained these species. Similarly, *Aeromonas* species were detected in 9 brands. But only 21.5% of the bottled water (21.8% of domestic and 10% of imported) and also 20% of tap water samples contained these organisms.

Table 2: **Colony forming units of heterotrophic bacteria observed from samples analyzed**

HPC per ml	Number (%) of samples tested
0 cfu	22 (33.9)
1-10 cfu	27 (41.5)
11-100 cfu	5 (7.7)
101-1000 cfu	5 (7.7)
>1000 cfu	6 (9.2)

Table 3: **Ranges of heterotrophic bacterial count in each bottled water brand**

Brand Code	Range of cfu of heterotrophic bacteria per mL
A	0 to 5
B	0 to 560
C	0 to > 1000
D	0 to > 1000
E	0 to > 1000
F	0 to 800
G	0 to > 1000
H	1 to 4
I	0 to 58
J	1 to > 1000
K	0 to 9
L	0 to > 1000
M	0 to 12
Tap	0 to 10

Discussion

***Heterotrophic Organisms*:**
In this assessment, heterotrophic organisms were detected from all brands, but in 66.2% of all samples. And yet, this figure does not mean that 66.2% of the samples were unfit for consumption, as drinking water containing below 100cfu per mL assumed to have no health risks (1). And that, as can be seen from Table 1 and 2, only 11 out of 65 samples (16.9%) could be considered unsafe for consumption. The rest 83.1% of the samples were assumed to be safe for human consumption. In a similar study conducted by Kassenga in Dar es Salaam, Tanzania, 92% of the bottled water samples analyzed contained heterotrophic organisms (12). Another study from India also reported that nearly 40% of the samples of bottled drinking water exceeded the limit of 100cfu of heterotrophic organisms per mL set by the Bureau of Indian Standards (21). Compared to the findings of Kassenga and Jeena et al., the microbiological quality of bottled water brands in this study seemed better (12, 21).

From 13 brands included in this study, only 5 of them fulfilled the requirement set by the WHO (i.e., < 100cfu per mL of water) in all five samples (1). Surprisingly, tap water samples also showed very low colony counts (an average of less than 10cfu). Yet again, some of the samples of the other eight brands also showed very low colony counts. One-sample *t*-test performed to compare the average colony forming units detected from each brand versus the national standard of 100cfu per mL of water indicated that the difference could not be considered statistically significant ($P > 0.05$). In comparison, 60% of samples of imported and 29% of domestic brands were free of heterotrophic organisms. Likewise, samples of 90% of imported and 81.8% of domestic brands showed less than 100cfu per mL, and thereby meeting the guidelines set by the WHO, the European Community and Ethiopian standards (1, 22, 23). However, Fisher's exact tests showed that the differences in colony counts between domestic and imported brands were not statistically significant ($P > 0.05$).

As can be understood from published literature, the presence of heterotrophic organisms is believed to show the overall efficiency of water treatment plants and general cleanliness and integrity of distribution systems (24). Despite the fact that the virulence factors of these organisms is considered very low (25) and that they do not pose a significant health risk (26), it is always safe to have very low counts of heterotrophic organisms in drinking water as their presence in water may include potential pathogens and opportunistic organisms (27). Data from epidemiological studies also suggest the presence of a positive correlation between gastrointestinal illnesses and heterotrophic plate counts at 35°C (28).

***Thermo-tolerant Coliforms and Intestinal Entrococci*:**
The good news here could be that colonies of thermo-tolerant coliforms were not observed from all samples of

domestic and imported brands. The tap water samples were also found to be free of these organisms. Thus, it can be said that the samples collected for this assessment did conform to the guidelines proposed by the WHO and the standard set by the Standard Agency of Ethiopia (1, 23), as both organizations demanded no thermo-tolerant coliforms to be detected in 100 mL sample of drinkable water (23, 29). Nevertheless, *Intestinal enterococci* were detected in one of the thirteen brands (Brand F), but only from 3% of samples. And the fact that *Intestinal enterococci* are mainly of fecal origin may raise questions about the safety of that particular brand.

Practically, thermo-tolerant coliforms could be detected in bottled waters as documented by a number of studies. Bharath et al. reported that from 344 bottled water samples tested in Trinidad, 1.5% of the samples contained E. coli or thermo-tolerant coliforms (10). Similarly, in a study conducted in Dar es Salaam, Tanzania, fecal coliforms were detected in 3.6% of analyzed samples (12). As thermo-tolerant coliforms or more specifically Escherichia coli are present in large numbers in the normal intestinal flora of humans and animals, it generally causes no harm. However, in other parts of the body, E. coli can cause serious diseases, such as urinary tract infection, bacteraemia and meningitis. Therefore, drinking-water needs to be free of these kinds of organisms.

***Pseudomonas and Aeromonas Species*:**
Regarding *Pseudomonas* species, it was found that 75.4% of the bottled water samples were contaminated with them. In comparison 76.4% of domestic and 70% of imported bottled water brands contained these species. However, no statistically significant differences were observed between domestic and imported brands concerning these organisms (Fisher's exact test, *P*-Value > 0.05). And yet, only 40% of tap water samples were found to contain *Pseudomonas* species. This huge difference between tap and bottled water samples may be due to high concentration of residual chlorine found in tap water samples as the residual chlorine concentrations of collected samples did even have apparent offensive smell due to chlorine. Besides, *Pseudomonas* species are well known in their ability to grow in nutrient limited environments including water.

This finding can also be supported by a longitudinal study conducted in Greece through the period 1995–2003 by Venieri et al. (11). According to Venieri et al., the most frequently isolated microorganisms during that period were *Pseudomonas* species. Bharath et al. also reported that 7.6% of the bottled water samples from Trinidad contained *Pseudomonas* species (10). A similar study from Zimbabwe also revealed that 6.7% of the analyzed samples had contaminations with *Pseudomonas* species (7). A report from Egypt by Abd El-Salam et al., however, reported a bit lower prevalence (3.6%) of *Pseudomonas* species from samples included in their study (13).

Though, there is no standard regarding *Pseudomonas* species in Ethiopia, the European Community Directive declares that bottled mineral water should be free from *Pseudomonas aeruginosa* (22). But, in this assessment, confirmatory tests for *Pseudomonas aeruginosa* were not performed as it is a pathogenic organism and normally not advisable to do it during quality monitoring

On the other hand, *Aeromonas* species were detected from only 21.5% (from 21.8% of domestic and 10% of imported) bottled water samples. Nevertheless, Fisher's exact test was performed and indicated that the difference between domestic and imported brands cannot be considered as statistically significant (*P*-value of > 0.05). *Aeromonas* species were also detected in tap water samples (20%). Various research groups tried to confirm the growth phenomenon of these bacteria by quantifying the bacteria present in natural mineral waters at the source and at several points in time after bottling and storage at different temperatures and also from environmental and clinical sources and the finding showed that environmental strains of *Aeromonas* had no ecological advantage over clinical isolates (30-32). But it is safe to say that bottled water can be used as vehicle of transmission for more dangerous strains like *Aeromonas* hydrophila (8, 9).

***Conclusion*:**
Obviously, it is hardly possible to provide microbe-free drinking water even after implementing the so-called multiple barrier concept or water safety plan (1) as some naturally occurring bacteria and yeast can survive all barriers. Despite such concerns, bottled water generally receives no further treatment by customers before consumption, as consumers are sure of its absolute quality. But as can be concluded from our study some of domestic bottled water brands failed to demonstrate compliance to HPC standards (though, all bottled water brands were free of thermo-tolerant coliforms and of *Intestinal enterococci* except one brand). Hence, some susceptible group of people could be at risk; unless regular microbiological monitoring instituted by regulatory bodies.

As can be witnessed by the contamination level of heterotrophic organisms and *Pseudomonas* species, the bottling companies were suspected to have faulty treatment or packaging practices. Thus, the companies would be advised to use water safety plans (WSP) from catchment to consumer. The WSP approach incorporates principles and concepts like the multiple-barrier approach and hazard assessment and critical control points (as used in the food industry). Consequently, it would be possible to continuously monitor the whole process cycle and implement corrective actions as necessary. On top of that, it is recommended that regular monitoring and testing for chemical compositions and microbial quality be made by the authorities concerned. We would like also to encourage future researchers to look into the association of microbial quality of bottled water with types of sources and environmental quality of the vicinity of the water sources.

Acknowledgments
We would like to thank the Ethiopian Public Health Institute for the financial help we got to undertake this study.

References

1. World Health Organization (WHO). Guidelines for drinking-water quality. Vol. 1, Recommendations, 4th ed. Geneva; WHO, 2011.
2. Rodwan JG. Bottled water 2011: The recovery continues - U.S. and International Developments and Statistics, 2012 [cited 2014]; Available at: URL:www.bottledwater.org.
3. Doria MF. Bottled water versus tap water: understanding consumers' preferences. *Journal of Water and Health* 2006;4(2):271-276
4. Zeenat A, Hatha AAM, Viola L, Vipra K. Bacteriological quality and risk assessment of the imported and domestic bottled mineral water sold in Fiji. *Journal of Water and Health* 2009;7(4):642-649.
5. Blake PA, Rosenberg ML, Florencia J, Costa JB, do Prado Quintino L, Gangarosa EJ. Cholera in Portugal, 1974. II. Transmission by bottled mineral water. *Am J Epidemiol* 1977;105(4):344–348.
6. Eckmanns T, Oppert M, Martin M, Amorosa R, Zuschneid I, Frei U, et al. An outbreak of hospital-acquired Pseudomonas aeruginosa infection caused by contaminated bottled water in intensive care units. *European Society of Clinical Microbiology and Infectious Diseases, CMI* 2008;14 (5):454–458.
7. Okagbue RN, Dlamini NR, Siwela M, Mpofu F. Microbiological quality of water processed and bottled in Zimbabwe. *Afr J Health Sci* 2002;9:99-103.
8. Manaia CM, Nunes OC, Morais P, Da Costa MS. Heterotrophic plate counts and the isolation of bacteria from mineral waters on selective and enrichment media. *J Appl Bacteriol* 1990;69:871–876.
9. Bharath J, Mosodeen M, Motilal S, Sandy S, Sharma S, Tessaro T, et al. Microbial quality of domestic and imported brands of bottled water in Trinidad. *International Journal of Food Microbiology* 2003;81:53– 62.
10. Venieri D, Vantarakis A, Komninou G, Papapetropoulou M. Microbiological evaluation of bottled non-carbonated (still) water from domestic brands in Greece. *International Journal of Food Microbiology* 2006;107:68-72.
11. Kassenga GR. The health-related microbiological quality of bottled drinking water sold in Dar es Salaam, Tanzania. *Journal of Water and Health 2007;5(1): 79-185.*
12. Abd El-Salam MMM, El-Ghitany EMA, Kassem MMM. Quality of Bottled Water Brands in Egypt Part II: Biological Water Examination. *J Egypt Public Health Assoc* 2008;83(5 & 6):467-486.
13. Abebe B. 26 bottled water brands still unaccredited. Capital 2014; 28 August 07:32.
14. Biadglegne F, Tessema B, Kibret M, Abera B, Huruy K, Anagaw B, et al. Physicochemical and bacteriological quality of bottled drinking water in three sites of Amhara Regional State, Ethiopia. *Ethiop Med J* 2009;47(4):277-84.
15. International Standard Organization [cited May 2014]; Available at: URL:http://www.iso.org/iso/catalogue_detail.htm?csnumber=36694.
16. Toranzos GA, McFeters GA, Borrego JJ. Detection of microorganisms in environmental freshwaters and drinking waters. In J. Hurst, R.L. Crawford, G.R. Knudsen, M.J. McInerney & L.D. Stetzenbach (Eds): Manual of Environmental Microbiology. 2nd edition. 2001:205–219.
17. American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF). Standard methods for the examination of water and wastewater. 20th edition. Washingtion DC: APHA, AWWA, WFA; 1998.
18. Environment Agency. The microbiology of drinking water - Part 7: Methods for the enumeration of heterotrophic bacteria. Environment Agency, 2012 [cited 2014]; Available at: URL:www.environment-agency.gov.uk/nls.
19. Environment Agency. The microbiology of drinking water - Part 4: Methods for the isolation and enumeration of coliform bacteria and Escherichia coli, 2009 [cited 2014]; Available at: URL:www.environment-agency.gov.uk/nls.
20. Environment Agency. The microbiology of drinking water - Part 8: The isolation and enumeration of Aeromonas and Pseudomonas aeruginosa by membrane filtration, 2010 [cited 2014]; Available at: URL:www.environment-agency.gov.uk/nls.
21. Jeena MI, Deepa P, Mujeeb Rahiman KM, Shanthi RT, Hatha AAM. Risk assessment of heterotrophic bacteria from bottled drinking water sold in Indian markets. *Int J Hyg Environ Health* 2006;209:191–196.
22. European Community Council. Directive no. 80/777/EEC of 15 July 1980 on the approximation of the laws of the member states relating to the

exploitation and marketing of natural mineral waters. Official J. European Communities L 229, 1980:1–10.

23. Ethiopian Standards 597. Bottled drinking water – specifications. Addis Ababa; Ethiopian Standards, 2001.
24. van der Kooij D. Importance and assessment of the biological stability of drinking water in the Netherlands. In: Craun GF (Ed): Safety of water disinfection: Balancing chemical and microbial risks. Craun, G.F. (Ed.) Washington DC: ILSI Press, 1993.
25. Edberg SC, Allen MJ. Virulence and risk from drinking water of heterotrophic plate count bacteria in human population groups. *International Journal of Food Microbiology* 2004;92:255–263.
26. Payment P, Coffin E, Paquette G. Blood agar to detect virulence factors in tap water heterotrophic bacteria. *Appl Env Microbiol* 1994; 60: 1179–1183.
27. Pavlov D, de Wet CME, Grabow WOK, Ehlers MM. Potentially pathogenic features of heterotrophic plate count bacteria isolated from treated and untreated drinking water. *International Journal of Food Microbiology* 2004;92:275–287.
28. Payment P, Franco E, Richardson L, Siemiatycki J. Gastrointestinal health effects associated with the consumption of drinking water produced by point-of-use domestic reverse-osmosis filtration units. *Appl Env Microbiol* 1991b;57:945–948.
29. WHO. The World Health Report: fighting disease, fostering development. *World Health Forum* 1996;18(1):1–8.
30. Warburton DW, Peterkin PI, Weiss KF, Johnston MA. Microbiological quality of bottled water sold in Canada. *Can J Microbiol* 1986;32:891–893.
31. Bischofberger T, Cha SK, Schmitt R, Konig B, Schmidt-Lorenz W. The bacterial flora of non-carbonated, natural mineral water from the springs to reservoir and glass and plastic bottles. International *Journal of Food Microbiology* 1990; 1: 1-72.
32. Mary P, Buchet G, Defives C, Hornez JP. Growth and survival of clinical vs. environmental species of Aeromonas in tap water. *International Journal of Food Microbiology* 2001;69:191–198.