Assessment of Drinking Water Quality from Bottled Water Coolers

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Abstract

Background: Drinking water quality can be deteriorated by microbial and toxic chemicals during transport, storage and handling before using by the consumer. This study was conducted to evaluate the microbial and physicochemical quality of drinking water from bottled water coolers.

Methods: A total of 64 water samples, over a 5-month period in 2012-2013, were collected from free standing bottled water coolers and water taps in Isfahan. Water samples were analyzed for heterotrophic plate count (HPC), temperature, pH, residual chlorine, turbidity, electrical conductivity (EC) and total organic carbon (TOC). Identification of predominant bacteria was also performed by sequence analysis of 16S rDNA.

Results: The mean HPC of water coolers was determined at 38864 CFU/ml which exceeded the acceptable level for drinking water in 62% of analyzed samples. The HPC from the water coolers was also found to be significantly ($P < 0.05$) higher than that of the tap waters. The statistical analysis showed no significant difference between the values of pH, EC, turbidity and TOC in water coolers and tap waters. According to sequence analysis eleven species of bacteria were identified.

Conclusion: A high HPC is indicative of microbial water quality deterioration in water coolers. The presence of some opportunistic pathogens in water coolers, furthermore, is a concern from a public health point of view. The results highlight the importance of a periodic disinfection procedure and monitoring system for water coolers in order to keep the level of microbial contamination under control.

Keywords: Drinking water, Water cooler, Water quality, Bacteria, HPC

Introduction

Drinking water quality is a worldwide concern and has the greatest impact on human health (1). Consumption of contaminated drinking water was associated with 80 percent of disease and one third of death in developing countries (2). Therefore, an essential basic requirement for health protection is to provide the public with adequate supply of drinking water that is safe (3).

Advances in water treatment have significantly increased the quality and specially the safety of water (4). However, drinking water quality can deteriorate by microbial and toxic chemicals during transport, storage and handling before reaching the consumer (5, 6). Distribution systems, service lines and home devices could influence the quality of drinking water (5). Water quality in

home devices is highly affected by biofilm formation (5). Prevailing conditions in the devices that influence bacterial proliferation include high surface to volume ratio, absence or very low of chlorine residual and relative long stagnation period (7).

Biofilms, which are well organized communities of microorganisms, are wide spread in the nature. They constitute a major problem in many environmental, industrial and medical settings (8). The presence of dissolved organic compounds in finished drinking water is responsible for growth of bacteria and colonization of water surfaces (5). Biofilm formation is also a concern from public health because it plays a key role in the persistence of bacteria in water systems and protects the bacteria from adverse environmental conditions, including disinfectants. Biofilms could also harbor pathogenic bacteria and support their proliferation which may contribute to the spread of waterborne diseases (9).

The heterotrophic plate count (HPC) is a parameter which could reflect the biofilm formation in water systems. It has been widely adopted as a standard and simple traditional technique for microbiological testing and safety management of drinking water (10).

Bottled water coolers are home devices which cool and dispense water. They are widely used in warm climates especially in public places and workplaces. The structure of water cooler could affect the bacterial quality of drinking water. Bacteria present in drinking water may attach to the bottle and dispenser surface of water coolers and form biofilm.

Chemical water pollution due to leaching of organic compounds from Polyethylene terephthalate (PET) bottles in drinking water is also a globally concern (11, 12). Therefore, bottled water coolers have the potential to release hazardous chemicals to the drinking water.

To our knowledge, very few studies have been conducted on the bacteriological quality of bottled water coolers (13, 14). Levesque et al. found a significantly higher proportion of water cooler samples resulted contaminated than tap water (13). Aerobic plate count was also higher in coolers compared with spring water used to supply the coolers (14). Similar results have also been reported about the microbial quality of drinking water dispensed from bottleless water coolers (water dispensers) (15-17). In general, the water dispensed from water coolers was found to be more contaminated than the water supplied to them.

Given the importance of drinking water safety and identification of potential microbial and chemical pollution sources of drinking water this study was conducted to evaluate the microbial and physicochemical quality of water from bottled water coolers. In particular, we studied some physicochemical factors that might influence the microbial quality of water from water coolers. Since, the bacteriological quality of drinking water is highly dependent on the bacterial species encountered; the identification of predominant bacteria was also performed.

Materials and Methods

Water samples

A total of 32 drinking water samples were collected, over a 5-month period in 2012-2013, from free standing bottled water coolers in office buildings in the city of Isfahan. Thirty two control samples were also obtained from the water taps representing the tap water used to fill the bottles. Almost all bottles were filled with drinking water from municipal tap water. All samples were collected in sterile glass bottles containing sodium thiosulfate to neutralize any residual disinfectant after the tap water was allowed to run for one minute. A water sample was also taken for physicochemical analyses.

Bacteriological Analysis

For total heterotrophic bacteria, the samples were agitated by vortexing for 15 s and ten-fold serial dilutions were prepared for each sample. From all, volumes of undiluted and diluted samples were spread plated on R2A agar medium (Merck, Germany) and incubated at 35 ℃ for 3-5 days as described in Standard Methods (18).Following incubation, plates were counted and results were ex-

pressed as colony-forming units per milliliter (CFU/ml). All the experiments were carried out in duplicates and the mean values were considered. Bacterial colonies were also characterized based on the colony and cell morphology on the agar plates and microscopic examination and the abundance percentage of different types of colonies were noted.

Physicochemical analyses

Water temperature, pH [Corning pH Meter] and residual free chlorine [METERRC] were determined at the time of sample collection. The electrical conductivity and turbidity of samples were determined in the laboratory by conductivity meter (SensIon7, Hach Company, USA) and turbidity meter (2100P, Hach Company, USA).
TOC concentration of water samples was measured by using of a photo catalytic oxidation process employed by the ANATOC series II software (SGE International Ltd., Ringwood, Australia). Titanium dioxide was employed as a catalyst with a continuous UV light source for its activation. All of the analyses were performed at 350 nm to allow photo catalytic oxidation of the organic carbon in the samples. Before any TOC analysis, any residual inorganic carbon present in the sample was converted to CO_2 by injecting 500 μl of sample into the acidified catalyst suspension and then TOC results were calculated and reported via ANATOC II software.

Molecular identification of microorganisms

Predominant bacteria were isolated and subcultured on to R2A agar plates based on their Gram stain and colony morphology. The isolated colonies were suspended in 100 μl of deionized water, and genomic DNA was extracted by boiling for 15 min and centrifugation at 13,000 rpm for 5 min. The supernatant was used for PCR amplification with Eubac 27F and 1492 R primers, which amplify a ~1,420 bp fragment of 16s rDNA. The PCR amplification was conducted in a final volume of 50 μl containing 2 μl of template DNA, 0.2 μM of each primer, 0.2 mM of each dNTP, 5 μl of 10× PCR buffer and 1.25 units of Taq polymerase. DNA sequencing of the amplified gene

was performed, and DNA sequences analysis was undertaken by BLAST algorithms and databases from the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis

Statistical analysis was performed with SPSS 20.0. Significant difference between the analyzed parameters in water coolers and control samples was tested using *t*-test. The effect of physicochemical parameters on microbial quality of water samples was tested by Pearson's correlation. A *P*-value of <0.05 was considered significant.

Results

The results of bacteriological analysis of water coolers and tap waters are presented in Table 1. The microbiological results indicated that the bacteria count was higher in 62% (20 of 32) of water cooler samples than the stated drinking water limits for HPC (≤500 CFU/ml) (5). The statistical analysis showed a significant difference between HPC number of water coolers and tap water samples. Table 2shows the mean and range values of the physicochemical parameters for water coolers and tap waters.

Table 1: Bacteriological results of water coolers and tap water sample

HPC (CFU/ml)	Water cooler	Tap water
Mean	38864	61
Maximum	300000	800
Minimum	0	0
HPC >500 CFU/ml (No. of samples)	20	1

The mean temperature of the water coolers and water taps were 9.9 °C and 24.2°C, respectively. Significant differences were observed between the amounts of temperature and residual chlorine concentrations in water coolers and water taps (*P*-value<0.05).However, the statistical analysis

showed no significant difference between the values of pH, EC, turbidity and TOC in water coolers and tap waters (*P*-value>0.05). The results of correlation analysis between measured parameters in water coolers are given in Table 3. The correlation analyses indicated that temperature and EC had a significant effect on the heterotrophic bacteria population in water coolers and also control samples. However, TOC did not show any significant effect on the microbial quality of water coolers. Frequency of isolated bacteria from water coolers and tap waters are shown in Fig. 1. This figure shows that bacilli were the predominant bacteria in all samples. According to 16s rRNA gene sequence analysis of predominant bacteria, eleven species of bacteria were identified in water coolers and tap waters. The species of identified bacteria are presented in Table 4.
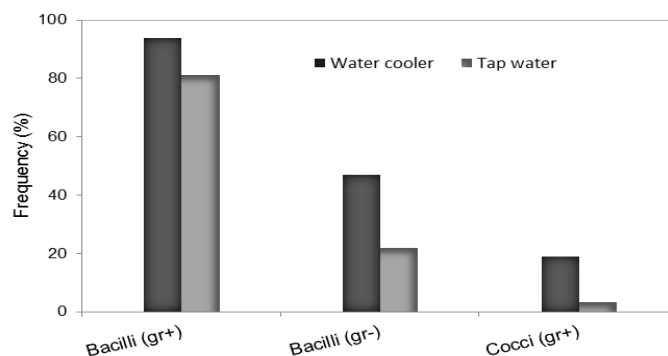

Fig. 1: Frequency of isolated bacteria from water coolers and tap waters

Table 2: Analytical results of the physicochemical parameters for water coolers and tap water samples

Parameter	Water cooler			Tap water		
	Max	Mean	Min	Max	Mean	Min
Temperature (°C)	29.9	9.9	5	29.7	24.2	19.9
Residual chlorine (mg/l)	0.12	0.03	0.01	0.24	0.15	0.01
EC (µs/cm)	554	357	59.1	590	395	58
pH	8.1	-	6.4	7.7	-	6.3
Turbidity (NTU)	2.47	0.6	0.15	1.72	0.4	0.1
TOC (mg/l)	8.7	2.9	0.16	5	2.2	0.09

Table 3: Correlation matrix of the analyzed parameters in water coolers

Parameter	Temperature (°C)	Residual chlorine (mg/l)	EC (µs/cm)	pH	Turbidity (NTU)	TOC (mg/l)	HPC (CFU/ml)
Temperature (°C)	......	-0.145	-0.014	-0.131	-0.335*	0.085	0.530**
Residual chlorine (mg/l)	......	.....	-0.283	0.128	0.079	-0.183	-0.267
EC (µs/cm)	......	.....	.....	-0.305*	0.121	0.094	0.330*
pH	......	.....	......		-0.044	-.210	0.017
Turbidity (NTU)	......	.....	.....	......	.....	-0.002	-0.186
TOC (mg/l)	......	.....	.....	......	.....	.....	0.085
HPC (CFU/ml)	......	.....	.....	.....	.....	......	.....

**Starred correlations are significant at *P*< 0.001 while *Starred correlations are significant at *P*< 0.05

Table 4: Predominant bacteria identified by 16S rDNA sequence analysis

Bacteria species	Water cooler	Tap water	Opportunistic pathogen
Bacillus sp.	+	.....	
Bacillus cereus	+	.....	*
Bacillus safensis	+	+	
Bacillus licheniformis	+	.....	*
Microbacteriumparaoxydans	+	.....	
Mycobacterium conceptionense	+	+	*
Sphingomonas sp.	+	.....	*
Sphingomonas ginsenosidimutans	+	.....	
Blastomonas natatoria	+	+	
Porphyrobacter donghaensis	+	+	
Phenylobacterium lituiforme	+	+	

Discussion

The mean HPC of water coolers was determined at 38864 CFU/ml which exceeded the acceptable level for drinking water in high percentage of the analyzed samples. Microbiological quality control of drinking water during the distribution from water treatment plants to the consumer's tap is a major challenge in drinking water safety management (10, 19). Therefore, monitoring of drinking water quality from source to point-of-use is essential to ensure compliance with quality standards and to protect public health. The results of the study showed that out of the 32 water cooler samples, 20 (62%) of the samples highly loaded with microbes at levels exceeding 500 CFU/ml which recommended for drinking water (Table 1). While in control samples, only one of the samples (3%) exceeded the recommended values for HPC (Table 1). This finding is similar to those of Levesque et al.(1994), who found a significantly higher number of HPC in dispenser of water coolers when compared to the municipal tap water. Their results indicated that the bacteria count was higher than 1000 CFU/ml in 62% samples of water coolers (13). Aerobic plate count was higher in coolers when compared with bottled samples of spring water used to supply the coolers (14). The present study supports the previous findings that the bacteriological water quality of water coolers is worse than the water used to supply these coolers and

that regrowth and biofilm formation appear to occur in the bottled water coolers.

Results in this study indicate that the surfaces of the bottle and dispensers favored excessive growth of bacteria and biofilm formation. Previous studies have shown surface materials highly influence biofilm formation. Plastic materials such as ethylene-propylene and latex surface support greater bacterial growth than either glass or stainless steel (8). Buffet-Bataillon et al. reported that rubber-lined hose contains high levels of plasticizer, which should encourage bacterial growth and replacing of these hoses with Teflon-lined hoses could decrease the risk of contamination (20). In addition, bacteria adhere more readily to rough surfaces (9, 21) and the characteristic of the plastic bottles used in water coolers is such that they could support biofilm formation. However, study of Taheri et al. showed no difference between the HPC counts in bottleless water coolers and tap waters (22). Indeed, they demonstrated that stainless steel small tanks in the bottleless coolers or fountains which were used for storage of chilled water did not support growth of biofim microorganisms. However, biofilm formation could potentially occur in bottleless coolers or dispensers due to the presence of plastic waterlines or water treating filters (15-17). The narrow bore water lines of dispensers which made of plastic material could provide a suitable surface for adhesion of the microorganisms on the inner surface

of the waterlines (15). Aerobic plate counts were higher in water plumbed in coolers from commercial stores compared with the tap water (16). The contamination may have been caused by the accumulation of small number of microorganisms from tap water or from faucet surface which are concentrated at filters used for bottleless coolers (16). High surface to volume ratio, absence or very low chlorine residual and relative long stagnation period are conditions which could also contribute to biofilm formation (5) in bottled water coolers. Therefore, water coolers must be thoroughly cleaned and disinfected in order to prevent the biofilm formation. The validity of this recommendation is supported by the study of Zanetti et al. that showed that the periodic disinfection of microfiltered water dispensers with hydrogen peroxide made it possible to obtain water with HPC levels conforming to Italian regulations for drinking water (≤100 CFU/ml) (17).

It is also known that a number of other factors such as temperature, pH and TOC could influence the growth of biofilms in aquatic surfaces (23).The correlation analyses indicated that temperature and EC had a significant effect on the heterotrophic bacteria population in water coolers (Table 3) and also control samples. Although, there was a significant difference between the temperature of water coolers and tap water samples, the lower temperatures in the water coolers did not influence bacterial growth. This result indicates that although higher temperature could speed up the bacterial growth but other conditions in water coolers were more effective on biofilm formation.

TOC did not show any significant effect on the microbial quality of water coolers (*P*-value>0.05) (Table 3). This may be related to this fact that presence of even microgram levels of dissolved organic compounds in aquatic systems allows growth of microorganisms and biofilm formation (24).

On the other hand the statistical analysis showed no significant difference between the values of pH, EC, turbidity and TOC in water coolers and tap waters (*P*-value>0.05). The amounts of TOC in coolers, as an indicator of organic compounds

suggest that there is no migration of organic carbon from bottles of water coolers to drinking water. Several studies reported that formaldehyde and acetaldehyde are the most relevant carbonyl compounds migrating from PET bottles to drinking water. Phthalates which used as plasticizer in plastic packaging have also been found in PET material and in PET-bottled water (12). However, more information on chemical mixtures and the effect observed in the water coolers is necessary.

The Gram staining of microorganisms showed that bacilli were the predominant bacteria in all samples. Gram-positive bacilli were present in 93% and 81% of water cooler and tap water samples, respectively. All samples were found to be completely free of gram-negative cocci (Fig. 1).

According to the16s rDNA sequence analysis of predominant bacteria in water coolers and tap waters, eleven species of bacteria were identified (Table 4). Five species of bacteria were detected in both the tap water and water coolers. However, six species were identified only in water coolers (Table 3). *Bacillus* spp. are ubiquitous gram-positive rod-shape bacteria which isolated from water distribution systems (5). They produce spores that are quite resistant to environmental stresses and disinfection. *Bacillus* includes both free living and pathogenic species (16, 25). *Microbacterium paraoxydans* and *Mycobacterium conceptionense* are opportunistic gram-positive pathogens which belong to the order Actinomycetales (26, 27). *Sphingomonas* spp. *Blastomonas natatoria*, *Porphyrobacter donghaensis* and *Phenylobacterium lituiforme*are gram negative bacteria which belong to the class Alphaproteobacteria (28). *Sphingomonas* spp. are a group of chemoheterotrophic strictly aerobic rod shaped bacteria that are widely distributed in nature and found in water distribution lines (16). Most of them are not clinically important, but some of species play a role in nosocomial infections. *Blastomonas natatoria* can colonize biofilm in water distribution system of neonatal intensive care unit (NICU) (20). The presence of *Porphyrobacter donghaensis* and *Phenylobacterium lituiforme* in sea water and subsurface aquifer, respectively also reported in other studies (29, 30).

Conclusion

High number of HPC in water coolers is indicative of microbial water quality deterioration in water coolers. The presence of some opportunistic pathogens in water coolers, furthermore, is of concern from a public health point of view; because of these microorganisms can lead to infection of vulnerable subpopulations. The results highlight the importance of a periodic disinfection procedure and monitoring system for water coolers in order to keep the level of microbial contamination under control.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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References

1. Fiebelkorn AP, Person B, Quick RE, Vindigni SM, Jhung M, Bowen A, Riley PL (2012). Systematic review of behavior change research on point-of-use water treatment interventions in countries categorized as low-to medium-development on the human development index. *Soc Sci Med*, 75 (4), 622-33.
2. WHO (1996). *Fighting Disease, Fostering Development.* The World Health Report, Geneva, Switzerland.
3. WHO (2011). *Guidelines for drinking-water quality.* World Health Organization, Geneva, Switzerland.
4. Betancourt WQ, Rose JB (2004). Drinking water treatment processes for removal of Cryptosporidium and Giardia. *Vet Parasitol*, 126 (1): 219-34.
5. Bitton G (2005). *Wastewater Microbiology.* 3rd ed. John Wiley & Sons Inc, Canada. pp. 419-455.
6. Arnal J, García-Fayos B, Sancho M, Verdú G, Lora J (2010). Design and installation of a decentralized drinking water system based on ultrafiltration in Mozambique. *Desalination*, 250 (2): 613-7.
7. Peter-Varbanets M, Zurbrügg C, Swartz C, Pronk W (2009). Decentralized systems for potable water and the potential of membrane technology. *Wat Res*, 43 (2): 245-65.
8. Szymańska J (2003) Biofilm and dental unit waterlines. *Ann Agric Environ Med,* **10** (2): 151-7.
9. Flemming H (2002). Biofouling in water system-cases, causes and countermeasures. *Appl Microbiol Biotech*, 59 (6): 629-40.
10. SU F, Luo M, Zhang F, Li P, Lou K, Xing X (2009). Performance of microbiological control by a point-of-use filter system for drinking water purification. *J Environ Sci,* 21 (9): 1237-46.
11. Leivadara SV, Nikolaou AD, Lekkas TD (2008). Determination of organic compounds in bottled waters. *Food Chem*, 108 (1): 277-86.
12. Bach C, Dauchy X, Chagnon MC, Etienne S (2012). Chemical compounds and toxicological assessments of drinking water stored in polyethylene terephthalate (PET) bottles: a source of controversy reviewed. *Water Res*, 46 (3): 571-83.
13. Lévesque B, Simard P, Gauvin D, Gingras S, Dewailly E, Letarte R (1994). Comparison of the microbiological quality of water coolers and that of municipal water systems. *Appl Environ Microbiol*, 60 (4): 1174-8.
14. Baumgartner A, Grand M (2006). Bacteriological quality of drinking water from dispensers (coolers) and possible control measures. *J Food Protect*, 69 (12): 3043-6.
15. Sacchetti R, De Luca G, Dormi A, Guberti E, Zanetti F (2013). Microbial quality of drinking water from microfiltered water dispensers. *Int J Hyg Environ Health*. **217** (2-3): 25-29.
16. Liguori G, Cavallotti I, Arnese A, Amiranda C, Anastasi D, Angelillo IF (2010). Microbiological quality of drinking water from dispensers in Italy. *BMC Microbiol*, 10 (1): 19.

17. Zanetti F, De Luca G, Sacchetti R (2009). Control of bacterial contamination in microfiltered water dispensers (MWDs) by disinfection. *Int J Food Microbiol,* 128 (3), 446-52.

18. APHA, WEF (2005). *Standard methods for the examination of water and wastewater.* 21st ed. Washington DC.

19. Lehtola MJ, Miettinen IT, Keinänen MM, Kekki TK, Laine O, Hirvonen A, Vartiainen T, Martikainen PJ (2004). Microbiology, chemistry and biofilm development in a pilot drinking water distribution system with copper and plastic pipes. *Water Res,* 38 (17): 3769-79.

20. Buffet-Bataillon S, Bonnaure-Mallet M, De La Pintière A, Defawe G, Gautier-Lerestif AL, Fauveau S, Minet J (2010). Heterotrophic bacterial growth on hoses in a neonatal water distribution system. *J Microbiol Biotech,* 20 (4): 779.

21. Ciston S, Lueptow RM, Gray KA (2008). Bacterial attachment on reactive ceramic ultrafiltration membranes. *J Member Sci,* 320 (1-2): 101-7.

22. Taheri E, Dastjerdi M, Hatamzadeh M, Hassanzadeh H, Nabari F. Nikaeen M (2010). Evaluation of The Influence of Conventional Water Coolers on Drinking Water Quality. *Iran J Health Environ,* 2 (4): 268-75.

23. Gibert O, Lefèvre B, Fernández M, Bernat X, Paraira M, Calderer M, Martínez-Lladó X (2012). Characterising biofilm development on granular activated carbon used for drinking water production. *Water Res,* 47 (3): 1101-10.

24. Butterfield PW, Camper AK, Ellis BD, Jones WL (2002). Chlorination of model drinking water biofilm: implications for growth and organic carbon removal. *Water Res,* 36 (17): 4391-405.

25. Madigan MT (2005). *Brock Biology of Microorganisms.* 11th ed, Prentice Hall. New Jersey.

26. Adékambi T, Stein A, Carvajal J, Raoult D, Drancourt M (2006). Description of *Mycobacterium conceptionense* sp. nov., a *Mycobacterium fortuitum* group organism isolated from a posttraumatic osteitis inflammation. *J Clin Microbiol,* 44 (4), 1268-73.

27. Miyamoto M, Sakurada T, Oishi D, Koitabashi K, Hanada K, Takemura H, Shibagaki Y, Yasuda T, Kimura K (2013). The first case report of peritoneal dialysis related peritonitis caused by *Microbacterium paraoxydans. Clin Nephrol,* 79 (5): 402-6.

28. Brenner D, Krieg N, Staley J (2005). The Proteobacteria (Part C): the Alpha, Beta, Delta and Epsilonproteobacteria. In: *Bergey's manual of systematic bacteriology.* Ed, Garrity. Second ed, Springer-Verlag. New York, pp. 1059-144.

29. Kanso S, Patel BK (2004). *Phenylobacterium lituiforme* sp. nov., a moderately thermophilic bacterium from a subsurface aquifer and emended description of the genus Phenylobacterium. *Int J Syst Evol Microbiol,* 54 (6): 2141-6.

30. Yoon JH, Lee MH, Oh TK (2004) *Porphyrobacter donghaensis* sp. nov., isolated from sea water of the East Sea in Korea. *Int J Syst Evol Microbiol,* 54 (6): 2231-5.