Assessment of microbial quality of household water output from desalination systems by the heterotrophic plate count method

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ABSTRACT

Point-of-use household water desalination systems (HWDSs) are becoming popular in Iran because of the deterioration of drinking water. This study aimed to determine the microbial quality of output water from HWDSs in Qom, Iran by using the heterotrophic plate count (HPC) method. Samples of input and output water from 30 HWDSs were collected over a six-month period. Heterotrophic bacteria were tested using the pour plate technique. At the first sampling stage, the HPC level in 23% of samples exceeded the 500 CFU/ml threshold level. On average, for 50% of samples, the HPC level of input samples was 0–10 CFU/ml, for 42% it was 10–100 CFU/ml and for 8% it was 100–500 CFU/ml. For output samples, for 25%, the level of HPC was 0–10 CFU/ml, for 43% it was 10–100 CFU/ml, for 24% it was 100–500 CFU/ml and for 8% it exceeded 500 CFU/ml. For total coliforms the most probable number test was positive for the first and third stages of sampling (3% input samples). The comparison of the averages with national standard values shows that in some cases, the contamination of output water from HWDSs in the city of Qom has been above the standard values.

Key words | desalination systems, drinking water, heterotrophic bacteria, toxic microbial quality

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INTRODUCTION

There has been great concern about water quality in recent years, because it is one of the most important needs of humans, animals and the environment (Alavi *et al.* 2016; Khosh Doost *et al.* 2016; Dobaradaran *et al.* 2018). According to WHO reports, drinking water must be suitable for all the typical household chores, human consumption and personal hygiene (WHO 2012). Heterotrophic plate count (HPC) is an indicator of the presence of all bacteria in water that are able to grow at 22–37 °C in the incubator (Burtscher *et al.* 2009; Falcone-Dias & Farache Filho 2013). The method is used to test the efficiency of water treatment processes in eliminating pathogens, to monitor

doi: 10.2166/wh.2018.082

the performance of filtration and disinfection systems and to evaluate the regrowth of microorganisms in the storage tank and distribution system (DS) (Azim & Little 2008). The main drinking water risks in developing countries are associated with microbial pollution (Peter-Varbanets *et al.* 2009).

The United States Environmental Protection Agency (USEPA) sets the maximum permissible level of heterotrophic bacteria in DS water at 500 CFU/ml (USEPA 2001). In Japan, Germany (Hambsch *et al.* 2004; Pavlov *et al.* 2004) and South Africa (Hambsch *et al.* 2004), the equivalent levels are 100 CFU/ml, and in Australia 500 CFU/ml (Bartram *et al.* 2003). In order to assess the filtration stages in Switzerland, Siebel mentioned that the legal level of heterotrophic bacteria in each ml of treated water was between 20 and 30 colonies (Siebel *et al.* 2008). The EPA determined that the HPC concentration of surface water or ground water mixed with surface water must be reduced to lower than 500 CFU/ml through treatment techniques (Francisque *et al.* 2009; Xin *et al.* 2018). Some heterotrophic bacteria such as *Escherichia coli* and groups such as *Pseudomonas* and *Aeromonas* are opportunistic and pathogenic, and may endanger the vulnerable groups (El-Rhman *et al.* 2009; Stoll *et al.* 2011; Igbinosa *et al.* 2012).

A water desalination system is the best way to supply fresh water from seawater and brackish water to growing populations. Desalination is a process that extracts mineral components from saline water. More generally, desalination refers to the removal of salts, microbial pollution and minerals from a target substance (Wu *et al.* 2018). Operating these systems is expensive and energy intensive (Chandrashekara & Yadav 2017). The adoption of a reversible thermodynamic process in any desalination system is most energy efficient and is independent of the mechanisms and the system used (Chandrashekara & Yadav 2017).

Due to high levels of total dissolved solids (TDS) in sources of drinking water in Qom city, Iran, household water desalination systems (HWDSs) are widely used in the town. The structure of these machines provides suitable conditions for the growth and development of biofilm (Luo *et al.* 2012; Ghaffour *et al.* 2013). Therefore, this study aimed to assess the impact of HWDS on the microbial quality of drinking water.

METHODS

This study adopts a descriptive-analytical approach and was conducted on domestic HWDSs from 2012 to 2013 in Qom, Iran. Thirty HWDSs were selected randomly as monitoring points in the city. The input and output water from these machines was sampled at three stages in a six-month period. Standard microbial methods were used to collect 180 samples in total. Temperature and residual chlorine were measured by a thermometer and N, N-diethyl-p-phenylenediamine (DPD) methods, respectively, at the sampling sites. Samples were preserved at 4 °C and transferred to the laboratory. The parameters of pH, turbidity, electrical conductivity (EC), most probable number (MPN), HPC and TDS were measured in accordance with standard methods of water and wastewater tests (APHA 2011). pH, EC and turbidity were measured using a Sartorius pH meter, portable EC meter Model WTW-LF90 and turbidity meter Model HACH A2100, respectively. The HPC test was carried out using R_2A Agar medium incubated for 48 hours at 35 °C, and the MPN method for coliforms was carried out using nutrient broth medium incubated for 24 hours at 35 °C. Finally, the fecal coliform test used *E. coli* broth incubated at 44 °C for 24 hours.

Statistical analysis

Data were analysed by Excel and SPSS. The average dispersion and standard deviation, *t*-test, Pearson correlation and linear regression were used to analyze data. Data averages were evaluated and compared with national and international standards of drinking water.

RESULTS AND DISCUSSION

The averages of the test results on input and output samples from HWDSs were classified into four levels of 0–10, 10–100, 100–500 and above 500 CFU/ml and are presented in Table 1. Table 2 presents the measured parameters at each stage of sampling with mean and standard deviation values.

As shown in Figure 1, some of the output samples had HPC values above the standard 500 CFU/ml, while in others the values were close to or lower than this level. As

can be seen, there is a significant difference between HPC levels of the input and output samples.

The Pearson correlation coefficients demonstrated that there was an inverse and strong correlation between HPC and pH, and an inverse and moderate correlation between HPC and temperature for input samples (Table 3). The results of linear regression analysis of input samples showed that pH was a good predictor for HPC changes ($P = 0.003$), such that the HPC underwent a −39.44 change with a one-unit rise of pH. The estimated regression model is:

$$E[HPC] = 316.641 - 39.44\ pH \quad (1)$$

The Pearson correlation coefficient showed an indirect and strong correlation between HPC and temperature. However, the linear regression results showed that the relationship between HPC and temperature was not significant (Table 4). Also, Table 5 shows the results of the paired t-test to determine the input-output differences.

Table 6 shows the MPN results. The values of input and output samples, with some possible exceptions, were negative for total coliforms. The numbers '38', '30' and '8' represent the number of bacteria with a positive MPN test (Table 6).

The results also showed 23% of HPC values in output samples at the first sampling stage were above 500 CFU/ml. This suggests the growth of heterotrophic bacteria on different inner surfaces of water desalination machines. Even when the number of bacterial colonies was lower in input samples, it was higher for outputs. Considering the

Table 1 | HPC microbial culture results classified by level and expressed as percentage (%) of total samples at each stage

	Sampling stage					
	First		Second		Third	
HPC (CFU/ml)	Input	Output	Input	Output	Input	Output
0–10	60	44	43	20	47	10
10–100	27	20	50	50	50	60
100–500	13	13	7	30	3	30
>500	0	23	0	0	0	0

Table 2 | Values of the measured parameters of HWDS samples

		Sampling stage					
		First		Second		Third	
Parameter		Input	Output	Input	Output	Input	Output
HPC (CFU/ml)	Mean ± SD	43.6 ± 97.5	351.8 ± 595.3	25.0 ± 29.8	92.6 ± 107.1	23.9 ± 26.6	91 ±87
	Minimum	0	0	0	0	0	0
	Maximum	450	2,351	114	326	120	280
pH	Mean ± SD	7.3 ± 0.3	7.1 ± 0.4	7.4 ± 0.4	6.7 ± 0.2	7.4 ± 0.4	7.1 ± 0.4
	Minimum	6.8	6.1	6.68	6	6.8	6.27
	Maximum	8.1	7.9	8.47	7.1	8.38	7.98
Residual chlorine (mg/l)	Mean ± SD	0.22 ± 0.43	0.01 ± 0.03	0.12 ± 0.13	0.01 ± 0.03	0.21 ± 0.18	0.04 ± 0.06
	Minimum	0	0	0	0	0	0
	Maximum	1.90	0.10	0.50	0.10	0.65	0.20
EC (μmoh/cm)	Mean ± SD	4,595 ± 547	357 ± 195	5,048 ± 775	566 ± 372	5,108 ± 602	580 ± 370
	Minimum	3,000	99	2,950	141	3,650	180
	Maximum	5,480	905	6,050	1,572	5,950	1,590
Turbidity (NTU)	Mean ± SD	0.90 ± 1.54	0.10 ± 0.14	0.50 ± 0.41	0.10 ± 0.07	0.57 ± 0.36	0.09 ± 0.07
	Minimum	0	0	0.05	0.01	0.06	0.01
	Maximum	7.12	0.67	1.44	0.39	1.21	0.28
TDS (mg/l)	Mean ± SD	4,136 ± 492	232 ± 127	4,544 ± 698	368 ± 242	4,597 ± 542	378 ± 240
	Minimum	2,700	64	2,655	92	3,285	117
	Maximum	4,932	588	5,445	1,022	5,355	1,034

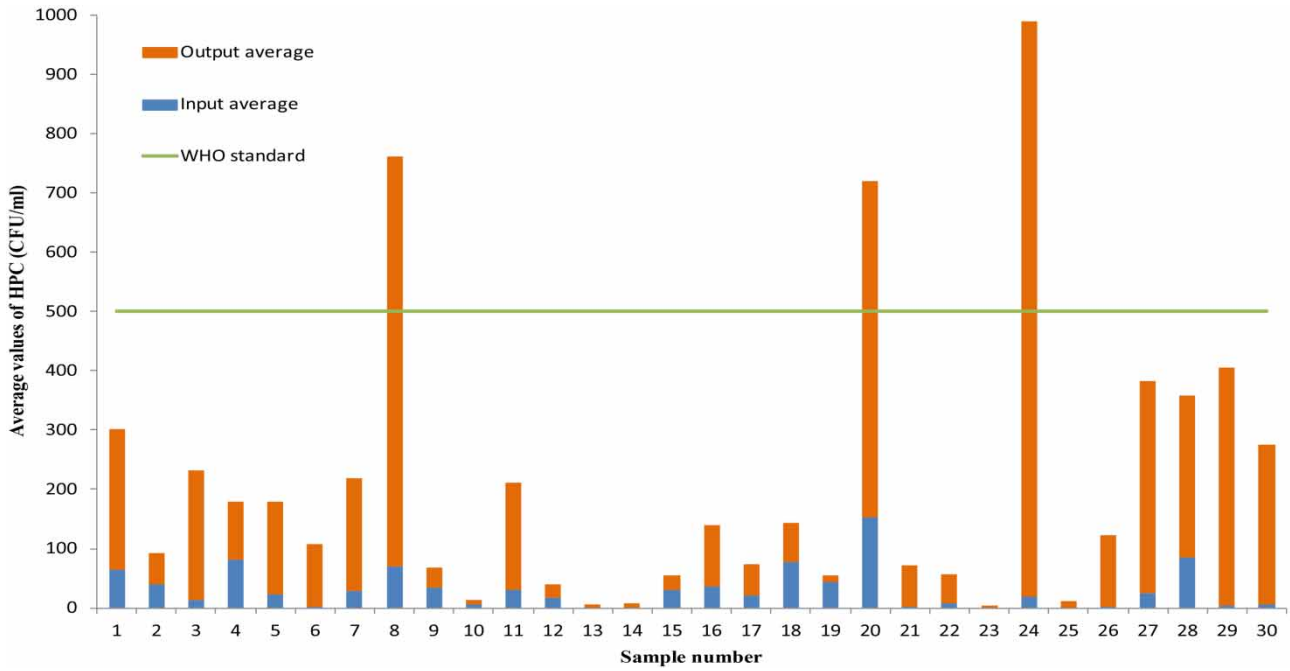

Figure 1 | Comparison of the HPC average values in input and output of HWDS samples against WHO standards.

Table 3 | Correlation between the variables of input samples

	HPC	Turbidity	Chlorine	Temperature	pH
HPC	1				
Turbidity	−0.231	1			
Chlorine	−0.278	0.224	1		
Temperature	−0.391*	0.362*	0.197	1	
pH	−0.543**	0.430*	0.294	0.457*	1

*Correlation is significant at the $\alpha = 0.05$ level.
**Correlation is significant at the 0.01 level (2-tailed).

average of data for three sampling stages, the HPC values for the input samples were between 0 and 10 CFU/ml for 50% of samples, between 10 and 100 CFU/ml for 42% of samples

Table 4 | Correlation between the variables of output samples

	HPC	Turbidity	Chlorine	Temperature	pH
HPC	1				
Turbidity	0.109	1			
Chlorine	0.032	0.168	1		
Temperature	−0.538**	−0.114	−0.08	1	
pH	0.101	0.238	−0.033	0.399*	1

*Correlation is significant at the $\alpha = 0.05$ level.
**Correlation is significant at the 0.01 level (2-tailed).

Table 5 | Results of the paired *t*-test to determine the input–output differences

	Input average	Output average	Average difference	*P*-value
HPC	30.9	178.4	−147.4	0.001
Turbidity	0.62	0.09	0.53	<0.0001
Chlorine	0.169	0.51	−0.34	<0.0001
Temperature	22.68	23.19	−0.5	<0.0001
pH	7.36	7.06	0.3	<0.0001

and between 100 and 500 CFU/ml for 8% of samples. For the output samples, the HPC values were between 0 and 10 CFU/ml for 25% of samples, between 10 and 100 CFU/ml for 43%, between 100 and 500 CFU/ml for 24% and higher than 500 CFU/ml for 8%. The values of other measured

Table 6 | Results of total coliform microbial culture in HWDS samples

	Sampling stage					
	First		Second		Third	
Total coliforms (MPN)	Input	Output	Input	Output	Input	Output
Total sample numbers (30)	38	30	0	0	8	0

parameters are shown in Table 2. The MPN values of input and output samples showed, apart from two cases, all samples were negative for all coliforms. None of the input and output samples were positive for fecal coliforms.

In accordance with current standards of the microbial quality of drinking water, fecal and total coliforms ought not to exist in DS water. In addition, Iranian and international standards allow the maximum level of HPC 500 CFU/ml in drinking water and DS water (Semerjian 2011; Institute of Standards and Industrial Research of Iran 2013). The results of the present study on samples of HWDSs in Qom city indicated that for some machines, the level of HPC exceeded the maximum permissible 500 CFU/ml threshold. Different sections of water desalination machines, particularly the reverse osmosis membrane, provide an ideal environment for bacteria to grow and multiply. If these machines are not checked and disinfected regularly, the gradual accumulation of microorganisms on the membrane can produce microbial biofilm (Herzberg & Elimelech 2007).

Although it is an ideal situation to reach the zero level of HPC in drinking water samples, in many cases, water treatment systems cannot eliminate 100% of heterotrophic bacteria. In addition, there are iron and sulfur bacteria in almost all DSs, contributing to the rise of HPC. Some countries use the optional (non-optional) maximum HPC of 500 CFU/ml at 35 °C (Bartram *et al.* 2003; APHA 2011). Although, heterotrophic bacteria are not regarded as indicators of the presence of microbial pathogens, their high density in drinking water may suggest dangerous conditions for vulnerable groups, so necessary actions should be taken to reduce these microorganisms.

For the total coliforms the MPN test was positive for the first stage of sampling (in 13% of input samples and in 13% of output samples) and for the third stage of sampling (in 3% of input samples). Additionally, no samples were positive for fecal coliforms. According to the WHO and Iranian standards, drinking water must be free from coliforms and fecal coliforms (WHO 2012; Institute of Standards and Industrial Research of Iran 2013). The high level of MPN values in some samples could be due to the low level of residual chlorine, stagnation, belated or improper checkups, and the dechlorination property of the desalination system. The importance of the level of HPC values in DS water is highlighted when dramatic changes occur, which may be indicative of poor disinfection performance, the presence of defects such as nicks, sedimentation or corrosions, leaks in the network or negative pressure in the DS. In all cases, the source of the problem must be detected and an appropriate measure should be taken (Shafiquzzaman *et al.* 2011). A study showed factors such as water stagnation in the system, the type and age of pipes, and water quality factors such as pH may affect the amount of released lead and iron from piping systems into drinking water (Lasheen *et al.* 2008). Another study, in villages in Saqqez County, Iran, showed there were no fecal coliforms in DS, and the drinking water of 88% of Saqqez's rural residents was not contaminated with fecal coliforms, whereas in the disinfected drinking water of some of the villages, up to 1,100 MPN/100 ml fecal coliforms were observed (Ghaderpoori *et al.* 2009).

Given the pH values of HWDS samples, it is necessary to state that, based on Iranian and world standards, the desired pH value is in the range 7–8.5, with the lowest permissible level of 6.5 and the highest of 9 (Institute of Standards and Industrial Research of Iran 2012; WHO 2012). The results of the study showed the minimum pH value in output samples was 6 and the maximum was 7.98. Compared with standard values, the pH values of some samples were lower than 6.5 (lowest permissible standard). At the first stage of sampling, 7% of samples had a pH value lower than the standard level, at the second, and at the third stages, 10% and 7% of the samples had pH values lower than 6.5, respectively. Also, 50% of output samples at the first stage of sampling, 30% at the second stage and 60% at the third stage were at the optimal range of pH 7–8.5. Considering the average pH of output samples at all three stages, 40% of them had optimal pH value in the range of 7–8.5 and the other 60% within the permissible range of 6.5–9.

The EC of output samples at the first, second and third stages were: 99–905, 141–1,572 and 180–1,590 μmoh/cm, respectively.

The Iranian National Standards has not defined a value of EC for drinking water, but the European standard is 400–1,000 μmoh/cm (Northern Ireland Environment Agency 2011). Water EC represents dissolved anions, cations; also, it shows which of them may lead to high salinity (Qasim

et al. 2000). Low values of EC result from water ions reduction and therefore there is no requirement for dilution to reach the range 400–1,000 μmoh/cm. In view of the average amount of EC of output samples at the three stages, it turns out that for 47% of samples the EC values were less than 400 μmoh/cm.

The threshold of TDS has no hygienic base or risk to human health by itself. Guidelines and standards proposed for this parameter are based on the taste of water. Accordingly, the TDS of salubrious drinking water is within the range of 100–500 mg/l. WHO also recommends the maximum permissible TDS of drinking water is 1,000 mg/l (WHO 2012). The EPA considers 1,500 mg/l of TDS as the acceptable maximum (EPA 2004). As for Iran, the National Standard sets 500 mg/l as the optimal maximum, 1,000 mg/l as the permissible maximum, and 1,500 mg/l in the absence of suitable alternatives (Institute of Standardsand Industrial Research of Iran 2012). The results of this study showed, for output samples, the value of TDS was between 64 and 588 mg/l at the first stage, 92 and 1,022 mg/l at the second stage and 117 and 1,034 mg/l at the third stage. The average amount of TDS for output samples was 99–789 mg/l, i.e. within the standard range. Taking 500 mg/l as the optimal maximum standard, a substantial 87% of sample TDS values were lower than 500 mg/l, and for a negligible 13% the average values of TDS were 500–1,000 mg/l.

As defined by the Iranian Standards, the maximum desirable turbidity of drinking water is 1 nephelometric turbidity unit (NTU) and the maximum allowable turbidity is 5 NTU (Institute of Standardsand Industrial Research of Iran 2012). High turbidity is important in view of aesthetics, disinfection interference and protection of microorganisms (Allen et al. 2008). As reported in previous studies, there is a direct relationship between turbidity and biological water quality including HPC and coliforms (Hammer 1986; Ghaderpoori et al. 2009). The results of this study indicate that the amount of turbidity in output samples did not exceed the desirable maximum of 1 NTU at any of the sampling stages. According to international standards, filtered water turbidity must be lower than 0.3 NTU for 95% of the measurements obtained over a one-month period and must never exceed 1 NTU (Kawamura 1991).

Residual chlorine is another parameter that is involved in determining the microbial quality of drinking water and microbial aggregation. In the disinfection process, an extra amount of disinfectant is always added for removing secondary pollutants. This amount is influenced by the pH, and varies from 0.5 to 0.8 mg/l. Higher pH values of drinking water require higher residual chlorine. The amount of residual chlorine in the water DS at homes must be approximately 0.5 mg/l (Institute of Standards and Industrial Research of Iran 2012). In this study, 10% of output samples at the first stage of sampling had 0.1 mg/l residual chlorine and for 90% the value was zero. Yet, at the third stage, residual chlorine was about 0.1–0.2 mg/l in 30% of samples and zero in 70%. As for the input samples, residual chlorine was zero for 37% of samples at the first and second stages of sampling and 13% at the third stage. According to the standards, DS water must have at least 0.5 mg/l residual chlorine at point of use (Institute of Standards and Industrial Research of Iran 2012).

The most suitable membranes for use in water desalination machines, i.e. reverse osmosis membranes, are prone to damage by free chlorine and, thus, in most of these machines an active carbon unit is set to remove free chlorine. In a study on turbidity, microbiological quality and residual chlorine concentration in drinking water of the rural areas of Kashan city in Iran, it was found that with an optimal amount of chlorine in drinking water and in respect of pH parameters, the amount of HPC could be reduced (Miranzadeh et al. 2011). As a result, by measuring and adjusting chlorine concentration, water disinfection can be made more efficient and water microbial quality can be enhanced (Stevens et al. 2003).

CONCLUSIONS

The results for output samples showed that the level of HPC values were in the range 0–10 CFU/ml for 25% of samples, 10–100 CFU/ml for 43%, and 100–500 CFU/ml for 24%, and were above 500 CFU/ml for 8%. Based on the results, desalination machines can increase the microbial population in the drinking water. However, there is no clear explanation for the variation of other parameters. Hence, the users of HWDSs should be aware of bacterial infection. It is suggested that the machines are regularly and

periodically checked by qualified technicians. Disinfection of the components, changing of filters and connecting tubes are the most important actions. Overall, it is proposed that the sellers of these machines must inform their customers about their proper operation.

CONFLICT OF INTERESTS

The authors have no conflicts of interest.

ACKNOWLEDGEMENTS

The authors of this research would like to extend their warm thanks to the Research Deputy and Management of Qom University of Medical Sciences for their financial support and great assistance. We are also deeply grateful to Qom Health Network's personnel and the Chemistry Laboratory's personnel in Environmental Health Engineering Department of the University of Qom.

FUNDING/SUPPORTS

This work was financially supported by grant: (88143) from Qom University of Medical Sciences.

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First received 6 April 2018; accepted in revised form 12 July 2018. Available online 3 September 2018