Original Article

Journal of Food Safety and Hygiene

journal homepage: http://jfsh.tums.ac.ir

A Survey on coliform contamination and chemical parameters of potable water from water dispensers in faculties of Mashhad University of Medical Sciences

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ARTICLE INFO

Article history:
Received 27 Jun. 2018
Received in revised form
21 Sep. 2018
Accepted 09 Nov. 2018

Keywords:
Drinking Water;
Water Cooler;
Total coliform;
Fecal coliform;
Residual free chlorine;
pH

ABSTRACT

Water is of great significance for living and access to safe water has been considered as an important goal in public health. However, water can transmit a wide range of pathogenic microorganisms. The aim of this study was to determine the bacteriological quality of drinking water dispensed by water coolers from different faculties of Mashhad University of Medical Sciences, on August 2016. A total of 22 samples from 9 water coolers of different faculties of Mashhad University of Medical Sciences were collected and some indicators of contamination including total coliform and fecal coliform, residual free chlorine and pH were evaluated. Results show that Coliform and Escherichia coli were not observed in any of the water samples. Residual chlorine content in drinking water in 50 percent of samples and pH in 12.5 percent of samples were out of Iranian standard levels range. There is no significant difference between the levels of pH and chlorine in water samples before and after water dispenser systems. The lack of coliform indicates the safety of drinking water from water coolers. However, the decrease in the residual free chlorine after water cooler may cause to grow some microorganisms in water coolers. Therefore, it is suggested that an appropriate and regular monitoring program should be established.

Citation: Ghorbanzadeh A, Peivasteh-roudsari L, Afshar Kohan N, Zangi R, Moradi F. **A Survey on coliform contamination and chemical parameters of potable water from water dispensers in faculties of Mashhad University of Medical Sciences.** J Food Safe & Hyg 2018; 4(3-4): 63-68.

1. Introduction

It is generally accepted that the quality of drinking water is a vital issue for human beings as it directly affects the life of all people, so identification of pathogens is one of the most important aspects of food safety and hygiene (1,2). The access to safe drinking water is thus essential for human health and is a basic human right. The safety of drinking water is now a major public health problem in many crises (3). The urbanization and population growth, industrialization, and the overuse of land resources have caused numerous environmental problems leading to the pollution of water resources as it's important consequence (4). Many developing countries suffer from a severe shortage of freshwater resources or pollution of existing water resources (5). According to the United Nations Children's Fund (UNICEF) report, about 80 million people do not have access to safe drinking water in Asia and Africa leading to a variety of diseases for many people (6). According to the United Nations (UN) report, the most population of Iranians, especially in rural areas, are at the risk of enteritis, diarrhea and contagious disease due to the lack of access to safe drinking water (2). According to the World Health Organization (WHO), the consumption of water containing microorganisms

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causes the death of 5 million people in the world, mostly children under the age of 5 years (7). Previous studies have reported a reduction of 17% to 85% of diarrheal diseases as a result of water purification and disinfection for household consumption (8).

Water can provide the possibility of transmitting a wide range of pathogenic microorganisms that are causes of gastrointestinal diseases. Therefore, safe drinking water is an important public health goal. Global standards have been developed to assess the microbial quality of drinking water to achieve a good quality. Due to previous studies and bacteriological findings, indicating the microbial burden of water dispensing systems, the Federal Office of Public Health has systematically and accurately studied and assessed microbiological hazards associated with water dispensing systems (9,10).

According to the WHO guidelines, the safe drinking water should not create any serious risk, such as a variety of diseases and allergies during the consumption and at different stages of human life. Infants, children, the elderly, or those with immunodeficiency as well as those, who live under unsanitary conditions, are generally the largest group at the risk of water-related diseases (3). In terms of health, the main objective of water quality management is to ensure that consumers do not catch pathogens. Protection of water resources has greatly reduced the risk of these diseases in developed countries. Parameters, which have been proposed by the WHO for monitoring drinking water resources, reduce the health status of water and the risk of water pathogens: 1. *Escherichia coli* and heat-resistant Coliforms; 2. The residual free chlorine; 3. pH; and 4. Turbidity (2).

Water dispensers are widely used in universities, offices and commercial places, especially in warm seasons; and thus the assessment of microbial quality of output water from these devices seems necessary to prevent the occurrence of water-borne outbreaks given the structure of these devices and reservoirs that can develop pathogenic microorganisms such as *Pseudomonas*, *Escherichia coli* and Coliform due to the water stagnation (10-12). Generally, the water dispensing pollution may be caused by issues such as the input water pollution, improper connection of water dispenser to the plumbing system, stagnation of water in the water dispensing tank, and the existence of cracks and fractures in its body (12,13).

Due to the extension of the environment and a higher number of students, universities of medical sciences should provide safe drinking water for students and staff and control its bacterial quality in order to prevent an outbreak and spread of waterborne diseases. The present study was conducted according to the importance of drinking water safety in order to determine its quality at faculties of Mashhad University of Medical Sciences in summer 2016 and compare the results with the permissible limits of the Institute of Standards and Industrial Research of Iran (ISIRI), and the World Health Organization (WHO).

2. Materials and methods

2.1. *Sample collection*

The research followed a descriptive and cross-sectional design. After field visits and according to the target population, sampling was done on metal water dispensers at all Mashhad faculties of medical sciences (except for the nursing faculty) including faculties of health (4 samples) and traditional medicine (2 samples) located in the University street, faculties of dentistry (4 samples), medicine (2 samples), pharmacy (2 samples), and paramedical faculty (4 samples) located in Vakilabad street, and Also the samples of input water to the university street (2 samples) and the input water to Vakilabad Street (2 samples) in two replications. Totally, 22 samples were collected from water dispensers of the faculties and their input water in summer 2016.

All necessary dishes and equipment for the research were disinfected by 70% alcohol and sterilized in an oven at 170°C for an hour. Samples were collected by glass containers with sandpaper heads containing 2 to 5 drops of 3% sodium thiosulfate solution for dechlorination. Samples in the cold box were transferred to the laboratory with dry ice in less than 2 h (the necessary temperature for transferring samples to the laboratory should be less than 10°C).

2.2. Detection of coliform

Most Probable Number test (MPN) or the 15-tube fermentation method by a standard method was used to measure the amount of contamination with Coliforms in samples, and Chromogen agar medium as the Pour Plate was utilized to detect *E.coli* for higher efficiency than other methods. *E.coli O157: H7* species were purple and other species of *E.coli* were blue or colorless in order to detect and distinguish bacteria in Chromogen agar medium according to Table 1. To detect the existence of coliform based on MPN test, one and two-concentration Lauryl sulfate broth (LSB) medium was used at the first stage; Brilliant Green Bile (BGB), Lactose Broth was applied to detect the total Coliform at an incubation temperature of 37°C during a 24-48 hour period at the second stage (confirmatory stage); and EC broth medium was used for detection of fecal coliform at a temperature of 44.5°C for 24 hours at the third stage (differential stage). If there was a positive tube at the confirmatory stage, the MacConkey agar (MAC) culture medium was used for a supplementary stage at 37°C and 24 h. Ultimately, the bacterial density was obtained using the MPN table based on the Poison distribution and the Thomas formula.

2.3. Chlorine test

The chlorine content of each sample was estimated using Chlorimetry laboratory kits and DPD tablets (N, N-diethyl-p-phenylenediamine).

Table 1. Detectable water borne pathogens by chromogenic agar medium (1)

Bacteria Species	Colony Color	Sensitivity (%)	Differential Ability (%)
E.coli O157	Purple	98	99
E.coli spp	Blue/colorless	98	99
Salmonella	Red	100	100
V. cholerae	Blue	99	98.5
S. aureus	Purple/red	95.5	99.4

2.4. pH test

The pH was reported by the pH meter paper and according to the observed color changes. All stages of the test were carried out in accordance with principles of Standard Methods for the Examination of Water and Wastewater book 21st edition (14) in the microbiology lab of the Faculty of Health at Mashhad University of Medical Sciences.

2.5. Statistical analysis

Finally, the obtained data were statistically analyzed by T-test and are presented as Mean±SD using Excel and SPSS version 16. P-value<0.05 was considered as the significant level.

3. Results

Results of MPN and Chromogen agar were negative in all samples from medical, paramedical, dentistry, pharmacy, and traditional medicine faculties as well as input water samples of water dispensers indicating the absence of fecal coliform and other coliform types in water. The obtained samples from the faculty of health at the confirmation stage of MPN were re-taken due to the suspicion, and all stages of the experiment were repeated and ultimately no coliform contamination was reported. Tables 2 and 3 present data on coliform contamination, mean pH, and the amount of free residual chlorine and their statistical analysis. According to obtained results, all water samples of tested water dispensers and the input water, had no total and fecal coliform.

Total pH of samples was 6.94 on average and at the range of 6.2-7.2. The pH of all samples was at the standard range (6.5-8.5) except for the sample of the faculty of pharmacy with an amount of 6.2 that was less than the standard limit. In terms of residual chlorine content, water samples of the faculties of health, dentistry, and traditional medicine, and water input 1 (university street) was outside the standard limit (0.2-0.8), but the residual samples were within the permitted range.

There was no significant difference between pH and the residual free chlorine of input water sample 1 (Vakilabad street) and its faculties (medicine, dentistry, pharmacy, and paramedicine) with the input water sample 1 (university street) and its faculties (traditional medicine and health school).

The average residual chlorine was 34±0.54 and 0.56±0.66 mg/l respectively, and the water pH was 0.36±6.86 and 7.06±0.08, respectively at faculties of Vakilabad region and University Street.

3. Discussion

The quality of drinking water may be affected by distribution lines, storage resources and installed cooling systems by consumers before the consumption, and there are concerns about the incidence of secondary microbial and chemical contamination during the water transmission and distribution (11). In places, where is transmitted water from one place to another, there is the possibility of out-of-control secondary

Table 2. Statistical Analysis results of water quality parameters

Sampling location	indicator	Average	Standard deviation	Significance	Minimum	Maximum
Vakilabad street and its faculties	Residual chlorine (mg/l)	0.54	0.34	0.461	0.2	1
	pH	6.86	0.35	0.124	6.2	7.2
University street and its faculties	Residual chlorine (mg/l)	0.56	0.66	0.18	0.1	1.5
	pH	7.06	0.08	0.42	7	7.2

Table 3. Coliform contamination according to MPN and Chromogen agar methods, mean pH and free residual Chlorine of potable water from water dispensers

Sampling location	Number of water coolers	Number of samples	MPN results	Chromogen agar results	Free Residual Chlorine (mg/l)	pH Mean
input water 1 (university street)	-	2	Negative	Negative	1.5±0.1	7±0.02
Health school	2	4	Negative	Negative	0.1±0.02	7.2±0.05
Traditional medicine school	1	2	Negative	Negative	0.1±0.05	7±0.03
input water 2 (Vakilabad street)	-	2	Negative	Negative	0.4±0.08	7.2±0.04
Medicine school	1	2	Negative	Negative	0.2±0.05	6.9±0.08
Paramedicine school	2	4	Negative	Negative	1±0.15	7.1±0.02
Pharmacy school	1	2	Negative	Negative	0.2±0.05	6.2±0.08
Dentistry school	2	4	Negative	Negative	0.9±0.12	6.9±0.05
Average	9	22	Negative	Negative	0.55±0.1	6.94±0.03

contamination due to the exposure to unwanted situations. The water dispenser reservoir and created biofilm are some of these cases (13,15). Since the pollution of drinking water by human and animal stools is directly or indirectly the most common way of transmitting these microorganisms (16), the isolation and detection of pathogenic microorganisms is a major aspect of the food health (1). Coliforms, especially *Escherichia coli*, are microbial indicators of water and food, and their presence in drinking water and food can be a sign of fecal contamination and the existence of other intestinal pathogens. The presence of these bacteria in water indicates the inadequacy of the purification process as well as the frequent and recent contamination of water with human and animal stools (17,18).

According to the ISIRI and WHO, the contamination with *Escherichia coli* or thermotolerant coliforms should be zero per 100 ml of drinking water or treated water in distribution systems (3,18). The optimal limit of pH should be at the range of 6.5 to 8.5 according to the Standard of Iran and the WHO and EPA (5,11,19). According to standard of Iran, the recommended residual free chlorine should be at least 0.5 to 0.8 mg/l at any point of the network after half an hour of contact in normal conditions, and at least 0.2 mg/l in the water consumption place. The minimum allowed amount of residual free chlorine should be at the range of 0.5-1 mg/l in emergencies, intestinal epidemics and natural disasters (19).

In the present study, there was no contamination with total coliform and *E. coli*. The amount of Chlorine exceeded the standard level in the input water sample 1 (university street), and it was lower than standard at faculties of that area, including traditional medicine and health faculties indicating the high contamination of water dispenser leading to a reduction of residual free chlorine in the output water of water dispensers. This was probably due to the old dispensing systems, inadequate water dispensing connection to the system, stagnation of water in the dispensing system reservoir, the existence of cracks in the dispensing system body, the lack of regular tank cleaning, and the biofilm formation in these systems.

In a study by Sattar-Mohammadi et al. (2013) with the aim to evaluate the bacteriological quality of drinking water in water dispensers of educational units at Shahid Beheshti University and Shahid Beheshti University of Medical Sciences, the assessment of 13 water dispensers based in different educational centers indicated that the range of residual chlorine in water was 0.4-0.8 ppm before entering the water dispenser and 0.2-0.6 ppm after entering the water dispenser. The pH of samples was 7.2-7.4 in samples before and after the water dispenser. A number of colonies of the heterotrophic bacteria were 1000±100 CFU/100ml in cultured samples before and after the water dispenser. No coliform bacteria were seen in samples (13). Taheri et al. (2009) evaluated the quality of water dispensers at Isfahan University of Medical Sciences and reported that heterotrophic bacteria count was less than the standard limit (500 CFU/ml) and no significant difference was observed between the input and output water samples of water dispensers (11).

Naghipour et al. (2016) studied the bacteriological quality of water in 13 water dispensers at hospitals in Rasht and found two cases of coliforms and all samples were less than 15 CFU/ml in terms of the heterotrophic contamination. The filtration system also reduced the amount of turbidity and residual free chlorine of output water of water dispensers (12).

A survey on the microbial quality of water at water dispensers of inter-urban buses of Bandar Abbas by Alipour et al. (2004) indicated that 6 out of 38 samples were contaminated with Coliforms. The residual chlorine content was zero in all samples and pH range was 7-7.5 (16). Vakilabadi et al. (2010) evaluated the bacterial quality of drinking water in inter-urban passenger buses of Bushehr Port and found that the residual chlorine content was zero in 97.5% of cases.

The pH of samples was at the range of 6.8 to 8.7 and the total amounts of the fecal coliform (E. coli) and total coliform (TC) were more than standard in 8.8% and 12.5% of samples respectively (20).

Levesque et al. (1994) studied and compared the bacterial contamination of public water dispensers with bottled water samples in different regions of Québec province in North America and found that 36% and 28% of water samples of tested water dispensers were at least contaminated with a coliform or a pathogenic bacterium in residential areas and commercial centers (21).

Liguori et al. (2011) found no contamination with E. coli and Enterococcus in drinking water samples of water dispensers and about 24% of samples were contaminated with Pseudomonas aeruginosa in commercial centers of southern Italy. It was also reported that the bottled water always had a higher bactericidal quality than drinking water in water dispensing systems (10).

The bacterial quality of water dispensing systems in educational units of Zanjan University of Medical Sciences was investigated by Chamandoust et al. in 2017 and their results indicated that 44.44% samples contained residual chlorine below the permissible level; 27.8% were contaminated with E. coli, and 44.44% were contaminated with mold or yeast (22).

It should be noted that conventional methods, which are used to isolate and detect some bacteria such as Escherichia coli, are time-consuming, and on the other hand, costs of culture media are very high. Chromogen culture medium (branded as Chrome Agar ECC) is among the fastest isolation methods that are based on the color change of applied material in the presence of specific enzymes of microorganisms. As a result of applying this medium, coliform bacteria can be detected in the shortest possible time within 24 hours (23). Conducted studies in different countries confirm that Chromogen medium is a fast and sensitive method that can easily detect the most important pathogenic microbes transmitting by water and food such as Staphylococcus Aureus, Escherichia coli, Salmonella and Listeria monocytogenes (17).

According to a research by Tavakoli et al. (2012), the duration of detecting E. coli in Chromogen medium was 18 hours and 20 minutes with the sensitivity of 99.61% on average, while the duration of its detection in a standard culture medium was 72 hours and 20 minutes with a sensitivity of 100% on average. Therefore, the Chromogen culture medium has a high speed, sensitivity, and specificity and does not require any confirmatory biochemical tests (1,24). In addition to the conventional MPN method, which is extremely time-consuming and costly, the present study utilized the Chromogen culture medium because of its availability in the laboratory and ensure the accuracy of work in order to detect E. coli O157:H7. It is suggested replacing conventional methods with Chromogen agar method in order to investigate the fecal coliform contamination.

5. Conclusions

According to the results of the present study, the consumed water of water dispensers based in Mashhad University of Medical Sciences had a good level of microbial quality and no serious problem was seen in qualitative parameters of drinking water due to the absence of bacteria in the coliform group. Given that the amount of residual free chlorine was not standard at some faculties, it is suggested measuring the residual chlorine levels during certain periods, and taking measures in the case of exceeding the standard limits. In general, the regular monitoring of the drinking water distribution system is significantly important for preserving student health and preventing any outbreak or epidemic.

Conflict of interest

There is no conflict of interest.

Acknowledgements

Special thanks go to Dr. Hossein Alidadi, (Director of Environmental Health Division, Health School, Mashhad University of Medical Sciences) And Dr. Monavvar Afzal Aghayi, (statistical expert, Epidemiology Division, Health School, Mashhad University of Medical Sciences) for their co-operation in all stages of this study.

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