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A survey of heterotrophic bacteria and coliforms in the water of old and new distribution networks

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Original Article

Abstract

Controlling the microbiological quality of water is important for its uses. Microbiological requirements of drinking water ensure the absence of coliforms, but may indicate the potential presence of other potentially pathogenic microorganisms and viruses of fecal origin. A total of 36 water samples, representing the drinking water of the whole city of Aradan, Semnan, Iran, were randomly collected from the old and new distribution systems of Aradan from December 2014 to June 2015.The hetroterophic plate count (HPC), coliform, residual chlorine, and pH of the samples were measured. Heterotrophic bacteria were measure using R2A and nutrients agar culture media, and the spread plate count method was used to determine HPC. Average concentration of total coliform in the old distribution network ($\frac{13/84}{100\ \text{ml}}$) was more than the new distribution network ($\frac{9/42}{100\ \text{ml}}$). The results of studying microbial load in the old and new distribution network showed that there is a significant difference between total coliforms in the two networks (P = 0.002). Moreover, the average concentration of heterotrophic bacteria in the old distribution network [1917.33 colony-forming unit (CFU)/100 ml] was more than that in the new distribution network (14.57 CFU/100 ml). There was a significant difference between the average concentration of heterotrophic bacteria in the old and new distribution networks (P = 0.003). The effects of seasons and the age of the water network on coliform bacteria and heterotrophic bacteria were also studied. The concentrations of heterotrophic bacteria, total coliform, and fecal coliforms were higher in the old distribution network in comparison to that in the new distribution network.

KEYWORDS: Heterotrophic Bacteria, Coliform, Aradan, Water Distribution System

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Introduction

In every society, healthy drinking water has the most effect on human health. Thus, access to healthy drinking water is of great importance in many countries of the world. The main concern of the World Health Organization (WHO) regarding water is its importance in disease transmission, because, according to the WHO, 2.2 million people die annually due to lack of access to healthy drinking water.[1-2]

Bactria, yeast, and molds are also heterotrophs, as they are unable to make all the nutrients they need to live, and grow. Plants, on the other hand, use the sun's energy to make their own food; therefore, they are called autotrophs. The heterotrophic plate count (HPC) is a procedure used to

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estimate the number of live herterotrophic bacteria present in a water sample.[1-3]

Different bacteria pose different risks to public health, microorganisms found though HPC tests generally include those that are part of natural (typically non-hazardous) microbial flora found in water. Some of the main bacteria genera detected in drinking water pose a higher risk for those people with a depleted immune system, such as the elderly and infants, or those with human immunodeficiency virus (HIV). Therefore, paying attention to the amount and type of these microorganisms is necessary to prevent infection transmission through drinking water.

The drinking water of a society must have a standard quantity and quality.[3] Therefore, awareness of physical, chemical, and microbial parameters of drinking water is particularly important in the condition of drinking water. Drinking water, generally, is ideal water lacking smell, taste,[1] pathogenic microorganisms, and harmful compounds.[4] Microbial quality is of great importance in drinking water because the infectious risks of drinking water are primarily related to fecal contamination.[4-5] According to the WHO recommendations and Iranian Standard, drinking water must be without fecal coliforms (MPN = 0), and in 95% of the cases, total coliforms must be zero in the studied samples.[5]

Different criteria such as temperature, retention time of water in the distribution network, bacterial quality of input water of the network, the material of the pipes, and the existence of the nutrients necessary for the growth of bacteria can influence the microbial load of water.[3]

To determine the microbial quality of drinking water, indicator organisms including total coliform and fecal coliforms are used.[1,4,6,7] It is worth mentioning that, lately, HPC test has been approved by the WHO as an indicator of the general quality of water in drinking water distribution network.[1,4,10] The maximum permitted HPC in Germany, USA, and Australia are 100, 500, and 500, respectively.[8] Various studies have been conducted on the microbial quality of drinking water in different parts of the world and Iran.[1-9] However, to the knowledge of the authors, no similar study has been conducted regarding the effect of the age of the distribution network on the amount of microorganisms in water in the city of Aradan, Semnan, Iran. Therefore, this study aims to analyze the effect of the age of the distribution network on the microbial quality of water.

Materials and Methods

In this descriptive cross-sectional study, the microbial quality of drinking water in the old and new systems of the distribution network in Aradan was analyzed from December 2014 to June 2015. Sampling was performed in 2 seasons and in different places of 6 different locations (36 times) (beginning, middle, and end) in the drinking water distribution network of the city. Of the 6 locations, 3 were in the old distribution networks and the other 3 in the new distribution networks. For sampling, 500 ml glass bottles, containing a few drops of a 1% solution (120 mg/l) of sodium thiosulfate, previously sterilized (at 121 °C for 15 minutes), and covered with sterile aluminum sheets were used. At each location, 200 ml of water were collected in the polystyrene bottles. To preserve and transfer samples to the internal laboratory, a cold box was used. During each time of sampling, pH and free residual chlorine were measured in situ. To evaluate pH, a pH meter kit and phenol red were used, and to evaluate the free residual chlorine, chlorine photometer kit and N, N diethyl parafynyly diamine (DPD) reagent were used.

Microbial test

The test included total coliform, fecal coliform, and HPC. To determine the total coliform and fecal coliform, multiple tube fermentation method (15-tube method) was used, and the standard method was used to test the water.[1-7] Multiple tube fermentation included possible, confirmatory, and complementary stages. Results were discussed based on the table colony-forming unit (CFU)/100 ml. The nutrient media used for HPC test is called R2A agar which is used at 35 °C for 48 hours. Then, the colonies created on the agar surface were counted using a colony counter and reported as CFU/100 ml.[6,7,10-12]

Statistical analysis was conducted using SPSS software (version 17, SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) and Tukey's post hoc test for pairwise comparisons were used to compare means in different stations. The one-sample Kolmogorov-Smirnov test was employed to evaluate the normality of the data. Kruskal-Wallis test was used as the nonparametric equivalence of one-way ANOVA if the normality assumption was redundant. The Mann-Whitney U test was employed for assessing significant difference of the bacterial concentrations in two seasons, and old and new distribution networks. All P values of 0.05 were considered significant. Pearson's rank correlation coefficient was used to determine the relationship between bacterial concentrations (HPC and coliform) and some parameters such as free residual chlorine.

Results and Discussion

The concentration of heterotrophic bacteria and coliforms in different distribution networks

According to table 1, the average concentrations of total coliforms and fecal coliforms in the old distribution network ($\frac{13/84}{100\ ml}$) were higher than the new networks. The comparison of the microbial load in the old and new distribution networks showed a significant difference between total coliforms in the two networks ($P < 0.05$). Fecal coliforms also showed the same pattern ($P < 0.05$). Table 1 also shows that the average concentration of heterotroph bacteria in the old distribution network was significantly higher than that in the new distribution network ($P < 0.05$). The higher concentration of studied bacteria in the old distribution network may be due to the material of the pipes that were metal. According to studies, since bacteria can be adsorbed onto iron oxides and organic compounds as well as pores in metal pipes, the growth of biofilms on the surfaces of metal pipes is better than polyvinyl chloride (PVC) pipes. A similar study conducted in Arak city, Iran, found that the amount of heterotrophic bacteria increased when damages increased, and the metal tubes were more than the PVC tubes.[13] It should be noted that burnout of pipes in the old distribution network can also be one of the reasons for the increasing microbial loads in such systems.

The total coliform concentrations at stations in different distribution networks are shown in figure 1.

Among all the studied stations, the highest concentration (15.82 CFU/100 ml) was observed at the end of the old water distribution network, while the lowest concentration (3.45 CFU/100 ml) was observed at the beginning of the new distribution network. This may be due to the decrease in chlorine concentration in parts of the system that were in end points or blinded areas of the network.

Table 1. Concentration of heterotrophic bacteria and coliforms (CFU/100 ml), in different distribution networks

Distribution network	Total coliform/100 ml	fecal coliform/100 ml	Heterotrophic Bacteria/100 ml
Old distribution network	13.84 ± 11.07	4.29 ± 2.02	1917.33 ± 2000.00
New distribution network	9. 42 ± 6.70	0.50 ± 1.05	57.14 ± 12
Significant difference	P = 0.002	P = 0.005	P = 0.003

CFU: Colony-forming unit

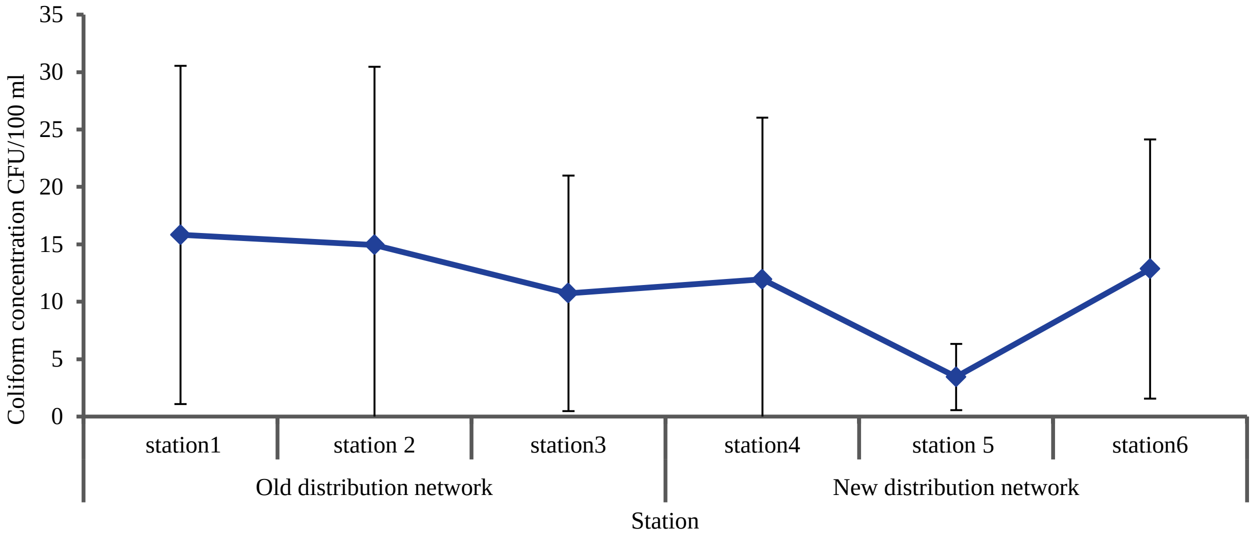

Figure 1. The average and standard deviation of total coliform concentration (CFU/100 ml) in water of the two distribution networks
CFU: Colony-forming unit

According to figure 2(a), the composition of total coliform in the old distribution network was 31% fecal coliforms and 69% other coliforms. However, according to figure 2(b), the composition of total coliforms in the new distribution network was 4% fecal coliforms and 96% other coliforms.

In similar studies conducted by Amin et al.[14], and Hamida et al.[15]. on bacteriological analysis of drinking water of Peshawar city, Pakistan, most samples were total coliform positive. These studies showed that only 8% of samples were found to be free of bacterial contamination, while the remaining 92% of samples were positive for bacterial contamination and unfit for drinking. In this study, high rates of chlorine loss were observed in the water distribution network compared to findings of Nagatani et al.[16] and Li et al..[17] This can be attributed to the poorer water quality (e.g. microbiology) and age of the network pipes in the old water distribution system of Aradan.

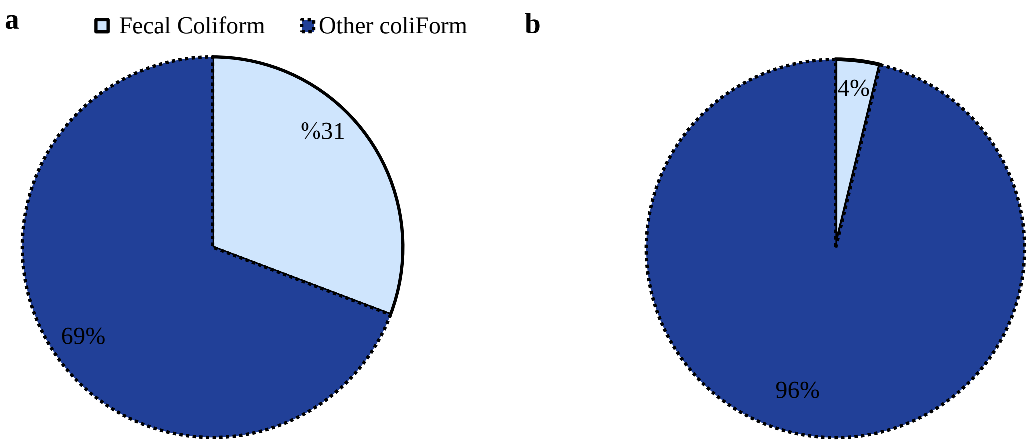

Figure 2. Composition of coliform in the (a) old and (b) new distribution networks

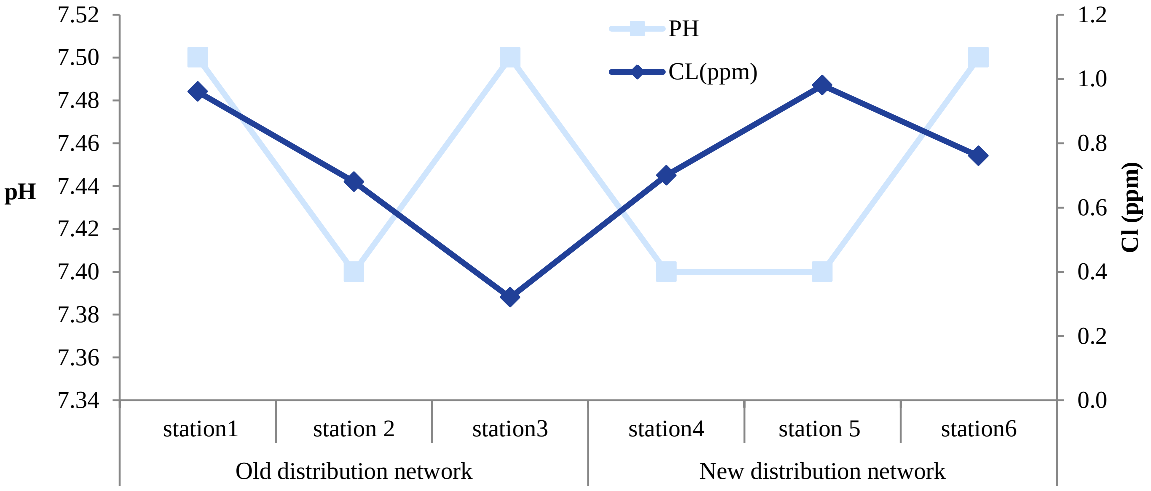

Figure 3. Temporal trends in the average of chlorine (Cl) and pH of the water distribution system during the study period in Aradan

Figure 3 shows the range of the free residual chlorine (Cl) and pH at different stations. The pH and Cl of water samples were found to, respectively, range from 7.4 to 7.5, and 0.3 to 1 ppm with a mean pH and Cl value of 7.45 and 0.73 ppm, respectively. The WHO recommended pH and Cl values for drinking water are 6.5 to 8.5, and 0.2 to 1.5 ppm, respectively. Interestingly, all pH and Cl values of analyzed water samples were within the permissible limits for drinking water.

Figure 4 shows the positive association between heterotrophic bacteria concentration and Cl concentration in the new and old distribution networks. Spearman's correlation between heterotrophic bacterial concentrations and Cl concentration for the new distribution network provided an r equal to 0.53 (n = 15). Nevertheless, for the old distribution network r was 0.60 (n = 15). In the study by Hilborn et al., no statistically significant correlation was observed between the CFU of and chlorine concentration in the distribution system.[18]

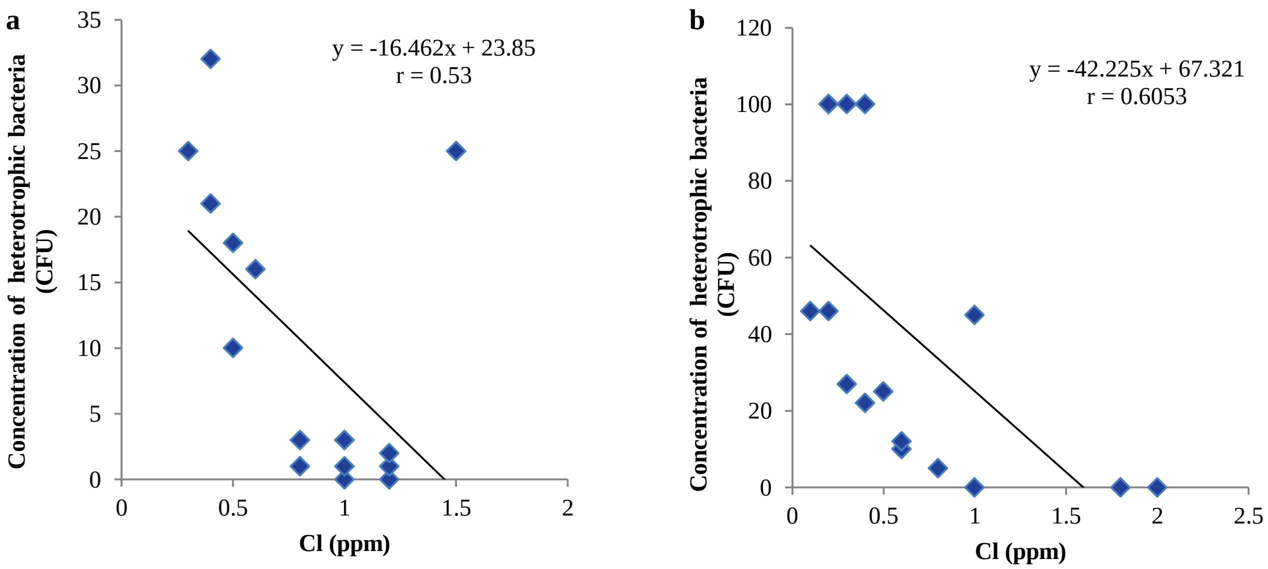

Figure 4. Scatter plot comparing heterotrophic bacteria concentration in the (a) new and (b) old distribution networks

CFU: Colony-forming unit; Cl: Chlorine

Table 2. Concentration of coliforms and heterotrophic bacteria (number /100 ml) in the water distribution network in two seasons

Seasons	Total coliform	Fecal coliform	Heterotrophic bacteria
Winter	81.16 ± 4.02	3.35 ± 2.50	1333.46 ± 1022
Spring	19.60 ± 18.00	1.80 ± 2.02	641.10 ± 588
Significant difference	P = 0.002	P = 0.045	P = 0.054

The presence of bacteria is important in terms of controlling the processes of water refinery and analyzing the engineering methods associated with appropriate refinery and distribution of water, health, and aesthetic issues of water. Heterotrophic bacteria are safe from disinfecting effects in biofilms and remaining deposits. Different factors are effective on the amount of heterotrophic bacteria including the existence of biofilm in deposits, preservative of remaining disinfectants, temperature of water, hydraulic conditions, pipe material and materials used in the distribution networks, and damages in the distribution network.[10] Studies have shown rapid increases in biofilm at the end point of distribution networks or in areas in which the water flow decreases and the pipe diameter changes.[11]

Seasonal survey of heterotrophic bacteria and coliforms

According to table 1, the average concentration of total coliforms in the old distribution network ($\frac{1917}{100\ ml}$) was more than the new distribution network ($\frac{14.57}{100\ \text{ml}}$). The results of microbial load analysis in the old and new distribution networks show a significant relationship between total coliform in the two networks ($P = 0.002$), but no significant relationship between fecal coliform in spring and winter ($P = 0.005$). According to table 2, in spring, the amount of studied bacteria was significantly lower than in winter ($P < 0.05$). Temperature is one of the parameters that have a strong effect on bacteria in water.[10] In spring, water has higher temperature than winter, which not only affects water treatment operations and procedures, but also has impact on bacterial growth in distribution networks.

The amount of biofilms in the old distribution network was higher than the standard amount recommended by the WHO, and further investigations in this regard are necessary. Studies have shown that 2% of heterotrophic bacteria are pathogenic. These bacteria are considered as the first and second pathogens. The presence of these bacteria (including Pseudomonas, Klebsiella, and Aeromonas) in the water is seriously dangerous for human health. Some of these pathogens cause opportunistic infection.[19,20]

Conclusion

The results indicate that drinking water is highly vulnerable to bacterial contamination. Water contamination may be due to leakage in pipes, cross-contamination between drinking water and wastewater, and poor constructed well head. It was also seen that the age of the water network is effective on the concentration of the bacteria that determine the microbial quality of the water.

Conflict of Interests

Authors have no conflict of interests.

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