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Original Article

The Interaction between Heterotrophic Bacteria and Coliform, Fecal Coliform, Fecal Streptococci Bacteria in the Water Supply Networks

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Abstract

Background: This study investigated the interaction between heterotrophic bacteria and coliform, fecal coliforms, fecal streptococci bacteria in water supply networks.

Methods: This study was conducted during 2013 on water supply distribution network in Aq Qala City, Golestan Province, Northern Iran and standard methods were applied for microbiological analysis. The surface method was applied to test the heterotrophic bacteria and MPN method was used for coliform, fecal coliform and fecal streptococci bacteria measurements.

Results: In 114 samples, heterotrophic bacteria count were over 500 CFU/ml, which the amount of fecal coliform, coliform, and fecal streptococci were 8, 32, and 20 CFU/100 ml, respectively. However, in the other 242 samples, with heterotrophic bacteria count being less than 500 CFU/ml, the amount of fecal coliform, coliform, and fecal streptococci was 7, 23, and 11 CFU/100ml, respectively. The relationship between heterotrophic bacteria, coliforms and fecal streptococci was highly significant ($P<0.05$). We observed the concentration of coliforms, fecal streptococci bacteria being high, whenever the concentration of heterotrophic bacteria in the water network systems was high.

Conclusion: Interaction between heterotrophic bacteria and coliform, fecal coliforms, fecal streptococci bacteria in the Aq Qala City water supply networks was not notable. It can be due to high concentrations of organic carbon, biofilms and nutrients, which are necessary for growth, and survival of all microorganisms.

Keywords: Heterotrophic bacteria, Coliform, Fecal coliforms, Fecal streptococci, Water networks

Introduction

Heterotrophic plate count (HPC), which is an aerobic – anaerobic bacteria test in water, can be used for detection of all bacteria, but cannot be used in fecal contamination test (1). Heterotrophic bacteria are natural inhabitant of the human body and animal. They have various types, such as Gram-negatives: *Proteus, Enterobacter, Aeromonas, Citrobacter, Pseudomonas, Klebsiella, Flavo bacterium, Sratya, Moraxella, Alcaligenese* and *Acinetobacter,* and gram-positives: *Bacillus* and *Micrococcus* (2). Heterotrophic bacteria are not indicators of pathogenic conditions but some of them like *Pseudomonas* is opportunists and can cause some infections in skin and lung and the other type like *Aeromonas* cause gastroenteritis (2-4).

Today heterotrophic bacteria are considered as an accessory indicator of measuring of coliform in water. Identification of heterotrophic bacteria is a very helpful method in the quality assessment of drinking water in storage tanks and in water distribution network. The concentrations of heterotrophic bacteria in the drinking water may vary from less than 1CFU/ml to over 10000 CFU/ml, which depends on water temperature, residual of chlorine and the amount of absorbable organic material. In the distribution system, the maximum permissible level of heterotrophic bacteria is 500 CFU/ml (5).

Changes in bacterial populations in the distribution network affect the aesthetic quality of water by changing the taste, odor and color by creating a sticky and slimy layer. Such bacterial populations can cause corrosion, deposition and decrease of water discharge, health effects by setting people at risk of pathogenicity (6), and corruption in main products such as food, water, medicines and cosmetics (7).

With high concentrations of heterotrophic bacteria in the water, it is hard to determine the fecal coliform and pathogenic contaminations (8). In addition, heterotrophic bacteria counts over 1,000 CFU/ml in water samples can cause low sensitivity in tube tests and the membrane filter. Organisms like *Pseudomonas* and *Flavo bacterium* can prevent the growth of total coliform and prevent the observed gas production in lactose fermentation and with dispersed growth on the membrane filter, and interfere with coliform on M-Endo medium, and therefore coliform detection could be hindered.

Increase in heterotrophic bacteria could be a sign of trouble in treatment, repair, installation or influence of microbial growth in the distribution system and presence of biofilm (9), and one reasons which may increase the risk of gastroenteritis (10).

In some cases of water distribution system with 0.6 mg/l of residual chlorine, more than 500 heterotrophic bacteria CFU/ml have been isolated. This indicates that some certain bacteria may survive in high concentrations of free residual chlorine (11). In the presence of more than 500 CFU/ml heterotrophic bacteria, coliforms are suppressed of 1 to 10 CFU/ml in the water distribution systems (12).

This study investigated the relationship between heterotrophic bacteria and coliform, fecal coliforms and fecal streptococci bacteria and their possible interactions in drinking water system (13).

Materials and Methods

Microorganism and experimental tests

Microorganisms are included as heterotrophic bacteria, coliform, fecal coliforms and fecal streptococci bacteria. The surface method was applied to test the heterotrophic bacteria and MPN method was used for coliform, fecal coliform and fecal streptococci bacterial test.

Sampling and culture

Because of high incidence of diarrheal diseases, taking samples was conducted in summer and early autumn. Overall, 324 samples were taken from rural areas and 32 samples from urban area in Aq Qala City, Golestan Province in 2013.

In order to get more accuracy in network water quality, 178 samples were taken from the beginning location of water network systems and 178 samples from the end points. Coliform, fecal coliform, fecal steptococci, and heterotrophic bacteria over 500 CFU/ml were under test. Sample taking were performed according to WHO guidelines (13). MPN method with 9 tubes was used to determine coliform. For bacterial growth, lactose broth medium was used in this method. The tubes were incubated in 0.5 ± 35 °C for 24-48 h and then, from each positive tube inoculate (by loop) was added to brilliant green and EC broth media. Then, the tubes with brilliant green medium were incubated at 0.5 ± 35 °C for 24-48 h and the tubes containing an EC medium incubated in serology water bath at 0.5 ± 44 °C for 24 h. Both media with gas were marked as positive. In order to determine fecal streptococci, 9-tube method was used. The tubes incubated at 0.5 ± 35 °C for 24-48 h. The tubes with turbidity were considered as positive and inoculated in PSE agar. After that, the plates were incubated at 0.5 ± 35 °C for 24 h. The plates having brown and black colonies and brown halo were indicators of fecal streptococci.

Surface method was used for heterotrophic bacteria (14) and the entire surface of plate with nutrient medium was covered by 0.1 ml of water sample. Then, the plates were placed at room temperature and were incubated at 0.5 ± 35 °C for 48 h. At last, all colonies were counted and multiplied by 10 to obtain CFU/ml.

Statistical analysis

In order to determine the significance relationships between variables Chi-square and Wilcoxon test were used and *P*-values less than 0.05 were considered statistically significant. All the available data were analyzed by a computer program (SPSS 18) (Chicago, IL, USA).

Results

Table 1 shows biological characteristics of water supply network samples in Aq Qala rural and urban areas. In this table, the average number of total coliform, fecal coliform, fecal streptococci, and heterotrophic bacteria in the water distribution networks in rural areas were 9.6, 0.76, 5.57 CFU/100 ml and 1042 CFU/ml, respectively. For urban areas, these parameters were 4.56, 0, 0.125 CFU/100 ml and 688 CFU/ml, respectively. Our finding demonstrated that with increase of heterotrophic bacteria in rural area, the other microorganisms were increased remarkably.

Table 1: Biological characteristics of water supply network samples in Aq Qala rural and urban areas, Golestan Province in 2013

Area	Heterotrophic bacteria /ml	Fecal streptococci MPN/100ml	Fecal Coliform MPN/100ml	total coliform MPN/100ml
Rural Areas	5000^{max}	313^{max}	19^{max}	126^{max}
	0^{min}	0^{min}	0^{min}	0^{min}
	1042^{ave}	5.57^{ave}	0.76^{ave}	9.6^{ave}
Urban areas	2045^{max}	0.57^{max}	0^{max}	31^{max}
	9^{min}	0^{min}	0	0^{min}
	688^{ave}	0.125^{ave}	0^{ave}	4.56^{ave}

In Table 2 the frequency of microorganisms in rural and urban areas of Aq Qala is shown. The results illustrated the comparison between rural and urban contamination. In rural areas incidence of all microorganism contamination were higher than urban areas.

Table 2: Frequency of all contaminations of rural and urban areas of Aq Qala, Golestan Province in 2013

Microorganisms	Rural areas		Urban areas	
	percent	number	percent	number
Coliform contamination	15	49	12.5	4
Fecal coliform contamination	4.6	15	0	0
Fecal streptococci contamination	8.9	29	3.1	1
Heterotrophic Bacteria ≥500 CFU/1ml	33	108	18.75	6
Total	100	324	100	32

Table 3 shows the biological characteristics of samples from the beginning and the end parts of water supply networks in Aq Qala rural and urban areas. Total coliforms, fecal coliform, fecal streptococci, and heterotrophic bacteria in the end points samples were more than the samples in beginning part. Table 4 shows all contaminations in samples with heterotrophic bacteria ≤ 500 and with > 500 CFU/ml. The results showed that nearly, in 114 samples from all samples, the heterotrophic bacteria counts were over 500 CFU/ml, which fecal coliform, coliform, and fecal streptococci were 8, 32, and 20 CFU/100 ml, respectively.

Table 3: Biological characteristics of samples from the beginning and the end parts of water supply networks in Aq Qala rural and urban areas, Golestan Province in 2013

Water supply networks	Heterotrophic bacteria CFU/ml	MPN/100ml		
		Fecal Streptococci	**Fecal Coliform**	**Total Coliform**
Beginning part	868	2.94	0.66	7.13
End part	1156	7.22	0.73	11.2
Total	1012	5.09	0.7	9.17

However, in 242 samples, the heterotrophic bacteria counts were less than 500 CFU/ml, and the fecal coliform, coliform, and fecal streptococci rates were 7, 23, and 11 CFU/100 ml, respectively. In the samples with high concentration of heterotrophic bacteria, the other types of bacteria also observed in high concentration.

Table 5 represents the correlation between heterotrophic bacteria with other variables in water supply networks. There is significant correlation between coliform and fecal streptococci bacteria with heterotrophic bacteria and there is low correlation between fecal coliform and heterotrophic bacteria.

Table 4: All contaminations in samples with heterotrophic bacteria ≤500 and < 500 CFU/ml

Heterotrophic bacteria < 500 CFU/ml	Heterotrophic bacteria ≥ 500CFU/ml
Fecal coliform (7) Coliform (23) Fecal streptococci (11)	fecal coliform (8) coliform (32) fecal streptococci (20)

Table 5: Correlation between heterotrophic bacteria with other variables in water supply networks

	Variables	Beginning point of Network ($P \leq 0.05$)	End point of Network ($P \leq 0.05$)	P
Heterotrophic bacteria	Total coliform	0.001	0.001	0.000
	Fecal Coliform	0.1	0.36	0.073
	Fecal streptococci	0.022	0.000	0.000

Discussion

Drinking water systems are the known locations for biofilms growing area, even if the environment is oligotrophic and contain disinfectants. Cellular activity is dependent on the carbon source. Humic substances are one of the carbon sources used for biofilm (15). Biofilms are mixed of microorganisms such as algae, yeasts, protozoa, bacteria. The bacterial growth in the network systems has been observed in the water without residual chlorine at 25 °C, where the system has received many biodegradable materials (16). Often coliform bacteria can be detached from biofilm and contaminate the water (17). Biofilms contain 34.9% carbon, 5.4% hydrogen and 5.4% nitrogen (18) that protects these bacteria against antimicrobial agents (11).

If biofilm grows on surface of ferrous pipes, it causes iron corrosion and releases iron particles in water (19). Shortage of nutrients in the pipe water can cause migration of bacteria toward biofilm that contain organic and inorganic substances. Therefore, biofilms are an ideal habitant for the survival and growth (20). Attached bacteria have a metabolic activity than free bacteria (21). Biofilm density is between 10 and 1.9 $*10^9$ bacteria /cm^2 and contains many bacteria such as *Pseudomonas*, *Klebsiella* and *Enterobacter* without any *salmonella* and *Shigella*. Pathogens that survived in the water treatment can grow and produce biofilm colonies that can be released into water flow. The larger water network systems have fewer pathogens while smaller water network_systems have a great numbers of gram-negative pathogens (22).

These results suggest that the heterotrophic bacteria in high concentrations in water do not have any effect on determining the fecal coliform and pathogens in the MPN method and do not suppress any coliforms, fecal coliforms and fecal streptococci in the Aq Qala City water supply network. We suppose it is due to high concentrations of organic carbon and nutrients in these systems.
Presence of coliforms in clean drinking water without any cracks in water treatment is considered as a major problem in the water industry. Coliforms in the distribution system may be proliferate and multiply in presence of organic carbon. Other nutrients such as nitrogen and phosphorus are necessary to support this proliferation. Carbon concentrations may be ranging from 0.05 to 12.2 mg/l in water supply (23). Organic and inorganic compounds in biofilms may provide more opportunities for bacteria to grow and release them to the liquid phase (24). In The distribution system, *K. pneumonia* in the presence of very low concentrations of organic carbon (0-0.1 mg/l), has a maximum specific growth in comparison with the other fecal coliforms. Organic carbon more than 5.44 mg/l in water allows growing of the *E. coli* bacteria. In presence of biofilm, growth of *K. pneumonia* has increased to 1 log higher. Coliforms are able to grow in low levels of organic matters in the water distribution system and water treatment facility at 4.8 to 21.9 °C. ETEC (One type of fecal coliforms) which can grow in low temperature and nutrition condition (18). Soluble carbon concentration between 10 to 50 μg/l, induces a significant growth of bacteria in the water network system (25). Even, concentration less than 3 μg/l can uptakes microorganisms (26).
Environmental conditions such as temperature above 15°C, neutral pH and assimilable organic carbon (AOC) are ideal condition for coliforms to colonize (9). Regrowth of coliform bacteria is related to some factors: filtration rate, temperature, disinfectant type, assimilable organic carbon, corrosion control and operational characteristics. Failure to control of corrosion and annual flushing program may lead to appearance of coliform bacteria in the network system. In water systems with free chlorine, increase in temperature from 5° C to over 20 °C, coliforms increase 18 times (27). Heterotrophic bacteria (*P. maltophilia*) in concentrations of 10^4 to 10^5 ml will cause decrease in growth of coliforms (0.5 log) (28).
Fecal coliform in the end point of water supply system partly increased than the beginning point. Despite environmental contaminants, fecal coliform has a slight increase, which may be due to higher sensitivity to the residual chlorine. Except Enterotoxigenic *E. coli*, other *E. coli* species are not able to regrow in the water network. In some cases, this bacterium attaches to the biofilm and multiply in the water distribution system (29). *E. coli* can be colonized in the absence of free residual chlorine in drinking water distribution systems at 20 °C. However, colonization of *E. coli* was temporary (30). Fecal coliform is very sensitive to disinfectants and their presence may be considered as a major deficiency in the water supply network systems and indicating the recent fecal contamination (26).
In the distribution system with $7*10^5$ CFU/ml of heterotrophic bacteria, there is no interfere in the AC test (the presence and absence of coliforms) (31). Unexpectedly, the number of fecal coliform isolated from the GAC water supply network is less than in nano filter network due to protozoan activity of bacteriophage (32). When biofilm is exposed to monochloramine or hypochlorous acid, fecal coliform can survive for at least 10 days, even at high levels of disinfectants (33). However, general agreement exists that in common water temperature and nutrients, the number of fecal coliform or pathogenic bacteria do not increase (34).
In the end points of water distribution network, fecal streptococcus observed more than fecal coliform due to the high resistance of these microorganisms to the environmental conditions and the free residual chlorine. Some samples with fecal streptococci and without fecal coliforms, confirm that this contamination is related to fecal. Fecal streptococci survival is longer in winter than other seasons in the water distribution network (35) and can tolerate moderately alkaline pH conditions and high sodium chloride (36).

The number of heterotrophic bacteria at the end points of water distribution network is greater than the beginning point. At present, eradication of biofilms in the distribution system is not possible, but it is necessary to control and minimize. Quality control of inlet water, including taking care of distribution system may be a good way to prevent the risk of infection. In addition, to avoid any contamination in the water supply, it is recommended to observe permanently the mechanisms that facilitate entering the pathogens to the distribution network, such as poor maintenance activities, long time water retaining and the availability of nutrients (33).

Conclusions

There was no interaction between heterotrophic bacteria with other microorganisms in the Aq Qala City water distribution network, except fecal coliform bacteria, which may occur because of the high sensitivity of fecal coliform. This interaction could not be confirmed due to fecal coliforms existence in water supply distribution network in the short time.

We suppose that high concentrations of organic carbon in water provide the necessary nutrient for all microorganisms in these water network systems. The average number of coliform bacteria, fecal coliforms, fecal streptococci and heterotrophic bacteria in the end point of water network systems is more than the beginning part. Thus, except the fecal coliforms at the end of the network with partial increasing, all of the microorganisms show significant increase, which may indicate a secondary growth of coliform and heterotrophic microorganisms in the biofilm. Biofilm serve as a protective layer for the other microorganisms such as fecal streptococci and fecal coliforms.

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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