

Original Article

Investigation of microbial contamination frequency in drinking water of buses at Sofeh terminal of Isfahan-Iran

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ABSTRACT

Aims: This study aimed to assess the microbial contamination status of drinking water supply among buses at the main bus terminal in Isfahan.

Materials and Methods: About 184 drinking water samples were taken randomly from buses arriving at the Sofeh terminal in Isfahan in 2011. For data analysis of one-sample, Kolmogorov-Smirnov test ($\alpha = 0.05$) and Mann-Whitney U-test with a significant difference ($\alpha = 0.05$) was used for the effect of setting insulated water tank in cities bus to reduce microbial contamination.

Results: Results showed that 15% of all samples were contaminated with total coliforms and 2.5% of samples contaminated with fecal coliforms. The most contaminated samples were related to buses arriving from Shiraz and Booshehr, (fecal MPN~4). About 16.66% of all samples collected from buses without insulated water tanks were contaminated with total coliforms and fecal coliforms.

Conclusion: Results indicated that the drinking water available on investigated public transportation was not safe for public health. However, the highest levels of microbial contamination were observed in samples taken from buses without insulated water tanks. In conclusion, a constant and systematic monitoring system is recommended to prevent contamination of drinking water available on buses.

Key words: Bus, drinking water, insulated water tank, Isfahan, microbial contamination

INTRODUCTION

Promoting community health without access to safe drinking water is not possible. Developed countries have solved a significant percentage of health problems, with recognition of this fact.^[1,2] The paramount importance of waterborne diseases has long been recognized, so the emphasis is on drinking water devoid of pathogenic microorganisms.^[1,3] The most common way of contaminating drinking water is through human or animal feces. For this reason, with regard to evaluating water safety, the coliform groups of

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bacteria are used as indicators of fecal contamination. Total coliforms and *Escherichia coli*, a member of the *Enterobacteriaceae* family, are a good indicator of probable fecal contamination in drinking water.^[3-6] According to the World Health Organization (WHO), treated and disinfected water must be devoid of any fecal coli-forms.^[7] Otherwise, the consumption of such water for drinking is undesirable and not recommended. According to WHO, about 1.7 million mainly children deaths a year worldwide, because of diarrheal diseases. In fact, half of these cases is attributed to contaminated drinking water.^[8] The need for water during travel is a priority for millions of people who travel around the world for personal and public purposes each year. Studies show that 50% of travelers suffer from diarrhea over several weeks, with bacteria (*E. coli* [enterotoxin producer]) being considered as the most common cause of this disease. Other factors such as viruses and parasites are also additional causes of this disease, with half of the cases suffering from mild diarrhea, and a quarter of them needing hospitalization.^[5,9] One of the issues that constantly threaten the health of the passenger is drinking water in public vehicles like buses, which becomes a more serious risk during the summer months.^[5] In the absence of available and regular air and rail transport systems in Iran, most of the traveling takes place by bus. Obviously, passengers are using drinking water available on these buses. Although, the drinking water provided is good for the welfare of passengers, but it could present a serious risk of spreading diseases if contaminated.

On most occasions, it has been observed that the bus services use inappropriate containers, un-healthy ice and drinking water for serving passengers. The results of similar studies on drinking water samples used in the buses of Kerman showed that 45% of such samples were contaminated with coliforms and 35% were contaminated with fecal coliform, also 73.75% of samples were undesirable due to free chlorine residues.^[10] Investigations carried out by Voojodi *et al.* showed that 23.3%, 37.8% and 90% of drinking water samples were contaminated with fecal coliform, total coliform and heterotroph bacteria in the water reservoir of the bus terminal in Mashhad, respectively, which were more than the Institute of Standard and Industrial Research of Iran level. Although, free chlorine residues found in 80% of the samples were lower than the standard rate.^[11] Furthermore, the results of a study by Vakilabadi *et al.* (2012) on the drinking water samples of buses in Booshehr indicated that 12.5% and 8.8% of samples were contaminated with the coliform and *E. coli*, respectively.^[12] However, providing safe drinking water is the most important health aspects during traveling. This study aimed to assess the microbial contamination status of drinking water supply among buses at the main bus terminal in Isfahan.

MATERIALS AND METHODS

This research was a cross-sectional study in which drinking water samples from buses entering the Sofeh terminal were examined. 184 samples were collected randomly from buses arriving at terminal in the morning and the afternoon time. Samples were taken directly from insulated water tanks after allowing the water to run for a while (at least 10 min). Water samples were collected in sterilized bottles and were subsequently tested for free chlorine residues. If there were only water reservoirs such as pitchers or barrels on the bus, the samples were then collected from those reservoirs according to the sampling procedure. The collected samples were transferred to the Microbiology Laboratory of the Public Health School at the Isfahan University of Medical Sciences. It should be noted that sampling and the subsequent culturing of the samples were carried out carefully to minimize any secondary contamination. The samples were analyzed using the multiple tube fermentation procedure according to the standard method.^[6] At first arrange the fermentation tubes in rows of three tubes each in a test tube rack. Inoculate each tube containing Lauryl Tryptose Broth medium (Mreck, Germany) with replicate sample valoums. Incubate inoculated tubes at $35 \pm 0.5^\circ\text{C}$ and examined incubated tubes at the end of $24 \pm -2-48 \pm 3$ h for production of gas or acidic growth in the tubes. The tubes were submitted with a positive presumptive reaction to the confirmed phase. The Brilliant Green Lactose Bile Broth for total coliforms and EC Broth for fecal coliforms confirm was used. The samples culture in a fermentation tube contains Brilliant Green Lactose Bile Broth. Then incubate the inoculated Brilliant Green Lactose Bile Broth tube for 48 ± 3 h at $35 \pm 0.5^\circ\text{C}$, formation of gas at any time within 48 ± 3 h constitutes a positive confirmed phase. Simultaneously, culture to a fermentation tube containing EC Broth elevated temperature (44.5°C) results as a positive completed test response.^[6]

Statistical analysis

For data distribution was used one-sample Kolmogorov-Smirnov test with a significant difference ($\alpha = 0.05$). For reveals the effect of setting insulated water tank in bus of cities in term of reducing microbial contamination was used the Mann-Whitney U-test with a significant difference ($\alpha = 0.05$). Also for determining the relationship of distance between Isfahan and other cities and also frequency of undesirable samples (due to the presence of coliform) using the Pearson correlation coefficient with the significance level ($\alpha = 0.05$).

In the present study according to the lack of insulated water tank risk in buses in term of present coliform in drinking water, the odds ratio (OR) was calculated using the following equation.

$$OR = \frac{\frac{21}{79}}{\frac{7}{77}} = 2.92$$

Where,

| | Samples with coliform | Samples without coliform |
|------------------------------|-----------------------|--------------------------|
| Without insulated water tank | 21 | 79 |
| With insulated water tank | 7 | 77 |

Therefore, the lack of the insulated water tank in buses could be a risk factor for prevalence water-borne-disease among passengers regarding $OR > 1$.

RESULTS

Results of microbial analyzes are depicted in Tables 1 and 2.

The results showed that 15.21% of the samples were contaminated with total coliforms, and 2.71% of them were contaminated with fecal coliforms. Although the highest level of total coliforms (36 MPN/100^{cc}) was from samples taken from buses without insulated water tank that originated from Shah-Reza city. The highest level of fecal coliforms (15 MPN/100^{cc}) was related to buses from Bushehr that were also without insulated water tanks. In fact, 16.66% and 14.27% of all samples belonging to buses without insulated water tanks were contaminated with total coliforms and fecal coliforms, respectively.

It should be noted that 49.24% of the buses were first class with appropriate water insulated water tanks, and the remaining 50.76% were ordinary buses without insulated water tanks or suitable containers for storing drinking water or ice. Furthermore, all samples collected from all of the buses lacked free chlorine residuals. However, the obtained results indicated that the frequency of contaminated samples with abnormal distribution ($P < 0.001$) and average frequency of contaminated samples with total coliform between buses with insulated water tank and without insulated water tank showed significant difference ($P = 0.048$) [Figure 1]. Hence that the average of samples with insulated water tank and without insulated water tank were 19.6% and 7.9%,

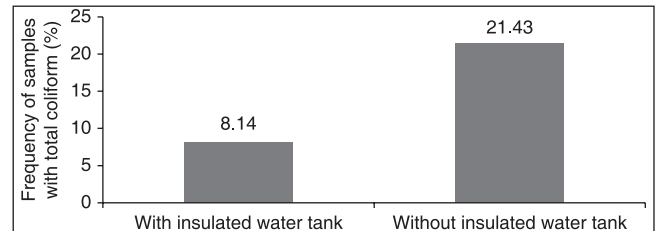


Figure 1: Percentage of samples frequency with total coliform

Table 1: Relative frequency of samples based on the total coliforms (MPN/100cc)

| Total coliforms % (number/ml) | Samples % |
|-------------------------------|-----------|
| 0 | 85 |
| 10 < | 12.5 |
| 11-50 | 2.5 |

Table 2: The results of microbial tests on drinking water samples from buses based on the origin of the city (MPN/100cc)

| City's bus of sampling | Distance between cities and Isfahan | Type of bus | | Samples without coliform (MPN = 0) | | Samples with coliform | | Samples with thermophilic coli forms | | Total samples |
|------------------------|-------------------------------------|---------------------------|------------------------------|------------------------------------|------------------------------|---------------------------|------------------------------|--------------------------------------|------------------------------|---------------|
| | | With insulated water tank | Without insulated water tank | With insulated water tank | Without insulated water tank | With insulated water tank | Without insulated water tank | With insulated water tank | Without insulated water tank | |
| Tehran | 429 | 8 | 2 | 8 | 2 | 0 | 0 | 0 | 0 | 10 |
| Ahvaz | 745 | 5 | 5 | 4 | 2 | 1 | 3 | 0 | 0 | 10 |
| Mahshahr | 450 | 5 | 7 | 7 | 5 | 0 | 0 | 0 | 0 | 12 |
| Yasooj | 299 | 4 | 5 | 5 | 4 | 0 | 0 | 0 | 0 | 9 |
| Bandarabas | 975 | 2 | 8 | 8 | 2 | 0 | 0 | 0 | 0 | 10 |
| Bushehr | 580 | 5 | 6 | 2 | 5 | 0 | 4 | 4 | 0 | 11 |
| Babolsar | 688 | 7 | 6 | 6 | 7 | 0 | 0 | 0 | 0 | 13 |
| Samiroom | 164 | 4 | 7 | 4 | 3 | 1 | 3 | 0 | 0 | 11 |
| Shiraz | 485 | 7 | 6 | 3 | 6 | 1 | 3 | 1 | 0 | 13 |
| Shah-Reza | 150 | 6 | 6 | 2 | 4 | 2 | 4 | 0 | 0 | 12 |
| Khoramshahr | 870 | 3 | 6 | 6 | 3 | 0 | 0 | 0 | 0 | 9 |
| Brojen | 143 | 3 | 5 | 5 | 3 | 0 | 0 | 0 | 0 | 8 |
| Kerman | 661 | 5 | 6 | 6 | 5 | 0 | 0 | 0 | 0 | 11 |
| Mobarakeh | 74 | 6 | 2 | 2 | 6 | 0 | 0 | 0 | 0 | 8 |
| Mmajlesi | 50 | 4 | 5 | 5 | 4 | 0 | 0 | 0 | 0 | 9 |
| Aasalevyeh | 904 | 5 | 5 | 5 | 5 | 0 | 0 | 0 | 0 | 10 |
| Zarinshahr | 189 | 3 | 5 | 5 | 3 | 0 | 0 | 0 | 0 | 8 |
| Omidyeh | 467 | 4 | 6 | 2 | 2 | 2 | 4 | 0 | 0 | 10 |
| Total | — | 82 | 102 | 77 | 79 | 7 | 21 | 0 | 5 | 184 |

respectively. Results showed that the relationship of distance between other cities and Isfahan also frequency of undesirable samples (due to presence of coliform) was significant due to presence of coliform ($P = 0.0378$), although this relationship was relatively poor about 0.21, which is closer to 0.0 of Pearson correlation coefficient (0-1).

DISCUSSION

Results obtained indicated that the contaminated samples belonged to the buses that did not have insulated water tanks. In fact, proper sanitation, storage of drinking water, and the use of suitable and clean insulated water tanks prevented contamination of water samples.^[13] The results obtained from the analysis of samples from the buses originating from both Bushehr and Shiraz were similar with regard to fecal coliforms, which were at the highest level of contamination when compared to buses from other cities. This was probably due to the presence of poor health facilities, using contaminated ice and polluted drinking water source and also a long distance between Shiraz-Isfahan (485 km) and Bushehr-Isfahan (580 km). The study, which carried out by Malakootian *et al.* (2008) showed that more polluted water samples are belonged to the buses that have long distances between origin and destination. It could be due to utilizing unsanitary ice and unhealthy water on the way.^[10] Whereas, the study of Dehghani *et al.* indicated that there was not a significant relationship between long distance of origin and destination and also drinking water polluted in the buses in spite of water sample contamination in these buses. It should be noted that the obtained results showed that to prevent drinking water contamination in the buses, don't use of contaminated ice, promotion the personal hygiene of the host, use of safe water sources and proper containers, daily and regular washing of containers especially the use of safe mineral water bottle is thus required. Furthermore, improved the knowledge of staff, regular chlorination and favorable relationship between bus terminal management and the health centers can be very effective.^[14]

Results showed that all the samples were lack of free chlorine residual, given that free chlorine residual known as a secondary safety factor in controlling microbial contamination of water, Therefore the absence of this factor could be another reason of the occurrence microbial contamination in drinking water.^[12] It could be stated that to obtain favorable effect of free chlorine residual in drinking water the physicochemical factors such as pH, temperature and turbidity should be controlled.^[15] In addition, other options that cause secondary drinking water contamination on buses are also included. These include the use of unhealthy water containers and barrels for storage of drinking water, lack of regular, proper cleaning and disinfection of containers or insulated water tanks, and type of containers ingredient. The study that conducted by Vakilabady *et al.* showed the samples collected from containers which made of metal ingredient have more fecal and total contamination than fiberglass type.^[12] Also,

Manshouri *et al.* indicated plastic containers can cause a decrease of water quality.^[16] Other factors such as entered the delivering container into the water reservoir and touched the bottom of the container with water of storage tanks, prepared unsafe water and ice from unsuitable places along the way.^[14] All of the aforementioned factors are linked to the growth of pathogens in water reservoirs and the eventual transmission of waterborne diseases. Other reasons, such as the lack of personal hygiene of the crew, location of the water supply were also effective in contaminating the drinking water on buses.^[17] Hence, it could be that a variety of infections observed in various studies might be due to each of those factors mentioned above. Similar studies have been carried out in the central provinces, Kermanshah, Bandar Abbas, Kerman, Mashhad and Boushehr, which have confirmed the contamination of drinking water in public transportation vehicles commuting between cities.^[11-13]

CONCLUSION

Considering the MPN count more than 10 coliform 100 ml and the presence of *E. coli*, the typical coliform indicates that the samples were not safe for drinking purposes due to fecal contamination.^[18] Therefore, the health authorities are obliged to pay more precise attention to the water distributors and monitoring health and safety conditions of water containers on the buses, so as to prevent the transmission of diseases to susceptible passengers. Furthermore, due to increased pollution in the water supply at the terminal, it is necessary to chlorinate the tanks manually as supervised by health authorities. Therefore, it is proposed that the cabin crew on buses must be granted health cards, and must be periodically replaced, and the cards must be examined routinely by traffic officers. In addition, using chlorine test kits, and associated measuring devices, the bus authorities must be trained to test chlorine residual in drinking water prior to the use of buses by the public. Therefore, it mandatory that all public vehicles equipped with cool water reservoirs must be washed and disinfected once a week using appropriate disinfectants such as chlorine at a concentration of 50 ppm.^[19]

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