ORIGINAL PAPER

Analysis of the microbial quality in drinking water distribution networks using the logistic regression model in Dasht-e Azadegan county, an arid region in the southwest of Iran

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ABSTRACT

The microbial quality of water plays a key role in community health. The present study aimed to determine the microbial quality of the drinking water distribution networks in the urban and rural areas of Dasht-e Azadegan County, Iran and assess the influential factors in the quality of drinking water. In this descriptive-analytical study, 907 drinking water samples were collected from the urban and rural regions in Dasht-e Azadegan County in 2017. Turbidity, free residual chlorine, pH, total coliforms, and fecal coliforms were measured using the Standard Methods for the Examination of Water and Wastewater, and the results were analyzed using the logistic regression model. The free residual chlorine was within the range of 0-3 mg/L (mean: 0.72 mg/L). The free chlorine residual in 58% of the samples was within the recommended range of the World Health Organization (WHO) for drinking water, and 29% of the samples had higher turbidity than the accepted limit of the WHO (5 NTU). In addition, 7.7 and 16% of the samples were infected with fecal and total coliforms, respectively. According to the results of the logistic regression analysis, coliform contamination was most significantly associated with free chlorine residual and turbidity, and reduced free chlorine residual was most effective in coliform contamination.

Keywords: Dasht-e Azadegan, Drinking water, Logistic regression, Microbial quality

Introduction

The microbial contamination of drinking water leads to numerous health issues, such as cholera and typhoid fever, which arise from the consumption of contaminated drinking water with germs.[1] Therefore, microbial quality is considered to be the foremost quality and health index of drinking water.[2] The microbial quality of drinking water largely concerns consumers, suppliers, legislators, and healthmanagers.[3] Some of the main pathogenic microorganisms that affect the microbial quality of water include bacteria, viruses, protozoa, and parasitic worms,[2] some of which cause diseases in humans, leading to irreparable health complications.[4] Evidently, numerous bacteria, viruses, and parasites could easily enter water supply networks, be transferred to the intestines, and give rise to acute diseases such as dysentery, typhoid, hepatitis, and chlorosis.[5]

According to reports, 500 waterborne pathogens could be found in drinking water sources, which have been detected by the US Environmental Protection Agency (EPA). In addition, studies in India have indicated that after the promotion of water distribution networks, the mortality rate of infectious diseases in the community has decreased significantly; for instance, the prevalence rate of

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cholera, diarrhea, typhoid, and deaths due to dysentery have reduced by 74.1, 42.7, 63.6, and 23.1%, respectively. Further research suggests that in developing countries, 80% of all diseases and 23% of deaths are due to the poor quality or contaminated water.[6]

Fecal and total coliforms are the most common indicator of the microbial contamination of drinking water. The identification of bacterial indicators in drinking water could show the presence of pathogenic microbes, which are the source of waterborne diseases.[7] Before water enters the distribution network, the quality of water and its standards are controlled through continuous monitoring, while after entering the distribution network, more protection is required due to vastness of the network. If a fracture occurs in a part of the network, it may lead to the entry or penetration of contaminants, including pathogenic pathogens.[7, 8] Therefore, the protection of water distribution networks is an essential measure, which is only possible through the continuous monitoring and measurement of the microbial quality parameters.

In North America and Europe, some disinfectants are preserved as persistent residues in drinking water to provide the microbial quality of drinking water and prevent pollution and microbial growth in water distribution networks.[9] Chlorine is a disinfectant that is most commonly used for such purposes, which is considered to be the most important protective barrier against contamination and microorganisms. In many countries, chlorine is added to water after water treatment before entering the distribution network. Its relative cost-effectiveness, simplicity of use, and high residual rate in water have resulted in the frequent application of free chlorine residual as a common indicator of water quality in drinking water distribution networks.[10]

Turbidity in water is another safe environment for the growth of microorganisms, which reduces the disinfection efficiency of chlorine and survival of microorganisms in water.[11] The evaluation of the mentioned factors in water distribution networks could properly assess the quality and health of drinking water.

Statistical techniques have the ability to evaluate water quality. In a study in this regard, the regression model was used to assess the influential factors in water quality, and the obtained results indicated that land use (residential) had the most significant effect on water quality.[12] In another research using the logistic regression model, the chemical quality of water was observed to depend on the aquifer type and season. However, few studies have used statistical techniques to evaluate the microbial quality of drinking water in drinking water distribution network.[13]

The present study aimed to investigate the microbial quality of drinking water in the urban and rural distribution networks of Dasht-e Azadegan County, located in Khuzestan province, Iran using the logistic regression model. Logistic regression provides the appropriate regression analysis when the dependent variable is dichotomous (binary). To date, no studies have been focused on the microbial quality of drinking water in Dasht-e Azadegan County using this method. We also evaluated the effects of pH, turbidity, and residual chlorine on microbial water quality using the logistic regression model. To accurately assess the turbidity parameter, free residual chlorine and pH were examined, along with total and fecal coliforms.

Materials and Methods

Study area

Dasht-e Azadegan County is one of the border counties in Khuzestan province, which consists of four towns, including Susangerd, Bostan, Abu-Homeyzeh, and Kut with the population of 92,000, as well as 66 villages with the population of 36,000. The sources of drinking water in these cities are Nissan and Karkhehnoor rivers.

Data collection

In this analytical, cross-sectional study, the water distribution networks of the mentioned cities and villages were evaluated, and the fixed points at the beginning and end of the distribution networks were selected to compare the effects of the length of the distribution network and pipelines. In addition, the points with the highest probability of contamination and lack of disinfectants were selected based on the sampling sites in the urban and rural networks. Sampling was performed during the first four days of the week, and four samples were harvested per day on average in 2017.

In total, 907 samples were collected from the locations in the urban and rural networks of Dasht-e Azadegan County. Sampling was carried out monthly during each season of the 2017 calendar. The number of the collected samples was determined based on the standard number 4208 of the Institute of Standards and Industrial Research of Iran (ISIRI). The samples were harvested in 250-milliliter sterile bottles with a smooth lid and containing sodium thiosulfate and transferred to the laboratory in hygienic conditions. Total coliforms, fecal coliforms, turbidity, free residual chlorine, and pH were measured. The samples were measured based on water and sewage testing as mentioned in the book 'The Standard Method', and the free residual chlorine was measured using diethyl-p phenylenediamine pills and a digital colorimetric kit. In addition, pH was measured using Aquatic mark pH meter at the sampling site.

To investigate the presence of total and fecal coliforms, the most probable number (MPN) test was applied in three phases of probabilistic, support, and supplementary with various objectives. In the probabilistic assay (phase one), the samples were cultured in lactose broth medium at the temperature of 37 °C for 48 h. Gas production in the samples was an indication of the positive probability of microbial contamination, and the positive samples were incubated immediately and directly on a culture medium (brilliant green lactose bile) at the temperature of 37 °C for 24-48 h. At the end of the cultivation period, gas production in the samples was an indication of the confirmed positive test.[14]

The Thomas's relationship was applied to calculate the probable number (100 mL) of the coliform and thermotolerant coliforms. In order to identify and count the fecal coliforms (*E. coli*), EC broth culture media were used, which were incubated at the temperature of 44.4 °C after inoculation for 24-48 h. The presence of gas after incubation in the EC broth was a positive marker of the test, confirming the presence of fecal coliforms. Following that, the MPN levels of fecal coliforms were calculated as well.

Statistical analysis

Data analysis was performed in SPSS version 22 using one-way analysis of variance (ANOVA) to compare the mean chlorine concentration and turbidity in four seasons. Tukey's post-hoc test was also used for the pair comparison of the mean values, and t-test was applied to compare the mean chlorine concentration and turbidity. Moreover, y-test and Chi-square were employed to determine the correlations between the variables and presence of coliforms in the water. Finally, the logistic regression mode was applied to determine the quality of water in the distribution networks, as well as the effect of each variable on the microbial quality of water. The variables with the significant level of less than 0.2 were analyzed using the model with the odds ratio and confidence interval considered as the basis of the statistical analysis.

Results and Discussion

Table 1 shows the descriptive and comparative statistics of residual chlorine, pH, and turbidity in the study are based on the months of the year.

Table 1. Descriptive statistics and comparison of chlorine, pH, and turbidity in 12 months of 2017 in drinking water of Dasht-e Azadegan County

Month	No.	Free chlorine residual		pH		Turbidity	
		Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
April	55	0.6±0.5	0-2	7.5±0.2		3±1.06	1-5
May	84	0.53±0.69	0-1.5	7.6±0.1	7.5-7.8	3.6±1.9	1-4.2
June	80	0.675±0.52	0-2	7.6±0.1	7.4-7.6	3±1.4	1-3.7
July	84	0.45±0.6	0-2.5	7.5±0.2	7.4-7.8	3.9±1.2	1-4.5
August	86	0.49±0.66	0-3	7.6±0.2	7.5-7.8	3.36±1.15	2-6
September	64	0.51±0.5	0-1.8	7.5±0.2	7.4-7.7	3.31±1.	1-2
October	72	0.92±0.71	0-3	7.5±0.1	7.4-7.6	3.26±0.86	2-5
November	92	0.73±0.65	0-2	7.5±0.2	7.2-7.6	3.46±1.3	1-5
December	81	0.88±0.64	0-2	7.6±0.1	7.4-7.6	3.77±1.16	1-6
January	61	0.84±0.8	0-3	7.6±0.1	7.4-7.6	3.5±1.88	1-6
February	75	1.02±0.76	0-3	7.5±0.2	7.4-7.7	3.5±2.13	1-5
March	67	1.13±0.79	0-2	7.5±0.1	7.4-7.6	3.06±1.53	1-4

Table 2 shows the mean pH, free residual chlorine, and turbidity in various cities. Accordingly, the mean free residual chlorine and pH varied widely in different cities ($P<0.05$), and the highest mean values of free residual chlorine and turbidity were observed in Kut and Bostan cities, respectively.

Table 2. Descriptive statistics on residual chlorine and turbidity in various cities

Variable	City	N	Mean	Std. deviation	P value
Free residual chlorine	Sosangerd	736	0.712	0.65	<0.001
	Bostan	86	0.96	0.82	
	Abohamize	25	0.2	0.43	
	Kut	47	0.8	0.72	
	Total	894	0.72	0.68	
Turbidity	Sosangerd	740	5.4595	3.04761	0.155
	Bostan	86	5.5465	2.90094	
	Abohamize	25	6.7	4.07349	
	Kut	47	6.4	3.01599	
	Total	898	5.4599	3.06835	
Total coliform	Sosangerd	736	3.65	7.88	<0.001
	Bostan	86	6.98	24.7	
	Abohamize	25	4.32	17.7	
	Kut	47	32.36	48.22	
	Total	894	7.27	24.86	
pH	Total	9.7	7.5	0.15	<0.001

Table 3. Comparison of parameters with 1053 standard

Sampling location	Free chlorine residual (mg/L)		Turbidity (NTU)		pH		No. of con. samples	
	Mean ±SD	Rang	Mean ±SD	Rang	Mean ±SD	Rang	Total coliforms	Fecal coliforms
Urban	0.53±0.5	0-2	3.32±3.1	1-4	7.6±0.1	7.5-7.8	40 (14.5%)	17 (6.2%)
Rural	0.82±0.7	0-3	3.96±2.9	1-6	7.5±0.1	7.5-7.8	106 (17.1%)	53 (8.5%)
1053 ISIRI Standard		0.2-0.8		≥1		6.5-8.5		

Table 3 shows the descriptive statistics and comparison of chlorine, pH, and turbidity of drinking water in the urban and rural areas of Dasht-e Azadegan County.

According to the obtained results, the mean pH and free residual chlorine were 7.5±0.1-7.6±0.1 and 0-3 mg/L, respectively. According to the World Health Organization (WHO), the recommended limits of pH and free residual chlorine in drinking water are 6.5-8.5 and 0.2-1.5

mg/L, respectively. In total, the free residual chlorine in 58% of the samples (530/907) was within the allowed range by the WHO for drinking water, with the lowest seasonal mean observed in warm seasons ($P<0.001$) (Table 1). Moreover, the free chlorine residual was within the range of 0.3-0.8 mg/L, which could decrease the growth of total coliforms in drinking water as these bacteria are highly sensitive to free residual chlorine.[14]

According to the findings, the free residual chlorine content was significantly higher in the rural area compared to the urban area. Furthermore, the drinking water samples with higher free residual chlorine concentrations had lower contamination rates with coliforms (0.2 mg/L of free residual chlorine or 0.5 mg/L of chloramine).[10] However, the excessive release of residual chlorine may lead to the formation of disinfection by-products in the water distribution networks.[15] However, lack of expertise and experienced operators and no monitoring of their performance compared to urban areas, as well as the use of manual chlorination by calcium hypochlorite powder could be another reason for the high concentration of free residual chlorine in the rural areas compared to the urban areas.

In a study in this regard, Lu and Zhang evaluated the influential factors in bacterial growth in distribution network, reporting that when the free residual chlorine content was less than 0.5-0.7 mg/L, the risk of microorganisms and their growth in the network was higher. In addition, the increased concentration of free residual chlorine led to the reduction of bacterial growth.[15] In the mentioned research, approximately 29% of the samples (262/907) had higher turbidity than the allowed limit of the WHO (5NTU). Turbidity could be used as an indicator to identify incoming contaminants and the hydraulic issues in drinking water distribution systems. The increased turbidity of drinking water distribution networks could protect microorganisms against disinfectants to promote the growth and multiplication of pathogens, while reducing the effectiveness of water disinfection. Moreover, turbidity provides the power supply of these networks, thereby facilitating their movement.[3]

In the present study, a significant difference was observed between the mean chlorine level and turbidity in the urban and rural areas, which could be due to the fact that water in rural areas is less purified than municipal water. In addition, the mean free residual chlorine in the urban and rural areas was 0.82 and 0.53 mg/L, respectively. The mean turbidity in the rural and urban areas was 3.96±2.9 and 3.32±3.1 NTU, respectively. According to the results of ANOVA, the mean turbidity was within the range of 3±1.06-3.77±1.16 (average: 3.44 NTU). Table 4 shows the mean total and fecal coliforms in the samples in different months.

Table 4. Microbial parameters of water distribution networks in Dasht-e Azadegan County, Iran

Month	No.	Total coliform		Fecal coliform	
		Mean±SD	Range	Mean±SD	Range
April	51	1.98±10.36	0-75	1.6±10	0-75
May	84	9.44±20.62	0-94	3.49±18.83	0-94
June	80	5.3±22.95	0-150	0.84±5.48	0-43
July	84	10.39±24.97	0-150	7.01±23.57	0-150
August	78	7.62±9.64	0-143	3±8.97	0-143
September	64	10.1±35.49	0-210	9.6±35.73	0-210
October	71	8.82±24.42	0-150	7.42±23.67	0-150
November	86	2.79±18.14	0-75	2.78±12.6	0-75
December	83	10.55±45.17	0-290	10.13±44.32	0-290
January	53	4.38±25.72	0-150	0.67±3.4	0-23
February	88	2.85±11.69	0-23	0.29±2.59	0-23
March	67	4.1±19.72	0-150	0.64±3.19	0-23

According to the results of the present study, 7.7% (70/907) and 16% of the samples (149/907) were infected with fecal and total coliforms, respectively, which could be due to the fractures in the pipelines of the distribution networks as some pipelines were broken since they were old. The samples contaminated with fecal and total coliforms had the mean free residual chlorine of 0.01 mg/L, while the samples with no fecal coliform had the mean free residual chlorine of 0.77 mg/L.

In the current research, the mean turbidity in the samples contaminated with fecal coliforms was 4.72 NTU versus the mean of 3.3 NTU in the non-contaminated samples (P=0.001). In these samples, the fecal coliforms originated from mammals and birds, and their presence in water may be an indication for intestinal pathogens.[16] According to the obtained results, fecal coliforms increased in the water samples at the endpoints compared to the elementary points of the drinking water distribution networks, which could be due to the fractures of the pipelines of the distribution networks. As mentioned earlier, some pipelines were broken in the studied distribution networks, and pollutants increased along the network through the connections or fractures, especially in the areas where pipelines were eroded, which led to increased contamination with fecal coliforms. In general, increased fecal coliforms in the drinking distribution networks is affected by the degree of filtration, temperature, type of disinfection, water turbidity, and eroded pipelines.[17]

According to the results of the present study, the highest and lowest levels of coliform contamination were observed in warm seasons (July, August, September, and October) and cold seasons (April, November, January, February, and March), respectively (P=0.059). In Dasht-e Azadegan County, the temperature reaches 50 °C in summer, influencing the processes and methods of water treatment, as well as bacterial growth in distribution networks.[18] For instance, increased temperature from 5 to 20 °C was associated with the higher total coliforms by 18 times.[17] Water temperature could provide the conditions for the growth of certain pathogenic species. For instance, the issues associated with the growth of *E. coli* in summer due to higher water temperature may increase the risk and reduce the efficiency of water treatment plants.[19]

In a study conducted in the Netherlands, an inverse correlation was observed between the concentration of free residual chlorine and plate microbial count.[10] Furthermore, the findings of Jaleilzadeh *et al.* regarding the heterotrophic and coliforms of old and new drinking water distribution networks indicated that the combination of coliforms in the old drinking water distribution network consisted of 31% fecal coliforms and 69% other coliforms, while in the new network, these values were 4% fecal coliforms and 96% other coliforms.[18]

According to the results obtained by Thani *et al.*, 83.4% of the samples were infected with total coliforms, and 60% were completely infected with fecal coliforms.[20] On the other hand, the findings of Chaidez *et al.* in Mexico city demonstrated that 43% of the drinking water samples were completely infected with total coliforms, while 26% were infected with fecal coliforms.[21] Table 5 shows the logistic regression analysis of the variability of bacterial contamination with total \ and fecal coliforms as the dependent variables.

Table 5. Logistic regression analysis of microbiological parameters

Variables	B	S.E	Wald	DF	Sig.	Exp (B)	95.0% C.I. for EXP(B)	
							Upper	Lower
Free Chlorine re.	-3.243	0.340	91.106	1	0.000	0.039	0.020	0.076
Turbidity	0.18	0.042	18.627	1	0.000	1.197	1.103	1.299
pH	0.12	0.24	4.36	1	0.04	0.45	0.31	0.56
Summer	0.011	0.288	0.002	1	0.969	1.011	0.575	1.778
Winter	0.537	0.333	2.606	1	0.106	1.712	0.891	3.287
City	0.336	0.379	0.789	1	0.375	1.400	0.666	2.942
Rural	1.186	0.480	6.110	1	0.013	3.275	1.278	8.390
Constant	-1.411	0.268	27.674	1	0.000	0.244	-	-

In order to investigate the correlations between the variables with total and fecal coliform contamination in the drinking water distribution network of Dasht-e Azadegan, analyses of one variable (total coliform and fecal coliform separately) was performed using t-test and Chi- square. Fig. 1 depicts the receiver operating characteristic (ROC) curve sensitivity to determine total and fecal coliform contamination, as well as the optimal cutoff point for residual free chlorine concentration.

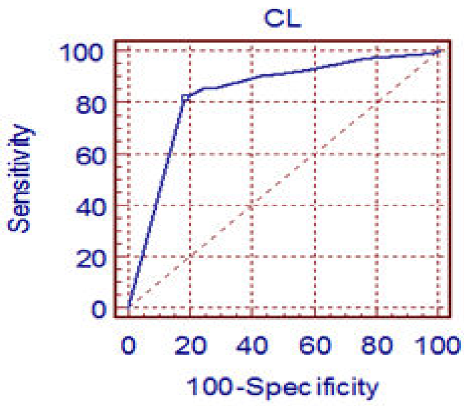

Fig. 1. ROC curve sensitivity of microbiological parameters to determine optimal cutoff point

The variables with the significance level of less than 0.2 were analyzed using the logistic regression model, and their odds ratio and confidence intervals were the basis for the statistical analysis. ROC curve analysis was also carried out to determine the optimal cutoff point for chlorine in predicting the presence of coliform contamination in water. Based on the ROC curve sensitivity, the surface area under the curve was approximately 83%, which was considered statistically significant. This point was confirmed at 95% confidence level, and the level of free residual chlorine could also confirm the presence or absence of total and fecal coliform contamination with high accuracy. Therefore, it could be inferred that the optimal cutoff point was zero with the sensitivity of 81 and 81.7%, which were considered statistically significant. A specific range of free residual chlorine is shown in Fig. 2 in order to determine the optimal cutoff point for this variable in preventing contamination with total and fecal coliforms.

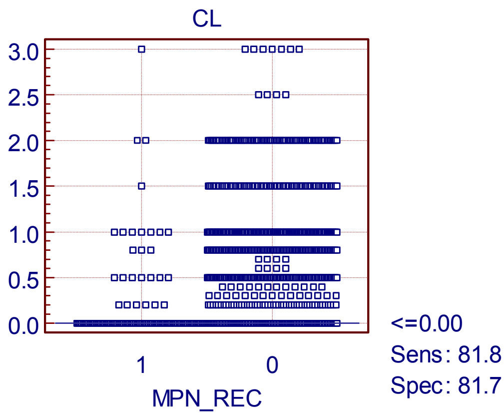

Fig. 2. Specific range of free residual chlorine to determine optimal cutoff point in prevention of total and fecal coliform contamination

As is depicted in Fig. 2, the free residual chlorine was within the range of 0.5-2 mg/L, indicating the optimal conditions for the prevention of microbial contamination. In the current resaerch, Tukey's post-hoc test was used to investigate the differences between the pair of the mean values, and the results indicated no significant difference between cities in Dasht-e Azadegan County in this regard, while the other comparisons showed significant differences.

According to the logistic regression model, contamination with total and fecal coliforms was correlated with free residual chlorine and turbidity, while seasons had no significant associations with coliform contamination. Moreover, the overall odds of total and fecal coliform contamination in Abu Homeyzeh city were approximately 27% higher than Susangerd city. On the other hand, turbidity increased the risk of contamination with total and fecal coliforms by 19%, while residual free chlorine reduced the risk of total and fecal coliform contamination by 97%. The accuracy of this statistical model was estimated at 85.6%. Therefore, it seems that free residual chlorine was the main factor in determining total and fecal coliform contamination, the higher concentrations of which could neutralize coliform contamination. Chlorine is considered to be an important barrier against the prevention of microbial contamination in water distribution networks with a significant impact on the microbial quality of water. Therefore, the logistic regression model confirmed the role and impact of the studied parameters (especially free residual chlorine) in maintaining the quality of drinking water.

The limitations of the present study were long distance of the rural areas and lack of a distribution network in a few villages.

Conclusion

According to the results, 262 and 377 the water samples did not meet the WHO guidelines in terms of turbidity and free residual chlorine, while pH was within the recommended limits of drinking water standards in Iran. In addition, 70 and 146 samples were infected with fecal coliforms and total coliforms, respectively. The results of the logistic regression analysis indicated that coliform contamination was associated with free residual chlorine and turbidity. Urban location was also correlated with coliform contamination, so that Abu Homeyzeh city had higher probability of 27%, and turbidity increased the risk of coliform contamination by up to 19%, while free residual chlorine reduced the risk by 97%.

Some of the effective measures to prevent the presence of pollutants in the distribution networks are controlling the quality of the water entering drinking water distribution networks, replacing old metal pipelines with PVC pipes and fittings, and consistent and accurate protection of distribution networks. In addition, mechanisms leading to the faster entry of pathogens into distribution networks (e.g., inattention to pipeline maintenance and improper design of distribution networks) might lead to the prolonged maintenance and long-term storage of water in distribution networks.

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Ethical Considerations

There were no ethical issues involved in performing this research project (e.g., plagiarism, data fabrication, and data falsification).

Authors' Contributions

All the authors equally contributed to the study and critically reviewed, refined, and approved the final manuscript.

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