

Assessment of the *Escherichia coli* pollution in drinking water and water sources in Sistan, Iran

Kambiz Nazemi, Saeed Salari and Majid Alipour Eskandani

ABSTRACT

Water should be free of dangerous agents and able to provide the nutritional needs of humans and animals. Adequate and reliable water supply is essential for healthy animal production. This study was carried out in Sistan region, Sistan and Baluchistan State, Iran, to evaluate the pollution rate of drinking water and water sources with fecal *Escherichia coli* via the most probable number (MPN) method. A total number of 100 water samples were collected and divided to drinking and non-potable water groups. The non-potable water was categorized according to flow type, irrigation usage, type of accessibility and geographic location. Mean number of MPN of fecal *E. coli* in non-potable and drinkable water were detected, 6.1×10^2 and 2.4 coliforms per 100 mL, respectively ($p < 0.05$). Running water was more contaminated than still and pipe water in the study area ($p < 0.05$). Significantly, the MPN of fecal coliforms, in non-potable water, collected from the east was higher than the west part of the study area ($p < 0.05$). Among potable water, in the study area, the west part was remarkably more polluted with fecal *E. coli* than the east part of the study area ($p < 0.05$). Results indicated the applicability of water for animal consumption and an alarming signal for human consumption and public health.

Key words | drinking water, fecal *E. coli*, Iran, Sistan, water sources

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INTRODUCTION

Water is an essential material for plant life and has a significant role for human and animal health. Water is a critical nutrient substance for livestock and is the most important vital urgent medium for life and is essential for the good health of human beings. In Europe and America, much attention has been paid to the problem of water purity (Fenwick 2006; WHO 2008). The people of developing countries are more exposed to water-borne diseases than those in developed countries. An adequate and safe water supply is essential to the production of healthy livestock (Hamner *et al.* 2007; Saravanan *et al.* 2011). The use of

water sources contaminated with human and animal excreta containing pathogenic organisms pose health risks to the population because of increased incidence of zoonotic water-borne diseases (Makoni *et al.* 2004; Mohammed 2014). Regardless of the availability of water, minimum quality standards should be considered, depending on the use of the water (human consumption, irrigation, or livestock). Polluted water is an important vehicle for the spread of diseases. Most outbreaks of water-borne diseases are caused by the contamination of drinking water with feces of infected animals or people (Medema *et al.* 2003; WHO 2008; Salem & Metaweia 2013). Drinking water can be the vector of viral, bacterial and parasitic diseases (Holmes & Gross 1990; Medema *et al.* 2003; Tavakkoli 2008). Microorganisms are of considerable importance in many aspects of water quality control. *Escherichia coli* is recommended by the United

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States Environmental Protection Agency (US EPA) as an indicator organism to screen freshwater systems and is a sensitive criteria of fecal pollution (ISIRI 2009; Rice *et al.* 2012). Detection and enumeration of *E. coli* in many types of water from different provinces of Iran has been previously reported (Rakhsh Khorshid *et al.* 2002; Jafari *et al.* 2006; Jafarzadeh Haghighifard *et al.* 2007; Khatib Haghighi *et al.* 2008; Mahmodi & Javanmardi 2009; Nasrolahi *et al.* 2011; Soltan Dallal *et al.* 2012; Momtaz *et al.* 2013; Yousefi *et al.* 2013; Shabankareh Fard *et al.* 2014). There is scant information on the microbial quality of water sources in Sistan region, Sistan and Baluchistan State, Iran. The aim of the present study is to investigate fecal *E. coli* contamination of water sources and drinking water by means of samples collected in Sistan, to assess the public health risks associated with water.

METHODS

Study area and design

As a descriptive cross-sectional study, this survey was conducted in different sites of Sistan in Sistan and Baluchistan province, Iran, from December 2014 to July 2015. The topography of the study area is located between latitudes 30 ° and 31 °N and longitudes 60 °50 and 61 °50'E (Figures 1 and 2). The fecal *E. coli* for human and livestock consumption and agricultural use were investigated.

Water sampling

In this study, a total number of 100 water samples were collected and categorized, when the water was available in the study area. Samples were collected in 300 mL sterile glass bottles for bacteriological investigation, according to standard guidelines (Pant 2004; WHO 2008). The water samples were collected as described by Duelge & Unruh (2002). Water samples were delivered to the Microbiology Laboratory, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran as soon as possible, or preferably stored in a refrigerator at approximately 4 °C.

Samples were divided into two main groups involving drinking ($n = 30$) and non-potable ($n = 50$) water. Five

sampling categories were defined for non-potable water samples including flow type (still water, $n = 31$; running water, $n = 19$; pipe water, $n = 30$), political divisions (Shibe Ab, $n = 11$; Poshte Ab, $n = 9$; Jazinak, $n = 28$), agricultural usage (irrigation, $n = 17$; non-irrigation, $n = 33$), type of accessibility (canal, $n = 10$; river, $n = 7$; pool, $n = 27$; well, $n = 2$; dam, $n = 4$) and geographic location (east, $n = 28$; west, $n = 20$). Eventually, drinking water samples were subdivided into two groups based on their geographic location (west part of city: $n = 12$; east part of city, $n = 8$).

Detection and enumeration of bacterium in water

Examination of the water samples was started within 24 h after collection, using most probable number (MPN), confirmatory and complementary techniques (WHO 2008). The fecal coliforms were enumerated using MPN as described by Iranian National Standards Organization, protocol No. 3759 (ISIRI 2009). Briefly, after determination of MPN by Brilliant Green Bile Lactose Broth (Merck, Germany), the tubes, showing growth, were inoculated onto MacConkey and EMB agar plates (Merck, Germany). After 24 h incubation at 44.5 ± 0.5 °C (Grabow 1996; Gould *et al.* 2013), suspected *E. coli* colonies were confirmed according to Quinn *et al.* (2002), using Triple Sugar Iron Agar, SIM, and IMViC tests.

Statistical analysis

The primary data were analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple range tests. The statistical analyses were calculated via Statistical Package for Social Sciences (SPSS version 17.0). $p < 0.05$ was the accepted significance level.

RESULTS

In the present study, 100 samples were investigated to detect the MPN of fecal coliforms among drinking water and water Sources in Sistan, Iran (Table 1). The results of this study showed that the running water was more polluted than still water, although there were no significance differences ($p > 0.05$). Drinkable water was significantly ($p < 0.05$)

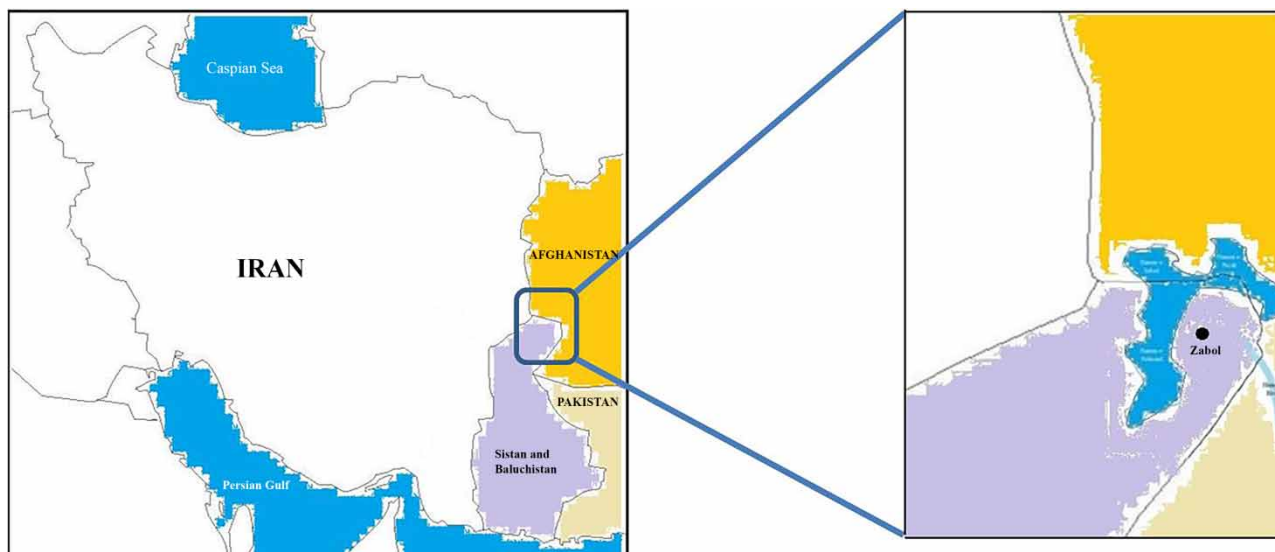


Figure 1 | Geographic location of study area.

clearer of fecal coliforms than non-potable water. However, drinkable water in the study area was not of the appropriate degree for drinking by the human population. The MPN differences of fecal *E. coli* of Shibe Ab, Poshte Ab and Jazinak were not significant ($p > 0.05$) but Jazinak has the highest MPN of fecal coliforms, while the lowest MPN was related to Shibe Ab. The highest MPN of fecal coliforms

was observed among water samples collected from canals followed by rivers, dam-wells, and eventually pools, with no significant differences ($p > 0.05$). Our results demonstrated that the drinkable water in the west part was, significantly, more polluted with fecal *E. coli* than the east part of the study area ($p < 0.05$). Moreover, significantly, the MPN of fecal coliforms in non-potable water collected from the east was higher than the west part of the study area ($p < 0.05$).

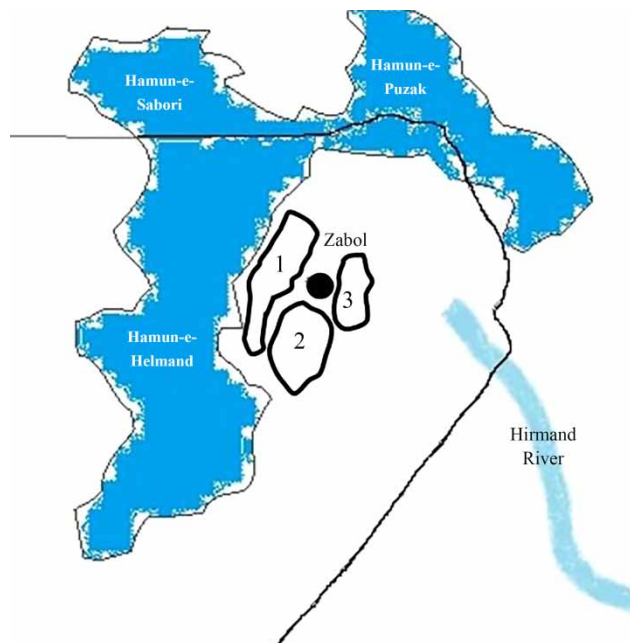


Figure 2 | Political division of Sistan (1: Poshte Ab; 2: Shibe Ab; 3: Jazinak).

DISCUSSION

Water is an important resource that supports all living organisms and is used for different purposes including drinking, cooking, washing and crop cultivation (Edberg 1996). At present, there is no economical substitute for water. Consequently, water conservation and water reuse are necessary (WHO 2008). The magnitude of the problem of bacterial contamination deserves more elaborative studies from the point of production of waste effluents to the point of consumption at all intermediary levels (Acha & Szyfres 2003). Based on our literature review, there was no comprehensive study to investigate the fecal *E. coli* contamination of water sources and drinking water of Sistan region, Sistan and Baluchistan State, Iran.

Table 1 | Minimum, maximum and mean number of MPN of fecal *E. coli* in collected samples

Type of sample	Minimum (per 100 mL)	Maximum (per 100 mL)	Mean (per 100 mL)
Still water	2	1.4×10^3	5.4×10^2
Running water	2	1.4×10^3	7.4×10^2
Drinkable water ^a	2	9.4	2.4
Non-potable water ^a	2	1.4×10^3	6.1×10^2
Shibe Ab	9.4	1.4×10^3	6.6×10^2
Poshte Ab	64	4.6×10^2	2.8×10^2
Jazinak	2	1.4×10^3	7.3×10^2
Irrigation usage	9.4	1.4×10^3	9.5×10^2
Non-irrigation usage	2	1.4×10^3	4.5×10^2
Canal	9.4	1.4×10^3	9.4×10^2
River	2	1.4×10^3	7×10^2
Pool	2	1.4×10^3	5×10^2
Dam	3	1.1×10^3	5.5×10^2
Well	3	1.1×10^3	5.5×10^2
East ^{b,d}	2	1.4×10^3	7.3×10^2
West ^{b,d}	9.4	1.4×10^3	5.1×10^2
East part of city ^{c,e}	2	2	2
West part of city ^{c,e}	3	9.4	3

^{a,d,e}Significance difference ($p < 0.05$); ^bindicates non-potable water; ^cdemonstrates drinking water.

E. coli could serve as a more specified indicator for fecal contamination of fresh water.

As standard methods in the detection and enumeration of coliforms, cultivation on the EMB agar plate and MPN method by Brilliant Green Lactose Bile Broth have been used (Holmes & Gross 1990; WHO 2008). MPN is a method to estimate the concentration of viable microorganisms in a sample by means of replicate liquid broth growth and is particularly useful with samples that contain particulate material that interferes with plate count enumeration methods (Tebbutt 1977; Borriello *et al.* 2005; Tavakkoli 2008).

The maximum limit of fecal coliforms in surface water has been considered to be 100, 200 and 200 coliforms per 100 mL, based on standards in Canada, North America and Europe, respectively (Rice *et al.* 2012; Health Canada 2006).

According to the above standards, the surface water of the present study area is harmful for human consumption. In other words, it is polluted by fecal coliforms, adversely. Significant differences were observed between drinking

water and non-potable water. Therefore, our results indicated that drinking water was being adequately treated to eliminate fecal *E. coli*. However, while drinking water was polluted with 2.4 coliform per 100 mL, it seems to be a problem for public health. Although our report represents the good performance of drinking water treatment plans in the studied area, more investigations should be carried out to determine other infectious agents in water to score the drinking water production systems.

The US EPA recommends that livestock water must contain less than 5,000 coliform organisms per 100 mL (Rice *et al.* 2012).

The results of the present study in comparison with US standards displayed that the water in the study area was suitable for livestock consumption, although all samples were positive for fecal coliforms.

Jafari *et al.* (2006) studied the water (source water, pipe water and drinking water) used for broiler farms in the rural area of Ahvaz, Khuzestan Province, Iran, for fecal coliforms and reported that the coliform counts by MPN in 20 farms (50%) were over the maximum acceptable level for poultry water and fecal coliform was verified in the water of all farms. Fifty fecal *E. coli* per 100 mL was assumed as the maximum acceptable level (Jafari *et al.* 2006).

Jafarzadeh Haghighifard *et al.* (2007) examined total coliform number from pools in drinking water production systems in Ahvaz using MPN. Their results showed that pools located south of Karon River were more polluted (Jafarzadeh Haghighifard *et al.* 2007).

Yousefi *et al.* (2013) tested water sources for fecal coliforms in Divandarreh, Kordestan Province, Iran. They collected samples from springheads, deep and semi-deep wells. 0.9–1 fecal coliforms for 95% of samples were reported (Yousefi *et al.* 2013).

Khatib Haghighi and Khodaparast (2010) surveyed the pollution of Gram-negative bacteria in some areas of Anzali pond. Maximum MPN for fecal coliforms was reported to be 135 ± 173 bacterium per 100 mL (Khatib Haghighi *et al.* 2008).

Shabankareh Fard *et al.* (2014) evaluated the microbial quality of the distribution network drinking water in Bushehr, Iran and noted fecal coliform of 0 MPN/100 mL (Shabankareh Fard *et al.* 2014).

Momtaaz *et al.* (2013) appraised *E. coli* and *Salmonella* species in tap water and bottled drinking water in Isfahan, Iran. In their survey, the culture method showed that 34 (7.58%) and four (0.89%) out of 448 water samples were positive for *E. coli* and *Salmonella* species, respectively. *E. coli* was detected in eight (2.63%) of 304 samples of bottled drinking water from five companies. Their study demonstrated the important public health problem in Isfahan, Iran (Momtaaz *et al.* 2013).

Nasrolahi *et al.* (2011) investigated the bacteriologic parameters of drinking water in Gorgan, Iran, by MPN methods and reported that drinking water had acceptable levels for human consumption (Nasrolahi *et al.* 2011).

We tried to review almost all of the published and accessible reports related to pollution of water with *E. coli* in Iran. Overall, according to investigations carried out in Iran and also based on the latest statute released by Iran Veterinary Organization, Executive order number 90/49753, released in 2011 (Iran Veterinary Organization 2011), the water of the study area is contaminated, harmfully, with coliform. The Executive order number 90/49753 is planned for water quality control of cage and shelves technologies for broiler farming in Iran. Based on this Executive order no. 90/49753, the presence of *E. coli* should be negative and, consequently, the water of study area is contaminated, dangerously, with *E. coli*, for cage and shelf types of broiler farming. Thus, we propose to review the rules and determine an accurate, permissible and admissible fecal coliform number in water drank by animals in Iran.

Albeit drinking water in the present study received a fair mark, the results showed that drinking water in the west part of the study area was significantly more polluted with fecal *E. coli* than the east. This could be related to the distance of the sampling locations from drinking water treatment systems which may lead to contamination of drinking water supplies. This theory needs future evaluation and control.

Mahmodi & Javanmardi (2009) sampled water from different areas of Lake Parishan, Kazeron, Fars Province, Iran, at regular intervals and estimated fecal *E. coli* by MPN. They investigated the correlation between rainfall and pollution rate of the lake; 100 CFU/100 mL of fecal coliform was been reported in the study area (Mahmodi & Javanmardi 2009).

Soltan Dalal *et al.* (2012) inspected the microbial pollution of wells of parks located in Tehran, Tehran Province, Iran, via the membrane filtration method. They outlined that 54.5% of samples were positive for *E. coli*. Wells located in the south part of Tehran were more polluted with *E. coli*. They proposed to apply an alarming screen board about contaminated water to notify people in the parks (Soltan Dallal *et al.* 2012).

Rakhsh Khorshid *et al.* (2002) determined the water microbial pollution trough coliforms and fecal coliforms as pollution indexes in bacteria testing in 2000, in Zahedan, Sistan and Baluchestan provine, Iran via MPN. Nineteen and 7.3% of tanker samples and water samples in dwellings were positive for fecal coliform, respectively. They concluded that the water of Zahedan was not suitable for drinking (Rakhsh Khorshid *et al.* 2002).

The results of previous investigations carried out in Iran regarding the presence of *E. coli* in water were compared and concluded with different standards. Although these studies involved quantitative outcomes and were perhaps carried out via different methods, our results in comparison to their results and local standards (Executive order number 90/49753) confirm the high contamination of water in the study area with fecal *E. coli*, based on the MPN of microorganisms for broiler farming by cage and shelves technologies, while based on global standards (Rice *et al.* 2012) it could be considered safe for animal use.

The present study explains that non-potable water in the east part of the study area was significantly more polluted with fecal *E. coli* than the west. It should be mentioned that the study area is affected by a seasonal wind known as the 120-day winds of Sistan which occurs from May to August. The speed of these winds may reach 150 km/h (Karimi *et al.* 2013). On the other hand, it could be concluded that the stormy climate of the study area leads to a collection of dust from some locations and also while flowing over the ground and surface water, moist dust picks up silt, particles of organic matter, and more bacteria from the topsoil and from decaying matter, some of which will ultimately enter the surface water. As a consequence, the effect of climate on water pollution by different microorganisms in the study area is hypothesized for future research.

CONCLUSIONS

All water samples in the present study are harmful for human consumption. In other words, the water in the study area is adversely polluted with fecal *E. coli*. Although all samples were positive for fecal coliforms, the water in the study area is suitable for livestock consumption. Our study indicated the important human public health problem in Sistan, Iran. We now suggest that the use of home filtration appliances in the study area would be strongly worthwhile and additional effort should be made to identify the possible actions to be applied to remedy and improve the current microbiological water quality situation.

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