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Relationship between Frequency of Escherichia Coli and Prevalence of Salmonella and Shigella Spp. in a Natural River

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ABSTRACT

Introduction: Microbial contamination prediction through detecting the indicator bacteria in natural waters is the first health and environmental step for preventing the transmission of water-associated diseases. This study was designed to determine the correlation between *Escherichia coli* as the indicator bacterium, on the one hand, and *Salmonella* and *Shigella* Spp. As the pathogenic bacteria, on the other hand.

Materials and Methods: Totally, 60 natural water samples were collected from natural rivers in Gorgan during summer and autumn of 2016. In order to detect *Escherichia coli*, the membrane filter method with Endo agar and IMVIC test was used and, in order to detect *Salmonella* and Shigella, the 9-tube fermentation method and biochemical tests with selenite F medium, XLD agar, TSI, urea, and SIM were utilized. For the statistical analysis, Pearson's correlation test was used at the significance coefficient of < 0.05.

Results: Results showed that all of the analyzed bacteria were detected in the water samples. The frequency of *Salmonella*, *Shigella*, *Escherichia coli*, *Klebsiella-Enterobacter*, and *Citrobacter* was 9.5, 22.2, 4.8, 22.2, and 65.1%, respectively. The statistical analysis demonstrated no statistical correlation between *Escherichia coli*, on the one hand, and *Salmonella* and *Shigella*, on the other hand; but the relationship between *Salmonella* and *Shigella* was significant.

Conclusion: Judging the microbial quality of water supplies cannot be sufficient only based on the presence or absence of *Escherichia coli* bacterium. Therefore, that the use of other secondary indicator bacteria such as fecal streptococci and supplementary sulphite-reducing clostridia will be advised.

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Introduction

Water microbial quality is determined based on the presence of certain types of microbes known as indicator microorganisms due to difficulties in counting all microorganisms, especially pathogens, in water [1]. Some of the most important characteristics considered for microorganisms are as follows: Being a member of normal flora of gastrointestinal tract of warm-blooded animals; being non-pathogenic; being present in higher numbers than pathogenic microbes; being absent in the non-contaminated samples with pathogens; being resistant like pathogenic microorganisms to adverse environmental conditions and disinfectants; not reproducing naturally in the environment; and being traced and

detected using simple and inexpensive methods [2]. Coliforms were the first group of microorganisms selected as indicators. These bacteria include bacilliform, non-spore-forming Gram-negative, facultative aerobic and anaerobic bacteria, and ferment lactose with gas production at 35°C. *Escherichia coli*, *Citrobacter*, *Enterobacter*, and *Klebsiella* are the coliform bacteria found in the digestive tract of humans, warm-blooded animals, plants, soil, and water. Fecal or thermotolerant coliforms are also a subset of coliforms that can produce the blue colonies at 44.5°C in 24 h in EC broth gas or M-FC medium [3]. It is important to note that although only *Escherichia coli* is fecal, *Citrobacter*, *Enterobacter*, and *Klebsiella* can grow and reproduce in fecal and non-fecal environments [4-5].

World Health Organization (WHO), Environmental Protection Agency (EPA), and many international organizations have a consensus on the use of fecal coliform bacteria and *Escherichia coli* as the fecal indicator bacteria. In Iran, the urban and rural water and wastewater companies (Ministry of Energy) as the custodian of water supply, Ministry of Health as the observer of health quality of drinking water, and Department of Environment as the custodian for the quality of natural waters use fecal coliform and *Escherichia coli* as the microbial indicators of water [6] . The results of reports on water-borne diseases show that the major challenges for indicator bacteria include lack of association with the presence of fecal pathogens [7-9], growth and proliferation in environments out of human feces (such as soil and leaves), regrowth in drinking water distribution network [10-11], and lack of correlation with pathogens, especially viral and protozoan agents [12].

Salmonella, *Shigella*, and *Escherichia coli* are the major bacterial species from the Enterobacteriaceae that play a significant role in the outbreak of water-associated diseases. *Salmonella* pathogenesis is mainly caused by the entry of fecal contamination originated from humans, livestock, and wild animals into water supplies and a large group of foods such as milk and meat. The control measures include protection of raw water supplies from animal and human wastewater as well as proper treatment and control of drinking water during distribution [13]. *Escherichia coli* or thermotolerant coliforms are generally reliable indicators for the detection of *Salmonella* in drinking water supplies [5]. The US Center for Disease Control and Prevention has reported that annually more than 400 thousand people in America are infected with *Salmonella* through the consumption of contaminated water [14]. According to WHO, various species of *Shigella* annually cause the infection of more than two million people with microbial dysentery, out of which about 600 thousand people die (especially in developing countries). Most of *Shigella* infection cases occur in children under 10 years old [5].

Results of epidemiological examinations, clinical signs, and analysis of human samples in the outbreaks of *Noroviruses* Pardis city, Tehran, showed that the fecal contamination of water supplies with *Noroviruses* was the main cause of the epidemics. The evidence related to the microbial quality of drinking water resulting from the findings of microbial tests of drinking water in the outbreak of the disease was negative in terms of fecal coliform contamination. However, in the analysis of fecal samples of the patients, the *Noroviruses* and Enterotoxigenic *Escherichia coli* were observed [15]. In the most of these cases, the use of *Escherichia coli* as the indicator bacteria is limited due to the inability in terms of complete prediction of pathogenic species. Thus, this study was designed to determine the association and correlation between the prevalence of *Escherichia coli* and *Salmonella* and *Shigella* Spp. in the natural river water that was exposed to all kinds of human, animal, and plant infections.

Materials and Methods

To perform this descriptive analytical study, 60 river water samples were taken according to the standard requirements for microbiological water examination, and by observing the cold chain conditions, they were transferred to Microbiology Laboratory, Faculty of Health, Gorgan University of Medical Sciences, within the time interval of

less than 2 h. Microbial parameters were analyzed immediately. In order to detect the coliform bacteria group, including *Escherichia coli*, *Enterobacter*, *Klebsiella*, and *Citrobacter*, the membrane filter method was used. First, 100 ml of the water sample was passed through a filter with 0.45 µm pores and cultured on EMB agar. After incubation (35 ± 0.5°C for 24 h), the nucleated red colonies (with or without metallic luster) and matte pink colonies (mucoid and non-nucleoid) were selected and counted as the positive colonies contaminated with fecal coliform. Then, using IMVIC test (indole, methyl red, VP, and citrate), various species of fecal coliform were detected [3].

In order to detect *Salmonella* and *Shigella*, 9-tube fermentation method was used. For this purpose, in the probability step, the samples were cultured in Selenite-F agar and incubated for 24-48 h at 35 ± 0.5°C. Then, the positive samples (pink to red) were linearly cultured in the XLD agar and, after incubation (35 ± 0.5°C for 24 h), all the red colonies with dark centers were considered as the positive samples containing *Salmonella* and *Shigella* Spp. Finally, through biochemical tests including TSI, urea, and SIM, *Salmonella* and *Shigella* Spp. were detected. The results of all the microbial tests were reported as MPN per 100 mL [3]. For the statistical analysis, SPSS 20.0 software and Pearson's correlation test were employed to examine the relationship between microbial parameters with each other at the significant level of $P < 0.05$.

Ethical Issues

This article was approved by Ethical Committee (ethical code; Ref: 94511) of Vice-Chancellor for Research and Technology, Golestan University of Medical Sciences.

Results

Results showed that all of the analyzed bacteria were detected in the water samples. The frequency of *Salmonella*, *Shigella*, *Escherichia coli*, *Klebsiella-Enterobacter*, and *Citrobacter* was 9.5, 22.2, 4.8, 22.2, and 65.1%, respectively. The statistical analysis showed no correlation between *Escherichia coli*, on the one hand and *Salmonella* and *Shigella*, on the other hand, while no statistically significant association was found between other microbial parameters. The results of Pearson's correlation test to determine the relationship between other analyzed bacteria are shown in Table 1.

Table 1: Correlation coefficient of pathogenic bacteria

Microbial Parameters	Citrobacter	Klebsiella-Enterobacter	Escherichia Coli	Shigella	Salmonella
Salmonella	0.004	0.109	0.100	0.006	
Shigella	0.040	0.069	0.037		
Escherichia coli	0.030	0.005			
Klebsiella-Enterobacter	0.038				
Citrobacter					

In Table 2, some of the water samples with negative *Escherichia coli*, but grown *Salmonella* and *Shigella* pathogenic bacteria, are demonstrated. According to the data, *Salmonella* and *Shigella* were observed respectively in 6 and 14 samples with negative *Escherichia coli*.

Table 2: Samples with negative Escherichia coli but positive Salmonella and Shigella

Sample No.	Salmonella	Shigella
1	+	+
2	-	+
3	+	+
5	-	+
6	-	+
7	+	+
8	+	+
9	-	+
10	-	+
12	-	+
15	+	-
24	-	+
25	-	+
26	-	+
37	-	+

Discussion

This study was aimed to compare the prevalence of indicator bacteria with pathogenic species to assess the microbial risk of water and predict the possible presence of pathogenic organisms and showed that the coliform bacteria can be found in more than half of the samples. The highest number of cases was respectively associated with *Citrobacter*, *Enterobacter*, *Klebsiella*, and *Escherichia coli*. However, in the separation of microorganisms from water samples, several factors such as type of medium, cultivation technique (multiple-tube fermentation or filtration), diluent, water turbidity, and competing bacteria in the environment were important. It should be noted that only *Escherichia coli* was fecal, with large numbers in the feces of humans, other mammals and birds, and rarely observed in water or soil without fecal contamination [2]. The origin of thermotolerant coliforms, except *Escherichia coli*, might be waters rich in organic matter, such as industrial wastewaters or decomposed plant material as well as soil. This means that the presence of this organism in warm waters or those rich in organic wastes does not necessarily indicate their human fecal contamination since they can enter the water through wild animals and birds. However, it should be emphasized that the presence of these microorganisms can show the entry of a microbial contamination source [16]. For example, *Klebsiella* is an opportunistic bacterium that can grow in many of the aquatic environments such as water distribution system and nutrient-rich waters and cause the disease in the elderly, children, and other people with defects in their immune system [5].

In this study, the number of detected *Salmonella* and *Shigella* Spp. was higher than the indicator bacterium, i.e. *Escherichia coli*. The comparison of results in terms of the correlation of microbiological parameters showed no significant relationship between the presence of *Escherichia coli* and *Salmonella* ($p = 0.663$) or Shigella ($p = 0.356$). In 20% of the samples where *Escherichia coli* was not detected, *Salmonella* and *Shigella* bacteria were found (Table 2). In contrast, only in 6.3% of the samples containing *Escherichia coli*, *Salmonella* and *Shigella* Spp. were identified. The results demonstrated that a higher number of pathogenic bacteria was identified in the samples than the indicator bacteria. In other words, the negative predictive value of *Escherichia coli* for *Salmonella* and *Shigella* was 90% and 76%, respectively; but the predictive value of *Escherichia coli* for both organisms was less than 50%. Therefore, the presence or absence of *Escherichia coli* does not necessarily mean the presence or absence of

pathogenic bacteria such as *Salmonella* and *Shigella*. Lack of significant correlation has also been observed between the presence of *Escherichia coli* and pathogenic microorganisms in the studies of other researchers [17- 18].

Reports by WHO have emphasized that although the use of *Escherichia coli* as the fecal contamination indicator is essential in water supplies, this group of bacteria are faced with limitations for the lack of relationship with the presence of fecal pathogens because some of them are more sensitive to environmental pressures and disinfection than viruses and parasitic protozoa. For example, each of the three bacteria examined in this study (*Escherichia coli*, Salmonella, and Shigella) differs in terms of resistance to adverse environmental conditions and survival length in natural waters [19]. *Escherichia coli* is more sensitive to environmental pressures and usually cannot grow outside the body of human and animals. This bacterium usually can be seen in recent and new infections. Moreover, the survival length of *Escherichia coli* in the water environment is less than that of *Salmonella* and *Shigella* bacteria [20].

Conclusion

Microbial quality of water supplies is judged based on the presence or absence of *Escherichia coli*. This study showed that the results of presence or absence of this indicator cannot be trusted with certainty and the secondary indicator bacteria such as fecal streptococci and sulphite-reducing clostridia can be used as supplementary tools. Also, the development of gene probe technology and polymerase chain reaction (PCR) in different natural sciences has raised hopes of using these techniques by the responsible monitoring systems of water quality management to evaluate the safety of water supplies. In particular, multiplex PCR can be utilized for simultaneous tracking of a wide range of pathogenic microorganisms and parasites in samples.

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Conflict of interests

No conflict of interest has been stated by the authors.

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