**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.

**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 water¹. 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

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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

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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.

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. 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>
<thead>
<tr>
<th>Microbial Parameters</th>
<th>Citrobacter</th>
<th>Klebsiella-Enterobacter</th>
<th>Escherichia Coli</th>
<th>Shigella</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td>0.004</td>
<td>0.109</td>
<td>0.100</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Shigella</td>
<td>0.040</td>
<td>0.069</td>
<td>0.037</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.030</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella-Enterobacter</td>
<td>0.038</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Salmonella</th>
<th>Shigella</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>26</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>37</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

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. The origin of thermostolerant 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. 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.

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.  

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. 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.

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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