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Short Communication

Exploring Relationships between Fecal Coliforms and Enterococci Groups as Microbial Indicators in Recreational Coastal Waters in Eastern Mazandaran, Iran - The Caspian Sea

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Exploring Relationships between Fecal Coliforms and Enterococci Groups as Microbial Indicators in Recreational Coastal Waters in Eastern Mazandaran, Iran - The Caspian Sea

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Abstract

Using microbial indicators is the most practical method in monitoring water quality. In this study, the correlation among the above-mentioned microbial indicators were investigated for the coastal waters of the Caspian Sea in the eastern part of the northern Iranian province of Mazandaran. Microbial analysis were conducted using the membrane filter (MF) procedures outlined in Standard Methods 9222D and EPA method 1600. The normality test of Skewness and Shapiro-Wilk Test, the Kruskal-Wallis test, and the Pearson correlation coefficient (PCC) were used for sample distribution, sampling stations and sampling time comparisons, and determining the relationship between the microbial indicators and the environmental parameters, respectively. Significant differences were observed among the sampling stations, with the highest pollution going to the Jafarabad and Daraee beaches. In addition, the observed percentages of pollution by enterococci and fecal coliforms were 25.55% and 22.59%, respectively; and the weight obtained for the former and the latter were 0.3806 and 0.3762, respectively, indicating very close results and the importance of enterococci. The weights obtained for the sampling stations show the higher importance of enterococci and fecal coliforms at eastern and western swimming stations, respectively.

Keywords: recreational coastal waters, microbial indicator, fecal coliform, enterococci, Caspian Sea, Mazandaran province

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Introduction

Coastal areas are rich in natural and aquaculture resources, and act as hubs for economic, social, tourism, and recreational activities [1-3]. Considering that the province of Mazandaran borders for 487 km with the Caspian Sea, the world's largest lake with an area of 436,000 km^2, a large number of tourists benefit from this provincial shoreline and from swimming in their coastal waters. Just like everywhere in the world, tourism development and the recreational importance of beaches have made the coastal water health for swimmers especially important and has a special impact on the economy especially in tourist areas [4]. Coastal waters are prone to pollution, and can act as a medium to transmit microbial diseases [4, 5]. Pathogens enter the waters via point sources such as sewage outlets, or coastal waters can become polluted by non-point sources like runoff, animal feces, and swimmers' beach huts [6, 7]. Tracking a variety of pathogens is very difficult, costly, and time-consuming [8] while measuring fecal indicators is easier, does not require elaborate instruments, and is fairly cheap, making the latter the most common practical approach [9]. Using bacteria as indicators of environmental water quality probably dates back to 1880 when Von Fritsch described Klebsiella pneumonia and Klebsiella rhinoscleromatis as micro-organisms characteristically found in human feces [10]. Since then, many studies have been conducted, and today, fecal indicator bacteria are globally used as the estimated mean for water quality monitoring and as an indirect mean for water-related health risks that may arise as a result of contact with recreational, surface, and drinking waters [5]. The levels of fecal indicator bacteria in fresh and marine water are strong indicators of fecal contamination and signals of the presence of disease-causing bacteria in the environment [11]. In the 1960's, total coliforms were the first fecal indicator, and in 1976, the U.S. Environmental Protection Agency recommended both total and fecal coliforms as the indicator bacteria. In the late 1970's and early 1980's, the EPA suggested epidemiologic studies for evaluating the fecal indicators and using multiple organisms including fecal coliforms, Escherichia coli, and enterococci as possible indicators for determining fecal contamination [12, 13]. The results from these studies showed that enterococci are effective predictors of gastrointestinal diseases in marine and fresh waters, and Escherichia coli are effective indicators of gastrointestinal diseases in fresh waters [14]. And today, the EPA recommends enterococci as the only indicator for marine and brackish waters [15]. Different indicators were recommended for different locations and at different times [16]. In Iran too, different indicators have been considered based on directives from the Ministry of Healthcare. During the initial years of monitoring, the preferred indicators were total and fecal coliforms, which has changed to enterococci in the recent years. The results obtained from microbial monitoring of the Caspian Sea recreational waters and consequently the contamination status announcements and public advisories varied. So, to eliminate these conflicts and ambiguities in swimming water quality advisories, and considering the environmental conditions of the region, the behaviors of the microbial indicators, and that the Caspian Sea is the world's largest brackish water lake that has one third the global sea salinity, despite the fact that coliforms are recommended as indicators for fresh waters and enterococci as indicators for marine waters due to their resistance to temperature, salinity, pH, etc. it seems necessary that each region be studied and monitored according to its own relevant conditions so that the right information is obtained and the right decisions are made for choosing the proper microbial indicator. Therefore, the aim of this study was to determine the suitable microbial indicator for swimming spots in the east of the province since selecting an organism as the indicator is an important step towards managing the aquatic resource in any country, and in predicting the quality status of these resources, and eventually in dispensing the right information to the public and protecting their health.

Material and Methods

The sampling was carried out at 30 swimming stations designated by the municipalities in the east of the province (Fig. 1) between 39 s 53°18'8.19" and 52°13'55" geographical coordinates in the cities of Behshahr, Miandroud, Sari, Jouybar, Babolsar, Freydonkenar and Mahmoudabad at weekly intervals during the swimming season from September until May 2019. The samples were taken manually and in 1liter sterile bottles at 15-30 cm depth below the surface in 1meter deep waters. The samples were transferred to the laboratory in ice chest at 5ºC, and the membrane filtration (MF) technique conforming with Standard Methods 9222D and EPA method 1600 was performed 8 hours after the sampling. The MF approach for enterococci was used based on the selective culture medium (mEI, Dickinson, Sparks, MD and Becton culture medium) and the samples were incubated at 41ºC for 24 hours. The colonies with blue halos were enumerated as enterococci. In the MF for fecal coliforms enumeration, the modified agar culture medium (MD, Dickinson, Sparks and Becton) was used. The samples were incubated at 44.5±0.5ºC for 24 hours, and the colonies with non-blue halos were counted as fecal coliforms. Concurrent with the sampling, the environmental parameters were measured using a portable instrument of multi-parameter water quality measurement Hack model 156. The results were compared with the EPA and the Iranian Ministry of Healthcare standards for swimming water quality status. Then, statistical analyses were performed using the SPSS statistics software (version 19, SPSS, 2010).

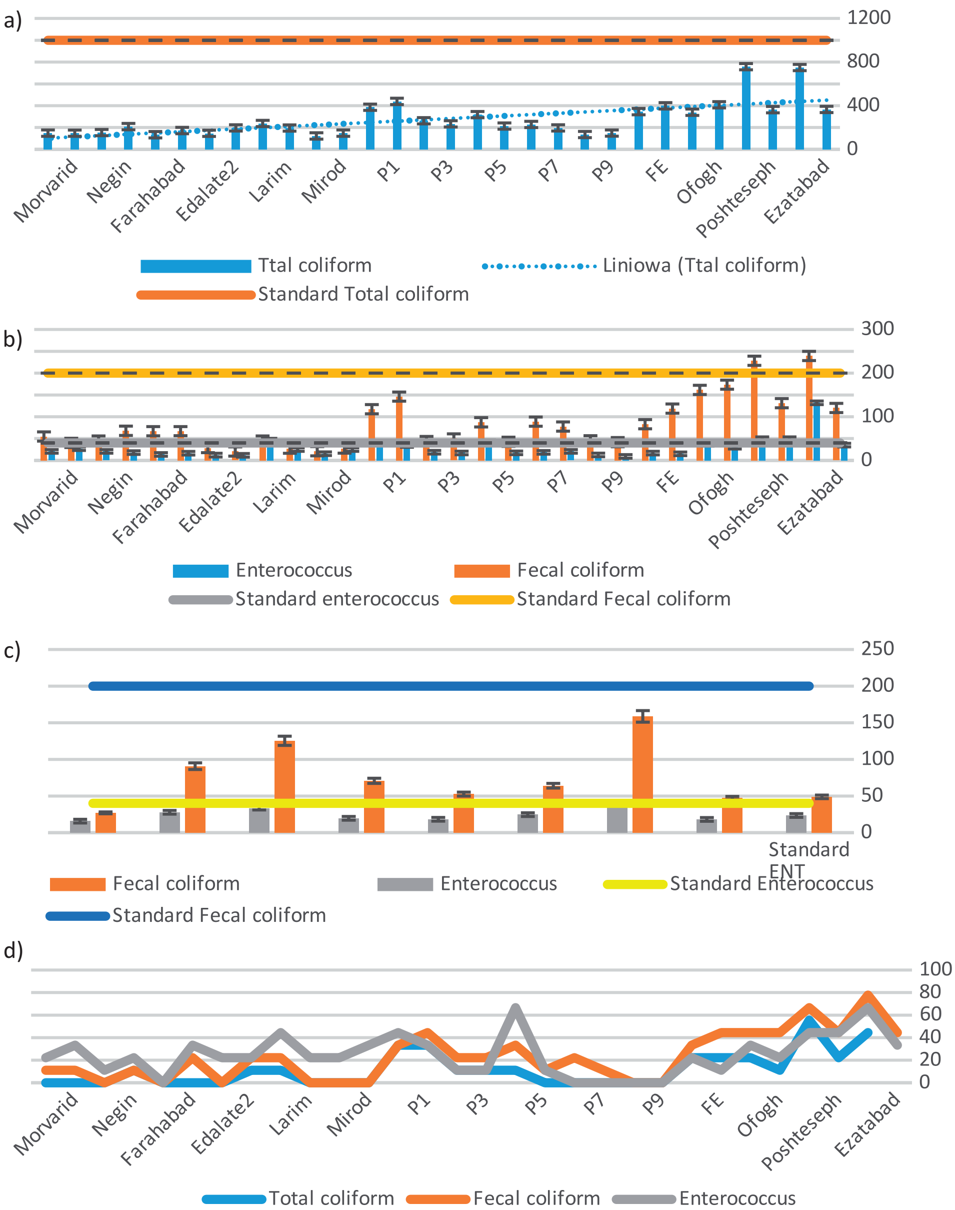

Fig. 1. a) Comparison of the total coliforms GM±SE at the sampling stations in eastern Mazandaran - The Caspian Sea coastal waters, b) Comparison of the fecal coliforms and enterococci GM±SE with standards at the sampling stations, c) Comparison of the total coliform, fecal coliform and enterococci, GM±SE at the sampling months, and d) Comparison of the observed percentages of pollution by total coliforms, fecal coliforms, and enterococci in sampling stations.

The sample distribution was performed using the Skewness and Shapiro-Wilk test of normality, and the usual descriptive statistics including the mean, the SD, the 75th percentile, and the geometric mean were done. The Kruskal-Wallis test was used for comparing the swimming stations and sampling times. The Pearson correlation coefficient was used for determining the relationship between the microbial results and the environmental parameters. Also, the Multiple-criteria decision making (MCDM) model, the Shannon entropy for weighting the microbial indicators, and The Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) for ranking the sampling stations were used.

Results and Discussion

As seen in Table 1, the means for the measured microbial indicators and the geometric mean for all three indicators matched the EPA and the Healthcare Ministry standards. The minimum enterococci and fecal coliform were 3 and the maximum was 1100 CFU/100. The results showed that the mean (standard deviation) of microbial indicators for Total coliform, Fecal coliform and Enterococci were 22.58231 (361.3481), 129.3778 (9.59933), and 44.0111 (6.12035), respectively. In addition, the highest and lowest microbial indicators were (2400 and 39), (1100 and 3) and (1100 and 3), respectively. The results were less than the Iranian standard range. The environmental parameters

Table 1. The values for the 75th percentiles of the microbial parameters of sampling stations.

Microbial indicators	Sadaf	Morvarid	Emam	Negin	Touska	Basij	E1	Edalat2	E4	Larim	Chaparoud	Miroud	P0	P1	P2
Total coliform	240	210	240	460	210	240	210	460	460	460	240	240	1100	1100	460
Fecal coliform	43	75	93	150	93	150	43	43	150	93	39	64	210	460	150
Enterococci	23	43	39	23	21	64	21	21	93	39	39	43	64	64	39
Microbial indicators	P3	P4	P5	P6	P7	P8	P9	FW	FE	Ghori	Ofogh	Daraee	Sepah	Jafarabad	Ezat abad
Total coliform	460	460	460	460	240	210	210	460	460	460	460	1100	460	1100	460
Fecal coliform	150	460	93	150	150	64	64	210	240	240	240	460	460	460	460
Enterococci	39	64	21	23	23	21	9	39	64	64	39	93	64	210	64

of temperature, salinity, and pH were measured, where the means for salinity and temperature were 10.2720±0.07 ppt and 26.1 348±0.15ºC and the ranges of salinity and temperature were 7.52-12.90 ppt and 20.20-29.8ºC, respectively. In addition to the geometric means, the 75th percentiles of the indicators were calculated. The 75th percentiles for enterococci, fecal coliforms, and total coliforms were 42.28, 175.1325, and 464 CFU/100, respectively, with enterococci being higher than the standard (Table 1). The Skewness and Shapiro-Wilk test showed that the population of the bacteria under study do not have normal distribution. Therefore, the Kruskal-Wallis test was used for comparing the sampling stations, which showed that these swimming zones are significantly different at $P<0.05$ in terms of total coliforms pollution (Chi-square = 78.287, df = 29 Sig = 0.000). Despite the observation of an almost increasing trend in the pollution of the swimming zones under study as we moved from east to west, the level of total coliforms was obtained corresponding to the standards, and the highest total coliforms levels belonged to Daraee and Jafarabad swimming zones with the geometric means being 757.5 and 749 CFU/100ml, respectively (Fig. 1a). The Kruskal-Wallis test showed that the stations are significantly different at $P<0.05$ in pollution by fecal coliforms (Chi-square = 81.086, df = 29 Sig = 0.000). Among the studied swimming zones, the two stations of Daraee and Jafarabad showed the highest pollution with the geometric mean being 239.4 and 228.4 CFU/100 ml, respectively. With pollution by enterococci too the Kruskal-Wallis test showed significant differences among the sampling stations. (Chi-square = 83.978, df= 29 Sig= 0.000). From the stations under study, the Jafarabad, Daraee, Sepah, Edalat4, P4 and P0 swimming zones were the most polluted of all the stations in all three indicators measured in the study (Fig 1b). The Kruskal-Wallis test showed that, in terms of the month of sampling, the swimming zones are significantly different at $P<0.05$ in total coliforms (Chi-square = 28.242 40.787, df = 8 Sig = 0.000), fecal coliforms (Chi-square = 81.086, df = 8 Sig = 0.000), and enterococci (Chi-square = 21.925, df = 29 Sig = 0.000). Among the months studied, only June H1 had enterococci higher than the standard, and at other sampling times the geometric means of the microbial parameters corresponded to the standards (Fig 1c). The results obtained from the indicators were superimposed on the Iranian and the EPA single sample standards. The comparison with those of the EPA indicates very low pollution at the swimming zones, and the observed pollution compared to the total samples were 1.1% (≥2400MPN/100ml), 8.5% (≥400MPN/100ml), and 5.9% (≥104MPN) for total coliforms, fecal coliforms, and enterococci, respectively. However, compared to the Iranian standards, examining the single samples showed 11.85% (≥ 1000 MPN/100 ml), 22.59% (≥ 200 MPN/100 ml (and 25.55 % (≥40 MPN/100 ml) pollutions for total coliforms, fecal coliforms, and enterococci, respectively.

Table 2. Ranking the sampling stations using the TOPSIS method.

Station	Rank	Pi	Station	Rank	Pi
P9	1	0.999968	Negin	16	0.993114
P8	2	0.999714	P2	17	0.991369
Emam	3	0.999323	Farahabad	18	0.990842
Touska	4	0.998699	FW	19	0.985319
Edalat1	5	0.997759	FE	20	0.983479
Larim	6	0.997414	P4	21	0.977004
Morvarid	7	0.996985	Ofogh	22	0.975857
Chapakroud	8	0.995782	Ezatabad	23	0.973606
Sadaf	9	0.995667	GHori	24	0.965062
P3	10	0.994437	Edalat4	25	0.964145
P6	11	0.994007	P1	26	0.956144
Miroud	12	0.995419	P0	27	0.921659
Edalat2	13	0.995401	POshtesepah	28	0.903181
P7	14	0.9936001	Draee	29	0.748024
P5	15	0.993408	Jafarabad	30	0.184558

Among the 13 sampling stations, the eastern stations belonging to the cities of Behshahr, Miandroud, Sari and Jouybar had an enterococci pollution of 78.78%, while as we go to the west, the pollution by fecal coliforms rises so much so that the swimming zones in the cities of Babolsar, Freydonkenar and Mahmoudabad had 56.84% and 43.16% of pollution by fecal coliforms and enterococci, respectively (Fig 1d). The MCDM models were used for determining the best indicator and ranking the stations. The entropy method was used for weighting the indicators, showing that the right indicators in swimming water pollution monitoring are in order of appearance enterococci, fecal coliforms, and total coliforms, with the importance of the first two as almost equal. In addition, with the fecal coliforms and enterococci indicators for the studied cities, the entropy method showed a higher importance of enterococci for the swimming zones in the eastern cities of Behshahr, Miandroud, Sari and Jouybar and a higher importance of fecal coliforms for those in the western cities of Babolsar, Freydonkenar and Mahmoudabad. The swimming zones were ranked based on the TOPSIS method where the P9 swimming zone ranked first and the Jafarabad and Daraee swimming zones became the most polluted (Table 2).

In this study, the geometric means for the microbial indicators were at expected ranges, but with single samples some cases of pollution were observed indicating the need for continuous monitoring for public health, understanding the environmental conditions and the quality of the coastal waters. Many studies have been conducted in the world. The EPA has announced enterococci as the marine water indicator [11]. Regarding coastal water quality monitoring among which we can name those conducted on Southern California waters by Cordero et al. in 2012 [17] and Doreen et al. in 2015 [18], and on Hawaii waters by Fujioka et al. 2015 [19], and on the Erie Lake by King et al., 2016 [20]. The most practical approach in coastal marine water monitoring is to use microbial indicators. The microbial indicators total coliforms, fecal coliforms, and enterococci are used to determine the water pollution status as these indicators are highly affected by fecal pollutions. In the results from the present study, the value for the weight vector of the geometric mean of enterococci was higher, indicating that enterococci are more important. But from the single samples of the swimming stations, pollutions were observed from one or several microbial indicators with difference from one city to another, so that in further east stations (Behshahr, Miandroud and Sari) the pollution arose from enterococci with lower fecal coliforms pollution while in stations further west (Babolsar, Freydonkenar and Mahmoudabad) there were pollutions exclusively from fecal coliforms or from fecal coliforms in addition to enterococci, supporting the need to determine with appropriate indicators, enterococci, fecal coliforms, or both, or even alternating indicators, as the right microbial indicator and the standards vary from country to country and require assessments [21-23]. The results from this study showed that the weight vector obtained for fecal coliforms is very close to that of enterococci. Also, at some swimming zones, fecal coliforms had a weight vector higher than that of enterococci, indicating the importance of this indicator in addition to that of enterococci in the coastal waters of this region. Anderson, 2005 stated that enterococci and fecal coliforms were recommended by the EPA as two indicators in assessing the microbial safety of waters, but they behave differently in different environments [24]. Hanes and Fragala 1967 suggested using multiple indicators for recreational waters [25]. In 2012, the EPA stated that new regulations are needed for protecting swimmers' health so that ambiguities and shortcomings in water regulations for indicator designation are eliminated. It asserted that further studies were required particularly in tropical climates and in waters affected by urban sewage and animal feces. In 2012, Recreational Water Quality Criteria (RWQC) by EPA stated the selection of an indicator as an important step in water resource management and water quality prediction [26]. Radojevic et al., 2012 claims that designating the appropriate indicator, accurate Based on the results obtained in this study, for the proper public advisories and particularly dispensing the right knowledge to swimmers and eliminating ambiguities and conflicts arising from different indicator results in this region, it is suggested that the geometric mean for enterococci be considered for the overall health of the water throughout the year, but considering the environmental conditions of the region, it is advised that, with single samples, in case the enterococci is negative, fecal coliforms be taken into account in the Healthcare Ministry regulations as the accompanying indicator of swimming water health especially in western swimming zones so that this indicator is also assessed in addition to enterococci. standards, and continuous monitoring are means to attain certainty about water health [26, 27]. The effect of environmental parameters was also studied since a combination of biological or chemical and physical factors of saline water affect the presence of the indicator bacteria [18].

Conclusions

The results showed that salinity is lower in the western swimming zones due to the multiplicity of rivers, the volumetric flow rate of river mouths, higher and heavier rainfalls, and the consequent increase in runoff. Salinity had a negative effect on the indicators, and the density of the indicators decreased as salinity increased, so that the swimming zones to the east had higher salinity and lower pollution than those to the west. Enterococci (0.178) had a higher resistance at 0.05 than fecal coliforms (0.282) and total coliforms (0.377). Also, dissolved oxygen affected the indicators negatively as these bacteria are facultative anaerobic and prefer anaerobic conditions. As enterococci were more resistant, these results support their higher durability in marine water. No significant relationship was found with temperature and pH as their variation range was slight.

Conflict of Interest

The authors declare no conflict of interest.

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