Original Article

Microbial Quality of Coastal Areas of Bandar Abbas City: Is there any Potential Risks for Swimmers?

Nayereh Besharatipour[1,2], Mahnaz Nikaeen[2], Bijan Bina[2,4], Sahar Gholipour[2], Akbar Hassanzadeh[3]

[1]Student Research Committee, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran,
[2]Department of Environmental Health Engineering, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran,
[3]Department of Biostatistics and Epidemiology, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran, [4]Environment Research Center, Research Institute for Primordial Prevention of Non-communicable disease, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Aim: This study was conducted on the microbial contamination of water of Bandar Abbas beaches in order to achieve comprehensive information for determining the quality of swimming coasts. **Materials and Methods:** After initial examination of the number and location of swimming coasts in terms of appearance and areas with the highest number of swimmers, 5 coastal swimming areas were selected as sampling sites. Sampling was done for 6 months and 10 samples per month. Physicochemical (water temperature, pH, turbidity, electrical conductivity, and salinity) and microbial indicators including total and fecal coliforms, *Escherichia coli*, fecal streptococci, *Clostridium perfringens* as well as *Salmonella* as a pathogenic microorganism were measured in each sampling period. **Results:** Results of this study showed that the mean number of streptococci, total coliforms, fecal coliforms, and *E. coli* was 930, 24,000, 9300, and 9300 most probable number (MPN)/100 ml, respectively. It was also found that the mean concentration of *C. perfringens* in the sampling stations was 250 CFU/100 ml. The frequency of detection of *Salmonella* in stations 2 and 3 was 16.7% and in station 5 was 8.3% and was not observed in other stations. According to the results, in most sampling stations, the concentration of the microbial indicators was higher than the standard. A significant relationship between different species of bacteria was observed. The results also showed a significant relationship between the amount of turbidity and microbial ($P < 0.05$). **Conclusion:** According to the results of the present study, it was observed that most of the swimming coasts of Bandar Abbas were not in a favorable microbial condition, due to the discharge of industrial and municipal sewage and waste disposal. The results highlight the potential risk of microbial pathogens for swimmers and the necessity of sanitation practices of coastal area to protect public health.

Keywords: *Clostridium perfringens*, fecal indicator bacteria, microbial quality, streptococci, swimming coasts

Introduction

Rapid population growth in cities, development of residential, industrial, and commercial centers around the coastal regions, discharge of municipal, and industrial wastewater at a high content of pathogens and other pollutants, such as heavy metals, to the swimming coasts, have been led to the contamination of natural swimming areas in recent years.[1,2] On the other hand, often a large amount of wastes is disposed into coastal areas, which can be dangerous to the health of swimmers, fishermen, sailors, etc., and affect the ecology.[3]

A variety of opportunistic and pathogenic microorganisms can enter the natural sources through urban wastewater, waste disposal as well as body of swimmers, due to illness and noncompliance with preswimming principles, and cause a variety of infections.[4,5] Some of the infections can cause acute and chronic diseases such as cutaneous, gastrointestinal and respiratory diseases.[6] According to the US Centers for Disease Control and Prevention, 26 out of 1363 types of diseases were reported to be due to the use of recreational water from 1986 to 1988, from which 6 cases were caused by the outbreak of lake-related diseases.[7] The studies carried out on the coasts of Hong Kong showed that the swimmers were at greater risk of gastrointestinal and respiratory diseases and infections of the eye, ear, and skin than others; the health problems were also higher on the contaminated coasts.[8]

Access this article online

Quick Response Code:

Website: www.ijehe.org

DOI: 10.4103/ijehe.ijehe_12_20

Address for correspondence: Prof. Bijan Bina, Department of Environmental Health Engineering, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: bbina123@yahoo.com

How to cite this article: Besharatipour N, Nikaeen M, Bina B, Gholipour S, Hassanzadeh A. Microbial quality of coastal areas of Bandar Abbas city: Is there any potential risks for swimmers? Int J Env Health Eng 2020;9:15.

Received: 01-02-2020, **Accepted:** 13-07-2020, **Published:** 31-12-2020

In total, the incidence of disease in swimming groups is higher than that of non-swimming groups, regardless of the water quality of the swimming areas.[9]

Among pathogens, bacteria are the most important microbial contaminants.[10] Fecal indicator bacteria (FIB) including total and fecal coliforms, *Escherichia coli*, *Streptococcus faecalis,* and *Clostridium perfringens* as well as opportunistic bacteria such as, *Pseudomonas aeruginosa,* and *Aeromonas hydrophila*[11,12] are used as the most important indictors to evaluate the microbial quality of coastal waters and consequently the health risk of swimmers. For example, Cabelli (1989) showed that, with an increase in the concentration of enterococci, the risk of gastrointestinal diseases increased among swimmers on the New Jersey coasts.[13] Brinks *et al*. also found a significant relationship between increased enterococci and gastrointestinal diseases among the swimmers.[3]

In order to determine the microbial quality of seawater for swimming, the World Health Organization (WHO) and European Economic Commission have specified some of the microbial agents (total and fecal coliforms, intestinal enterococci, and *Pseudomonas*) as indicator.[11] Concerning the best indicator for water of coastal and recreational swimming areas, WHO has introduced intestinal enterococci as a suitable indicator with the average number equal to or smaller than 40 per 100 ml. Environmental Protection Agency's guideline, pointed out the *E. coli* and intestinal enterococci as the good indicators for gastroenteritis associated with swimming.[7]

Coastal areas of Bandar Abbas are among the most important swimming areas in IRAN. However, one of the major challenges is the entry of municipal and industrial wastewater into some coasts of Bandar Abbas, which may affect the coastal swimming areas of the city and lead to health hazards for swimmers. Therefore, the present study was carried out to investigate the microbial quality of swimming areas of Bandar Abbas to assess the potential health hazards to swimmers.

Materials and Methods

This study was conducted in Bandar Abbas city to determine the microbial quality of sea water in terms of FIB including total coliforms, thermophilic or fecal coliforms, *E. coli*, fecal streptococci as well as *C. perfringens* as an indicator for the presence of protozoa. The presence of *Salmonella* as a pathogenic microorganism in coastal swimming areas was also investigated. After investigation about the number and location of the swimming areas based on the appearance and areas with the highest number of swimmers, 5 coastal swimming areas were selected as the sampling sites.

Samples were taken from the swimming areas during 6 months, every two weeks, in spring and summer, in accordance with the *Standard methods* at the distance of 1–1.5 m from the coast and depth of 20–30 cm below the water level. In total, 60 samples were collected. Sterilized glass bottles were used for sampling and the samples were stored in a cold chain (within a cold box) at 4°C until reaching the laboratory.[6]

To measure the physicochemical parameters (temperature, pH, EC, salinity, and turbidity), the multi-parameter meter (WTW, Germany) was used in the sampling time.

According to the proposed Standard Methods and National Standard No. 3759–3760, the culture medium of lauryl tryptose broth (probable stage), brilliant green bile broth (confirmatory stage), and EC broth were used for the presence of total and fecal coliforms,[6] LMX agar was used for the detection of *E. coli*. After incubation time, the number of FIB in positive samples were reported as the MPN per 100 ml.[14]

According to the proposed Standard Methods and National Standard No. 3619, detection of fecal streptococci was performed by glucose azide for the enrichment stage. Bile esculin azide agar and the catalase test were used for confirmatory and complementary step, respectively, and the results were reported as MPN per 100 ml.[6]

Membrane filter and TSC agar were used to identify *C. perfringens* as described previously.[15] The results were presented as colony forming units per 100 ml (cfu/100 ml). To confirm the suspected colonies considered as *C. perfringens*, polymerase chain reaction (PCR) using specific primers [Table 1] was performed on bacterial isolates.

To test the presence of *Salmonella*, 100 ml of samples was filtered through a membrane filter and then filter was transferred to the peptone water. After primary enrichment, samples were transferred to selenite F and then onto SS agar medium (*Salmonella*-Shigella Agar).[6,16]

To confirm the detection of *Salmonella* and *C. perfringens,* a loopful of each colony was inoculated into 100 µl of deionized water. DNA was extracted by boiling for 15 min and centrifugation at 13,000 rpm for 10 min. Supernatant was used for PCR amplification. All PCR reactions were performed in a final volume of 15 µl containing 7.5 µl of 2× premix (2 mM MgCl2, Ampliqon-Denmark), 0.2 µM of each primer, and 2 µl template DNA. The PCR cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of 30 s at 94°C, primer annealing at varied temperatures [Table 1] for 30 s, primer extension at 72°C for 30 s, and final extension at 72°C for 10 min. All PCR assays included positive (*Salmonella*, *C. perfringens*) and negative controls (deionized water). PCR products were analyzed by electrophoresis using 1.5% (w/v) agarose gel. Gels were analyzed using an ultraviolet (UV) transilluminator (UV Tech, France).

Statistical analyses

After performing microbial tests on each sample, the descriptive analysis was performed on microbial information. Pearson's correlation and ANOVA were performed for analysis

of correlation between the microbial groups and difference between the microbial and physicochemical quality of coastal areas, respectively. The results were analyzed using SPSS 22 (International Business Machines Corporation, IBM, Armonk, New York, USA). The probability level was also evaluated with coefficient of 99.95% and significant level of 0.05.

Results

This study was conducted to investigate the microbial quality of water of coastal areas of Bandar Abbas city. In the period of sampling time, 19 coasts had ebb current (31.7%) and 41 coasts had flow current (68.3%).

As shown in Table 2, the physicochemical parameters of sampling stations are presented. According to the results, there was no significant difference between the various stations in terms of physicochemical parameters.

The microbial quality of Coastal areas of Bandar Abbas is summerized in Table 3.

The results showed that the number of bacteria in station 3 was significantly different from that of other stations ($P < 0.05$). The highest values of bacteria were measured in this station with concentration of 24,000, 9300, 9300, and 930 (MPN/100 ml) for total coliforms, fecal coliforms, *E. coli,* and fecal streptococcus, respectively, as well as 250 cfu/100 ml for *Clostridium*.

As shown in Figure 1, in station 5, none of the samples had concentration exceeding the standard for any of the indicator bacteria.

Regarding the fecal streptococcus, station 3 had the highest frequency of the samples at the concentrations above the standard, but in the case of total coliforms and fecal coliforms, station 2 had the highest frequency. Stations 2 and 3 had similar frequencies in terms of *E. coli* concentrations above the standard limit.

The presence or absence of *Salmonella* in coastal areas is summerized in Table 4.

According to the statistical analysis, the frequency of *Salmonella* detection did not differ significantly between the stations ($P > 0.05$).

Table 5 shows the correlation between analyzed parameters.

Discussion

Coastal water contamination causes many health problems. One of the important parameters for determining the quality of swimming beaches is determining the microbial quality.

As shown in Table 2, the physicochemical parameters of sampling stations are presented. According to the results, stations 2 and 4 had the maximum pH of 8.38. Stations 4 and 5 also had the highest electrical conductivity of 57 and station 3 had the highest turbidity. The results also showed [Table 3] that the number of bacteria in station 3 was significantly different from other stations ($P < 0.05$).

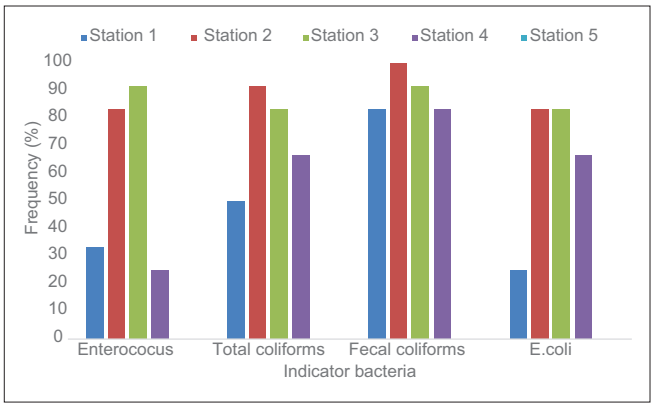

Figure 1: Frequency of microbial indicators above the standard in the five studied stations (*Escherichia coli* standard: 100 MPN/100 ml, fecal coliforms: 100 MPN/100 ml, total coliforms: 460 MPN/100 ml and fecal streptococcus: 40 MPN/100 ml

Table 1: Primer sets for DNA amplification

Bacteria (target gene)	Oligonucleotide sequences	Amplification length (bp)	Annealing temperature	References
Salmonella (invA)	5'- GTGAAATTATCGCCACGTTCGGGCA-3'	284	59	[16]
	5'- TCATCGCACCGTCAAAGGAACC-3'			
Clostridium (16s rRNA)	5'- GGGGGTTTCAACACCTCC-3'	170	59	[17]
	5'- GCAAGGGATGTCAAGT-3'			

Table 2: Mean (standard deviation) of physicochemical parameters of sampling stations

Sampling station (coastal area)	Turbidity (NTU)	Salinity (%)	EC (ms/cm)	pH	Temperature (°C)
1	10.67 (5.3)	38.13 (0.46)	56.62 (0.73)	7.98-8.45	36.09 (2.94)
2	14.58 (4.1)	38.07 (0.14)	56.53 (0.75)	8.27-8.55	35.58 (2.94)
3	16.17 (2.89)	37.26 (2.21)	55.58 (2.96)	8-8.55	35.63 (2.83)
4	12.67 (5.05)	38.49 (0.58)	57.13 (0.62)	8.2-8.69	35.73 (2.74)
5	7.42 (3.8)	38.68 (0.75)	57.14 (0.68)	8.25-8.55	35.35 (2.88)
Total	12.3 (5.19)	38.13 (1.18)	56.6 (1.52)	7.98-8.69	35.68 (2.78)

EC: Electrical conductivity

Table 3: Mean (standard deviation) of microbial indicators in sampling stations

Sampling station	*Clostridium* (CFU/100)	*E. coli* (MPN/100 ml)	Fecal coliform (MPN/100 ml)	Total coliforms (MPN/100 ml)	*Enterococci* (MPN/100 ml)
1	24.58 (16.29)	230.72 (451.44)	326.33 (412.8)	1163 (1293.15)	28.73 (33.63)
2	63.08 (87.91)	580.5 (655.81)	649.17 (608.96)	2380.83 (2325.34)	74.93 (46.84)
3	105.42 (107.26)	2535.5 (3417.12)	2706.08 (3331.81)	6877.5 (7031.73)	233.59 (257.57)
4	43 (70.19)	278.83 (298.38)	307.33 (276.74)	1471.7 (1247.4)	31.69 (34.12)
5	14.5 (7.9)	26.5 (35.2)	41.84 (32.34)	82.76 (79)	7.08 (5.97)
Total	50.11 (74.98)	730.41 (1781.11)	806.15 (1772.14)	2395.15 (4060.3)	75.2 (141.71)

E. coli: Escherichia coli

Table 4: Frequency distribution of *Salmonella* detection at different stations

Station	Frequency (%)
1	0
2	16.7
3	16.7
4	0
5	8.3

The frequency of *Salmonella* detection in stations 2 and 3 was 16.7% and in station 5 was 8.3% and was not observed in the other stations. From the causes of microbial contamination of stations 1–4, the discharge of untreated wastewater, effluent disposal of water treatment plant as well as disposal of household wastes into seafronts can be mentioned; however, in station 5, due to the proper conditions of disposal and treatment of wastewater in the upstream area and connection of upstream areas to the wastewater network, less pollutant sources entered the station, which leads to better quality of water in this station. In the proximity of the sampling stations, there were a number of outlets that discharged sanitary and industrial wastewater as well as a large volume of urban wastes, which finally lead to the contamination of these areas. Moreover, large numbers of users and swimmers in the area, especially in the warm season, as well as lack of adequate sanitation facilities, such as toilets and showers are effective in causing such contamination. The discharge of contaminants into water sources could increases the rate of turbidity which prevent the penetration of UV radiation into water sources and consequently increase the load of microbial contamination in the swimming coasts. In the study of Shahryari *et al*. about the microbial contamination of the Caspian Sea in Gorgan Gulf, the results showed that the mean concentration of total and fecal coliforms were 1555 and 817 per 100 ml, respectively, which had better quality than the sampling sites of the present work.[17] The study by Binesh Barahmand *et al*. for evaluating the quality of water on the southern coast of the Caspian Sea also showed that the mean concentration of total coliforms in two stations and the mean thermophilic coliforms in six stations were above the standard. With respect to the standards related to swimming areas they concluded that there is a potential risk for swimmers.[18] In a similar study conducted by Noroozi *et al*. in Bushehr province, the mean concentration of total and fecal coliforms and *Pseudomonas* were 540, 165.56, and 6 MPN per 100 ml, respectively,[11] which had better quality than the results of the present study. This difference could be due to the less discharge of wastes into the studied coasts. In the present study, Pearson's statistical test was used to determine the relationship between microbial and physicochemical factors [Table 5]. The results showed a significant relationship between different species of bacteria, which was consistent with the results of Praveena *et al*. and Viau *et al*.,[19,20] The results also demonstrated the negative relationship of temperature, pH, EC, and salinity with the amount of examined bacteria, but due to the low range of values of these parameters, no specific outcome can be explained. The results of the present study were in agreement with those of Moresco *et al*., showing that with an increase in salinity and temperature, the concentration of organisms on swimming coasts is decreased.[21] The results of this study also showed a significant relationship between the amount of turbidity and microbial factors, which was consistent with the results of Binesh Barahmand *et al*.[22]

Conclusion

This study aimed to investigate the microbial indicators in swimming stations of Bandar Abbas city. According to the results, the swimming coasts of Bandar Abbas were not in a favorable microbial condition, due to the discharge of industrial and municipal sewage and waste disposal. Based on the results obtained from most of the sampling stations, the concentration of microbial indicators was above the standard; therefore, it is essential to plan for marine sanitation and preventing the entry of wastewater and effluent into the stations to protect public health. Further studies are also needed to investigate the presence of other pathogens in coastal regions and the risks posed to swimmers health.

Acknowledgment

We would like to thank the workers and the head of Department of Environment Hormozgan Provincial Directorate.

Financial support and sponsorship

This research was supported by the Vice Chancellery for Research at the Isfahan University of Medical Sciences (Grant

Table 5: Correlation matrix of parameters analyzed in sampling stations

Variables	Enterococci	Total coliforms	Fecal coliform	*E. coli*	Clostridium	Temperature	pH	EC	Salinity	Turbidity
Enterococci	1									
Total coliforms	0.791**	1								
Fecal coliform	0.689**	0.898**	1							
E. coli	0.649**	0.872**	0.99**	1						
Clostridium	0.407**	0.587**	0.512**	**0.468	1					
Temperature	-0.002	-0.237	-0.292*	-0.302*	-0.132	1				
pH	-0.398**	-0.192	-0.208	-0.207	-0.088	-0.269*	1			
EC	-0.74**	-0.76**	-0.753**	-0.755**	-0.378**	0.177	0.314**	1		
Salinity	-0.737**	-0.786	-0.761**	-0.756**	-0.48**	0.254*	0.267*	0.859**	1	
Turbidity	0.344**	0.377**	0.288*	0.277*	0.344**	0.353**	-0.105	-0.174	-0.228	1

EC: Electrical conductivity, *E. coli: Escherichia coli*

No.) 396886. "Ethics Code" of this study was IR. MUI. REC.1396.3.886.

Conflicts of interest

There are no conflicts of interest.

References

1. Bedri Z, Corkery A, O'Sullivan JJ, Deering LA, Demeter K, Meijer WG, *et al*. Evaluating a microbial water quality prediction model for beach management under the revised EU Bathing Water Directive. J Environ Manage 2016;167:49-58.
2. Pond KR, Cronin AA, Pedley S. Recreational water quality in the Caspian Sea. J Water Health 2005;3:129-38.
3. Brinks MV, Dwight RH, Osgood ND, Sharavanakumar G, Turbow DJ, El-Gohary M, *et al*. Health risk of bathing in Southern California coastal waters. Arch Environ Occup Health 2008;63:123-35.
4. Dakka HA, Aljaroucha AE, Allam NG, Shaltout KH. Epidemiological and etiological agents of water borne infections among the bathers in the Mediterranean Sea water in Gaza Strip. Egyp J Experim Biol (Botany) 2018;14:299-306.
5. Sunderland D, Graczyk TK, Tamang L, Breysse PN. Impact of bathers on levels of Cryptosporidium parvum oocysts and Giardia lamblia cysts in recreational beach waters. Water Res 2007;41:3483-9.
6. APHA. Standard Methods for the Examination of Water and Wastewater. 21st ed. USA: APHA; 2013.
7. World Health Organization. Guidelines for Safe Recreational Water Environments: Coastal and Fresh Waters. Switzerland: World Health Organization; 2003.
8. Thoe W, Lee OHK, Leung KF, Lee T, Ashbolt NJ, Yang RR, *et al*. Twenty five years of beach monitoring in Hong Kong: A re-examination of the beach water quality classification scheme from a comparative and global perspective. Mar Pollut Bull 2018;131:793-803.
9. Delille D, Gleizon F. Distribution of enteric bacteria in Antarctic seawater surrounding the Port-aux-Français permanent station (Kerguelen Island). Mar Pollut Bull 2003;46:1179-83.
10. Papadopoulou C, Economou V, Sakkas H, Gousia P, Giannakopoulos X, Dontorou C, *et al*. Microbiological quality of indoor and outdoor swimming pools in Greece: Investigation of the antibiotic resistance of the bacterial isolates. Int J Hyg Environ Health 2008;211:385-97.
11. Noroozi Karbasdehi V, Dobaadaran S, Mohamadi SR, Mokhtari H, Darabi H, Faraji F. Survey of microbiological and chemical quality of the swimming beaches along the persian gulf in port. Iran South Med J 2015;18:393-408.
12. Zakaryaee M, Sefatian S, Saeedi AA, Nasrolahzadeh Saravi H, Adel M. Microbiological quality of some swimming water in the Caspian Sea in Mazandaran province beaches, Iran. J Mazandaran Univ Med Sci 2014;23:159-63.
13. Cabelli VJ. Swimming-associated illness and recreational water quality criteria. Water Sci Technol 1989;21:13-21.
14. Nikaeen M, Pejhan A, Jalali M. Rapid monitoring of indicator coliforms in drinking water by an enzymatic assay. J Environ Health Sci Eng 2009;6:7e10.
15. Shahryari A, Nikaeen M, Khiadani M, Nabavi F, Hatamzadeh M, Hassanzadeh A. Applicability of universal Bacteroidales genetic marker for microbial monitoring of drinking water sources in comparison to conventional indicators. Environ Monitoring Assessment 2014;186:7055-62.
16. Farhadkhani M, Nikaeen M, Yadegarfar G, Hatamzadeh M, Pourmohammadbagher H, Sahbaei Z, *et al*. Effects of irrigation with secondary treated wastewater on physicochemical and microbial properties of soil and produce safety in a semi-arid area. Water Res 2018;144:356-64.
17. Shahryari A, Kabir MJ. Kolsoom golfirozy evaluation of microbial pollution of Caspian Sea at the Gorgan Gulf. J Gorgan Univ Med Sci 2008;10:69-73.
18. Binesh Barahmand M, Nabizadeh R, Nadafi K. Qualitative analysis of coastal waters in the Caspian Sea in Guilan province: Determining the environmental health indicators in swimming areas. J Mazandaran Univ Med Sci 2012;22:41-52.
19. Praveena SM, Chen KS, Ismail SN. Indicators of microbial beach water quality: Preliminary findings from Teluk Kemang beach, Port Dickson (Malaysia). Mar Pollut Bull 2013;76:417-9.
20. Viau EJ, Goodwin KD, Yamahara KM, Layton BA, Sassoubre LM, Burns SL, *et al*. Bacterial pathogens in Hawaiian coastal streams-associations with fecal indicators, land cover, and water quality. Water Res 2011;45:3279-90.
21. Moresco V, Viancelli A, Nascimento MA, Souza DS, Ramos AP, Garcia LA, *et al*. Microbiological and physicochemical analysis of the coastal waters of southern Brazil. Mar Pollut Bull 2012;64:40-8.
22. Nagpal R, Ogata K, Tsuji H, Matsuda K, Takahashi T, Nomoto K, *et al*. Sensitive quantification of *Clostridium perfringens* in human feces by quantitative real-time PCR targeting alpha-toxin and enterotoxin genes. BMC Microbiol 2015;15:219.