Evaluating spatial-temporal variations and correlation between fecal indicator bacteria (FIB) in marine bathing beaches

Jingfeng Fan, Hongxia Ming, Lili Li and Jie Su

ABSTRACT

The horizontal distribution and temporal variation of bacterial indicators (total coliforms (TC), fecal coliforms (FC), enterococcus (EC) and *Escherichia coli* (*E. coli*)) were investigated to identify the proper bacterial indicators for a marine bathing beach in China. Two different sampling efforts were conducted during dry weather and two large rain events at Xinghai Bathing Beach in Dalian, China. Samples were collected from three different water depths and analyzed for the four indicator bacteria. The results indicated that all four bacterial indicators exceeded the single sample standards at different levels. Specifically, the water quality exceeded the standard for TC, FC, EC and *E. coli* in 7%, 28%, 38% and 10% of the samples, respectively. Comparison of the rate of the indicators before and after rainfall revealed a significant increasing post-rainfall. The concentrations of bacteria differed significantly with distance from the shoreline, with knee-depth near the shore exceeding the standard most frequently. This was primarily due to contamination by excessive sewage discharge and rainfall. Based upon the concentration of indicators and exceedance rates, as well as the correlation between indicators, both EC and FC should be evaluated at the same time as fecal pollution bacterial indicators in marine bathing beaches in China.

Key words | bacterial indicators, bathing beach, *Escherichia coli*, fecal coliforms, total coliforms

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INTRODUCTION

There is increasing concern regarding the dumping of urban and industrial wastewater into the sea owing to the high level of pathogenic and other polluting agents they contain and their potential consequences on both health and ecology. Gastrointestinal (GI) pathogenic organisms pose a great threat to water systems used for recreation. Numerous studies have demonstrated a relationship between fecal indicator bacteria (FIB) in marine beaches and swimming-related illnesses (Prüss 1998; Wade *et al.* 2003; US EPA 2012). Epidemiological studies of the health risks associated with recreational water have focused on the identification of water quality indicators that can effectively predict illness (Haile *et al.* 1999). Monitoring of FIB in recreational water systems is used to assess the microbiological safety of water that swimmers come into contact with at such sites (He & He 2008). The FIB concentrations of seawater have been used for decades to assess the risk of swimming-related illnesses (Colford *et al.* 2007). Based on the epidemiological studies conducted to date, it is evident that no single indicator can predict illness consistently in all environments at all times. Because many beaches have multiple contamination sources, such as animal vs. human sources, the size of the contributing population depends on environmental contamination. Nevertheless, FIB levels in recreational water systems have been by far shown the most frequently correlated with GI illness in swimmers (US EPA 2012).

It is important to select adequate bacterial indicators and indicator levels that directly affect the results as standards to assess recreational water quality. Owing to differences in the regional characteristics of bacteria, different indicator bacteria and different indicator levels have been used as standards by water quality programs in different states,

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countries, and regions. However, there is no universal agreement regarding which indicator organism(s) is most useful owing to the effects of varying sampling collection times, locations, and rainfall. Samples collected at different times of the day and different locations can lead to significantly different levels because of a number of environmental factors, including radiation, rainfall, tide, and the presence of bathers (Enns *et al.* 2012; Zhang *et al.* 2013).

This study was conducted to investigate the spatio-temporal variation and correlation of four bacterial indicators (total coliforms (TC), fecal coliforms (FC), enterococci (EC) and *Escherichia coli* (*E. coli*)) at Xinghai Bathing Beach in Dalian, China, to select the proper FIB and facilitate establishment of recreational water quality standards (WQSs) in China.

METHODS

Sample collection

Xinghai Bathing Beach of Dalian is located in Xinghai Park, Dalian, China. The area is chronically listed as one of the most polluted beaches in Dalian (www.dlemc.gov.cn/). Surface water samples were collected from a depth of 0.3 m on incoming waves. Spatial differences were evaluated by collecting samples from three water depths. Specifically, samples were collected from 50, 150, and 300 m away from the shoreline, corresponding to knee-depth (KD, Sites A1, A2, A3), waist-depth (WD, Sites B1, B2, B3), and chest-depth (CD, Sites C1, C2, C3), respectively. In addition, samples were collected from a reference site (site CS) located about 1,000 m from the shoreline, where few or no bathers were observed, and this site was considered too distant to have a consistent effect by human activity on water quality. In addition, samples were collected from a site (drain outlet, site DO) near fresh water outlets that drain land-based runoff to the ocean, this site was considered as the largest and most direct FIB source to Xinghai Bathing Beach (Figure 1). The parameters of sampling sites are shown in Table 1. The spacing of different depth was designed to reflect variability that might arise between different monitoring stations in the same area in any given sampling date. About 200-mL seawater samples were collected in each site, which were packed in sterilized bottles and refrigerated during shipment. Water samples were transferred to the laboratory under cold storage and analyzed within 6 h.

Figure 1 | Sampling locations at Xinghai Bathing Beach in Dalian, China. A1, A2, A3: knee-depth; B1, B2, B3: waist-depth; C1, C2, C3: chest-depth; CS: control site; DO: drain outlet.

Table 1 | The parameters of sampling sites in Xinghai Bathing Beach

Site	Latitude and longitude	Depth
A1	38°52′29.74″ N121°33′35.26″E	KD, 50 m away from shoreline
B1	38°52′27.75″ N121°33′36.90″E	WD, 150 m away from shoreline
C1	38°52′22.13″ N121°33′40.96″E	CD, 300 m away from shoreline
A2	38°52′33.05″ N121°33′41.17″E	KD, 50 m away from shoreline
B2	38°52′31.02″ N121°33′42.64″E	Waist-depth, 150 m away from shoreline
C2	38°52′24.76″ N121°33′47.22″E	CD, 300 m away from shoreline
CS	38°52′19.87″ N121°33′51.18″E	WD, 150 m away from shoreline
A3	38°52′34.98″ N121°33′48.49″E	KD, 50 m away from shoreline
B3	38°52′33.09″ N121°33′49.90″E	Waist-depth, 150 m away from shoreline
C3	38°52′27.13″ N121°33′53.87″E	CD, 300 m away from shoreline
DO	38°52′25.46″ N121°33′30.65″E	100 m-depth, 1,000 m away from shoreline

Temporal differences in water quality were evaluated by collecting samples at different times before and after rainfall over a 13-day period (9 August to 21 August 2011). The first 7 days corresponded to dry weather and the latter six to wet weather; accordingly, they were defined as before-rainfall and post-rainfall, respectively. In this study, post-rainfall was defined as the period between 0 and 72 h after a rainfall event of more than 20 mm. We collected samples at 9:00 a.m. and 3:00 p.m. before rain. There was an average of more than 20 mm of rainfall on 16 and 20 August; therefore, samples were collected seven times post-rainfall on each day at the site A2, B2, and C2. Samples were still only collected twice a day at site CS on these dates. Samples were placed into a 200 mL sterilized bottle and sent to the laboratory for measurement of TC, FC, EC, and *E. coli* within 24 h. Samples from site DO were only collected at 9:00 a.m. every day. The details of sampling time and rain are shown in Table 2.

Table 2 | Sampling time and rain in Xinghai Bathing Beach

Date	Sampling time	Rain (mm)
9 August	9:00	0
	15:00	0
10 August	9:00	0
	15:00	0
11 August	9:00	0
	15:00	0
12 August	9:00	0
	15:00	0
13 August	9:00	0
	3:00	0
14 August	9:00	0
	13:00	5
15 August	15:00	0
	17:00	0
	19:00	0
16 August	7:00	28
	9:00	0
	11:00	0
	13:00	0
	15:00	0
	17:00	0
	19:00	0
17 August	7:00	0
	9:00	0
	11:00	0
	13:00	0
	15:00	0
	17:00	0
	19:00	0
18 August	7:00	0
	9:00	0
	11:00	21
19 August	7:00	0
	9:00	0
	11:00	0
	13:00	0
	15:00	0
	17:00	0
	19:00	0
20 August	7:00	0
	9:00	0
	11:00	0
	13:00	0
	15:00	0
	17:00	0
	19:00	0
21 August	7:00	0
	9:00	0
	11:00	0
	13:00	0
	15:00	0
	17:00	0
	19:00	0

Microbial analysis

Upon collection, 3M Petrifilm test kit (3M Ltd, China) were used to enumerate TC, FC, and *E. coli* according to the manufacturer's instructions. Briefly, the top layer was lifted to expose the plating surface, and with a pipette, 1 mL of each water sample was aseptically added, then slowly rolled down the top film. Samples were incubated at $37.5 \pm 0.5\,^{\circ}$C for 24 h to measure TC and *E. coli*, or at $44.5 \pm 0.5\,^{\circ}$C for 24 h to measure FC. After the incubation period, colonies appeared either as splotches, spots which were surrounded by bubbles, or a combination of both. EC was analyzed by membrane filtration according to EPA method 1600 (US EPA 2006). Each sample was inoculated with three replicates.

Standards for the quality of recreational seawater

The results obtained as above were compared with the single sample standards. Specifically, the TC and FC values were compared with the Chinese standards (TC < 1,000 most probable number (MPN) or CFU/100 mL; FC < 200 MPN or CFU/100 mL) (Chinese GB 3097-1997), while EC and *E. coli* were compared with the US EPA standards (EC < 104 CFU/mL, *E. coli* < 235 MPN or CFU/100 mL) (US EPA 2012).

Statistical analysis

Statistical analysis was performed using SPSS 19.0. Paired *t*-tests were employed to analyze the impact of each parameter among depths and between dry days and wet days. Partial correlations were used to evaluate the relationships between the four bacterial indicators. Statistical analyses were conducted based on the Alpha 95% credible intervals for regression parameters. Box plots were also performed using SPSS 19.0 to reflect the concentrations of bacterial indicators.

RESULTS

Total coliforms, fecal coliforms, enterococci, and *Escherichia coli* concentrations

A total of 325 water samples were collected and analyzed for TC, EC, FC, and *E. coli*. The ranges of the four bacterial indicators were considerably large and the concentrations varied by several orders-of-magnitude among organisms.

TC concentrations ranged from <100 CFU/100 mL to 10^5 CFU/100 mL, with the maximum concentration for TC being 100 times higher than the standard. At least 7% of the samples exceeded the single sample marine WQS of 1,000 CFU/100 mL for TC based on the Chinese standard (GB 3097-1997).

FC concentrations ranged from no more than 10 CFU/100 mL to 28, 500 CFU/100 mL, and the maximum concentration for FC was 28 times higher than the standard. At least 28% of the samples exceeded the single sample marine WQS of 200 CFU/100 mL for FC based on the Chinese standard (GB 3097-1997).

EC concentrations ranged from no more than 15 to 5,352 CFU/100 mL, and the maximum concentration for EC was 130 times higher than the standard. At least 38% of the samples exceeded single sample marine WQS of 104 CFU/100 mL for EC as determined by EPA Method 1600 (US EPA 2006). EC levels were consistently higher in the supratidal sand than the intertidal sand and subtidal sand.

Escherichia coli concentrations ranged from no more than 100 to 6,200 CFU/100 mL. The maximum concentration of *E. coli* was 26 times higher than the standard, and at least 10% of the samples exceeded the single sample marine WQS of 235 CFU/100 mL for *E. coli* based on the US EPA standard (US EPA 2012).

The maximum concentration for all four bacterial indicators was detected near the drain (site DO), while the minimum concentration for all four bacterial indicators was observed at the control site (CS). All samples collected from CS contained bacteria at levels below the standards, except for one collected on 17 August at 9:00 a.m. (12 h after rainfall stopped), for which all four bacterial indicators exceeded the standards.

Spatial variation

The concentrations of all four bacterial indicators differed with water depth during the study period. The bacterial abundance occurred in the order of CD < WD < KD. The concentrations of all four bacterial indicators at KD were two to three times higher than at WD. The highest rate of standard failures for all four bacterial indicators was found at KD as well. The median values, ranges and typical rates of standard failures of the four bacterial indicator levels at different water depths are compared in Figure 2 and Table 3. As shown in Table 3, the typical rates of standard failures of the four bacterial indicators at KD were comparatively higher than at other depths.

Comparison of microbial concentration and rate of standards failure before and after rainfall

Monitoring results showed that except for *E. coli*, the concentrations of indicator bacteria increased by more than an order of magnitude post-rainfall (Figure 3), and the geometric mean concentrations post-rainfall for all four bacterial indicators were 4–20 times higher than before rainfall. As shown in our study, the mean concentrations for all bacterial indicators were below the standards before rainfall at all sampling sites except for the drain site. However, with the exception of TC, the bacterial indicators exceeded the standards post-rainfall, especially the mean concentration of FC and EC, which were 1,475 and 690/100 mL post-rainfall compared with only 143

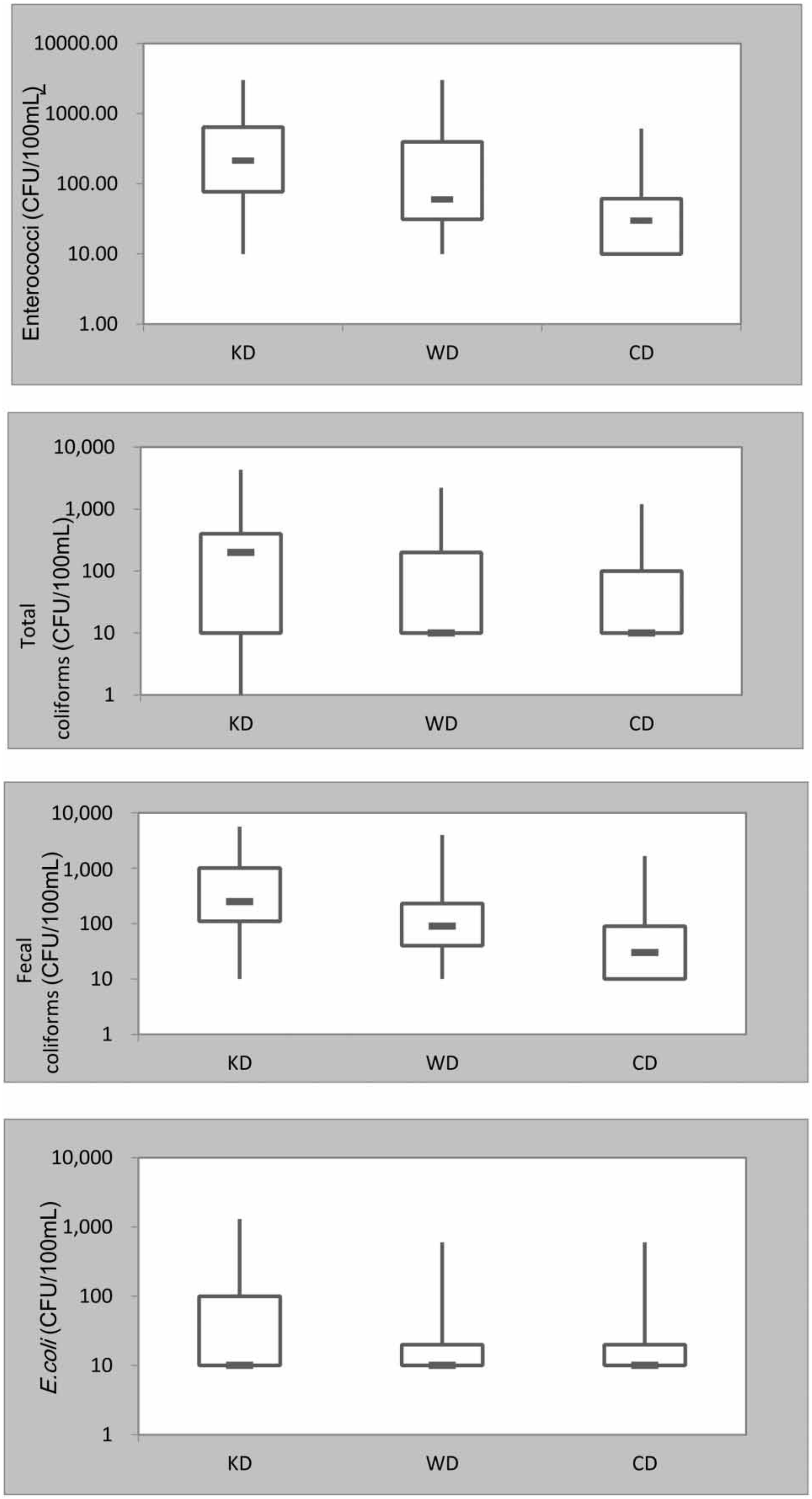

Figure 2 | Box plot of KD, WD, and CD water enterococci, TC, FC and *E. coli* levels. The center line in the boxes indicates the median value. KD: knee-depth; WD: waist-depth; CD: chest-depth.

Table 3 | The rate of standards failure at different water depths

Bacterial indicator	**KD (%)**	**WD (%)**	**CD (%)**
Total coliforms (/100 mL)	10	7	4
Fecal coliforms (/100 mL)	51	21	12
Enterococci (/100 mL)	58	36	23
E. coli (/100 mL)	13	8	2

and 19 CFU/100 mL before rainfall. The rates of standards failure for all four indicator bacteria were higher post-rainfall than before rainfall ($p < 0.01$) (Table 4). Indicator microbe concentrations were observed at the outlet, and all samples exceeded the standards by more than 100 times, regardless of whether they were collected pre- or post-rainfall.

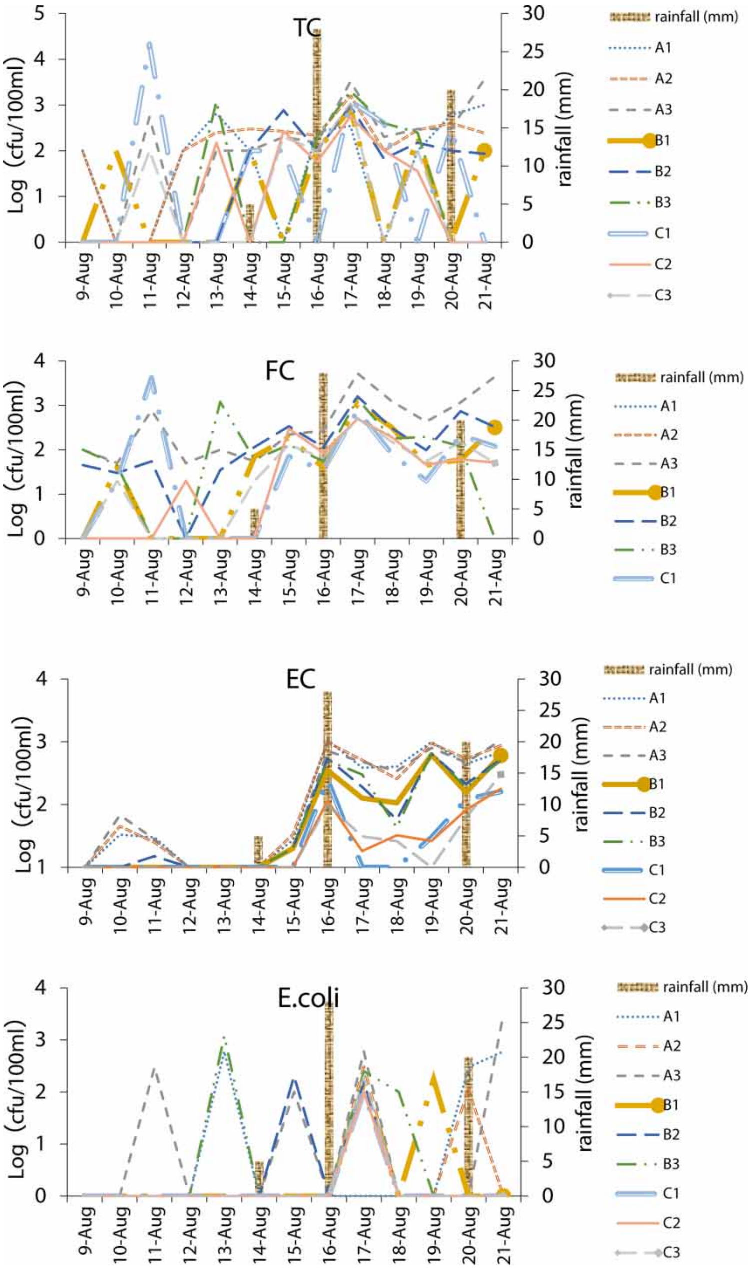

Figure 3 | Concentration of TC, FC, EC and *E. coli* (MPN/100 mL) at each site during the 13-day period.

Table 4 | Rates of standards failure for four bacterial indicators

	Total coliforms			**Fecal coliforms**			**Enterococci**			***E. coli***		
Indicator	**KD (%)**	**WD (%)**	**CD (%)**	**KD (%)**	**WD (%)**	**CD (%)**	**KD (%)**	**WD (%)**	**CD (%)**	**KD (%)**	**WD (%)**	**CD (%)**
Before rainfall	6	3	3	17	3	0	8	8	0	6	3	3
After rainfall	13	9	6	79	34	21	73	44	29	8	5	2
Mean values		7			28			39			10	
Standards		China			China			EPA			EPA	

Correlation of all four bacterial indicators

The Pearson–Moment correlation procedure was used to analyze the relationships among the four investigated bacterial indicators (Table 5). The results suggested that EC was poorly correlated with other bacterial indicators before rainfall ($r = 0.061–0.24$, $p > 0.05$), but well correlated with TC and *E. coli* ($r = 0.434$ and 0.328, respectively, $p < 0.01$). In addition, the concentrations of FC and *E. coli* were strongly correlated before rainfall ($r = 0.858$, $p < 0.01$), but FC showed a poorer relationship with TC before rainfall ($r = 0.430$, $p < 0.01$). Finally, all indicators were significantly correlated post-rainfall.

DISCUSSION

China and international institutions have established several directives or guidelines to protect the environment and public health by reducing pollution of bathing waters (China State Oceanic Administration 2010; EEC 1976; WHO 2003). Different indicators and indicator levels are used as standards by water quality programs in various states, countries, and regions (Corbett *et al.* 1993). For example, the USA currently monitors enterococcus or coliforms, Hong Kong monitors *E. coli*, and the UK monitors fecal streptococci. Prieto *et al.* (2001) suggested that TC is the best predictor of the analyzed symptoms, such as GI, respiratory, and dermatological afflictions; however, they only compared the total coliforms, fecal coliforms and fecal streptococcus, and did not evaluate EC or *E. coli* (Prieto *et al.* 2001). In 2006, the European Commission adopted a new directive in which they decreased their standards from 19 to two bacterial indicators, *E. coli* and intestinal enterococci. Intestinal enterococci are used as indicators at marine beaches used for swimming in the EU (EC 2006). In 2012, EPA revised recreational water quality criteria, which also recommended indicators for fresh water are the bacteria enterococci and *E. coli* and for marine water are enterococci (US EPA 2012). Sea WQSs (Chinese GB 3097-1997) were established in 1997 in China to provide limits ensuring the sanitary quality of recreational waters based on the levels of indicator microorganisms. In this present study, we applied international directives to evaluate microbiological quality standards for systems for which they have not yet been established.

As shown in this study, the rates of standard failure for all four bacterial indicators at Xinghai Bathing Beach of Dalian were high, indicating relatively serious contamination during our study period. The mean concentrations of all bacterial indicators post-rain were 4–20 times higher than before rain. Moreover, EC exceeded the single sample standards most often in our study (38%). In July 2007, the EC

Table 5 | Correlation of bacterial indicators

	TC			**FC**			**EC**		
Indicator	**FC**	**EC**	***E. coli***	**TC**	**EC**	***E. coli***	**TC**	**FC**	***E. coli***
Before rainfall	0.430**	0.24	0.385*	0.430**	0.203	0.858**	0.24	0.203	0.061
Post-rainfall	0.694**	0.434**	0.882**	0.694**	0.756**	0.641**	0.434**	0.756**	0.328**

*$0.01 < p < 0.05$; **$p < 0.01/7$.

concentration ranged from 0 to 77 CFU/100 mL. Although both of these sampling times were peak recreational seasons, significantly higher EC values were observed after rainfall. Moreover, a significant correlation was observed between the concentration of bacterial indicators and precipitation. Our results are consistent with those of Noble *et al.* (2003), Hsu & Huang (2008) and Zhang *et al.* (2013), who suggested that there was a higher level of consistency among indicators on rainfall days. Accordingly, the increase of bacteriological indicators post-rainfall may be due to the increasing flow of sewage water, as well as the mixed level of increased and indicator bacteria washed out of sand into shoreline waters. There was also an outlet near the Xinghai bathing beach, where the maximum concentrations of all four bacterial indicators were detected. The main sources of contamination at the study site were sewage discharge and hospital wastewater (Wang *et al.* 1990). On rainy days, the discharge of sewage increased, which is in accordance with the results of a previous study (Zhang *et al.* 2013). As shown in the present study, small rainfall events influence the water quality for 24 h, while storm events (≥20 mm) may influence the water quality for 72 h. If another storm follows the former one, the pollution would be severe; therefore, swimming post-rainfall can increase the health risk. Given the importance of rainfall events to the dynamics of contamination, rainfall should be considered when performing routine monitoring surveys.

Analysis of data from different water depths showed that the mean concentrations of bacterial indicators differed at the three water depths and gradually decreased with distance from the shore. Overall, 79 and 73% of all standard failures were detected when FC and EC were used as the indicators in knee deep water 50 m away from the shore, while only 34 and 44% of the failures occurred in waist deep water 150 m away from the shore, respectively. The results showed that swimming closer to shore may result in a higher exposure to FIB. A possible explanation for this differential failure is the difference in the degree of effect by sewage, tidal action and human activity. Hartz *et al.* (2008) and Shibata *et al.* (2004) reported that enterococci and other bacterial indicators exhibited increased survivability and growth in sand relative to seawater. Colford *et al.* (2012) reported that those bathers exposed to high concentrations of bacterial indicators showed a significant increase in the risk of GI problems. The beach investigated in the present study attracts up to 30,000 visitors a day; therefore, we inferred there is a potential threat to a high number of visitors at this beach.

The results of this study revealed that it was more conservative to select EC and FC as indicators of fecal contamination. This is because the rate of EC standard failures was higher than that of other samples. One explanation for this is that EC survive longer in the marine environment than TC or FC (McQuaig *et al.* 2006). Hanes & Fragala (1967) found that *E. coli* survival in marine water was 0.8 d, while EC could survive 2.4 d. Sinton *et al.* (1994) found that *E. coli* degraded more rapidly with increased sunlight intensity than EC in bacterial samples from southern California. Moreover, studies have demonstrated the relationship between enterococci at marine beaches and swimming-related illnesses. Haile *et al.* (1999) reported an association between enterococcus, fecal, and total coliforms and swimming-related illnesses in Santa Monica Bay, California. Numerous marine beach epidemiological studies found EC was correlated better with swimming-associated gastroenteritis at marine bathing beaches subjected to wastewater contamination or other fecal contamination that generally contains pathogenic microorganisms (Ashbolt & Bruno 2003; EC 2006; Wade *et al.* 2006; Colford *et al.* 2012; US EPA 2012; Yau *et al.* 2014), while the Guidelines for Canadian Recreational Water Quality (2012) pointed out that a strong correlation has been demonstrated between the concentration of *E. coli* in fresh waters and the risk of GI illness among swimmers. The World Health Organization (WHO 2003) also pointed that *E. coli* is intrinsically suitable as a regulatory parameter of public health significance for recreational fresh waters but not marine water. Besides, compared with before-rainfall, there was no obvious increase about the concentration and exceedance rate of *E. coli* on post-rainfall days. Therefore, EC but not *E. coli* was chosen as the FIB in this present study. However, EC and other indicators concentrations were less well correlated in our study before rainfall, and did not reflect the level of other indicators. Therefore, it is necessary to use an additional bacterial indicator to evaluate fecal contamination. We recommend FC as the second indicator because it showed a higher failure rate than the other indicators and was significantly correlated with other indicators before (except for EC) and after rainfall. Currently, FC is the only indicator considered during routine monitoring of beaches used for swimming in China, which may neglect the risks associated with EC. Accordingly, it is highly recommended that both EC and FC be used concurrently as indicators of fecal pollution during routine monitoring.

CONCLUSIONS

The present study investigated pollution based on fecal bacterial indicators, and the results revealed a significant increase after rainfall. In addition, the concentrations of bacteria differed significantly with distance from the shoreline, with KD near the shore exceeding the standard most frequently. Therefore, swimming in marine bathing beach 24 h after small rainfall events or 72 h after storm events is not recommended, especially near the shore. Based on the concentration of indicators and their exceedance rates, as well as the correlation between indicators, both enterococci and fecal coliforms should be evaluated at the same time in marine bathing beaches in China.

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