Monitoring of faecal indicator bacteria at the recreational beaches of South Korea

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ABSTRACT

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The densities of *Enterococcus*, *Escherichia coli* and total coliform bacteria at the beach sand, swash zone, and the waist-high marine water were monitored using Enterolert® and Colilert®-18 kits (IDEXX Laboratories Inc., Westbrook, Maine) at the Gyeongpo and the Jumunjin recreational beaches of the eastern coast of Korea. The samples were collected weekly from July 19, 2011 to Aug. 30, 2011. The densities of *Enterococcus* and total coliform bacteria were the highest in the beach sand. The density of *Escherichia coli* was the highest in the swash zone. The frequencies above the U.S. EPA single-sample criteria were 29 at the Gyeongpo beach and 16 at the Jumunjin beach among the 42 samples of swash zone and marine water. The densities of faecal indicator bacteria were higher in the swash zone than the marine water. The beach sand may play as a bacteria reservoir of marine water. Regular monitoring of faecal indicator bacteria in beach sand is necessary during the beach opening. Alarming about the level of faecal contamination of the beach sand will be an important health problem. The guidelines of faecal indicator bacteria in beach sand should be made for the purpose of beach health.

ADDITIONAL INDEX WORDS: *Enterococcus, Escherichia coli, Total coliform bacteria, Enterolert, Colilert, beach sand, swash zone, Korea.*

INTRODUCTION

Beach-goers are exposed to diverse beach environments. Beach sand is important environment to beach-goers, but beach sand also accumulates microorganisms shed from humans and animals (Papadakisi *et al.*, 1997). Faecal contamination at bathing beaches can be hazardous to humans because faeces may contain bacteria, viruses, and protozoa that can be ingested and cause intestinal disease. The U.S. Environmental Protection Agency (EPA) has estimated that up to 3.5 million people become ill from contact with raw sewage from sanitary sewer overflows each year (U.S. EPA, 2001). It is estimated that each year more than 120 million cases of gastrointestinal disease and 50 million cases of severe respiratory diseases are caused by swimming and bathing in wastewater-polluted coastal waters (Shuval, 2003). Faecal indicator bacteria serves as surrogates for microbial pathogens.

Faecal indicator bacteria, such as *Enterococcus* species, faecal coliform bacteria, total coliform bacteria and *Escherichia coli* are ordinarily harmless microbes. They are commonly found in sewage and indicate the faecal contamination. Historically, faecal coliform and *E. Coli* have been used as indicators of choice to monitor the recreational water quality. *Enterococcus* are stronger correlated with swimming-associated gastrointestinal illness in both marine and fresh waters than other bacterial indicator organisms and are less likely to die off in saltwater. So, the *Enterococcus* is more recommended as a water quality indicator at marine water (U.S. EPA, 1986). The Guidelines for recreational marine water recommended by U.S. EPA are provided in Table 1.

The levels of these faecal indicator bacteria are statistically associated with gastrointestinal illness in recreational water (Wade *et al.*, 2003). The adjusted risk of gastrointestinal illness among those who dug in the sand was 1.13 times the risk of gastrointestinal illness among those who did not dig in the sand. The adjusted risk of diarrhoea among those who dug in the sand was 1.20 times the risk among those who did not dig in the sand. The crude incidence of upper respiratory illness was higher among those who dug in the sand compared with those who did not dig in the sand (Heaney *et al.*, 2009).

Children have lower immunity to gastrointestinal pathogens contained in beach sand than adults and tend to dig the beach sand more than adults. Children at 10 years or younger were at greater risk from swimming-associated gastrointestinal illness following

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exposure at freshwater beaches polluted by sewage discharge (Wade *et al.*, 2008).

Faecal indicator bacteria may replicate and possibly colonize in beach sand. Beach sand provide considerable protection from UV exposure. Beach sand act as a reservoir of faecal indicator bacteria (Beversdorf *et al.*, 2007). The swash zone is that area of the beach where waves continuously wash up on the sand. Swash zone generally harbours higher densities of microorganisms and faecal indicator bacteria than adjacent waters (World Health Organization, 2003). Considerable exposure is a likely occurrence as a significant percentage of time is spent by bathers, particularly children, on the beach itself rather than in the water. It is suggested that there are great potential health risks in beach sand and swash zone. The monitoring of faecal indicator bacteria at both sites seems to be important.

Beach closings owing to faecal pollution are a global public health issue. U.S. natural resources defence council's annual analysis showed that the number of beach closing and advisory days reached totalling 23,481 days in 2011. More than two-thirds of closings and advisories were issued because bacteria levels in beach water exceeded public health standards, indicating the presence of human or animal waste in the water. More rapid reports of faecal indicator bacteria can make officials to do preventive actions.

There are two standard methods of counting the faecal indicator bacteria at the beach sand and marine water. One is a membrane filtration method and the other is a multiple-tube fermentation technique. In membrane filtration method, the sample is passed under vacuum pressure through specially designed membrane. The membrane is transferred to the prepared selective agar plates and incubated for 24 hours at 41 ℃. However, the membrane filtration method has limitations, particularly when testing waters with high turbidity or non-coliform background bacteria like beach sand and swash zone water. For such samples, it is desirable to conduct tests with a multiple-tube fermentation technique. When multiple tubes are used in the fermentation technique, results of the examination of replicate tubes and dilutions are reported in terms of the Most Probable Number (MPN) of organisms present. For *E. coli*, MPN method is based on the defined substrate technology using *o*-nitrophenyl-β-D-galactopyranoside and 4-methylumbelli feryl-β-glucuronide as the growth substrates. For *Enterococcus*, MPN method is based on the defined substrate technology using *o*-methylumbel-liferon-β-D-glucoside and 4-methylumbelli feryl-β-glucuronide as the growth substrates (Kinzelman *et al.*, 2003).

Table 1. Guidelines for recreational marine water recommended by U.S. EPA.

Indicator microbe	**Guidelines**
E. coli	Not recommended for marine water. For freshwater a geometric mean of ≤ 126/100 ml and ≤ 235/100 ml on a single day
Enterococci	A geometric mean of ≤ 35/100 ml and 104/100 ml on a single day
Faecal coliform	A monthly average (geometric mean) of 200/100ml, ≤ 400/100 ml in 10% of samples, and ≤ 800/100 ml on a single day
Total coliform	A monthly average (geometric mean) of ≤ 1000/100 ml, ≤ 1000/100 ml in 20% of samples, and ≤ 2400/100 ml on a single day

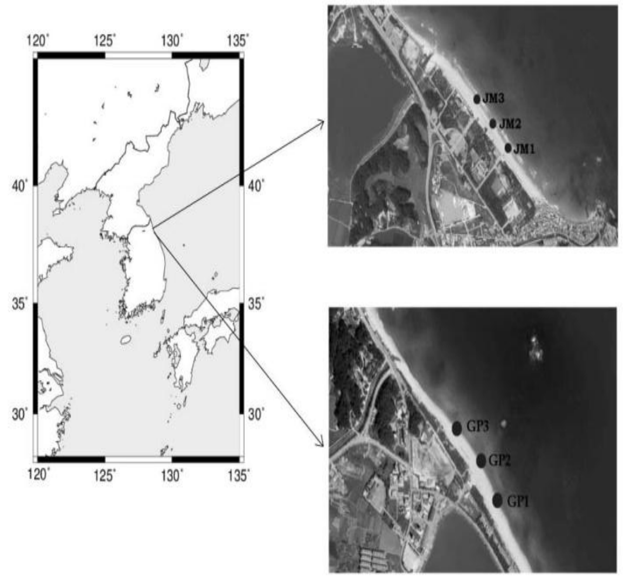

Figure 1. The beaches and sampling sites of the Jumunjin beach (JM1, JM2, JM3) and the Gyeongpo beach (GP1, GP2, GP3).

A proprietary medium formulation and testing package incorporating these substrates has been marketed by IDEXX Laboratories (Westbrook, Maine) under the product name Colilert®-18 kit and Enterolert® kit. MPN method using these kits is well correlated with a membrane filtration method (Abbott *et al*., 1998; Eckner, 1998).

The recreational beaches are open about 7 weeks from the middle of July till the late August in Korea. The monitoring was performed in the opening period of beaches. Most people, especially children, play on the beach sand and shallow waters. The monitoring was carried out by Colilert®-18 kit and Enterolert® kit in beach sand, swash zone and marine water of eastern coast of Korean peninsula.

METHODS

Monitoring beaches and sampling sites

Two the most popular beaches of the eastern coast of Korea were monitored (Figure 1). Gyeongpo beach has more than 8 million visitors and Jumunjin beach has more than 2 million visitors per year. Gyeongpo beach is near from the center of Gangneung city and looked for by many people at the usual time. The lagoon is nearby and commercial buildings locate very close. Jumunjin beach is 15 km north of the Gyeongpo beach.

The center points of each beach were selected using GPS. Two other points were selected 200 meters apart from center point north and south. Three sampling sites were appointed vertical to shoreline at each point. First site was waist-high marine water. Second site was the swash zone. Third site was the borderline of wet and dry sand.

Sample collection and analysis

The beaches were open from July 8, 2011 till August 20, 2011. Samples were collected seven times from July 19, 2011 till August 30, 2011 at 9:00 AM of every Tuesday. Each beach had 3 points and each point had 3 sampling sites, so 18 samples were collected at every 100 ml marine water were collected at autoclaved polypropylene bottles at each site. The samples were placed in coolers and maintained on ice at 4 °C. The temperatures of air and marine water were recorded. The pH of marine water was measured. The samples were carried to the laboratory immediately. Dilution, inoculation and incubation were started less than 4 hours after sampling.

The marine water samples were diluted by 1:10 (90 ml of sterilized distilled water and 10 ml of marine water). The 25 grams beach sand was mixed with 250 ml of sterilized distilled water and shaken by vortex mixer for 30 minutes (Boehm *et al.*, 2009). Detection of faecal indicator bacteria was done according to the manufacturer's instruction. The Colilert and Enterolert reagents of the kits were added to the water samples and mixed. The mixed samples were transferred to the wells of tray. The trays were incubated for 18 hours at 35.5°C for Colilert®-18 kit and 24 hours at 41°C for Enterolert® kit. The positive wells turn the medium yellow by long-wave 366nm UV light. The MPN chart provided by the manufacturer was used for enumeration.

RESULTS

The average pH and temperature of marine water were 8.24 (7.88 – 8.38) and 23.2°C (20.1 – 27.6). The average temperature were 24.3°C (20.0 – 30.0). Three days were sunny, three days were cloudy and one day was rainy.

The MPN numbers of *Enterococcus* at each site are provided in Figure 2. The numbers of event exceeding the criteria are provided in Table 2. Only 1 out of 21 (4.78%) samples of Jumunjin beach and none of the 21 samples of Gyeongpo beach exceeded the U.S. EPA single-sample criterion for waist-high water. Only 1 out of 21 (4.78%) samples of Jumunjin beach and 5 out of 21 (23.8%) samples of Gyeongpo beach exceeded the criterion in the swash zone. By the criterion of *Enterococcus* in marine water, both beaches were seemed to be bacteriologically safe. The mean densities of *Enterococcus* in the Gyeongpo beach were 8.93 MPN/100ml for marine water, 75.05 MPN/100 ml in the swash zone and 151.07 MPN/100 ml in beach sand (Table 3). The mean densities of *Enterococcus* in Jumunjin beach were 21.71 MPN/100ml for marine water, 25.57 MPN/100 ml in the swash zone and 74.10 MPN/100 ml in beach sand. The mean density of *Enterococcus* of both beaches was the highest in beach sand, then swash zone and marine water in decreasing order. The samples exceeding the criterion appeared 4weeks after beach opening at both beaches.

Table 2. The numbers of event exceeding the U.S. EPA single-sample criterion. W = waist-high water; and Z = swash zone.

Bacteria	Beach	Site	Frequency
	Gyeongpo	W	0
Enterococcus		Z	5
	Jumunjin	W	1
		Z	1
	Gyeongpo	W	6
E. coli		Z	9
	Jumunjin	W	4
		Z	7
Total coliform bacteria	Gyeongpo	W	3
		Z	6
	Jumunjin	W	1
		Z	2

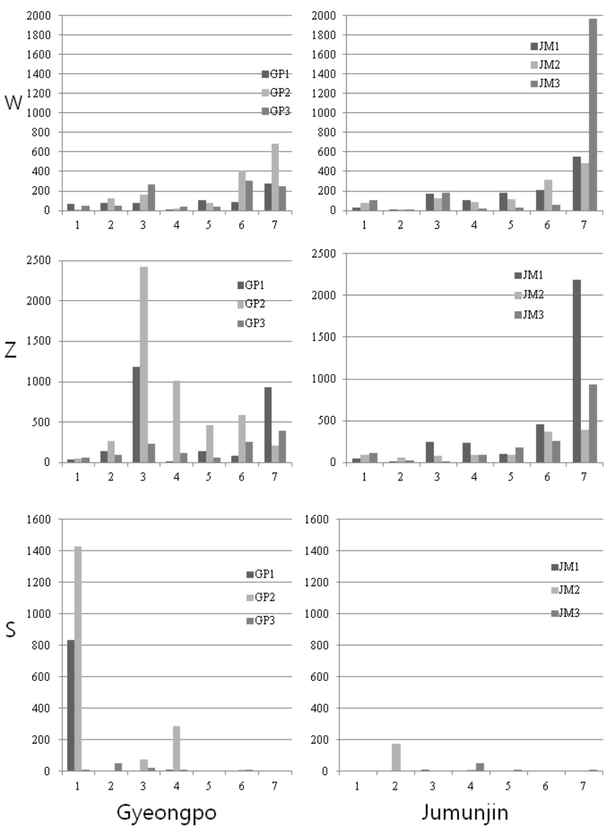

Figure 3. The MPN/100ml of *Escherichia coli* at each site. Day 1 = Jul. 19; 2 = Jul. 26; 3 = Aug. 2; 4 = Aug. 9; 5= Aug. 16; 6 = Aug. 23; 7 = Aug. 30; W = waist-high water; Z = swash zone; and S = beach sand.

The MPN numbers of *E. coli* at each site are provided in Figure 3 and shows that 4 out of 21 (19%) samples of Jumunjin beach and 6 out of 21 (28.6%) samples of Gyeongpo beach exceeded the U.S. EPA single-sample criterion in waist-high water. In addition, 7 out of 21 (33.3%) of Jumunjin beach and 9 out of 21 (42.6%) samples of Gyeongpo beach exceeded the criterion in the swash zone. Both beaches showed high population of *E. coli*. Beach biological safety were more frequently violated by the criteria of *E. coli* than *Enterococcus*. The mean densities of *E. coli* in Gyeongpo beach were 151.52 MPN/100 ml in marine water, 418.40 MPN/100 ml in the swash zone and 131.10 MPN/100 ml in beach sand. The mean densities of *E. coli* in Jumunjin beach were 230.74 MPN/100 ml in marine water, 286.33 MPN/100 ml in the swash zone and 13.17 MPN/100 ml in beach sand. The mean density of *E. coli* was the highest at swash zone in both beaches. The density of *E. coli* was 6-10 times higher than *Enterococcus* in marine water and swash zone.

The MPN numbers of total coliform bacteria at each site are provided in Figure 4. Only 1 out of 21 (4.78%) samples of Jumunjin beach and 3 out of 21 (14.2%) samples of Gyeongpo beach exceeded the U.S. EPA single-sample criterion in waist-high water. Only 2 out of 21 (9.5%) of the Jumunjin beach and 6 out of 21 (28.6%) samples of the Gyeongpo beach exceeded the criterion in the swash zone. The mean densities of total coliform bacteria in Gyeongpo beach were 1084.33 MPN/100ml in marine water, 1979.24 MPN/100 ml in the swash zone and 3741.47 MPN/100 ml in beach sand. The mean densities of total coliform bacteria in Jumunjin beach were 858.67 MPN/100 ml in marine water, 861.93 MPN/100 ml in the swash zone and 3440.38

MPN/100 ml in beach sand. The mean density of total coliform bacteria was the highest in beach sand in both beaches. High population of total coliform bacteria occurred in early period of beach opening.

In summary, *E. coli* was the most frequently exceeding the criteria in both beaches. *Enterococcus* and total coliform bacteria showed the highest density in beach sand. *E. coli* showed the highest density in the swash zone in both beaches. The swash zone showed higher density of faecal indicator bacteria than the marine water.

DISCUSSION

Wet and dry beach sands in diverse climates and with diverse geographies are reservoirs of *Enterococcus* and *E. coli.* (Alm et al., 2003; Yamahara et al., 2007). The source of *E. coli* and *Enterococcus* in sand is often uncertain. In some cases and these organisms may originate from exogenous sources, such as animal feces, runoff, or spilled sewage. In other cases, where an exogenous source cannot be readily identified, it has been suggested that they may represent indigenous populations adapted to living and growing in beach sands.

The important components of beach sand are diverse bacteria, bacteriophages, viruses, protozoan predators, particles and nutrients. Faecal indicator bacteria are exposed to salinity and moisture in beach sand. Faecal indicator bacteria would be killed by bacteriophages or predated by protozoa or died-off from bad

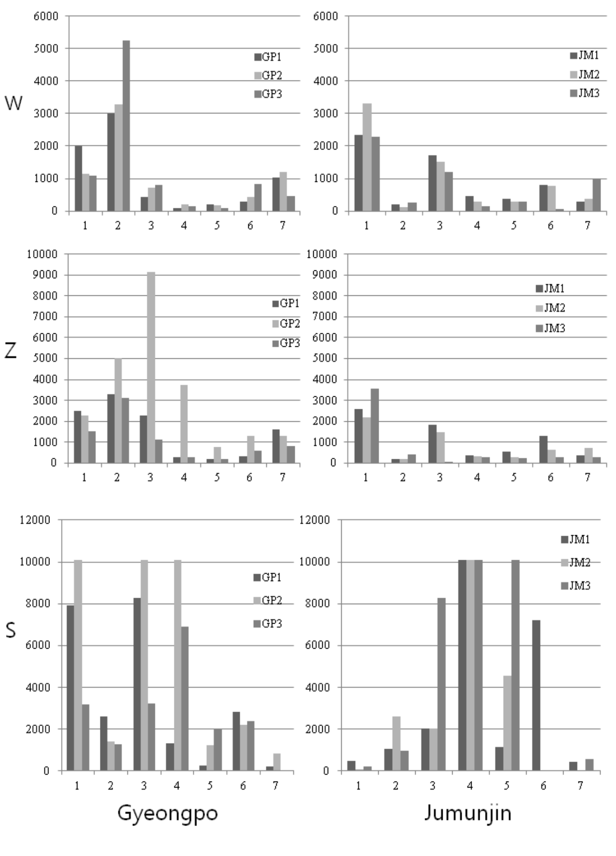

Figure 4. The MPN/100 ml of total coliform bacteria at each site. Day 1= Jul. 19; 2 = Jul. 26; 3 = Aug. 2; 4 = Aug. 9; 5= Aug. 16; 6 = Aug. 23; 7 = Aug. 30; W = waist-high water; Z = swash zone; and S = beach sand.

environments. But, beach sand provides the protection of sunlight exposure to faecal indicator bacteria. Solar inactivation alone is relatively ineffective in sand relative to water. A controlled exposure to UV light achieved 94–99% reduction of *E. coli* in pure culture. But, the same UV light application had no significant effect on the levels of *E. coli* in sand microcosms (Mika *et al.*, 2009). *E. coli* and *Enterococcus* exhibited increased survivability and growth in sand relative to seawater. Faecal bacteria are capable of replicating in sand. The results suggest that sand may be an important reservoir of metabolically active faecal organisms (Hartz *et al.*, 2008).

The results from the sterile sediment mixing experiment showed that *E. coli* and *Enterococcus* are capable of multiplying under simulated environmental conditions. Inactivating the indigenous microbes in the sediment provides a favorable environment for *E. coli* and *Enterococcus* to grow depending on increasing the nutrient availability or decreasing the predation. The microbe populations were increased at sediment of higher organic contents (Desmarais et al., 2002). This result suggests that the main source of faecal indicator bacteria is beach sand (U.S. EPA, 1999). The beach sand disperses by wave or run-off. The density of faecal indicator bacteria is decreased by die-off or dilution by ocean water. This is supported by the report of the spatial decrease in *E. coli* counts from foreshore sand to water with increasing depth (Whitman *et al.*, 2003).

The densities of *Enterococcus* and total coliform bacteria were the highest in beach sand**.** But, the density of *E. coli* was the highest in swash zone. *Enterococcus* may be more tolerant to desiccation in a sandy matrix than *E. coli*, and less tolerant to increased moisture. Levels of *E. coli* in soil have been shown to decrease dramatically with decreasing water content (Mika *et al.*, 2009). *E. coli* can be inactivated through desiccation, some cells can recover and regrow upon the addition of new moisture. Increased presistence of *E. coli* was observed in raked wet sand that was then compacted, presumably owing to slower dessication (Kinzelman *et al.*, 2004). The concentration of *E. coli* is the most high in wet sand by assumption from these reports. The beach sand samples were collected at the borderline of dry and wet sand in this study. If the sand samples were collected at wet sand, the density of *E. coli* would be the highest at beach sand.

The densities of all three faecal indicator bacteria were higher in swash zone than in marine water**.** Beach sand and swash zone have higher population of faecal indicator bacteria than marine water. But, they are often overlooked. Most people enjoy the recreational beach on beach sand or at shallow water. Digging in the sand or having one's body buried in the sand was associated with an elevated risks of gastrointestinal illness and diarrhea. Especially, children tend to dig the wet sand and put hands into mouth.

The events of exceeding the criteria of *Enterococcus* and *E. coli* appeared 4 weeks after beach opening. But, the events of total coliform bacteria exceeding the criteria appeared in the early period of beach opening. The concentration of *Enterococcus* and *E. coli* may be related with the accumulative number of beach-goers and/or temperature and/or rain. Total coliform bacteria includes diverse strains of bacteria such as Citrobacter, Enterobacter, Hafnia, Klebsiella, Serratia, faecal coliform and *Escherichia*. The diversity of members may cause that the total population is not affected by a single environmental change such as human exposure or seasonal environments..

The densities of faecal indicator bacteria in Gyeongpo beach were higher than Jumunjin beach. Gyeongpo beach is located near the city center and has many commercial buildings nearby. Many people visit the beach throughout the year. Gyeongpo beach needs the control of point and nonpoint pollution sources.

The main reservoir of faecal indicator bacteria is the beach sand. Regular monitoring of faecal indicator bacteria in wet and dry sand is necessary during the beach opening. Alarming about the level of faecal contamination of the beach will be an important health problem. The guidelines of faecal indicator bacteria in beach sand should be made for the purpose of beach health.

CONCLUDING REMARKS

Escherichia coli was the most frequently exceeding the U.S. EPA single-sample criterion. The beach sand is a reservoir of faecal indicator bacteria. Regular monitoring of the beach sand during beach opening is necessary. The guideline of beach sand faecal indicator bacteria should be made for the purpose of beach health.

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Table 3. The mean densities (MPN/100ml) of faecal indicator bacteria at each site. W = waist-high water; Z = swash zone; and S = beach sand.

Bacteria	Beach	Site	MPN/100ml
Enterococcus	Gyeongpo	W	8.93±12.85
		Z	75.05±107.57
		S	151.07±247.86
	Jumunjin	W	21.71±37.57
		Z	25.57±32.51
		S	74.10±192.15
E. coli	Gyeongpo	W	151.52±165.45
		Z	418.40±572.68
		S	131.10±351.66
	Jumunjin	W	230.74±424.47
		Z	286.33±485.00
		S	13.17±39.24
Total coliform bacteria	Gyeongpo	W	1084.33±1304.64
		Z	1979.24±2107.26
		S	3741.47±3533.07
	Jumunjin	W	858.67±893.61
		Z	861.93±953.81
		S	3440.38±4007.88

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