Long-Term Monitoring of Noxious Bacteria for Construction of Assurance Management System of Water Resources in Natural Status of the Republic of Korea

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Introduction

Life on Earth are greatly affected by the dynamics of climate system, especially the Earth’s surface climate. In particular, infectious pathogens are emerging as a source of issue as many aspects of public health accompanying the climate change are widely recognized [1, 2]. The term pathogen covers a wide range of disease agents, such as virus, bacteria, parasitic germs, and fungi that can affect human beings either directly or indirectly through influencing the habitat, environment, or by competing with other pathogens. Climate change is a global phenomenon and is expected to accelerate in the future, especially in situations where the extent of climate change on Korean peninsula is relatively large (e.g., temperature rise, rainfall change, etc.) [3]. The annual mean temperature has been increasing at a rate of 0.52°C per decade and is significantly larger over urbanized areas [4], and it is anticipated that the incidence and geographic distribution of vector-borne diseases will change as a result [5].

Shigella is a genus of gram-negative pathogenic enterobacteria and a pathogenic variant of *Escherichia coli* comprising four groups, *Shigella boydii*, *S. dysenteriae*, *S. sonnei*, and *S. flexneri* [6]. *Shigella* species are water-
The Moolgeum region of Nakdong River, which is a
environment in response to changes in the landscape according to the climate change scenario by
(Daejeon), and two water intake plants (the Guui region on Han River in Seoul Special City (Seoul) and the
province), Hyundo region (near Hyundo Bridge of Geum River at Shintanjindong in Daejeon Metropolitan City
five surface water sampling locations: two lakes (Lake Soyang in Gangwon province and Lake Juam in Jeollanam
and

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borne and food-borne agents of bacillary gastrointestinal dysentery or shigellosis responsible for an estimated 80-
165 million cases worldwide and account for a primary cause of childhood morbidity and mortality [7]. S. sonnei
and S. flexneri result in most Shigelllosis cases, with S. sonnei causing over 80% of all shigellosis infections and is
increasingly found in developing countries [8]. In developing countries, especially where exist various public health
problem caused by poor hygiene standards, a safe supply of drinking water influences the risk of public health.

Enterohemorrhagic E. coli O157 is a subtype of shiga toxin-producing E. coli and a primary food-borne pathogen
causing the severe diseases in human such as hemolytic uremic syndrome, thrombotic thrombocytopenic
purpura, and hemorrhagic colitis worldwide, although elderly and children are more expugnable [9].

Salmonellae are facultative anaerobic gram-negative bacteria belonging to the family Enterobacteriaceae and are
a medically pivotal pathogen; two main species are Salmonella bongorin and S. enterica [10]. S. enterica has six
subspecies that are composed of over 1500 subtypes some of which have profound medical significance [11].
Salmonella is an international food-borne intravacular pathogen causing a huge number of deaths and has a
substantial cost burden. S. enterica subsp. enterica is responsible of more than 99% of human salmonellosis cases
[12].

Legionella pneumohila, which causes community-acquired pneumonia that requires hospitalization, is an
opportunistic pathogen that is omnipresent in aquatic environments in which it replicates in free-living amoebae
[13, 14]. L. pneumophila pneumonia is strongly associated with high morbidity. Moreover, legionellosis is
consistently reported as one of the top three most identified respiratory pathogens in community-acquired
pneumonia, along with the hospital-acquired pneumonia [15].

Camplyobacterota, formerly identified as Epsilon proteobacteria, are a whole bunch of gram-negative motile
bacteria found in manifold ecological habitats [16]. Camplyobacterota are pivotal clinical pathogens in human;
the gut of half of the human beings is mass-dwelled with the stomach ulcer-causing bacterium Helicobacter pylori,
while Camplyobacter jejuni is a ubiquitous gastrointestinal pathogen and one of the majorly diagnosed bacterial
food-borne pathogens in human and remains among the most common causes of bacterial gastroenteritis in
many areas of world [17]. Camplyobacteria infection causes the development of miscarriage, septicaemia,
gastroenteritis, proctitis, meningitis, and many neurological diseases, the foremost of which is Guillain–Barré
syndrome among other central nervous system (CNS) diseases with similar acute progresses. Large global
population (1-10% of the whole) can influence the risk of campylobacteriosis annually [18]. The non-food-borne
transmission pathways for Camplyobacter to human are birds and animals in which C. jejuni is part of normal
flora, with the major pathway of transmission being the ingestion of contaminated food or drinking water [19].

Vibrio cholerae is a motile, aquatic curved-rod facultative gram-negative anaerobe belonging to the family of
Vibrionaceae. Cholera caused by an etiological agent, V. cholerae, has been a serious epidemic secretary diarrheal
disease that can quickly lead to severe dehydration and prove fatal within hours if untreated. Strains of V. cholerae
inhabit both marine and freshwater ecosystems [20, 21]. Despite great betterments in hygiene, water quality, and
sanitization, as well as in the clinical treatment, the disease is still estimated to cause about 100,000 deaths every
year.

Climate change is causing water scarcity not only through increased temperatures and prolonged drought times
but also through the degradation of water resources caused by increasing levels of pathogens and other
contaminants posing significant health risks [22]. Thus, there is clearly a strong need for establishing management
strategies and constant monitoring the water resources based on the results of testing water for contamination
from relevant sources. Herein, we report the results on long-term (six-year) surveillance of noxious bacteria
(E. coli O157, S. enterica, L. pneumohila, S. sonnei, C. jejuni, and V. cholera) from August 2013 to February 2019 at
various locations in Republic of Korea (Korea) to help the establishment of the management systems to maintain
water quality and security.

Material and Methods

Collection Sites

A catchment scale investigation of the prevalence of E. coli O157, S. enterica, L. pneumohila, S. sonnei, C. jejuni,
and V. cholera was carried out. Water samples were collected 20 times from August 2013 to February 2019 from
five surface water sampling locations: two lakes (Lake Soyang in Gangwon province and Lake Juam in Jeollanam
province), Hyundo region (near Hyundo Bridge of Geum River at Shintanjin-dong in Daejeon Metropolitan City
(Daejeon)), and two water intake plants (the Guui region on Han River in Seoul Special City (Seoul) and the
Moolgeum region on Nakdong River in Gimhae-si) (Table 1). These five locations were selected to reflect the
environment in response to changes in the landscape according to the climate change scenario by
Intergovernmental Panel on Climate Change (IPCC): the Moolgeum region of Nakdong River, which is a

| Table 1. Climate characteristics and geographic indexes the sample collection sites. |
|---------------------------------|------------------------------|-----------------|-----------------|-----------------|
| Collection sites                | Climate classification       | Geographic indexes | Characteristics |
| Lake Soyang                    | Exceptional Subtropic Zone   | 37.5654/127.4855 | Lake            |
| The Guui region on River Han in Seoul | RCP 8.5 Subtropic Zone       | 37.3305/127.0641 | River           |
| The Hyundo region (near Hyundo Bridge of Geum River at Shintanjindong in Daejeon) | RCP 8.5 Subtropic Zone       | 36.2724/127.2544 | River           |
| Lake Juam                      | RCP 4.5 Subtropic Zone       | 35.0340/127.1412 | Lake            |
| The Moolgeum Region on the Nakdong River | Subtropic Zone              | 35.1834/128.5837 | River           |
subtropical zone; Lake Juam, which is classified as Representative Concentration Pathway (RCP) 4.5 proceeding to subtropical zone; the Guui region of Han River and the Hyundo region of Geum River, which are RCP 8.5 zones; and Lake Soyang, which is considered as non-subtropical zone in Korea.

**Testing of Indicator Bacteria**

Water samples were collected from each location. *E. coli* contamination were measured in samples using a Most Probable Number (MPN) assay. One hundred ml aliquots of water samples from each location were evaluated for total coliform (TC), fecal coliform (FC) and *E. coli* contamination and an IDEXX Colilert-18 and Quanti-Tray System (IDEXX Laboratories, USA). The collected water samples were placed immediately in a refrigerator (4°C) upon collection using sterile bags and transported to private laboratory in Kyonggi University for further processing. Briefly, the process started by adding a Colilert-18 reagent to each sample until it fully dissolved. The mixture was placed in a Quanti-Tray, which was sealed and incubated at 35°C and 44.5°C for 24 h each. Following incubation and positive well counts, the results were obtained using the IDEXX results table, where the number of colored and fluorescing large and small cells determined the MPN for coliform bacteria and *E. coli*. For each test sample, appropriate dilutions were prepared. This system is based on the MPN technique [23] and is a semiautomatic enzyme-based assay reduced to multi-wells. Control samples of commercially available sterile water were included along with the samples to evaluate cross- contamination.

**Quality of the Collected Water Samples**

Physiochemical parameters such as pH, total dissolved solids, dissolved oxygen (DO), total nitrogen, ammonia, nitrate, nitrite, phosphate, and sulfate were analyzed according to the Standard Methods for the Examination of Water and Wastewater [24]. Turbidity and conductivity were measured with a HACH 1900C portable turbidity meter (HACH, USA) and a HACH sension 5 conductivity meter (HACH), respectively. pH was measured on-site using individually calibrated portable testers. Chemical oxygen demand (COD) and ammonium content (NH₄-N) were measured according to standard method [25, 26].

**Sample Collection and Analysis**

The targeted bacteria were *S. sonnei*, *E. coli* O157, *S. enterica* spp., *L. pneumophila*, *C. jejuni*, and *V. cholerae*. Spatially distributed samples were aseptically collected at five locations using sterile containers (Table 1). Samples were simultaneously and in parallel examined for the detection of 6 noxious bacteria. One-liter aliquots from each of five consecutive sampling were filtered using 0.2 mm filters to collect particulates. The filters were processed and extracted DNA using GeneAll Exgene Soil DNA kits (GeneAll Biotechnology, Korea) according to the manufacturer's recommendation. The concentration of the extracted DNA was determined by measuring

**Table 2. Primer set sequences used for noxious bacteria and cycling parameters in this study.**

| Species         | Target gene | Primer sequences                      | Product size | Cycling parameters                      |
|-----------------|-------------|---------------------------------------|--------------|-----------------------------------------|
| *Shigella sonnei* | Hypothetical protein | F: 5’-AGCGGTGTTAAAAGATGATGCCTGTT-3’<br>R: 5’-TGCCCGCTAAAAATCTCTCTGCTCCT-3’ | 325 bp       | Initial denaturation: 95°C (2.5 min)<br> 45 cycles of 95°C (10 sec), 60°C (20 sec)<br> Denaturation: 95°C (10 sec)<br>Slope range: 65-95°C for melting curve and melting peak |
| *E. coli* O157  | Hypothetical protein | F: 5’-GCCGTACATGCTGAGAGTC-3’<br>R: 5’-TAGCCCCCATATAGCGTAAGAAT-3’ | 215 bp       | Initial denaturation: 95°C (2.5 min)<br> 45 cycles of 99°C (10 sec), 39°C (20 sec)<br> Denaturation: 95°C (10 sec)<br>Slope range: 65-95°C for melting curve and melting peak |
| *Salmonella enterica* | Hypothetical protein | F: 5’-CGCGTGCGCTGCTGCTGAGAGTC-3’<br>R: 5’-TAGCCCCCATATAGCGTAAGAAT-3’ | 353 bp       | Initial denaturation: 95°C (2.5 min)<br> 45 cycles of 99°C (10 sec), 39°C (20 sec)<br> Denaturation: 95°C (10 sec)<br>Slope range: 65-95°C for melting curve and melting peak |
| *Legionella pneumophila* | Hypothetical protein | F: 5’-ACAGGTGTAAGAGAGGAGTTAG-3’<br>R: 5’-ACAAGCTCATTTCAATGCCC-3’ | 264 bp       | Initial denaturation: 95°C (2.5 min)<br> 45 cycles of 95°C (10 sec), 59°C (20 sec)<br> Denaturation: 95°C (10 sec)<br>Slope range: 65-95°C for melting curve and melting peak |
| *Vibrio cholerae* | Hypothetical protein | F: 5’-CCGTTGAGCGGAGTTGTTGAGTAAGAAT-3’<br>R: 5’-GTGGCGGGCGGGGAGAACTATATATAGT-3’ | 195 bp       | Initial denaturation: 95°C (2.5 min)<br> 45 cycles of 95°C (10 sec), 52°C (20 sec)<br> Denaturation: 95°C (10 sec)<br>Slope range: 65-95°C for melting curve and melting peak |
| *Campylobacter jejuni* | Hypothetical protein | F: 5’-AAAAAGAGATTATATGATACAAAAA-3’<br>R: 5’-GCTTAATTGTATAATGGTTATATATAT-3’ | 177 bp       | Initial denaturation: 95°C (2.5 min)<br> 45 cycles of 95°C (10 sec), 53°C (20 sec)<br> Denaturation: 95°C (10 sec)<br>Slope range: 65-95°C for melting curve and melting peak |
ultraviolet absorbance at 260 nm using a spectrophotometer (NanoDrop ND-1000, Thermo Fisher Scientific, USA), after which the samples were stored at -70°C before use.

Real-time PCR analysis was conducted with 10 ul of SYBR green master mix (Thermo Fisher Scientific) and 10 pM specific primer sets in a reaction volume of 20 ul using CFX96 Real-time PCR system (Bio-Rad Laboratories, USA). The primer set sequences and reaction conditions for each targeted noxious bacterium and the amplified target sizes are in Table 2. The specificity of the primers was confirmed using a BLAST search in GenBank database from NCBI. For each bacterium tested in this monitoring, the BLASTn searches yield no solid match to any of the other identified bacterium reference sequences. Specificity tests were performed using conventional PCR techniques for each species or subspecies primer set against DNA samples from various bacterium strains [27]. Matches between the cyclic quantification (Cq) value and each of noxious bacteria detection was verified, and positive estimation was determined for a single peak using the Cq value. The positive samples were analyzed and confirmed by sequencing the 16s rDNA fragments by Macrogen Inc. (Korea). Analysis of the derived nucleotide sequences was performed for matching genotypes using the NCBI-BLAST service to target the noxious bacteria.

Results

Physicochemical Parameters in the Water Samples

There were slight variations in physicochemical parameters among the water sample collecting sites. The water temperature at the Guui region and Lake Juam tended to increase slightly, while no definite trend was observed for precipitation (Data not shown). The other collection sites did not show a definite tendency in the parameters of precipitation or water temperature. Table 3 summarizes the physicochemical parameters of water samples from the five sample collecting sites during the periods from August 2013 to February 2019. All the parameters other than precipitation and water temperature fluctuated continuously throughout the year. The pH values are in the range of 6.5 to 8.5 (in a descending order: Guui region > Moolgeum region > Hyundo region > Lake Soyang > Lake Juam), which according to the World Health Organization (WHO) guidelines for drinking water [28], the pH values at the surface fall within the normal limit. Most of the monitoring locations had a sufficient DO level (more than 7 mg/l), although one was borderline (Lake Juam). The conductivity values of the collected water samples were ranged from 70 to 350 mS/cm on average, which are well within the unpolluted freshwater range of 10 to 1,000 mS/cm. The average amount of total nitrogen in the water samples from the collection sites was 1.898 ± 0.850 mg/l, while ammonical nitrogen (NH₃ or NH₄⁺) did not consistently exceed 0.3 mg/l. The acceptable amount of nitrates in drinking water is up to around 44 mg/l [29], so the samples from the locations were well within this (0.4-2.1 mg/l). The phosphate level in the samples was 0.025 ± 0.021 mg/l, which is well within the WHO guideline of 1 mg/l. In general, the Moolgeum region on Nakdong River had the highest values for BOD, COD, conductivity, total nitrogen, total phosphorus, and phosphate, while the Guui region on Han River in Seoul had highest values for pH, DO, ammonia, and nitrate.

Indicator Bacteria and Water-Quality Monitoring Stations

The monitoring points in this study were selected for the water quality measurements due to the needs for long-term monitoring and management of Korean rivers and lakes and the links between the nearby water quality measuring network points according to the prediction scenario for climate change: Lake Soyang in the exceptional subtropical zone and the Guui region on the Han River in Seoul, Hyundo region (near Hyundo Bridge of Geum River at Shintanjin-dong in Daejeon), Lake Juam, and the Moolgeum region on Nakdong River in the subtropical zone.

In practice, it is impossible to enumerate all pathogens in water-source because of the absence of specific

| Parameter | Temp. (°C) | pH | Dissolved oxygen (mg/l) | BOD (mg/l) | COD (mg/l) | Conductivity (mS/cm at 20°C) | Total nitrogen (mg/l) | Ammonia (mg/l) | Nitrate (mg/l) | Total phosphorus (mg/l) | Phosphate (mg/l) |
|-----------|------------|----|-------------------------|------------|------------|-----------------------------|----------------------|---------------|---------------|----------------------|-----------------|
| The Guui region on the Han River in Seoul | 12.71 ± 9.11 | 8.125 ± 0.17 | 12.24 ± 1.89 | 1.515 ± 0.51 | 4.115 ± 0.61 | 209.3 ± 47.96 | 2.684 ± 0.47 | 0.097 ± 0.091 | 2.028 ± 0.33 | ± 0.019 ± 0.019 | ± 0.0065 ± 0.008 |
| The Moolgeum region on the Kakdong River | 15.06 ± 9.50 | 8.075 ± 0.37 | 10.925 ± 2.45 | 1.955 ± 0.52 | 6.265 ± 1.22 | 336.25 ± 104.24 | 2.742 ± 0.57 | 0.086 ± 0.035 | 1.959 ± 0.60 | ± 0.027 ± 0.013 | ± 0.0112 ± 0.005 |
| The Hyundo region (near Hyundo Bridge of Geum River in Daejeon) | 13.26 ± 6.85 | 7.905 ± 0.23 | 10.335 ± 2.50 | 0.73 ± 0.25 | 3.875 ± 0.61 | 167.35 ± 26.31 | 1.479 ± 0.25 | 0.106 ± 0.273 | 1.088 ± 0.34 | ± 0.00 ± 0.005 | ± 0.008 ± 0.005 |
| Lake Soyang | 9.45 ± 4.70 | 7.33 ± 0.35 | 8.395 ± 1.65 | 1.18 ± 0.26 | 2.09 ± 0.32 | 76.2 ± 5.73 | 1.867 ± 0.31 | 0.024 ± 0.014 | 1.377 ± 0.17 | ± 0.006 ± 0.006 | ± 0.0025 ± 0.002 |
| Lake Juam | 11.93 ± 5.40 | 6.82 ± 0.37 | 6.985 ± 3.14 | 0.82 ± 0.19 | 2.94 ± 0.47 | 79.6 ± 8.08 | 0.718 ± 0.11 | 0.069 ± 0.062 | 0.472 ± 0.12 | ± 0.005 ± 0.003 | ± 0.003 ± 0.003 |
Table 4. Total indicator bacteria (MPN/100 ml) including TC, FC, and *E. coli* during the monitoring period.

| Indicator bacteria | Collection sites | Aug, 2013 | Oct, 2013 | Dec, 2013 | Feb, 2014 | Apr, 2014 | Oct, 2014 | Dec, 2014 | Feb, 2015 | Apr, 2015 | Oct, 2015 | Dec, 2015 | Feb, 2016 | Apr, 2016 | Jun, 2016 | Aug, 2016 | Oct, 2017 | Dec, 2017 | Feb, 2018 | Apr, 2018 | Aug, 2018 | Oct, 2018 | Dec, 2018 | Feb, 2019 | Average |
|-------------------|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Total Coliforms (TC) | Lake Soyang | 90,000 | 12,000 | 5,400 | 100 | 610 | 2,400 | 550 | 67 | 32 | 70 | 150 | 1,270 | 3,700 | 1,300 | 160 | 7 | 0 | 3,400 | 170 | 65 | 6,027.55 |
|                    | Han River | 190,000 | 85,000 | 5,500 | 200 | 5,200 | 1,200 | 370 | 130 | 1,700 | 1,400 | 480 | 1,100 | 160,000 | 1,700 | 2,900 | 4,400 | 14,000 | 1,200 | 610 | 300 | 23,869.5 |
|                    | Geum River | 63,000 | 11,000 | 5,800 | 1,000 | 1,100 | 37 | 2,000 | 140 | 290 | 1,300 | 17,000 | 2,500 | 24,000 | 22,000 | 650 | 410 | 0 | 34,000 | 2,900 | 770 | 9,494.85 |
|                    | Lake Juam | 110,000 | 5,900 | 5,400 | 1,400 | 6,000 | 5,500 | 130 | 14 | 150 | 17 | 260 | 980 | 7 | 2,400 | 20 | 0 | 1,000 | 5,200 | 390 | 16 | 7,239.2 |
| Fecal Coliforms (FC) | Lake Soyang | 190,000 | 48,000 | 1,900 | 100 | 2,300 | 9,200 | 460 | 56 | 12 | 240 | 1,900 | 3,500 | 3,700 | 8,700 | 1,000 | 220 | 6,900 | 3,900 | 520 | 18 | 14,139.4 |
|                    | Han River | 1 | 1 | 0 | 0 | 1 | 2 | 2 | 0 | 7 | 3 | 0 | 1 | 0 | 1 | 2 | 2 | 1 | 4 | 4.91 |
|                    | Geum River | 13 | 37 | 40 | 12 | 30 | 120 | 25 | 29 | 79 | 32 | 38 | 200 | 48,000 | 290 | 96 | 820 | 650 | 54 | 30 | 2530.3 |
|                    | Lake Juam | 51 | 2 | 5.1 | 19 | 0 | 6 | 0 | 3 | 3 | 670 | 31 | 290 | 2,100 | 96 | 22 | 9 | 0 | 150 | 120 | 1 | 183.905 |
|                    | Nakdong River | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.405 |
| *E. coli* | Lake Soyang | 4.1 | 1 | 1 | 0 | 5.2 | 3.1 | 0 | 0 | 310 | 2 | 0 | 0 | 8 | 0 | 0 | 68 | 2 | 200 | 3 | 0 | 30.115 |
|                    | Han River | 1 | 1 | 0 | 0 | 0 | 4.1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0.405 |
|                    | Geum River | 8.5 | 20 | 38 | 8.6 | 30 | 73 | 6.3 | 5 | 71 | 32 | 23 | 96 | 29,000 | 100 | 28 | 820 | 20 | 33 | 23 | 9 | 1,522.22 |
|                    | Lake Juam | 0 | 1 | 5.1 | 1 | 1 | 0 | 15 | 0 | 3 | 3 | 19 | 210 | 42 | 28 | 9 | 0 | 120 | 96 | 3 | 29.405 |
|                    | Nakdong River | 1 | 3 | 3.1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.61 |
|                    | Han River | 2 | 1 | 1 | 0 | 4.1 | 240 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14.705 |

Detection of Noxious Bacteria in Monitoring Sites

Most of the targeted bacteria were found in 77% of the samples and at least one of the target bacteria was detected (65%) (Fig. 1, Table 5). Among all the detected bacteria, *E. coli* O157 were the most prevalent with a detection frequency of 22% (22/100), while *S. sonnei* was the least prevalent with a detection frequency of 2% (2/100 samples). Nearly all of the bacteria (except for *S. sonnei*) were present in samples from Lake Soyang, Lake Juam, and Nakdong River (Fig. 2), while *C. jejuni* was detected in those from Han River. During the six-year detection techniques. Thus, indicator bacteria including TC, FC and *E. coli* are traditionally used to indicate the presence of a pathogens, especially in wastewater as well as other intestinal pathogens [30]. The presence of TC and FC is indicative of human fecal contamination. TC, FC, and/or *E. coli* were detected in almost samples collected across 6 year monitoring and 23% of the samples exceeded the regulations provided by the Pennsylvania Department of Environmental Protection (PA DEP) form TC (5,000 CFU/100 ml) at concentration ranging from 0 to 1.9 × 10⁷ MPN/100 ml (CFU and MPU are equivalent), which were mainly observed between August and October [31]. Concentrations of the FC in 13% exceeded the PA DEP regulations for fecal coliforms (200 CFU/100 ml) during the investigation period [31]. Spatially, the Guui region of Han River was the highest contaminated place among the monitored sites in this study, followed by Moolgeum region of Nakdong River. During the period under investigation, the TC (average: 1.2 × 10⁴ MPN/100 ml) rather than FC (average: 563 MPN/100 ml) was detected in almost samples from Han River. During the six-year monitoring period, the detection rates of FC and *E. coli* were detected in almost all the samples and at least one of the target bacteria was detected (65%) (Table 5). Among all the detected bacteria, *E. coli* O157 were the most prevalent with a detection frequency of 22% (22/100), while *S. sonnei* was the least prevalent with a detection frequency of 2% (2/100 samples). Nearly all of the bacteria (except for *S. sonnei*) were present in samples from Lake Soyang, Lake Juam, and Nakdong River (Fig. 2), while *C. jejuni* was detected in those from Han River. During the six-year
sampling period, individual targeted noxious bacteria in water samples exhibited seasonal patterns in their occurrence that were different from the indicator bacteria levels in the water samples. The occurrence of noxious bacteria in the samples was higher during the colder months (October, December, and February) than the warmer ones. However, after April 2016, the occurrences of noxious bacteria in the water samples dramatically decreased to 10.39%. This can be attributed to the authorities’ effort, such as sewage system management, to improve the water quality. Detection of TC, FC and *E. coli* in the water samples could not predict the total noxious bacteria presence.

Table 5. Positive incidence cases of each noxious bacterium at the five-water sample collecting sites during the monitoring period in this study.

| Site          | Aug. | Oct. | Dec. | Feb. | Aug. | Oct. | Dec. | Feb. | Aug. | Oct. | Dec. | Feb. | Total |
|---------------|------|------|------|------|------|------|------|------|------|------|------|------|-------|
| Lake Soyang   | 0    | 6    | 2    | 1    | 4    | 2    | 1    | 1    | 3    | 2    | 0    | 1    | 1104 |
| S. sonnei     | 0    |      | 3    |      | 1    | 1    |      | 1    |      |      |      |      |       |
| E. coli O157  |      |      |      |      | 4    | 4    | 2    |      | 3    | 4    | 1    |      |       |
| S. enterica   |      |      |      |      | 3    | 1    |      |      |      |      |      |      |       |
| S. cholerae   |      |      |      |      |      |      |      |      |      |      |      |      |       |
| C. jejuni     |      |      |      |      |      |      |      |      |      |      |      |      |       |
| Han River     | 1    |      |      |      | 1    |      |      |      | 3    | 4    |      |      |       |
| S. sonnei     |      |      |      |      |      |      |      |      |      |      |      |      |       |
| E. coli O157  |      |      |      |      |      |      |      |      |      |      |      |      |       |
| S. enterica   |      |      |      |      |      |      |      |      |      |      |      |      |       |
| S. cholerae   |      |      |      |      |      |      |      |      |      |      |      |      |       |
| C. jejuni     |      |      |      |      |      |      |      |      |      |      |      |      |       |
| Geum River    | 3    |      |      |      | 1    |      |      |      | 4    |      |      |      |       |
| S. sonnei     |      |      |      |      |      |      |      |      |      |      |      |      |       |
| E. coli O157  |      |      |      |      |      |      |      |      |      |      |      |      |       |
| S. enterica   |      |      |      |      |      |      |      |      |      |      |      |      |       |
| S. cholerae   |      |      |      |      |      |      |      |      |      |      |      |      |       |
| C. jejuni     |      |      |      |      |      |      |      |      |      |      |      |      |       |
| Lake Juam     | 0    |      |      |      | 3    |      |      |      | 3    |      |      |      |       |
| S. sonnei     |      |      |      |      |      |      |      |      |      |      |      |      |       |
| E. coli O157  |      |      |      |      |      |      |      |      |      |      |      |      |       |
| S. enterica   |      |      |      |      |      |      |      |      |      |      |      |      |       |
| S. cholerae   |      |      |      |      |      |      |      |      |      |      |      |      |       |
| C. jejuni     |      |      |      |      |      |      |      |      |      |      |      |      |       |
| Nakdong River | 0    |      |      |      | 3    |      |      |      | 3    |      |      |      |       |
| S. sonnei     |      |      |      |      |      |      |      |      |      |      |      |      |       |
| E. coli O157  |      |      |      |      |      |      |      |      |      |      |      |      |       |
| S. enterica   |      |      |      |      |      |      |      |      |      |      |      |      |       |
| S. cholerae   |      |      |      |      |      |      |      |      |      |      |      |      |       |
| C. jejuni     |      |      |      |      |      |      |      |      |      |      |      |      |       |
| Total         | 6    | 20   | 13   | 1    | 0    | 4    | 7    | 7    | 3    | 0    | 0    | 0    | 2    | 0    | 1    | 0    | 2    | 77  |
Statistical Analysis of Correlation between Indicator Bacteria and the Tested Noxious Bacteria

We performed correlation tests between the monitored six noxious bacteria and the tested three indicator bacteria (TC, FC, *E. coli*) using permutation technique [33]. Testing results are summarized in Table 6. Only between *S. enterica* and TC has *p*-value less than 0.05. All the other relationships were not able to look at a significant association. Even in the case of the association between *S. enterica* and TC, when their *p*-value were adjusted by the Bonferroni calibration, it did not produce any significant results. After Bonferroni adjustment, all the combinations show *p*-values larger than 0.05. Thus, we concluded that the current results show no statistically significant association in any combination.

Discussion

The worldwide burden of infectious waterborne disease is considerable, and the bacterial pathogens are strongly resistant in the water environment and to most disinfectants. Some bacterial agents such as *S. sonnei*, *C. jejuni* and *E. coli* O157 can contaminate pristine waters through wildlife and human activities. In addition, climate variables such as precipitation, temperature that have changed significantly as a result of global climate change are major driving forces of food- and waterborne diseases and alter the exposure pathways. These determinants could influence the fate and transport of pathogens, as well as their stability, reproduction rates, and viability in the environment. Therefore, sophisticated and consistent surveillance systems and means should be put in place to monitor the targeted pathogen candidates for serious waterborne diseases.

Some of the noxious bacteria exhibited spatial and seasonal patterns at the collecting sites in this study. The presence of *C. jejuni* in samples from four of the targeted sampling collection sites (except the Guui region of Han River) indicates that positive cases are in fall and winter (October, December and February) but not in spring and summer seasons (August, April, and June), which coincides with previously reported studies [34, 35].

Table 6. A statistical association between the monitored noxious bacteria and the tested indicator bacteria.

| Noxious bacteria | Indicator bacteria | *p*-value | Bonferroni-adjusted *p*-value |
|------------------|--------------------|-----------|-------------------------------|
| *S. sonnei*      | TC                 | 0.148     | 1                             |
|                  | FC                 | 0.304     | 1                             |
|                  | *E. coli*          | 0.38      | 1                             |
| *E. coli* 0157   | TC                 | 0.268     | 1                             |
|                  | FC                 | 0.065     | 1                             |
|                  | *E. coli*          | 0.905     | 1                             |
| *S. enterica*    | TC                 | 0.014     | 0.252                         |
|                  | FC                 | 0.262     | 1                             |
|                  | *E. coli*          | 0.28      | 1                             |
| *L. pneumophila* | TC                 | 0.326     | 1                             |
|                  | FC                 | 0.806     | 1                             |
|                  | *E. coli*          | 0.327     | 1                             |
| *V. cholerae*    | TC                 | 0.54      | 1                             |
|                  | FC                 | 0.102     | 1                             |
|                  | *E. coli*          | 0.97      | 1                             |
| *C. jejuni*      | TC                 | 0.876     | 1                             |
|                  | FC                 | 0.204     | 1                             |
|                  | *E. coli*          | 0.264     | 1                             |
the positive cases for *L. pneumophila* are in winter except for one in August 2013 at the Moolgeum region site. In the case of *S. sonnei*, there were only two positive cases of samples from the Guui region and the Hyundo region in October 2013 were not linked to seasonality. Similarly, the indicator bacteria TC, FC and *E. coli* were not consistently and significantly correlated with the detection of the targeted noxious bacteria (Table 6). These data indicate that indicator bacteria and physiochemical parameters used in this study are not potential candidates for consistently and significantly correlated with the detection of the targeted noxious bacteria (Table 6). These data were not linked to seasonality. Similarly, the indicator bacteria TC, FC and *E. coli* were not consistently and significantly correlated with the detection of the targeted noxious bacteria (Table 6). These data indicate that indicator bacteria and physiochemical parameters used in this study are not potential candidates for consistently and significantly correlated with the detection of the targeted noxious bacteria (Table 6). These data were not linked to seasonality.

According to the results of the monitoring in this study, the occurrences of noxious bacteria in water samples were dramatically decreased after April 2016. Although it is difficult to elucidate the specific cause, this could be attributed to the authorities’ effort, such as sewerage system management and social good-informed cognition, to improve the water quality. Korea achieved 92.1% penetration rate of sewage into the advanced countries through the first National Sewage Comprehensive Plan (NSCP) (2007-2015) through continuous expansion of sewage treatment facilities and sewage systems, improved sewage maintenance, enhancement of sewerage and sewerage management, establishment of water resource circulation utilization systems, and improved sewage treatment technology and sewage sludge treatment [36].

This study has a critical limitation. First of all, in some years during the study, the collection of surface water samples has limitations that have not been carried out as originally planned and thus we were not able to proceed with consistent sample collection and monitoring during the summer season. In addition, since this study was only based on the genetic analysis using PCR methods, we were not able to determine the infectivity and pathogenesis despite the positive detection. Nevertheless, this study was designed and practiced at these specific sites as a project of the National Institute of Environmental Research funded by the Ministry of Environment of the Republic of Korea. In fact, despite the growing interest in monitoring nosocomial microorganisms, it is difficult to find a case of research on their distribution and monitoring related to climate change at Korea or abroad. The Ministry of Environment of the Republic of Korea recognized the need for this research to provide public health security and secure drinking water stability because of water temperature rise, flooding, drought and heat waves due to climate change increase the prevalence of noxious microbes. The Ministry of Environment had set a Priority Management List (PML) of 20 noxious microbes in groups including TC bacteria, FC bacteria, pathogenic *E. coli*, enterococci, fecal *Streptococci*, *Pseudomonas* as concerns about unregulated waterborne microbes increase.

In conclusion, it was not possible to determine the infectivity and pathogenicity on the six noxious bacteria examined in this study, and it was difficult to precisely identify any noticeable seasonal or regional effects. However, the fact that they were detected in the five Korea’s representative water environments comprising lakes, rivers, and drinking water collecting sites make it necessary to establish the chemical and biological analysis for noxious bacteria and sophisticated management systems in response to climate change. Thus, relying on predictive models and monitoring for timely warning can protect the health of the public.

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**Conflict of Interests**

The authors have no financial conflicts of interest to declare.

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