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Enteropathogenic Bacteria Contamination of Unchlorinated Drinking Water in Korea, 2010

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Objectives: The purpose of this study was to assess the microbiological quality of unchlorinated drinking water in Korea, 2010. One hundred and eighty unchlorinated drinking water samples were collected from various sites in Seoul and Gyeonggi province.

Methods: To investigate bacterial presence, the pour plate method was used with cultures grown on selective media for total bacteria, total coliforms, and *Staphylococcus* spp., respectively.

Results: In the 180 total bacteria investigation, 72 samples from Seoul and 33 samples from Gyeonggi province were of an unacceptable quality (>10^2 CFU/mL). Of all the samples tested, total coliforms were detected in 28 samples (15.6%) and *Staphylococcus* spp. in 12 samples (6.7%). Most of the coliform isolates exhibited high-level resistance to cefazolin (88.2%), cefonicid (64.7%) and ceftazidime (20.6%). In addition, *Staphylococcus* spp. isolates exhibited high-level resistance to mupirocin (42%). Species of *Pseudomonas, Acinetobacter, Cupriavidus, Hafnia, Rahnella, Serratia,* and *Yersinia* were isolated from the water samples.

Conclusions: The results of this study suggest that consumption of unchlorinated drinking water could represent a notable risk to the health of consumers. As such, there is need for continuous monitoring of these water sources and to establish standards.

Key words: Antimicrobial resistance, Enteropathogenic bacteria, Microbiological quality, Unchlorinated drinking water

INTRODUCTION

The microbial quality of drinking water is a topic of continued interest in a range of disciplines. The presence of microorganisms in drinking water has received increased attention in recent years because they have been linked to enteric infection. Water quality is usually measured according to the degree of bacterial contamination and the microbial quality of drinking water should meet local water standards, which may differ from nation to nation [1,2]. According to the World Health Organization, the microbiological quality of drinking water should be decided using bacterial indicators of contamination, such as *Escherichia coli*, enterococci, and aerobic bacteria presence (colony count) [3]. Coliform bacteria are not likely to cause disease; however, their presence in drinking water indicates that pathogenic organisms may also be in the water system by the Washington State Department of Health. The water-borne pathogenic bacteria that pose the most significant threat to human health are *Salmonella*, *Campylobacter* and *Yersinia* [3]. Pathogenic bacteria such as *E. coli* and *Salmonella* spp. have been demonstrated to not only be capable of surviving in drinking but have the potential to cause outbreaks among consumers [4]. Most people in

Korea use purified water as drinking water; but, according to a public awareness study by the Korean Ministry of Environment, 7.7% of people surveyed in May 2005, and 5.0% in June and July 2008, still drank unchlorinated drinking water. In this study, unchlorinated drinking water was defined as: water drunk, after being obtained from mountainous regions or an underground source, without being subjected to heat or other disinfection treatment [5]. The aims of the present study were: to assess the microbiological quality of unchlorinated drinking water in Korea; and to investigate the antimicrobial resistance of unchlorinated drinking water isolates.

Table 1. MIC interpretive standards according to the CLSI

(μg/mL)

Antimicrobial agent	Sensitive	Intermediate	Resistance
Enterobacteriaceae			
Cefonicid	≤8	16	≥32
Cefazolin	≤8	16	≥32
Ceftazidime	≤8	16	≥32
Meropenem	≤4	8	≥16
Tigecycline	≤2	4	≥8
Ciprofloxacin	≤1	2	≥4
Tobramycin	≤4	8	≥16
Staphylococcus spp.			
Oxacillin	≤2	-	≥4
Vancomycin	≤4	8-16	≥32
Teicoplanin	≤8	16	≥32
Daptomycin	≤1	-	-
Tigecycline	≤0.5	-	-
Ciprofloxacin	≤1	2	≥4
Linezolid	≤4	-	-
Mupirocin	-	-	≥25
Quinupristin/Dalfopristin	≤1	2	≥4
Gentamycin	≤4	8	≥16

MIC: minimal inhibitory concentration, CLSI: Clinical Laboratory and Standards Institute.

Table 2. Microbiological limits for acceptable drinking water according to the Korean Ministry of Environment

Criterion	Tap water*	Mineral water [†]
Total bacteriaa	< 10^2	< 10^2
Total coliforms	0	0
Fecal coliforms	0	0
Escherichia coli	0	0
Staphylococcus spp.	-	-

*colony-forming units per 100 mL, [†]colony-forming units per 250 mL.
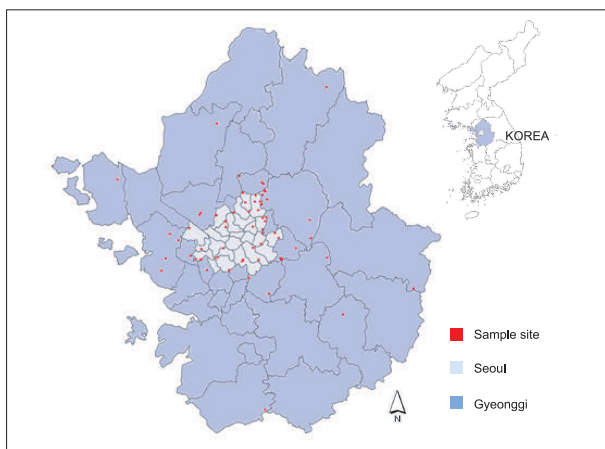

Figure 1. A map of sampling sites in Seoul and Gyeonggi province, Korea.

MATERIALS AND METHODS

l. Unchlorinated Drinking Water Samples

A total of 180 unchlorinated drinking water samples, from 61 sites, were analyzed. The sampling sites were mostly

Table 3. Bacterial traits by sources isolated from unchlorinated drinking water samples

(n=180)

Samples	n (%)	Distribution of bacterial count (CFU/mL)		
		10^0 - <10^1	10^1 - <10^2	> 10^2
Total bacteria	166 (92.2)	33 (18.3)	28 (15.6)	105 (58.3)
Total coliforms	28 (15.6)	14 (7.8)	6 (3.3)	8 (4.4)
Staphylococcus spp.	12 (6.7)	12 (6.7)	0 (0.0)	0 (0.0)

CFU: colony forming units.

situated in the mountainous regions of Seoul and Gyeonggi province. The location of the sampling sites is shown in Figure. 1.

All samples were gathered between March and May, 2010. Each sample was put in an attested 50 mL conical tube to prevent contamination. Collected samples were kept refrigerated at 4°C during the journey back to the laboratory [1].

ll. Microbiological Quality

Bacterial presence within the samples was quantified via the pour plate method and was recorded in colony forming units (CFU) per milliliter of unchlorinated drinking water [2]. For total bacteria counts, samples were plated on R2A agar (Difco, USA.) followed by incubation at 37°C for 48 hours. For the selective isolation of *Staphylococcus* spp. and total coliforms, the test media were Baird-Parker agar (Difco, USA.) and ENDO agar (Merck, Germany), respectively. The plates were incubated aerobically at 37°C for 24-48 hours. Total coliform counts were determined as the number of colonies that were red to reddish in colour with a permanent metallic sheen, or that were hemispherical or mucoid, according to the product catalog.

lll. Antimicrobial Susceptibility Test

Colonies sifted from the pre-cultures were grown overnight on Mueller Hinton broth (Difco, USA.) at 37°C for 16-18 hours, and were tested for resistance to various antibiotics (for total coliforms: cefonicid (Guk-Dong), cefazolin (Yuhan), ceftazidime (GlaxoSmithKline), meropenem (Yuhan), tigecycline (Wyeth Pharmaceuticals), ciprofloxacin (Il-Dong), and tobramycin (Dae-woong); and for *Staphylococcus* spp.: oxacillin (Sigma), vancomycin (Lilly), teicoplanin (Gruppo Lepetit S.P.A.), daptomycin (Cubist Pharmaceuticals), tigecycline (Wyeth Pharmaceuticals), ciprofloxacin (Il-Dong), linezolid (Pharmacia), mupirocin (Hanol), quinupristin/dalfopristin (Rhone-Poulenc Rorer), and gentamicin (Kuk-Je)). Minimal inhibitory concentrations (MICs) of the various agents were decided by the agar dilution method, according to the guidelines established by the Clinical Laboratory Standards Institute (CLSI) [6]. For susceptibility to daptomycin, the test medium was Muller Hinton broth adjusted to contain calcium at a concentration of 50 μg/mL; as recommended by

Table 4. Comparison of microbiological quality for unchlorinated drinking water samples according to month of collection and province (Seoul and Gyeonggi)

(n=180)

Samples	n (%)	Distribution of bacterial count (CFU/mL)		
		10^0 - $<10^1$	10^1 - $<10^2$	$> 10^2$
March (n=90)				
Total bacteria	82 (91.1)	8 (8.9)	12 (13.3)	62 (68.9)
Total coliforms	9 (10.0)	5 (5.6)	1 (1.1)	3 (3.3)
Staphylococcus spp.	3 (3.3)	3 (3.3)	0 (0.0)	0 (0.0)
May (n=90)				
Total bacteria	83 (92.2)	24 (26.6)	17 (18.9)	42 (46.7)
Total coliforms	19 (21.1)	9 (10.0)	5 (5.6)	5 (5.6)
Staphylococcus spp.	9 (10.0)	9 (10.0)	0 (0.0)	0 (0.0)
Seoul (n=116)				
Total bacteria	110 (94.8)	20 (17.2)	18 (15.5)	72 (62.1)
Total coliforms	16 (13.8)	7 (6.0)	4 (3.4)	5 (4.3)
Staphylococcus spp.	9 (7.8)	9 (7.8)	0 (0.0)	0 (0.0)
Gyeonggi (n=64)				
Total bacteria	56 (87.5)	13 (20.3)	10 (15.6)	33 (51.6)
Total coliforms	12 (18.8)	7 (10.9)	2 (3.1)	3 (4.7)
Staphylococcus spp.	3 (4.7)	3 (4.7)	0 (0.0)	0 (0.0)

CFU: colony forming units.

Table 5. MIC distributions for antimicrobial agents of *Staphylococcus* spp. and coliforms isolated from unchlorinated drinking water

n (%)

Antimicrobial agent	MIC (μg/mL)			Sensitive	Intermediate	Resistance
	Range	50%	90%			
Staphylococcus spp. (n=12)						
Oxacillin	<0.06 - >128	< 0.06	1	11 (92)	0 (0)	1 (8)
Vancomycin	≤0.06 - >128	≤0.06	0.5	11 (92)	0 (0)	1 (8)
Teicoplanin	<0.016 - >128	< 0.06	4	11 (92)	0 (0)	1 (8)
Daptomycin	<0.06 - 128	< 0.06	16	10 (83)	-	-
Tigecycline	<0.06 - 1	< 0.06	0.25	11 (92)	-	-
Ciprofloxacin	<0.06 - 4	< 0.06	<0.06	11 (92)	0 (0)	1 (8)
Linezolid	<0.06 - >128	< 0.06	16	10 (83)	-	-
Mupirocin	<0.06 - >128	< 0.06	>128	-	-	5 (42)
Quinupristin/dalfopristin	≤0.06 - >128	≤0.06	0.5	11 (92)	0 (0)	1 (8)
Gentamycin	≤0.06 - 8	≤0.06	0.5	11 (92)	1 (8)	0 (0)
Coliforms (n=28)						
Cefonicid	8 - >128	64	>128	6 (17.6)	6 (17.6)	22 (64.7)
Cefazolin	<0.06 - >128	> 128	>128	4 (11.8)	0 (0)	30 (88.2)
Ceftazidime	<0.06 - >128	0.25	32	27 (79.4)	0 (0)	7 (20.6)
Meropenem	<0.06 - 8	0.5	8	30 (88.2)	4 (11.8)	0 (0)
Tigecycline	<0.06 - 8	1	4	30 (88.2)	2 (5.9)	2 (5.9)
Ciprofloxacin	<0.06 - 4	0.12	1	32 (94.1)	0 (0)	2 (5.9)
Tobramycin	<0.06 - 8	0.5	1	33 (97.1)	1 (2.9)	0 (0)

MIC: minimal inhibitory concentration.

drug description. The resistance of samples was characterized according to the guidelines established by the CLSI (Table 1). But, no interpretive criteria have been approved for tigecycline and daptomycin resistance, according to the existence of definitions by drug description [7]. The resistant isolates were identified by Samkwang Medical Laboratories.

RESULTS

1. Microbiological Quality

One hundred eighty samples of water mainly used for human drinking were collected in various places of Seoul and Gyeonggi province in Korea during March and May, 2010. The microbiological quality of unchlorinated drinking water samples was determined with reference to the values in Table 2.

The respective prevalence of total bacteria, total coliforms and *Staphylococcus* spp. are shown in Table 3. A total of

Table 6. Antimicrobial agent resistant bacteria isolated from unchlorinated drinking water

Strain	n (%)
Achromobacter denitrificans	1 (2.9)
Acinetobacter haemolyticus	1 (2.9)
Acinetobacter ursingii	1 (2.9)
Acinetobacter lwoffii	2 (5.9)
Acinetobacter baumannii	1 (2.9)
Cupriavidus pauculus	1 (2.9)
Hafnia alvei	2 (5.4)
Pseudomonas oryzihabitans	3 (8.8)
Pseudomonas fluorescens	9 (26.5)
Pseudomonas aeruginosa	7 (20.6)
Rahnella aquatilis	1 (2.9)
Serratia fonticola	2 (5.9)
Serratia liquefaciens	2 (5.9)
Yersinia enterocolitica	1 (2.9)

180 samples, Total bacteria, total coliforms, and *Staphylococcus* spp. were detected in 166 (92.2%), 28 (15.6%), and 12 (6.7%) unchlorinated drinking water samples, respectively. Of the 180 sample tested, 105 (58.3%) and 28 (15.6%) were of an unacceptable quality according to the total bacteria count and total coliforms count, respectively.

A comparison of the microbiological quality of samples from Seoul and Gyeonggi province is provided in Table 4. The water was of an unacceptable quality in 72 (62.1%) out of 116 samples from Seoul and 33 (51.6%) out of 64 samples in Gyeonggi. The prevalence of total bacteria and of *Staphylococcus* spp. was higher in the Seoul samples than those from Gyeonggi, except for total coliforms.

The prevalence of total coliforms in samples collected during May was twice as high as in March (Table 4).

II. Antimicrobial Resistance

The resistance to various antibiotics was tested in the 12 *Staphylococcus* spp. isolates and 28 coliform isolates.

Staphylococcus spp. isolates were susceptible to daptomycin, tigecycline, and linezolid; however, isolates from some samples exhibited a high-level of resistance to mupirocin (42.0%), oxacillin (8.0%), vancomycin (8.0%), teicoplanin (8.0%), ciprofloxacin (8.0%), quinupristin /dalfopristin (8.0%) (Table 5). Mupirocin is used as a topical treatment for bacterial skin infections. Therefore, it is useful in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) [8]. Resistance to mupirocin among *Staphylococcus* spp. is an emerging problem worldwide and may be contributing to failures regarding the extermination of MRSA [9].

The MIC$_{50}$, MIC$_{90}$, and MIC ranges, of the coliforms isolated from the water samples, to the seven antimicrobials tested for are shown in Table 5. Most of the coliform isolates exhibited high-level resistance to cefazolin (88.2%), cefonicid (64.7%), ceftazidime (20.6%), and ciprofloxacin

(5.9%). Additionally, these isolates were resistance to new antimicrobial agents such as tigecycline (5.9%). However, resistance to meropenem and tobramycin were not observed in any coliforms isolates (MIC$_{90}$, 1 μg/mL) (Table 5).

Identification of unchlorinated drinking water isolates confirmed various antibiotic-resistant strains of the bacterial species, such as *Serratia fonticola, Serratia liquefaciens, Hafnia alvei*, and *Rahnella aquatilis* (Table 6); furthermore, other bacteria that are known to opportunistic pathogens were isolated: *Pseudomonas aruginosa* (7 samples), *Pseudomonas fluorenscens* (9 samples), and *Pseudomonas oryziabitants* (3 samples). *Acinetobacter* spp. were also isolated from 5 samples: *Acinetobacter baumannii* (1 sample), *Acinetobacter haemolyticus* (1 sample), *Acinetobacter lwoffii* (2 samples), and *Acinetobacter ursingii* (1 sample).

DISCUSSION

Contaminated drinking water has become a cause of numerous waterborne disease outbreaks in the developing area [10]. Many studies have found these microorganisms to be those most frequently discovered in drinking water [11-12]. In this study, total bacteria, staphylococci and coliform counts were determined to investigate the microbiological quality of unchlorinated drinking water samples. Among 180 water samples, 92.2%, 15.6% and 6.7% were shown to unacceptable quality based on total bacteria, total coliforms and staphylococci counts, respectively. This suggests a relatively high prevalence of antibiotic resistant bacteria as compared to other study. (The occurrence rate of total bacteria (92.2%), coliform (15.6%), staphylococci (6.7%) were found to relatively high.) Sohn et al. [13] observed antimicrobial resistance of coliform isolates from unchlorinated natural drinking water in northern Gyeongbuk province, Korea and found that 9.7% of the isolates were resistant to the antibiotics tested. Messi et al. [14] investigated antibiotic resistance in heterotrophic bacteria from mineral water. *Pseudomonas* spp. and *Acinetobacter* spp. were isolated, amongst others; of these isolates were resistant to chloramphenicol (60%), to ampicillin (55.0%) and to carbenicillin (27.5%). Strains with multi-antibiotic resistance were detected in 55.0% of the isolates. *Acinetobacter* species have been involved in hospital outbreaks and are common inhabitants of some water sources [15]. *Acinetobacter* spp. (especially *Acinetobacter baumannii*) have recently emerged as pathogens of considerable interest, particularly as the causative agents of nosocomial pneumonia or of complications in immunocompromised patients [7].

Water is an essential part of life and a satisfactory supply must be provided to everyone [16]. To date, in Korea, there is no legislation on microbiological standards for

unchlorinated drinking water. Many people believe that unchlorinated drinking water is superior to tap water because it does not contain microorganism. However, this investigation shows this belief to be incorrect. The results of this study suggest, according to current standards regarding the presence of coliforms in drinking water, consumers of unchlorinated drinking water in Korea should be concerned about water-borne disease. Due to the presence of opportunistic pathogens and enteropathogenic bacteria detected in this study we believe in the need for more detailed studies on the potential health risks posed by unchlorinated drinking water. Therefore, we suggest the need for continuous monitoring of unchlorinated drinking water and to establish practicable standards for the safety of consumers.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare on this study.

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