



ORIGINAL ARTICLE

Enteric Bacteria Isolated from Diarrheal Patients in Korea in 2014

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Abstract

Objectives: The aim of this study was to characterize the pathogens responsible for causing diarrhea according to season, region of isolation, patient age, and sex as well as to provide useful data for the prevention of diarrheal disease.

Methods: Stool specimens from 14,886 patients with diarrhea were collected to identify pathogenic bacteria from January 2014 to December 2014 in Korea. A total of 3,526 pathogenic bacteria were isolated and analyzed according to season, region of isolation, and the age and sex of the patient.

Results: The breakdown of the isolated pathogenic bacteria were as follows: *Salmonella* spp. 476 (13.5%), pathogenic *Escherichia coli* 777 (22.0%), *Vibrio parahaemolyticus* 26 (0.74%), *Shigella* spp. 13 (0.37%), *Campylobacter* spp. 215 (6.10%), *Clostridium perfringens* 508 (14.4%), *Staphylococcus aureus* 1,144 (32.4%), *Bacillus cereus* 356 (10.1%), *Listeria monocytogenes* 1 (0.03%), and *Yersinia enterocolitica* 10 (0.3%). The isolation rate trend showed the highest ratio in the summer season from June to September for most of the pathogenic bacteria except the Gram-positive bacteria. The isolation rate of most of the pathogenic bacteria by patient age showed highest ratio in the 0–19 year age range. For isolation rate by region, 56.2% were isolated from cities and 43.8% were isolated from provinces.

Conclusion: Hygiene education should be addressed for diarrheal disease-susceptible groups, such as those younger than 10 years, aged 10–19 years, and older than 70 years, and monitoring for the pathogens is still required. In addition, an efficient laboratory surveillance system for infection control should be continued.

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1. Introduction

An estimated 9.4 million foodborne illnesses caused by a known pathogen occur annually in the United States [1]. It has been reported that ~2 million diarrhea disease patients die per year throughout the world [2–4]. Considering the public health importance of acute diarrhea diseases, laboratory surveillance of acute diarrhea diseases is utilized in many countries. In the United States, the Foodborne Diseases Active Surveillance Network (FoodNet, www.cdc.gov/foodnet) tracks important foodborne illnesses and generates information that provides a foundation for food safety policies and prevention efforts [5]. Australia has a supervising system for foodborne diseases known as OzFoodNet (www.ozfoodnet.gov.au) [6]. In Canada, FoodNet-Canada (www.phac-aspc.gc.ca/foodnetcanada), which is an area-based monitoring system, is used.

In Korea, the acute diarrheal disease laboratory surveillance system (Enter-Net) is used. The Enter-Net system is coordinated by the Korea National Institute of Health (KNIH), and comprises 17 local Public Health Institutes (PHIs) and 68 participating hospitals. Since 2003 when Enter-Net was established, the program has provided information that contributes to public health by monitoring laboratory-confirmed infections caused by pathogens, attributing illnesses to specific pathogens, and disseminating information [7,8].

Since its founding, Enter-Net has been an excellent example of partnership between KNIH and the local PHIs. Enter-Net has monitored laboratory-confirmed infections caused by ten bacterial pathogens (pathogenic *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio parahaemolyticus*, *Campylobacter* spp., *Staphylococcus aureus*, *Clostridium perfringens*, *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Bacillus cereus*) and five viruses (rota, noro, adeno, sapo, and astro virus) [1,9,10].

In this surveillance study, we analyzed isolated bacterial pathogens by season, patient age and sex, and region of isolation. This report will be useful for the prevention of diarrheal disease and to improve the public health care system and sanitation in Korea.

2. Materials and methods

2.1. Stool sample collection

A total of 14,886 stool samples were collected by the acute diarrheal disease laboratory surveillance system (Enter-Net) in 2014. The Enter-Net system is coordinated by the Korea NIH and comprises 17 local PHIs and 68 participating hospitals. A stool sample was collected from patients who had diarrhea symptoms (diarrhea was defined as “the passage of three or more unformed stools per 24 hour period, with at least one passage accompanied by symptoms of nausea, vomiting,

abdominal cramps or pain, fever or blood in the stool” [11,12]).

2.2. Bacterial isolation and primary identification

Different selective agar plates were used to isolate the pathogenic bacteria. MacConkey agar was used for the detection of *E. coli*, *Salmonella*, and *Shigella* species. Thiosulfate-citrate-bile salts-sucrose (TCBS; Oxoid, Basingstoke, UK) agar was used for the detection of *V. parahaemolyticus*, Mannitol-Salt Agar (MSA; Oxoid) for *S. aureus*, Tryptose-Sulfite-Cycloserine (TSC; Oxoid) for *C. perfringens*, Modified Campylobacter Blood-Free Selective Agar Base (mCCDA; Oxoid) for *Campylobacter* species, Listeria Selective Agar (LSA; Oxoid) for *L. monocytogenes*, Cefsulodin-Irgasan-Novobiocin (CIN; Oxoid) for *Y. enterocolitica*, and Mannitol-Egg Yolk-Polymixin (MYP; Oxoid) for *B. cereus*. For the primary identification of *E. coli*, *Salmonella*, *Shigella*, *Vibrio*, and *Yersinia*, the standard physiological and biochemical tests with an API 20E and VITEKII (BioMérieux, Marcy l’Etoile, France) were employed. For the identification of *Campylobacter*, *C. perfringens*, *Bacillus cereus*, *Listeria*, and *Staphylococcus*, API Campy, API 20A, 50CHB/E, API Listeria, and API Staphy were used, respectively.

2.3. DNA manipulations and genetic techniques

PCR was also used to screen the bacterial pathogens. A loopful of the human stool sample was directly inoculated into 3 mL of Tryptic Soy Broth (TSB; Oxoid) for enrichment and was incubated overnight at 37°C with shaking. After incubation, the enriched broth culture was used for isolation of chromosomal DNA. The template DNA for PCR was extracted by the conventional boiling method. All of the PCRs were performed with the Expanded High Fidelity Polymerase System (Roche) or *Taq* polymerase (Takara, Shiga, Japan) according to the manufacturer’s instructions. The primers used for amplification are listed in Tables S1 and S2.

2.4. Serotyping

The serotyping of *Salmonella*, *E. coli*, *Vibrio*, and *Shigella* spp. was carried out according to the manufacturer’s instructions (Denka Seiken, Tokyo, Japan and Difco, New Jersey, USA).

2.5. Statistical analysis

The collected data were analyzed using the SPSS 20.0 statistical package (IBM, Seoul, Korea). The patients’ age and sex, and the month and regional distributions of the isolated pathogens were analyzed using the Chi-square or Chi-square trend test. For the statistical analysis, $p < 0.05$ was taken to be significant.

Table 1. The monthly isolation rate of the bacterial pathogens.

Pathogens	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	
<i>Salmonella</i>	Enteritidis	3	2	4	3	5	10	37	14	14	5	5	5	107
	Typhimurium	1	3	1	4	12	8	7	8	5	0	3	3	55
	others	13	10	14	12	17	33	57	45	61	27	18	7	314
	Subtotal	17	15	19	19	34	51	101	67	80	32	26	15	476
<i>Escherichia coli</i>	EHEC	0	1	0	3	2	8	12	5	3	2	2	0	38
	ETEC	1	1	0	6	2	8	12	19	19	5	1	5	79
	EAEC	15	10	11	6	3	7	12	18	22	11	10	14	139
	EPEC	26	13	19	14	19	45	111	83	80	42	28	35	515
	EIEC	2	0	0	0	0	0	1	2	1	0	0	0	6
	Subtotal	44	25	30	29	26	68	148	127	125	60	41	54	777
<i>Vibrio parahaemolyticus</i>	0	0	1	1	0	0	0	4	8	1	7	1	23	
<i>Shigella</i> spp.	3	1	0	0	0	3	0	1	0	0	3	5	16	
<i>Campylobacter</i> spp.	6	5	3	2	14	27	59	52	12	9	15	11	215	
<i>Clostridium perfringens</i>	82	59	60	64	41	31	35	33	12	42	32	17	508	
<i>Staphylococcus aureus</i>	93	66	79	81	86	88	120	124	127	85	91	104	1,144	
<i>Bacillus cereus</i>	17	9	8	25	22	39	60	56	42	32	19	27	356	
<i>Listeria monocytogenes</i>	0	0	0	0	0	0	1	0	0	0	0	0	1	
<i>Yersinia enterocolitica</i>	0	1	0	4	2	0	0	2	0	0	1	0	10	
Isolations (n)	262	181	200	225	225	307	524	466	406	261	235	224	3,526	
Specimens (n)	1,503	1,049	1,166	1,361	1,080	1,242	1,608	1,284	1,313	976	1,036	1,268	14,886	
Isolation rate (%)	17.4	17.3	17.2	16.5	20.8	24.7	32.6	36.3	30.9	26.7	22.7	17.7	23.7	

EAEC = Enteroaggregative *E. coli*; EHEC = Enterohaemorrhagic *E. coli*; EIEC = Enteroinvasive *E. coli*; EPEC = Enteropathogenic *E. coli*; ETEC = Enterotoxigenic *E. coli*.

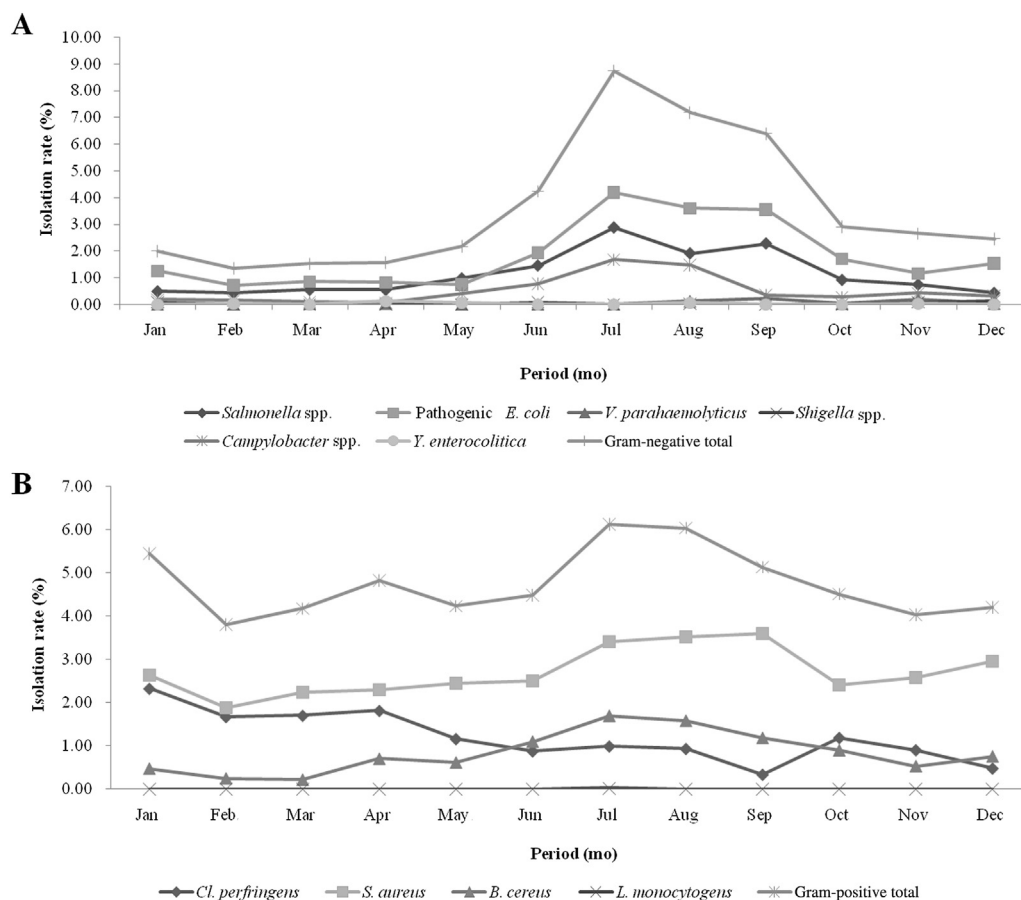


Figure 1. Seasonal isolation rate patterns. (A) Gram-negative bacterial pathogens and (B) Gram-positive bacterial pathogens are shown.

3. Results

3.1. Pathogenic bacteria isolation

From January 2014 to December 2014, stool specimens were collected from 14,886 diarrheal patients to identify the pathogenic bacteria involved. Among the 14,886 stool specimens, 3,526 pathogenic bacteria (23.7%) known to cause diarrhea were isolated from the stool specimens. The breakdown of the 3,526 bacteria isolates was as follows: *Salmonella* spp. 476 (13.5%), pathogenic *E. coli* 777 (22.0%), *V. parahaemolyticus* 26 (0.74%), *Shigella* spp. 13 (0.37%), *Campylobacter* spp. 215 (6.10%), *C. perfringens* 508 (14.4%), *S. aureus* 1,144 (32.4%), *B. cereus* 356 (10.1%), *L. monocytogenes* 1 (0.03%), and *Y. enterocolitica* 10 (0.3%); [Table 1](#)). In additional analysis, 208 (1.40%) infected patients were found to represent duplicate cases. Among the isolated *Salmonella* strains, the representation of *S. Enteritidis*, *S. Typhimurium* and *Salmonella* spp. was 120 (25.2%), 74 (15.5%), and 282 (59.2%), respectively. In the pathogenic *E. coli* group, Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETEC), Enterohaemorrhagic *E. coli* (EHEC), and Enteroinvasive *E.*

coli (EIEC) were represented by 513 (66.0%), 139 (17.9%), 80 (10.3%), 39 (5.0%), and 6 (0.8%) isolates, respectively ([Table 1](#)).

3.2. Seasonal prevalence of the isolated bacterial pathogens

We analyzed the pathogenic bacteria isolation rate by month (from January to December). An average isolation rate of 10 pathogenic bacteria gradually increased from May and was highest in August at 36.29% (summer) and decreased to the lowest rate of 16.53% by April (spring; [Table 1](#) and [Fig. 1](#)). Among the 10 pathogenic bacteria, Gram-negative bacteria, *Salmonella* spp., pathogenic *E. coli*, and *Campylobacter* spp., showed an increasing pattern by temperature, which increased from May and was highest from June to July. *Salmonella* spp., pathogenic *E. coli*, *Campylobacter* spp., showed the highest prevalence in July. Interestingly, *Shigella* spp. was isolated mostly in the winter season in November and December ([Table 1](#) and [Fig. 1A](#)). However, with Gram-positive bacteria, *C. perfringens* and *S. aureus*, the average isolation rate tended to be even throughout the year. Only *B. cereus* showed a similar trend to that of the Gram-negative

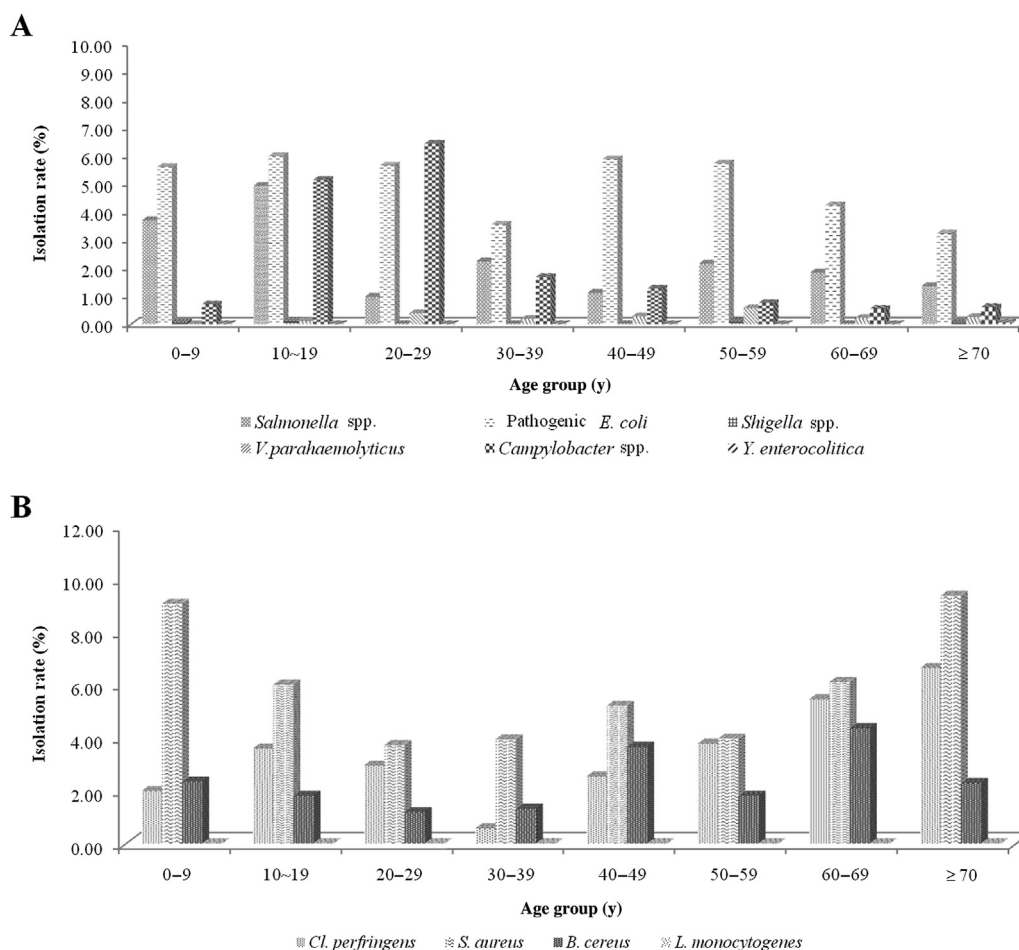


Figure 2. Isolation rates. (A) Gram-negative and (B) Gram-positive bacterial pathogens on the basis of age are shown.

bacteria, specifically the pathogenic *E. coli* (Table 1 and Fig. 1B).

3.3. Age and sex-specific prevalence of the isolated bacterial pathogens

Next, we analyzed the pathogenic bacteria isolation rate based on eight patient age categories: < 10 years, 10–19 years, 20–29 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, and ≥ 70 years (Fig. 2). An average isolation rate of the 10 pathogenic bacteria by age was highest among patients aged < 10 years (44.5%) and ≥ 70 years (13.4%), and the difference among the age classes was significant based on the Chi-square test ($p < 0.05$). Among the 10 pathogenic bacteria, Gram-negative pathogenic bacteria (*Salmonella* spp., pathogenic *E. coli*, *V. parahaemolyticus*, *Shigella* spp., and *Campylobacter* spp.) showed a similar pattern with the average isolation rate. Gram-positive pathogenic bacteria (*S. aureus* and *B. cereus*) showed a similar pattern to the Gram-negative bacteria, and the isolation rate was highest in the < 10 years group and decreased gradually with age. However, only *C. perfringens* showed an increasing pattern with age (Table 2). Next we examined the isolation rate by sex. An average isolation rate of the 10 pathogenic bacteria by sex was 45.0% for men and 38.3% for women (Table 2); however, the Chi-square test result was not significant ($p \geq 0.05$).

3.4. Regional prevalence of the isolated bacterial pathogens

A total of 14,886 total samples obtained from patients with acute diarrhea were analyzed by regional prevalence (cities or rural provinces). Of the isolated samples, 1,982 (56.2%) pathogenic bacteria were isolated from 6,823 urban patients, and 1,544 (43.8%) were isolated from 8,063 rural patients. As shown in Table 3, the number of isolated bacteria ranged from 138 (3.9%) to 565 (16.0%) in cities, while in the rural province region, it ranged from 63 (1.8%) to 418 (11.9%; Table 3).

4. Discussion

Diarrheal disease continues to be an important problem in the world [13]. In our surveillance results of bacterial pathogens, pathogenic *E. coli* (22.0%) and *Salmonella* spp. (13.5%) were the major Gram-negative bacteria isolated and *S. aureus* (32.4%) and *C. perfringens* (14.4%) were the major Gram-positive bacteria isolated.

In the pathogenic *E. coli* group, EPEC was the most frequently isolated pathogen (66.3% in pathogenic *E. coli*, 14.6% in isolated pathogens), which was followed by EAEC (17.9% in pathogenic *E. coli*, 3.9% in isolated pathogens). High frequencies of EPEC isolation were also found in another countries, such as Chile (38.3%)

Table 2. The isolation rate of the bacterial pathogens by age and sex.

Age (y)0–9	No. of specimens (n = 14,886)	No. of isolates (n = 3,526)	Pathogenic									
			<i>Salmonella</i> spp. (n = 476)	<i>Escherichia coli</i> spp. (n = 777)	<i>Shigella</i> spp. (n = 13)	<i>Vibrio parahaemolyticus</i> (n = 26)	<i>Campylobacter</i> spp. (n = 215)	<i>Clostridium perfringens</i> (n = 508)	<i>Staphylococcus aureus</i> (n = 1,144)	<i>Bacillus cereus</i> (n = 356)	<i>Listeria monocytogenes</i> (n = 1)	<i>Yersinia enterocolitica</i> (n = 10)
10–19	951	263	47	57	1	1	49	34	606	156	0	0
20–29	512	109	5	29	0	2	33	15	57	17	0	0
30–39	535	72	12	19	0	1	9	3	19	6	0	0
40–49	713	142	8	42	0	2	9	18	21	7	0	0
50–59	1,063	200	23	61	1	6	8	40	37	26	0	0
60–69	920	209	17	39	0	2	5	50	42	19	0	0
≥ 70	1,979	471	27	64	1	5	12	131	56	40	0	0
Unknown	1,510	490	88	90	6	7	43	85	185	45	0	1
Sex												
Male	6,787	1,588	221	354	5	8	95	200	121	40	1	9
Female	5,993	1,352	172	286	3	12	77	183	547	153	0	5
Unknown	2,106	586	83	137	5	6	43	125	463	154	0	2
									134	49	1	3

Table 3. Regional distribution of the isolated bacterial pathogens.

	Regions	Specimens	<i>Salmonella</i>	<i>Shigella</i>	<i>Vibrio</i>	<i>Campylobacter</i>	<i>Clostridium</i>	<i>Staphylococcus</i>	<i>Bacillus</i>	<i>Listeria</i>	<i>Yersinia</i>	Isolation Total	
			spp.	<i>Escherichia coli</i> spp.	<i>parahaemolyticus</i>	spp.	<i>perfringens</i>	<i>aureus</i>	<i>cereus</i>	<i>monocytogenes</i>	<i>enterocolitica</i>		
Cities	Seoul	676	27	73	1	0	42	121	20	10	0	0	294
	Busan	868	21	30	0	0	8	72	44	11	0	0	186
	Daegu	821	12	48	0	0	7	52	23	8	0	0	150
	Incheon	1,237	81	115	7	2	40	187	60	72	0	1	565
	Gwangju	1,483	104	103	0	0	27	113	57	61	0	1	466
	Daejeon	812	8	40	0	2	14	13	43	17	1	0	138
	Ulsan	926	8	73	0	1	30	62	7	2	0	0	183
	Subtotal	6,823	261	482	8	5	168	620	254	181	1	2	1,982
Rural province	Gyeonggi	1,495	28	75	0	6	11	99	21	36	0	0	276
	Gangwon	1,342	60	56	0	2	1	41	4	22	0	0	186
	Chungnam	788	11	26	1	3	5	36	69	8	0	0	159
	Chungbuk	764	7	35	0	1	5	51	64	18	0	0	181
	Jeonnam	409	21	16	3	0	1	18	11	3	0	0	73
	Jeonbuk	1,815	19	38	0	0	4	231	34	84	0	8	418
	Gyeongnam	565	6	28	1	9	0	14	28	0	0	0	86
	Gyeongbuk	370	16	11	0	0	0	30	2	4	0	0	63
	Jeju	515	47	10	0	0	20	4	21	0	0	0	102
	Subtotal	8,063	215	295	5	21	47	524	254	175	0	8	1,544
Total	14,886	476	777	13	26	215	1,144	508	356	1	10	3,526	

Chungbuk = Chungcheongbuk-do; Chungnam = Chungcheongnam-do; Gyeongbuk = Gyeongsangbuk-do; Gyeongnam = Gyeongsangnam-do; Jeonbuk = Jeonllabuk-do; Jeonnam = Jeollanam-do.

and Brazil (34.0%), but low frequencies were observed in Thailand (5.5%) [14–16]. Based on a recent report, EAEC was the most frequent diarrheagenic *E. coli* category in Brazil [17]. EHEC and ETEC isolates represented < 10% of the pathogenic *E. coli* with 3.7% and 9.5% representation, respectively (0.9% and 2.5% of the total isolated pathogens, respectively). In the USA FoodNet Annual Report, EHEC O157 and EHEC non-O157 represented 2.7% and 2.8% of the isolated pathogens, respectively (www.cdc.gov/foodnet).

Salmonella represented the second largest pathogen group (13.5%). Among *Salmonella* strains, there were twice as many *S. Enteritidis* isolates as *S. Typhimurium* isolates, which is similar to the pattern described in a previous report [18]. Additionally, there are reports that a similar rate (11%) of *Salmonella* species isolation was detected through the surveillance of childhood diarrheal diseases in Asia and Hong Kong, but another study indicated that the rate of enteric infection from *Salmonella* species is 1.8% in Bangladesh, which is lower than our report [19,20].

Campylobacter is one of the most common causes of human diarrheal disease in the US (34.7% in isolated pathogens; 2012 Annual Report), but cases of human Campylobacteriosis may have been underestimated in Korea at 6.1% [21–23]. Pathogenic *E. coli*, *Salmonella*, and *Campylobacter* spp. showed an increasing prevalence pattern by temperature with an increase from May to a peak at June–July.

There were only 16 *Shigella* spp. and 10 *Y. enterocolitica* isolates identified in Korea in 2014. *Shigella* spp. pathogens were isolated mainly in the winter season, which was unlike the other Gram-negative bacteria.

S. aureus is widely distributed in the surrounding environment and can be the cause of severe infectious diseases. As it is tolerant of harsh environments, such as human skin and various foods, *S. aureus* is easily isolated and represented the highest frequency of any pathogen in our study at 32.4%. Additionally, *S. aureus* was the fourth most frequent pathogen associated with foodborne illness in Korea (www.kfda.go.kr/e-stat/).

The isolation rate of Gram-positive pathogenic bacteria by season tended to be distributed evenly throughout the year. In addition, the isolation rate was highest under the age of 10 years and decreased gradually as age increased. However, *C. perfringens* isolation increased with age. Of note, the isolation rate of *S. aureus*, *C. perfringens*, and *B. cereus* strains in this study may have been overestimated because the Enter-Net surveillance system diagnoses these pathogens by presence of the enterotoxin genes by PCR.

There are few comparable studies concerning adult patients with diarrhea, but there are considerably more studies regarding childhood diarrhea [24–28]. In British and Swedish studies, the presence of at least one enteropathogen was detected in 45% and 56% of patients, respectively [29,30]. The difference between

previous research and our surveillance results may be due to many different factors, including the sampling procedures, diagnostic methods, and treatment with antibiotics shortly after the onset of diarrhea. We plan to analyze more than a 1 year time frame using epidemiological and clinical data in the near future. Our analysis consists of surveillance of sporadic cases of illness caused by these pathogens, which largely excludes cases of foodborne outbreaks.

In conclusion, hygiene education should be addressed for diarrheal disease-susceptible groups, such as those preschool children, young generation, and the old and weak class, and monitoring for the pathogens is still required. As most diarrheal illnesses are preventable, an efficient laboratory surveillance system for monitoring prevention progress and infection control should be continued.

Conflicts of interest

All authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.phrp.2015.07.005>.

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