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Surveillance of Bacterial Pathogens Associated with Acute Diarrheal Disease in the Republic of Korea During One Year, 2003

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An epidemiological survey of human enterobacterial infections was conducted to determine the prevalence of enteropathogens in the Republic of Korea during one year, 2003. We tested for infectious diseases in 26,992 stool samples obtained from people who visited clinics located in six big cities and six rural provinces. From these samples, we isolated 1,291 cases of enteritis bacterial infection (4.8%). In the urban areas, 821 cases of bacterial infection (6.4%) were identified and, in the rural areas, 479 bacterial strains (3.3%) were isolated. Seasonal patterns were seen for diarrhea associated with *S. aureus*, *E. coli* and *V. parahaemolyticus*, while *Salmonella* and *Shigella* infections showed slight seasonal variation. We found that *S. aureus* and *Salmonella* were more frequently isolated from children and the elderly; however, the prevalence of *E. coli*, *V. parahaemolyticus*, and *Shigella* were similar in different age groups. Routine monitoring of these infections is considered a worthwhile means by which to elucidate their epidemiology and modes of transmission and ultimately to control them more effectively. Continuous laboratory-based surveillance for findings of enteritis bacterial infection should be emphasized in the prevention of these infections.

Keywords: epidemiology, enteropathogens, *Staphylococcus aureus*, *Salmonella*

Diarrhea continues to be one of the most common causes of morbidity and mortality among infants and children in developing countries (Bern *et al.*, 1992; Nataro and Kaper, 1998; Clarke, 2001). Acute diarrhea is an extraordinarily common disease with worldwide distribution and a significant impact of public health. Diarrheal diseases are the cause of almost three million deaths annually (Baudry *et al.*, 1990; Black, 1993; Guerrant *et al.*, 2002), mainly among children younger than five years of age. Approximately 35% of the deaths can be attributed to acute non-dysenteric diarrhea and an estimated 45% occur in children with persistent diarrhea (Murray and Lopez, 1996).

Universal surveillance for diarrhea in patients has not been conducted adequately in the Republic of Korea, except for in infants and children. In the Republic of Korea, infant mortality, which is highly correlated with diarrheal disease mortality, declined from 53 per 1,000 live births to 14 per 1,000 live births over the past 20 years (Korean Ministry of Health and Society Affairs, 1984; Hong, 1985; Kim *et al.*, 1989). Nevertheless, among all children in hospitals, diarrhea frequency has been high, ranging from 10% to 18%. Few studies have been conducted in the Republic of Korea to determine the incidence of the different enteric pathogens associated with diarrhea. In addition to the classical pathogenic agents *Salmonella*, *Shigella*, enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *E. coli* (ETEC), and enteroinvasive *E. coli* (EIEC) (Germani *et al.*, 1994), the following recently recognized agents are of interest: *Vibrio cholerae* O1, O139, *Campylobacter jejuni* (Varavithya *et al.*, 1990), *Clostridium difficile*, *Aeromonas hydrophila*, enteroadherent *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), and rotavirus (Albert *et al.*, 1999).

The Korean National Institute of Health (KNIH), Center for Infectious Diseases, Division of Enteric Bacterial Infections, located in Seoul, is a national standards laboratory that is utilized for identification of bacteria related to intestinal infectious diseases, including identification and confirmation of the isolates. To obtain useful information representative of

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all patients, a surveillance system in which stool specimens are studied in detail, for etiological agents of diarrhea and other conditions, was instigated by the KNIH in 2003.

In order to determine the significance of bacterial species as possible pathogenic microorganisms that cause diarrhea, we determined the prevalence of seasonal, age-specific and regional patterns. The data obtained have been specifically analyzed for a possible association between the urban and rural locations of patients and the incidence of diarrhea.

Materials and Methods

Surveillance study design

The survey was carried out as part of a national program for the control of diarrheal disease. The surveillance study was planned by the Laboratory of Enteric Infections of the Korean Center for Disease Control and Prevention. The plan was based on six big cities and six provinces in the Republic of Korea. Public health institutes (PHIs) of these cities and provinces, which acted the infectious agent surveillance centers, collected stool samples from diarrheal patients and performed laboratory examinations to isolate clinical specimens from the stools. In a one-year period, from January to December 2003, a total of 26,992 stools were collected from patients with diarrhea.

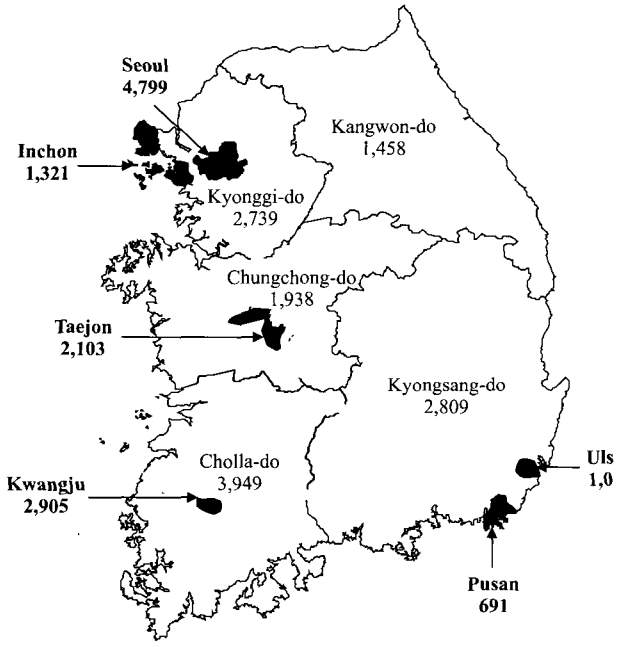

Fig. 1. Number of stool samples obtained from large urban centers (bold writing) and rural provinces in Korea.

Stools and bacteria isolated from the stools

The stools were collected in the different geographical areas: six big cities (Seoul, Pusan, Inchon, Kwangju, Taejon, and Ulsan) and six provinces (Kyonggi-do, Kangwon-do, Chungchong-do, Cholla-do, Kyongsang-do, and Cheju-do). As shown in Fig. 1, 12,882 stools were collected from patients in the six big cities and 14,110 stools were collected in the six rural provinces. The bacterial pathogens found on selective agar plates – pathogenic *E. coli, Salmonella* spp., *Shigella* spp., *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Campylobacter jejuni* and *Listeria monocytogenes* – were assayed by API test and polymerase chain reaction.

Media for the isolation of bacteria from stools

Bacteria from stool samples were cultivated on eight different selective agar plates in order to isolate the microorganisms. MacConkey agar was used for the detection of *E. coli* and *Salmonella* and *Shigella* species. Thiosulfate-citrate-bile salts-sucrose (TCBS) agar was used for the detection of *Vibrio* species, Mannitol-Salt Agar (MSA) for *Staphylococcus aureus,* Tryptose-Sulfite-Cycloserine (TSC) for *Clostridium perfringens*, Campylobacter Blood-Free Selective Agar Base (CCDA) for *Campylobacter jejuni*, Listeria Selective Agar (LSA) for *Listeria monocytogenes*, Cefsulodin-Irgasan-Novobiocin (CIN) for *Yersinia enterocolitica*, and Mannitol-Egg Yolk-Polymixin (MYP) for *Bacillus cereus*.

Detection of target genes by polymerase chain reaction

A loopful of human stool sample was used to directly inoculate 3 ml of LB broth for enrichment, and the both was then incubated with shaking overnight at 37°C. After incubation, enriched broth culture was centrifuged at 13,000 rpm (Sorvall® Biofuge Pico, Germany) for 1 min and the pellet was heated at 100°C for 10 min. Following centrifugation of the lysate, 5 μl of the supernatant was used for PCR. To detect pathogen target genes, PCR assays were performed using the primers shown in Table 1. PCR assays were carried out in 50 μl with 2U DNA Taq polymerase (Takara Ex Taq™, Japan) in a thermal cycler (PTC-100; MJ Research, USA) under the following conditions: initial denaturation for 5 min at 94°C; 30 cycles of 1 min each for denaturation (94°C), annealing and extension (72°C); and then a final cycle at 72°C for 5 min. Amplified PCR products were analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide, visualized using UV illumination, and imaged with the Gel Doc 2000 documentation system (Bio-Rad, USA).

Table 1. Primers used in this study

Pathogen	Target gene	Primer sequence (5' to 3')	Size of the PCR product (bp)
Salmonella spp.	*inv*	ATTAATTATGGAAGCGCTCGCATT	247
		GTAATGAGATCCATCAAATTAGCG	
Shigella spp.	*ial*	GTTGCGCTTGATGGGTGGGGTATC	356
		GAAATGTCCATCAAACCCCACTC	
STEC	Shiga toxin 1 (*stx*1)	CGTACGGGGATGCAGATAAATCGC	210
		CAGTCATTACATAAGAACGCCCAC	
	Shiga toxin 2 (*stx*2)	GTTCTGCGTTTTGTCACTGTCAC	326
		GTCGCCAGTTATCTGACATTCTGG	
EAEC	Heat-stable enterotoxin (*east*1)	ATGCCATCAACACAGTATATCCG	119
		TCAGGTCGCGAGTGACGGCTTT	
EPEC	Attaching and effacing (*eae*A)	ATGCTGGCATTTGGTCAGGTCGG	233
		TGACTCATGCCAGCCGCTCATGCG	
ETEC	Heat-labile toxin (*lt*)	GATCACGCGAGAGGAACACAAACC	366
		ATCTGTAACCATCCTCTGCCGGAG	
	Heat-stable toxin (*st*)	CTTTCCCCTCTTTTAGTCAGTC	167
		CACAGGCAGGATTACAACAAAGT	
EIEC	Invasion-associated locus (*ial*)	GTTGCGCTTGATGGGTGGGGTATC	356
		GAAATGTCCATCAAACCCCACTC	
S. aureus	*sea*	GCAGGGAACAGCTTTAGGC	520
		GTTCTGTAGAAGTATGAAACACG	
	seb	ATGTAATTTTGATATTCGCAGTG	683
		TGCAGGCATCATATCATACCA	
	sec	CTTGTATGTATGGAGGAATAACAA	283
		TGCAGGCATCATATCATACCA	
	sed	GTGGTGAAATAGATAGGACTGC	384
		ATATGAAGGTGCTCTGTGG	
	see	TACCAATTAACTTGTGGATAGAC	170
		CTCTTTGCACCTTACCGC	
	seg	CGTCTCCACCTGTTGAAGG	327
		CCAAGTGATTGTCTATTGTCG	
	seh	CAACTGCTGATTTAGCTGAG	360
		GTCGAATGAGTAATCTCTAGG	
	sei	CAACTCGAATTTTCAACAGGTAC	465
		CAGGCAGTCCATCTCCTG	
	sej	CATCAGAACTGTTGTTCCGCTAG	142
		CTGAATTTTACCATCAAAGGTAC	
	sek	ATGGCGGAGTCACAGCTACT	197
		TGCCGTTATGTCCATAAATGTT	
	sel	CACCAGAATCACACCGCTTA	410
		TCCCCTTATCAAAACCGCTAT	
	sem	CTATTAATCTTTGGGTTAATGGAGAAC	325
		TTCAGTTTCGACAGTTTTGTTGTCAT	
	sen	ACGTGGCAATTAGACGAGTC	475
		GATTGATCTTGATGATTATGAG	
	seo	AGTTTGTGTAAGAAGTCAAGTGTAGA	179
		TTTAAATTCAGCAGATATTCCATCTAAC	
	sep	CTGAATTGCAGGGAACTGCT	187
		ATTGGCGGTGTCTTTTGAAC	
	seq	GAACCTGAAAAGCTTCAAGGA	209
		ATTCGCCAACGTAATTCCAC	
V. parahaemolyticus	*tdh*	CTTCCATCTGTCCCTTTTCCTGCC	217
		ATGTTCACAGTCATGTAGGATGTC	
Y. enterocolitica	*ail*	TTATCAATTGCGTCTGTTAATGTG	449
		GACTTTGGAGTATTCATATGAAGC	

Bacterial strain identification using API test

One well-isolated colony from each culture was used to inoculate 5 ml of 0.85% NaCl medium, pH 5.5 to 7.0. A humid atmosphere was provided, and, to identify the organisms, a kit (Biomerieux, France) was used according to the instructions of the manufacturer. After 18 to 24 h, all reactions were analyzed according to the interpretation chart included in the package insert. Reagents were added to the TDA, Voges-Proskauer, and IND tubes, and the reactions were recorded. The oxidase test was also performed with oxidase reagent. After the results for all biochemicals were obtained, the identification of the organism was made by using the seven-digit number generated (e.g., 5205573) and the analytical profile index.

Serotyping

Serotyping of *Salmonella* and *Shigella* spp. was carried out according to the instructions of the manufacturer (Denka Seiken, Japan)

Antibiotic susceptibility test

Antibiotic susceptibility of the three most isolated species groups, *S. aureus*, *Salmonella* spp., and pathogenic *E. coli* strains, was determined by NCCLS methodology (NCCLS, 1997). *S. aureus* strains were tested for susceptibilities to the following antibiotics: penicillin, oxacillin, trimethoprim and sulfamethoxazole, erythromycin, vancomycin, amikacin, and clindamycin. *Salmonella* spp. and pathogenic *E. coli* strains were examined for their susceptibilities to ampicillin, amikacin, cefazolin, cephalothin, gentamicin, cefepime, cefotetan, cefotaxime, ciprofloxacin, imipenem, trimethoprim and sulfamethoxazole, chloramphenicol, tetracycline, nalidixic acid, ampicillin and sulbactam, and ticarcillin.

Results

Comparison of enteritis bacteria isolated from diarrheal patients in rural and urban regions in the Republic of Korea

A total of 26,992 stool samples obtained from patients with acute diarrhea were analyzed during the one-year study period. Of these samples, 12,882 were collected from big cities and 14,110 were from rural provinces (Fig. 1). According to our diagnostic procedure, 1,291 (4.8%) of the samples were positive for at least one of the pathogens being tested for. Bacteria were isolated from the stool samples of 821 (6.4%) of the urban patients, and from the stool samples of 470 (3.3%) of the rural patients. As shown in Table 2, the percentage of samples from each region from which bacteria could be isolated was different. This percentage ranged from 3.4% to 12.9% in the urban regions, while in the rural region, it ranges from 1.3% to 4.7%.

Table 2. Stool samples and isolated pathogens using samples from big cities and rural provinces

	PHI	No. of stools	No.of isolates	% of isolates
Cities	Seoul	4,799	230	4.8
	Pusan	691	89	12.9
	Inchon	1,321	61	4.6
	Kwangju	2,905	231	8.0
	Taejon	2,103	72	3.4
	Ulsan	1,063	138	13.0
	total	12,882	821	6.4
Local	Gyunggi-do	2,739	38	1.4
	Kangwon-do	1,458	43	2.9
	Chungchong-do	1,938	91	4.7
	Cholla-do	3,949	157	4.0
	Kyongsang-do	2,809	104	3.7
	Cheju-do	1,217	37	3.0
	total	14,110	470	3.3
Total		26,992	1,291	4.8

Table 3. Prevalence of enteritis bacteria in the urban and rural regions

Bacteria	No. of isolated bacteria (%)	
	urban	rural
S. aureus	333 (40.6)	86 (18.3)
Salmonella spp.	186 (22.7)	190 (40.4)
E. coli	165 (20.1)	60 (12.8)
V. parahaemolyticus	74 (9.0)	42 (8.9)
Shigella spp.	56 (6.8)	70 (14.9)
Y. enterocolitica	2 (0.2)	20 (4.3)
C. jejuni	3 (0.4)	1 (0.2)
L. monocytogenes	2 (0.2)	1 (0.2)

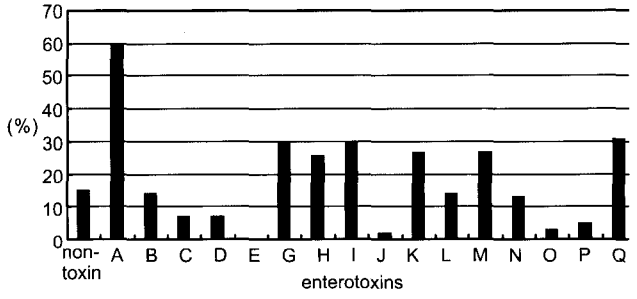

Fig. 2. Detection of staphylococcal enterotoxins (SE) of *S. aureus* strains.

Prevalence of enteritis bacteria isolated from diarrheal patients in Republic of Korea

The prevalence of enteritis bacteria obtained from diarrheal patients was different between urban and rural regions. As shown in Table 3, *Staphylococcus aureus* was the most frequently identified pathogen in the urban regions. Identified causes of diarrhea in urban regions were as follows: *Staphylococcus aureus*, *Salmonella* species, pathogenic *Escherichia coli*, *Vibrio parahaemolyticus*, and *Shigella* species. In the rural regions, *Salmonella* species were the most common isolates, followed by *S. aureus*, *Shigella* species, pathogenic *E. coli*, and *Vibrio parahaemolyticus* isolates.

Enterotoxins of *S. aureus* strains were tested with the primers (enterotoxin A to Q) as shown in Table 1. Of 419 *S. aureus* strains, 356 strains (85%) possessed enterotoxins, while no toxins were found in 63 (15%) of the strains (Fig. 2). In this study, the enterotoxin-negative *S. aureus* strains were added to the total number of isolated *S. aureus* strains.

Salmonella and *Shigella* isolates were analyzed for serotype (data not shown). The serotype analysis of *Salmonella* isolates indicated that the most commonly isolated serotype was *Salmonella* Enteritidis (47%) followed by *Salmonella* Typhimurium (18%) and *Salmonella* Bardo (11%). In the *Shigella* isolates, the most commonly isolated serogroup was *Shigella sonnei* (59%) followed by *Shigella flexneri* (40%).

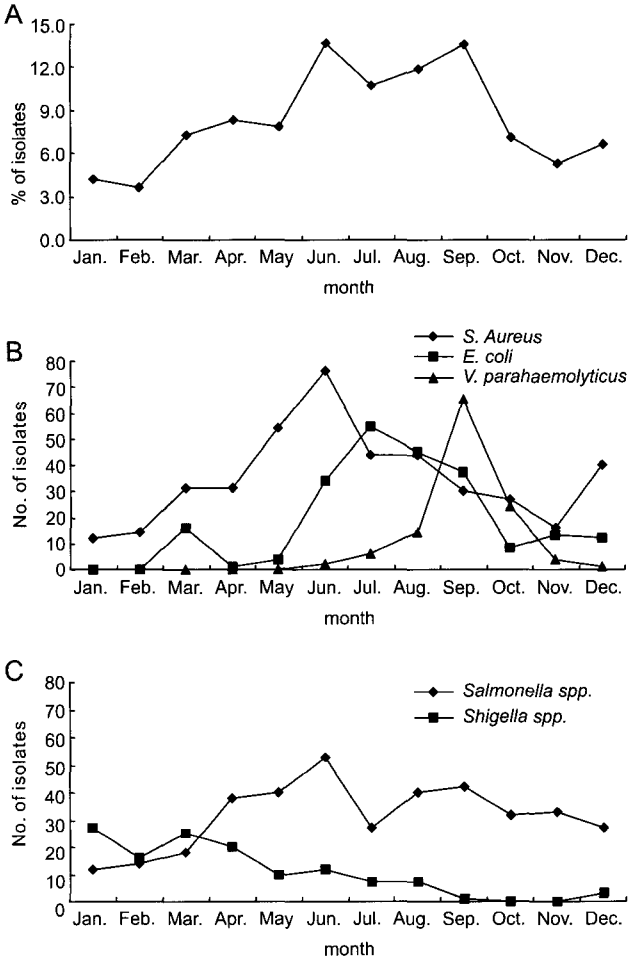

Fig. 3. Monthly isolation of enteropathogens: isolation rates of total pathogens (A), *S. aureus*, *E. coli*, and *V. parahaemolyticus* (B), and *Salmonella* spp. and *Shigella* spp. (C).

Seasonal analysis of prevalence of enteritis bacteria

The seasonal prevalence of the isolated bacterial species was analyzed. In the Republic of Korea, the months from June to August are hot, wet summer months. A high prevalence of infection was seen in June, July, August and September. The rate of infection peaked in June at 13.7% of total isolated bacteria (177 of 1,291). The prevalence decreased to 10.7% in July; however, it increased again to 11.9% in August and to 13.5% in September (Fig. 3A).

As indicated in Figs. 3B and C, seasonality of *S. aureus*, *E. coli*, and *V. parahaemolyticus* infection was observed, while infections with *Salmonella* spp. and

Table 4. Seasonal prevalence of each pathogenic pattern of *E. coli*

	No. of monthly isolation of pathogenic *E. coli*													Rate of isolates (%)
	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	total	
EAEC	2	1	4	4	7	9	7	4	1	2	32	10	83	36.89
EHEC					2	1	1	1					5	2.22
ETEC			2	3	1	12	3	12	7	1	3	2	46	20.44
EIEC						1							1	0.44
EPEC	1	2	1	3	9	32	16	18	4	2	2		90	40.00
total	3	3	7	10	19	55	27	35	12	5	37	12	225	100

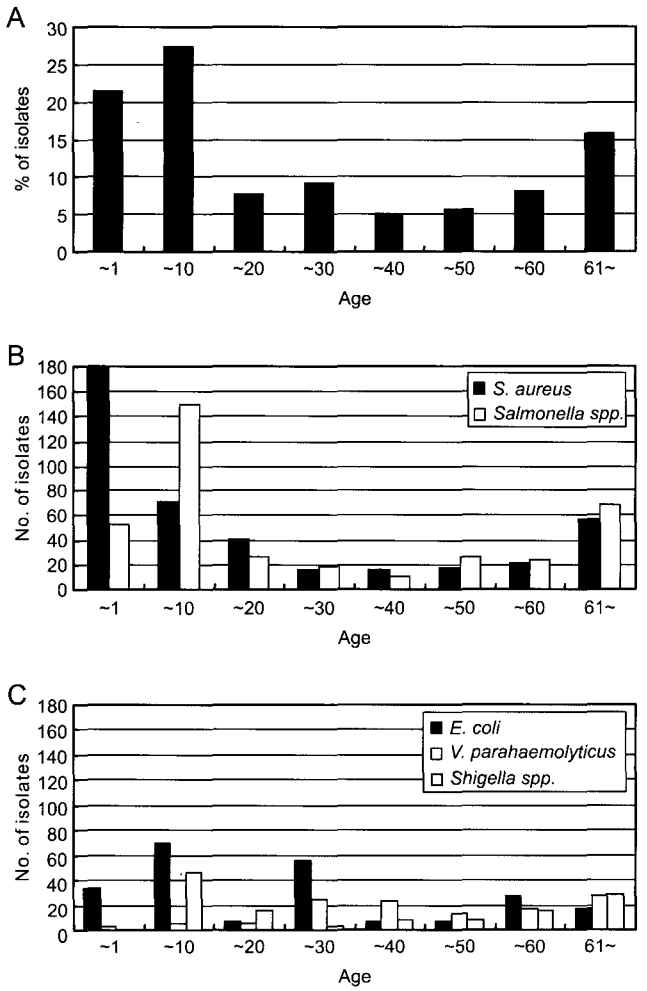

Fig. 4. Isolation of enteropathogens in different age groups: isolation rates of total pathogens (A), *S. aureus* and *Salmonella* spp. (B), and *E. coli*, *V. parahaemolyticus*, and *Shigella* spp. (C).

Shigella spp. were present throughout the year. *S. aureus* infections were more frequent from May to August. The highest prevalence of infection due to *S. aureus* was seen in June. Pathogenic *E. coli* infection showed high prevalence in the summer months from June to September. Additionally, the seasonal prevalence of each of the *E. coli* strains with specific pathogenic patterns is shown in Table 4. Infection with *V. parahaemolyticus* peaked in September. However, infection due to *Salmonella* spp. was prevalent throughout the year, showing a slight increase in the infection rate during the summer months. *Shigella* spp. infection was isolated fairly frequently throughout the year, with slight seasonal variation.

Age-specific prevalence of enteritis bacteria

As shown in Fig. 4, age-specific patterns were observed in these bacterial infections, with a high rate of prevalence in children and the elderly. Among children under 10 years of age, bacteria were isolated from 630 (48.8%). The rate of bacterial isolation was drastically lower in adults, especially in people from the 31- to 40-year-old age group, where it was 5%. However, this climbed to 15.7% in people greater than 60 years of age (Fig. 4A).

Data on infections with single organisms are shown in Figs. 4B and C. Infections due to *S. aureus* and *Salmonella* spp. were more frequent in children and the elderly than in other adults, while the prevalence of *E. coli*, *V. parahaemolyticus*, and *Shigella* spp. showed no significant differences among age strata.

Antibiotic susceptibility of pathogens

The results of disk diffusion testing for *S. aureus*, *Salmonella* spp., and pathogenic *E. coli* with MHA were compared to the NCCLS reference range.

The antibiotic susceptibility of 419 strains of *S. aureus*, 376 strains of *Salmonella* spp., and 225 strains of pathogenic *E. coli* was tested. Of the *S. aureus* strains, 90% were resistant to penicillin, 43% were erythromycin-resistant, 28% were oxacillin-resistant, 20% were clindamycin-resistant, 17% were amikacin-resistant, and 4% were resistant to a

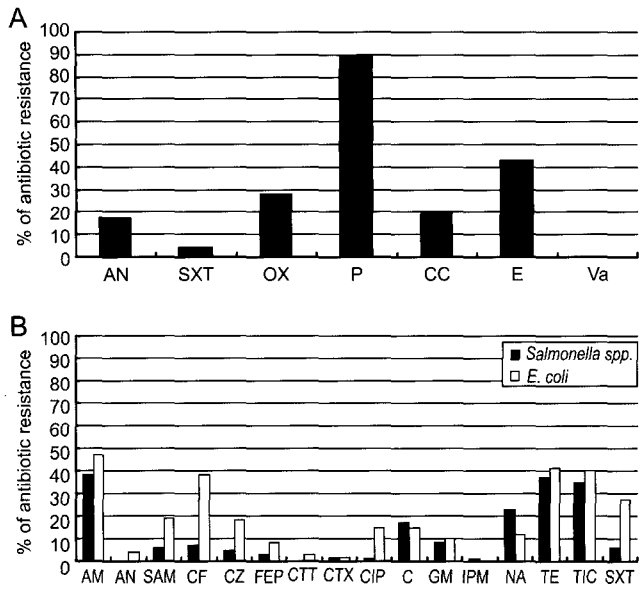

Fig. 5. Antibiotic susceptibility patterns of *S. aureus* (A) and *Salmonella* spp. and pathogenic *E. coli* (B).
OX; Oxacillin, P; Penicillin, CC; Clindamycin, E; Erythromycin, Va; Vancomycin, AM; ampicillin, AN; amikacin, SAM; ampicillin and sulbactam, CF; cephalothin, CZ; cefazolin, FEP; cefepime, CTT; cefotetan, CTX; cefotaxime, CIP; ciprofloxacin, C; chloramphenicol, GM; gentamicin, IPM; imipenem, NA; nalidixic acid, TE; tetracycline, TIC; ticarcillin, SXT; trimethoprime and sulfamethoxazole.

combination of trimethoprim and sulfamethoxazole. However, strains resistant to vancomycin were not found (Fig. 5A). The antibiotic susceptibility patterns of *Salmonella* spp. and pathogenic *E. coli* were similar. In both pathogenic groups, the resistance to ampicillin, tetracycline, and ticarcillin was higher than to other antibiotics. The resistance to cephalothin in pathogenic *E. coli* strains was high, while almost all *Salmonella* spp. strains were susceptible to this antibiotic (Fig. 5B).

Discussion

Reports of diarrheal illness in the Republic of Korea are rare. In this prospective study, an extensive laboratory investigation was performed to establish the rate of isolation of enteropathogenic agents from stools of patients with diarrhea who visited clinics for treatment. This report is the first document of systematic surveillance of the prevalence of bacteria isolated from the stools of diarrheal patients in the Republic of Korea. In this surveillance study, eight bacterial species (*S. aureus*, *Salmonella* spp., pathogenic *E. coli*, *V. parahaemolyticus*, *Shigella* spp., *Y. enterocolitica*, *C. jejuni*, and *L. monocytogenes*) were isolated from diarrheal patients.

A potential enteropathogen was detected in 4.8% of all patients screened. There are few comparable studies concerning adult patients with diarrhea, while considerably more studies have been conducted regarding childhood diarrhea (Baqui *et al.*, 1992; Caprioli *et al.*, 1996; Munk Petersen *et al.*, 1996; Barnes *et al.*, 1998; Presterl *et al.*, 1999). In recent British and Swedish studies, the presence of at least one enteropathogen was detected in 45% and 56% of patients, respectively (Wheeler *et al.*, 1999; Svenungsson *et al.*, 2000). Many factors might explain why the diagnostic yield was not higher in the present study; these factors may include, the sampling procedure, sensitivity of diagnostic methods, and treatment with antibiotics shortly after the onset of diarrhea.

The prevalence data for enteritis bacteria showed that *S. aureus* and *Salmonella* spp. were isolated at relatively high rates from diarrheal patients in both urban and rural regions of the Republic of Korea. Enterotoxigenic *S. aureus* is well known to be a food contaminant that causes symptoms of food poisoning (Balaban and Rasooly, 2000; Loir *et al.*, 2003). However, diagnosis of the cause of diarrhea in terms of enterotoxin is difficult. Our study indicated that 85% of the strains isolated from diarrheal patients possessed enterotoxins. The number of *S. aureus* strains in this study could have been overestimated, because the enterotoxin-negative strains were included in the total number of isolated strains of *S. aureus*. There are conflicting reports regarding the association of *Salmonella* species with acute diarrhea. Nelson et al. (Nelson *et al.*, 2004) reported that the high rates (11%) of *Salmonella* species isolation were detected through the surveillance of childhood diarrheal diseases in Hong Kong, while another study indicated that, in Bangladesh, the rate of enteric infection from *Salmonella* species is low (Albert *et al.*, 1999). Pathogenic *E. coli* strains isolated in this study were characterized by their toxin patterns (data not shown). As reported previously (Albert *et al.*, 1995; Akinyemi *et al.*, 1998), we found an association between diarrhea and EPEC and ETEC. Of the pathogenic *E. coli*, EPEC was the most frequently identified pathogen (56%), and ETEC strains were isolated in 17% of patients infected with pathogenic *E. coli*. The highest frequencies of isolation of the EPEC group were found in Chile (38.3%) (Levine *et al.*, 1988) and São Paulo, Brazil (34.0%) (Gomes *et al.*, 1991), and low frequencies were observed in Somalia (4.0%) (Casalino *et al.*, 1988) and Thailand (5.5%) (Echeverria *et al.*, 1991). The rates of EHEC and EAEC infection were 16% and 10%. In a recent report (Regua-Mangia *et al.*, 2004), EAEC was the most frequent diarrheagenic *E. coli* category.

The pathogens were analyzed for seasonality of infection and association with age. The incidence of *Salmonella* infection was significantly greater in children with diarrhea than in adults, but infectious seasonality was not found. However, in another study (Casalino *et al.*, 1988), *Salmonella* spp. were rarely identified in children, but were predominantly identified in samples from adults. Interestingly, *S. aureus* was also present in a significantly higher proportion of children than in adults. Moreover, this pathogen showed seasonal peaks in the summer months. Although analysis of data according to age strata indicated no age-specific isolation of *E. coli* or *V. parahaemolyticus,* seasonal patterns of these pathogens were revealed by this study. *E. coli* had seasonal peaks in the hot and wet summer months from June to September with *V. parahaemolyticus* peaking in September. Several previous studies have shown that ETEC shows a seasonal pattern of incidence (Albert *et al.*, 1995; Albert *et al.*, 1999) and EAEC has been recognized as a causative organism of persistent diarrhea in children (Baudry *et al.*, 1990; Itoh *et al.*, 1997). In this study, *Shigella* species showed no seasonality or age-specific patterns, although, in other studies, *Shigella* spp. were closely associated with diarrhea in children in a relatively older age group (Casalino *et al.*, 1988; Echeverria *et al.*, 1991) and in adults (Germani *et al.*, 1994).

We examined *S. aureus*, *Salmonella* species, and pathogenic *E. coli* for antibiotic susceptibility. The resistance to sulfamethoxazole in combination with trimethoprim in *S. aureus* was very low; therefore, this antibiotic combination is recommended as the drug of choice for the treatment of *S. aureus*. The drugs of choice for the treatment of *Salmonella* species and pathogenic *E. coli* were β-lactams. Both *Salmonella* species and pathogenic *E. coli* were susceptible to several β-lactams, including the cephalosporins (cefotetan, cefotaxime, cefepime) and carbapenem (imipenem). However, the resistance of *E. coli* to another cephalosporin (cephalothin) was somewhat high (Table 3).

The present study has identified bacterial pathogens that are significantly associated with diarrhea. This new knowledge regarding the etiology of diarrhea in the surveyed patients will help us to plan studies to investigate various aspects of diarrheal disease.

In summary, clinical features were not helpful in predicting the etiology of individual cases. This further emphasizes the need for more rapid, sensitive, and simple methods to improve diagnostic yield. General recommendations for optimal sampling should be established so that cost-effective routines can be designed for both epidemiological investigations and clinical use. Progress in the field of molecular analytic techniques will probably lead to the addition of new microorganisms to the already long list of potentially enteropathogenic agents in the near future.

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