The Journal of Microbiology, June 2008, p. 325-330
DOI 10.1007/s12275-008-0015-4

Vol. 46, No. 3

Enteric Bacteria Isolated from Acute Diarrheal Patients in the Republic of Korea between the Year 2004 and 2006

Seung-Hak Cho, Hyun-Ho Shin, Yeon-Hwa Choi, Mi-Sun Park, and Bok-Kwon Lee*

Division of Enteric Bacterial Infections, Center for Infectious Diseases, National Institute of Health, Seoul 122-701, Republic of Korea

(Received January 14, 2008 / Accepted April 4, 2008)

In an epidemiological survey of human enterobacterial infections in the Republic of Korea during three years from 2004 to 2006, we isolated 1,784 (6.2%, isolation rate of enteropathogens from stool samples) in 2004, 2,547 (9.5%) in 2005 and 3,506 bacteria (12.3%) from people who visited clinics. Among the isolated bacteria, pathogenic *Escherichia coli*, especially, EAEC was the most frequently identified pathogen in both urban and rural regions followed by *Staphylococcus aureus*, *Salmonella* species, *Bacillus cereus*, *Vibrio parahaemolyticus*, *Campylobacter jejuni*, *Clostridium perfringens*, and *Shigella* species. Distinct seasonality was found in *V. parahaemolyticus* species, while this pathogen showed no age-specific patterns. However, other bacteria, i.e., pathogenic *E. coli*, *S. aureus*, *Salmonella* spp., and *B. cereus* showed similar seasonality throughout the year, showing a slight increase in the infection rate during the summer months and high prevalence among children under 10 years of age and elder-age people. The antibiotic susceptibility patterns of pathogenic *E. coli*, *Salmonella* spp., and *S. aureus* showed high resistance to penicillins. However, both pathogenic *E. coli* and *Salmonella* spp. were susceptible to several cephems, imipenem, and amikacin. Moreover, *S. aureus* strains resistant to vancomycin were not found. In conclusion, these surveillances can play an important role for the control and prevention to the diseases originated by enteritis bacteria.

Keywords: epidemiology, enterobacterial infections, pathogenic *E. coli*

Diarrhea diseases are known to be one of the most common causes of morbidity and mortality among infants and children in developing countries (Guerrant *et al*., 2002). Infant mortality in the Republic of Korea (ROK) is also highly correlated with diarrheal disease (Korean Ministry of Health and Society Affairs, 1984; Kim *et al*., 1989; Kim *et al*., 2005). Recently, the information on the surveillance was reported for identification of bacteria related to intestinal infectious diseases among the patients that visited hospitals in due to diarrhea in the ROK in the year of 2003 (Cho *et al*., 2006).

In order to determine the trend of bacterial species as possible pathogenic microorganisms that cause diarrhea in the ROK, continuous nation-wide surveillance for diarrhea in patients between the year 2004 and 2006 has been conducted by the National Institute of Health (NIH), Division of Enteric Bacterial Infections. The data obtained from this surveillance have been specifically analyzed for the prevalence of regional, seasonal and age-specific patterns.

Materials and Methods

Participating centers

The surveillance study was planned by the Division of Enteric Bacterial Infections in the National Institute of Health. Seventeen Public Health Institutes (PHIs) of seven big cities (Seoul, Pusan, Incheon, Gwangju, Daegu, Daejeon, and Ulsan) and six provinces (Gyeonggi-do, Gangwon-do, Chungcheong-do, Jeolla-do, Gyeongsang-do, and Jeju-do) were participated in this surveillance.

Stools and identification of the bacteria isolated from the stools

The PHIs of these cities and provinces collected stool samples from diarrheal patients and performed laboratory examinations to isolate clinical specimens from the 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*, *Salmonella*, and *Shigella* species. Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar was used for the detection of *Vibrio* species, Mannitol-Salt Agar (MSA) for *S. aureus*, Tryptose-Sulfite-Cycloserine (TSC) for *C. perfringens*, Campylobacter Blood-Free Selective Agar Base (CCDA) for *C. jejuni*, Listeria Selective Agar (LSA) for *Listeria monocytogenes*, Cefsulodin-Irgasan-Novobiocin (CIN) for *Yersinia enterocolitica*, and Mannitol-Egg Yolk-Polymixin (MYP) for *B. cereus*. The bacteria isolated from the stools were identified by API test (Biomerieux Co. Ltd, Marcyl' Etoile, France).

PCR primers and amplification

For the amplification, same primers as shown in previous study (Cho *et al*., 2006) were used. PCR assays were carried out in 50 μl with 2 U DNA *Taq* polymerase (TaKaRa Ex Taq^{TM}, Japan) in a thermal cycler (PTC-100; MJ Research, USA) under the following conditions: initial denaturation

* To whom correspondence should be addressed.
(Tel) 82-2-380-1462; (Fax) 82-2-352-4767
(E-mail) bokrates@nih.go.kr

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).

Serotyping

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

Antibiotic susceptibility test

Antibiotic susceptibility of the three most isolated species groups, pathogenic *E. coli*, *Salmonella* spp., and *S. aureus* strains, was determined using the method of disk diffusion test by CLSI methodology (CLSI, 2007). Pathogenic *E. coli* and *Salmonella* spp. strains were examined for their susceptibilities to ampicillin, amikacin, cefazolin, cephalothin, gentamicin, cefepime, cefotetan, cefotaxime, ciprofloxacin, imipenem, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, ticarcillin, nalidixic acid, and ampicillin-sulbactam. *S. aureus* strains were tested for susceptibilities to the following antibiotics: penicillin, oxacillin, trimethoprim-sulfamethoxazole, erythromycin, vancomycin, amikacin, and clindamycin.

Results

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

During the year 2004 and 2006, a total of 84,406 stool samples obtained from patients with acute diarrhea were collected. From these samples, 1,784 (6.2%) in 2004, 2,547 (9.5%) in 2005 and 3,506 bacteria (12.3%) in 2006 were isolated. As shown in Table 1, pathogenic *E. coli* was the most frequently identified pathogen. Among the pathogenic *E. coli*, the prevalence of EAEC was highest. Identified major causes of diarrhea in both regions were as follows: pathogenic *E. coli*, *S. aureus*, *Salmonella* species, *B. cereus*, *V. parahaemolyticus*, *C. jejuni*, *C. perfringens*, and *Shigella* species. Interestingly, *S. aureus* was more isolated in the urban regions than in the rural regions, while the prevalence of *V. parahaemolyticus* in the rural regions was higher than in the urban regions.

The serotype analysis of *Salmonella* isolates indicated that the most commonly isolated serotype was *S.* Enteritidis (259 of 629 strains, 41%) followed by *S.* Typhimurium (115 of 629 strains, 18%) and *S.* Bardo (67 of 629 strains, 11%). In the *Shigella* isolates, the most commonly isolated serogroup was *S. sonnei* (48%) followed by *S. flexneri* (46%) and *S. boydii* (5%).

Seasonal and age-specific prevalence of enteritis bacteria

As indicated in Fig. 1, distinct seasonality was observed in the infection of *V. parahaemolyticus*. The *V. parahaemolyticus* infection increased from July, peaked in August (in the year of 2004) and September (in the year of 2005 and 2006) and decreased in October again. However, infections with pathogenic *E. coli*, *S. aureus*, *Salmonella* spp., and *B. cereus* showed similar seasonality throughout the year. The prevalence of these infections showed slight peak in the summer months from June to August.

Age-specific patterns were observed in the infection of pathogenic *E. coli*, *S. aureus*, *Salmonella* spp., and *B. cereus*. Among children under 10 years of age and elder-age people,

Table 1. Stool samples and isolated pathogens using samples from urban and rural regions

Year	2004		2005		2006	
Region	Urban	Rural	Urban	Rural	Urban	Rural
No. of stools	13,703	15,221	13,942	12,872	13,778	14,890
No. of isolates	803	981	1,245	1,302	1,798	1,708
% of isolates	5.9	6.4	8.9	10.1	13	12.3
E. coli	298 (37)	293 (30)	681 (55)	654 (50)	836 (46)	1,201 (60)
EAEC	160	51	457	235	523	415
EPEC	93	167	180	271	238	496
ETEC	39	66	30	141	65	278
EHEC	4	9	13	5	8	11
EIEC	2	0	1	2	2	1
S. aureus	253 (31)	136 (14)	246 (20)	160 (12)	607 (34)	234 (14)
Salmonella spp.	122 (15)	237 (24)	139 (11)	131 (10)	165 (9)	163 (10)
B. cereus	50 (6)	52 (5)	93 (8)	149 (11)	95 (5)	157 (9)
V. parahaemolyticus	26 (3)	179 (18)	26 (2)	128 (10)	56 (3)	98 (6)
C. jejuni	21 (3)	30 (3)	16 (1)	14 (1)	17 (1)	13 (1)
Shigella spp.	12 (2)	30 (3)	9 (1)	28 (2)	19 (1)	13 (1)
C. perfringens	15 (2)	14 (1)	26 (2)	31 (2)	51 (3)	60 (4)

( ): % of isolates

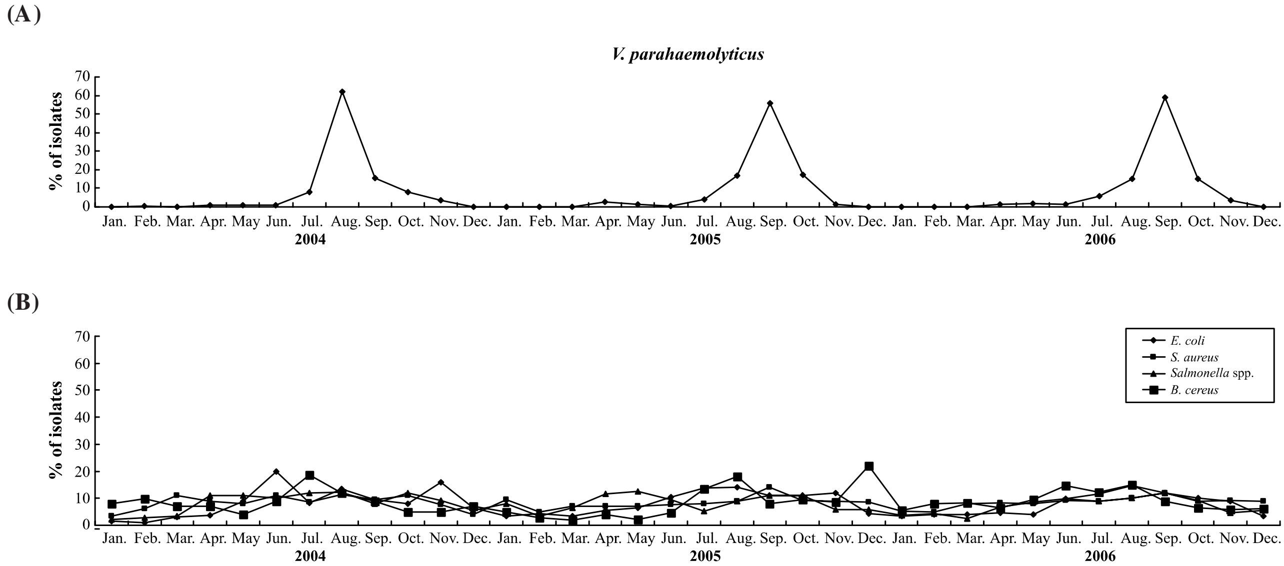

Fig. 1. Monthly isolation of enteropathogens: isolation rates of *V. parahaemolyticus* (A), and pathogenic *E. coli*, *S. aureus*, *Salmonella* spp., and *B. cereus* (B).

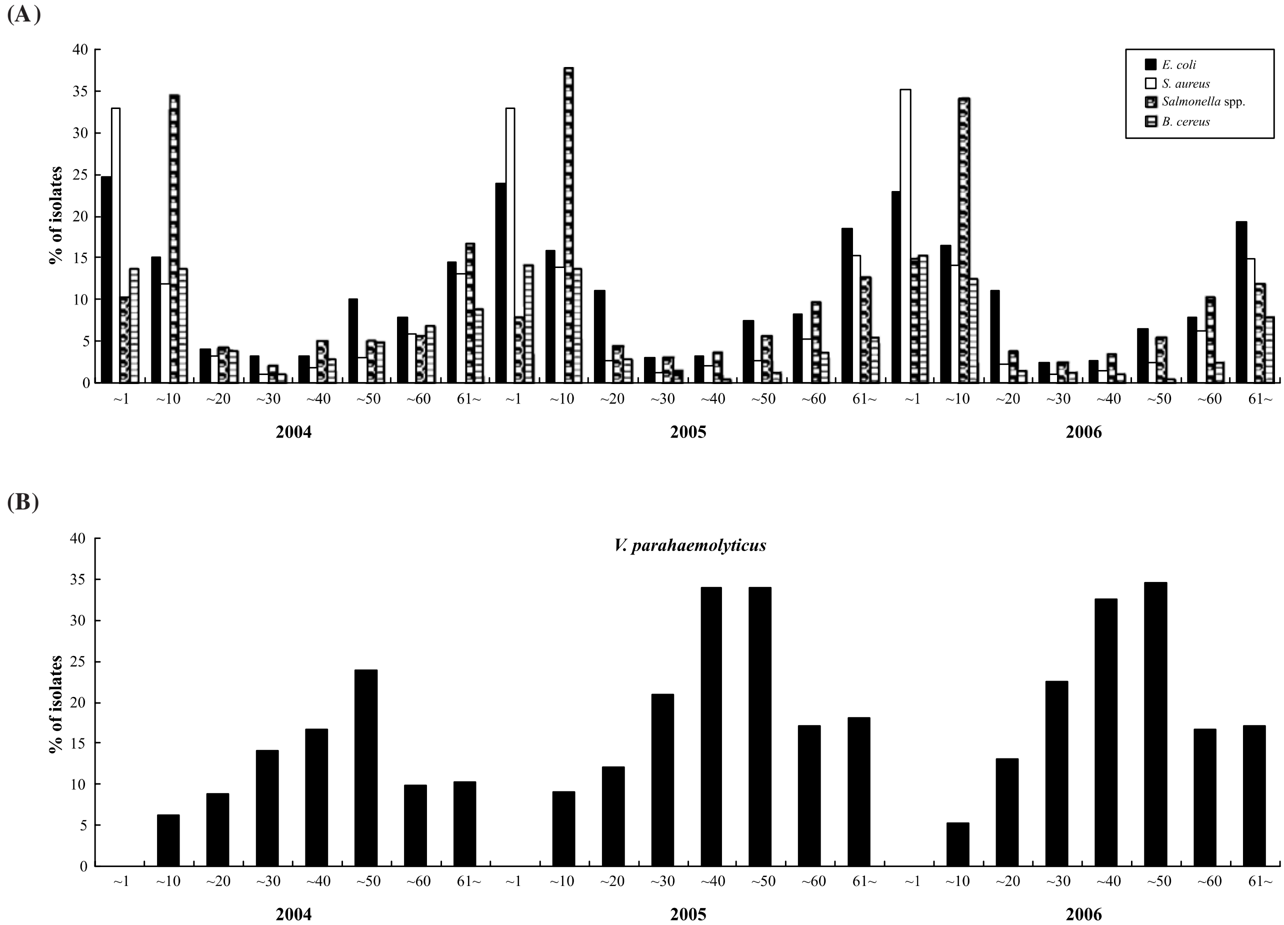

Fig. 2. Isolation of enteropathogens in different age groups: isolation rates of pathogenic *E. coli*, *S. aureus*, *Salmonella* spp., and *B. cereus* (A) and *V. parahaemolyticus* (B).

Table 2. Antibiotic susceptibility patterns of pathogenic *E. coli*, *Salmonella* spp., and *S. aureus*

Antimicrobial agents	% of resistant strains to antibiotics		
	Pathogenic *E. coli*	*Salmonella* spp.	*Staphylococcus aureus*
Penicillins			
Ampicillin	52	40	
Ticarcillin	41	35	
Penicillin			90
Oxacillin			43
ß-Lactam/ß-Lactamase inhibitor combinations			
Ampicillin-sulbactam	16	4	
Cephems			
Cephalothin	23	7	
Cefazolin	15	3	
Cefepime	8	3	
Cefotetan	2	0	
Cefotaxime	2	2	
Carbapenems			
Imipenem	0	1	
Aminoglycosides			
Amikacin	4	0	14
Gentamicin	13	5	
Tetracyclines			
Tetracycline	44	39	
Fluoroquinolones			
Ciprofloxacin	15	1	
Quinolones			
Nalidixic acid	12	23	
Phenicols			
Chloramphenicol	15	14	
Folate pathway inhibitors			
Trimethoprim-sulfamethoxazole	27	6	3
Lincosamides			
Clindamycin			14
Macrolides			
Erythromycin			37
Glycopeptides			
Vancomycin			0

these bacteria were isolated with a high prevalence, while the rate of bacterial isolation was drastically lower in adults. Interestingly, the prevalence of *Slamonella* spp. between 1 year and 10 years of age was higher than under 1 year of age. However, the prevalence of *V. parahaemolyticus* showed no significant differences among age strata (Fig. 2).

Antibiotic susceptibility of pathogens

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

As shown in Table 2, the antibiotic susceptibility patterns of pathogenic *E. coli* and *Salmonella* spp. were similar, although the resistance to each antibiotic in pathogenic *E. coli* was some higher than *Salmonella* spp. In both pathogenic groups, the resistances to penicillins (ampicillin and ticarcillin) and tetracycline were high. These strains were susceptible (resistance rate of ≤10%) to several cephems (cefotetan, cefotaxime, cefepime) and carbapenem (imipenem), and aminoglycosides (amikacin). Of the *S. aureus* strains, the resistances to penicillins (penicillin-resistance of 90% and oxacillin-resistance of 28%) and to macrolides (43%

of erythromycin-resistance) were high. However, strains resistant to vancomycin were not found.

Discussion

Recently, the information about the surveillance during the year of 2003 was reported for the identification of bacteria related to intestinal infectious diseases among the patients that visited hospitals in due to diarrhea in the ROK (Cho *et al*., 2006). Here, we report the data of continuous laboratory-based surveillance for enteritis bacterial infection conducted for three years from 2004 to 2006.

In this study, it was found that the isolation rates of potential enteropathogens (6.2% in 2004, 9.5% in 2005, and 12.3% in 2006) were higher than the rate (4.8%) reported by a surveillance conducted in 2003, although those were lower than other countries (Caprioli *et al*., 1996; Petersen *et al*., 1996; Presterl *et al*., 1999; Wheeler *et al*., 1999; Svenungsson *et al*., 2000). However, in this study, the surveillance for viral and protozoa infections was also performed. Actually, the prevalence of viral infections (22%) was higher than that of bacterial and protozoa infections (unpublished data).

The prevalence data from 2004 to 2006 showed that pathogenic *E. coli*, *Salmonella* spp., and *S. aureus* strains were isolated at relatively high rates from diarrheal patients in both urban and rural regions in the ROK. Our results of the prevalence of pathogenic *E. coli* are consistent with previous study which indicated that EAEC was the most frequent diarrheagenic *E. coli* category (Regua-Mangia *et al*., 2004), and EPEC and ETEC strains were identified as high rates (Gomes *et al*., 1991; Cho *et al*., 2006). The prevalence of *Salmonella* species with acute diarrhea was also high in Hong Kong (Nelson *et al*., 2004), while another study indicated that, in Bangladesh, the rate of enteric infection from *Salmonella* species is low (Albert *et al*., 1999). All of the *S. aureus* strains isolated from diarrheal patients in this surveillance possessed enterotoxins (data not shown). 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). In this study, *B. cereus* showed high prevalence. All of these strains produced enterotoxin T (*bce*T). *bce*T was reported to encode a single component toxin (BceT) which exhibited Vero cell cytotoxicity and to which has also been attributed a role in the diarrheal syndrome (Agata *et al*., 1995). It is well known that *B. cereus* is worldwide spread and cause food poisoning. It was indicated in other study of us that 73 *B. cereus* isolates (25%) from 293 cereals possessed enterotoxins (unpublished data).

The character of four seasons in the ROK is very distinct. Especially, the months from June to August are hot, wet summer months. Microorganism showing distinct seasonality adequate for this climatic character was *V. parahaemolyticus*. On the contrary, however, pathogenic *E. coli* in this study indicated weak seasonality, while several previous studies reported that pathogenic *E. coli* showed a seasonal pattern of incidence (Albert *et al*., 1995; Albert *et al*., 1999; Cho *et al*., 2006). *Salmonella* and *S. aureus* infections showed no seasonality in this study. However, in our previous report in 2003, strong seasonality was found in *S. aureus* infections (more frequent from May to August), while *Salmonella* infections showed no seasonality.

Pathogenic *E. coli* showed age-specific patterns, as previously reported that EAEC has been recognized as a causative organism of persistent diarrhea in children (Baudry *et al*., 1990; Itoh *et al*., 1997). Also, both *Salmonella* and *S. aureus* infections showed age-specific patterns as same as the data in 2003.

Antibiotic resistance of pathogenic *E. coli*, *Salmonella* species, and *S. aureus* was similar to the data of the year 2003. It was found, however, that MRSA strains were more isolated in this study showing resistance rates of 43%, while the rates were 28% in the year of 2003. Interestingly, the resistance to sulfamethoxazole in combination with trimethoprim in *S. aureus* was very low as shown in the data of the year 2003.

In conclusion, the results of this study provide evidence for identified bacterial pathogens that are significantly associated with diarrhea and will help us to plan studies to investigate various aspects of diarrheal disease, for example, diagnosis, prevention and medical treatment. Actually, during the past 4 years from 2003 to 2006, the isolation rates of pathogens that cause diarrheal infections became higher every year in due to rapid, sensitive, and simple diagnostic methods, for example, development of multiplex PCR for the detection of pathogenic *E. coli* toxins. These continuous laboratory-based surveillances for enteritis bacterial infections play an important role for the control and prevention to the diseases originated by enteritis bacteria.

Acknowledgements

This study was supported by a grant from National Institute of Health (NIH) and Food and Drug Administration (FDA), Seoul, Republic of Korea.

References

Agata, N., M. Ohta, Y. Arakawa, and M. Mori. 1995. The *bceT* gene of *Bacillus cereus* encodes an enterotoxic protein. *Microbiology* 141, 983-988.

Albert, M.J., S.M. Faruque, A.S.G. Faruque, P.K.B. Neogi, M. Ansaruzzaman, N.A. Bhuiyan, K. Alam, and M.S. Akbar. 1995. Controlled study of *Escherichia coli* diarrheal infections in Bangladesh children. *J. Clin. Microbiol*. 33, 973-977.

Albert, M.J., A.S.G. Faruque, S.M. Faruque, R.B. Sack, and D. Mahalanabis. 1999. Case-control study of enteropathogens associated with childhood in Dhaka, Bangladesh. *J. Clin. Microbiol*. 37, 3458-3464.

Balaban, N. and A. Rasooly. 2000. Staphylococcal enterotoxins. *Int. J. Food Microbiol. Rev*. 61, 1-10.

Baudry, B., S.J. Savarino, P. Vial, J.B. Kaper, and M.M. Levine. 1990. A sensitive and specific DNA probe to identify enteroaggregative *Escherichia coli*, a recently discovered diarrheal pathogen. *J. Infect. Dis*. 161, 1249-1251.

Caprioli, A., C. Pezzella, R. Morelli, A. Giammanco, S. Arista, D. Crotti, M. Facchini, P. Guglielmetti, C. Piersimoni, and I. Luzzi. 1996. Enteropathogens associated with childhood diarrhea in Italy. *Pediatr. Infect. Dis. J*. 15, 876-883.

Cho, S.H., J.H. Kim, J.C. Kim, Y.H. Kang, and B.K. Lee. 2006. Surveillance of bacterial pathogens associated with acute diarrheal disease in the Republic of Korea, during one year, 2003.

J. Microbiol. 44, 327-335.

Clinical and Laboratory Standards Institute (CLSI). 2007. Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement.

Gomes, T.A.T., V. Rassi, K.L. MacDonald, S.R.T.S. Ramos, L.R. Trabulsi, M.A.M. Vieira, B.E.C. Guth, J.A.N. Candeias, C. Ivey, M.R.F. Toledo, and P.A. Blake. 1991. Enteropathogens associated with acute diarrheal disease in urban infants in São Paulo, Brazil. *J. Infect. Dis*. 164, 331-337.

Guerrant, R.L., M. Kosek, A.A. Lima, B. Lorntz, and H.L. Guyatt. 2002. Updating the DALYs for diarrhoeal disease. *Trends Parasitol*. 18, 191-193.

Itoh, Y., I. Nagano, M. Kunishima, and T. Ezaki. 1997. Laboratory investigation of enteroaggregative *Escherichia coli* O untypeable: H10 associated with a massive outbreak of gastrointestinal illness. *J. Clin. Microbiol*. 35, 2546-2550.

Kim, J.S., J.O. Kang, S.C. Cho, Y.T. Jang, S.A. Min, T.H. Park, B. Nymbat, D.S. Jo, J. Gentsch, J.S. Bresee, T.C. Mast, and O.E. Kilqore. 2005. Epidemiological profile of rotavirus infection in the Republic of Korea: Results from prospective surveillance in the Jeongeub district, 1 July 2002 through 30 June 2004. *J. Infect. Dis.* 192, 49-56.

Kim, K.H., I.S. Suh, J.M. Kim, C.W. Kim, and Y.J. Cho. 1989. Etiology of childhood diarrhea in Korea. *J. Clin. Microbiol*. 27, 1192-1196.

Korean Ministry of Health and Social Affairs. 1984. Public health. Yearbook of Health and Social Statistics. 30, 11-23.

Loir, Y.L., F. Baron, and M. Gautier. 2003. *Staphylococcus aureus* and food poisoning. *Genet. Mol. Res*. 2, 63-76.

Nelson, E.A., J.S. Tam, L.M. Yu, R.I. Glass, U.D. Parashar, and T.F. Fok. 2004. Surveillance of childhood diarrhoeal disease in Hong Kong, using standardized hospital discharge data. *Epidemiol. Infect*. 132, 619-626.

Petersen, A.M., S.V. Nielsen, D. Meyer, P. Ganer, and K. Ladefoged. 1996. Bacterial gastroenteritis among hospitalized patients in a Danish county, 1991-93. *Scand. J. Gastroenterol*. 31, 906-911.

Presterl, E., R. Nadrchal, D. Wolf, M. Rotter, and A.M. Hirschl. 1999. Enteroaggregative and enterotoxigenic *Escherichia coli* among isolates from patients with diarrhea in Austria. *Eur. J. Clin. Microbiol. Infect. Dis*. 18, 209-212.

Regua-Mangia, A.H., T.A. Gomes, M.A. Vieira, J.R. Andrade, K. Irino, and L.M. Teixeira. 2004. Frequency and characteristics of diarrhoeagenic *Escherichia coli* strains isolated from children with and without diarrhoea in Rio de Janeiro, Brazil. *J. Infect*. 48, 161-167.

Svenungsson, B., A. Lagergren, E. Ekwall, B. Evengard, K.O. Hedlund, A. Karnell, S. Lofdahl, L. Svensson, and A Weintraub. 2000. Enteropathogens in adult patients with diarrhea and healthy control subjects: a 1-year prospective study in a Swedish clinic for infectious diseases. *Clin. Infect. Dis*. 30, 770-778.

Wheeler, J.G., D. Sethi, J.M. Cowden, P.G. Wall, L.C. Rodrigues, D.S. Tompkins, M.J. Hudson, and P.J. Roderick. 1999. Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. *BMJ* 318, 1046-1050.