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| 보 문 |

Antibiotic Resistance Among *Salmonella* spp. Isolated from Feces of Patients with Acute Diarrhea in Gwangju Area, Korea, during 2000-2009

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2000년-2009년 광주 광역시 지역의 급성설사환자의 분변에서 분리한 살모넬라 균주에서의 항생제 내성률 조사

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Antibiotic susceptibility was examined for 596 *Salmonella* isolates from patients with acute gastroenteritis during 2000–2009 in Gwangju area in South Korea. Of 16 antibiotics tested, ampicillin resistance (43%) was the most commonly observed resistance among the 596 *Salmonella* sp. isolates, followed by tetracycline (35.9%), nalidixic acid (31.5%), and chloramphenicol (26.2%). Antibiotic resistance varied among serotypes: The highest resistance of *S.* Enteritidis and *S.* Typhimurium was to ampicillin (51.1%) and tetracycline (77.9%), respectively. A total of 89 resistance patterns were observed, and 26% (155/596) of *Salmonella* isolates were susceptible to all antibiotics tested in this study. About 21% (127/596) and 15% (87/596) of the isolates were resistant to one and two antibiotics, respectively. The rest of *Salmonella* isolates (227/596, 38%) were resistant to three or more antibiotic agents. The highest multi-drug resistance (MDR) was observed in serotype *S.* Paratyphi B (76.5%), followed by *S.* Typhimurium (58.2%), and *S.* Enteritidis (40.2%). The most common resistance pattern of MDR isolates was ampicillin-chloramphenicol-nalidixic acid-ticarcillin (36/227, 15.8%), most of which (35/36, 97.2%) were *S.* Enteritidis.

***Keywords*:** *Salmonella*, antibiotic resistance, gastroenteritis

Salmonella enterica is one of the most common pathogens to contaminate food and cause diarrhea in humans. It was reported that there are globally 94 million cases of gastroenteritis and 155,000 deaths due to *Salmonella* each year (Majowicz *et al*., 2010). *Salmonella* infections also account for a great part of gastroenteritis in the Republic of Korea (hereafter to be referred as Korea). During 1981–1990, 23% of outbreaks of bacterial foodborne diseases in Korea were caused by *Salmonella* sp. (Lee *et al*., 1996). In Korea, the surveillance for *Salmonella* species has been carried out by Korean Center for Diseases Control and Prevention (KCDC) in association with 16 Public Health and Environment Institutes (PHEI) and 106 collaborative hospitals nationwide since 1972, as part of a national program for monitoring acute diarrheal disease. Results of the surveillance were summarized and published on the KCDC website as a comprehensive overview of the prevalence and characteristics of bacteria causing acute diarrhea in Korea. *Salmonella* sp. was reported to be the most frequently isolated bacteria causing acute diarrhea in Korea during 2009 (KCDC, 2010).

Although approximately 2,000 serotypes of *Salmonella* have been associated with enterocolitis, most human infections are caused by limited number of serotypes. *Salmonella* Typhimurium

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(*S*. Typhimurium) and *Salmonella* Enteritidis (*S*. Enteritidis) are two major causative agents of food-borne salmonellosis in humans (WHO, 1997; Baay and Huis in't Veld, 2000). In our previous report (Kim *et al*., 2012), we also described that *S*. Enteritidis (47.9%) and *S*. Typhimurium (20.4%) were the most common serotypes among 596 *Salmonella* isolated from 29,896 fecal samples of patients with acute diarrhea during 2000–2009 in Gwangju area in Korea.

In addition, the global prevalence of antibiotic-resistant *Salmonella* has increased markedly in the past years (WHO, 1997). Especially, the emergence of multi-drug resistant (MDR) *Salmonella* and *S*. Typhimurium Definitive Type 104 (DT104) poses threats to public health worldwide (Gebreyes *et al*., 2004; Rayamajhi *et al*., 2008). Since the pattern of bacterial resistance is constantly changing, continual surveillance of antibiotic resistance in *Salmonella* isolates is critical for both managing resistance problem and selecting effective agents. However, data are scarce on antibiotic resistance in *Salmonella* recovered from Gwangju area after 2000s. The aim of the study was, therefore, to assess the antibiotic susceptibility of *S. enterica* subsp. *enterica* strains isolated from fecal samples of patients with acute diarrhea in Gwangju area in Korea during 2000–2009.

Materials and Methods

Sample collection

This study was conducted by PHEI of Gwangju metropolitan city, which is located in southwestern part of Korea, with cooperation of 12 local hospitals and clinics in the area. Physicians were asked to submit stool samples from patients with diarrhea and other symptoms of acute gastroenteritis to microbiological laboratory in PHEI, Gwangju. Information was also collected on all patients whose stool samples were submitted, including age, gender, and date of specimen collection. Although some of this information for some of the patients was missing, samples from those patients were still included in this study.

Isolation and identification of *Salmonella* spp.

During the 10-year study period from January 2000 to December 2009, a total of 29,896 stool samples were collected from local hospitals and clinics in Gwangju area. A standard technique was used to isolate and identify *Salmonella* strains as follows: 1 g of each freshly collected diarrheal stool sample was diluted in 10 ml of phosphate buffered saline (PBS, pH 7.2, Sigma, USA) and 500 μl of this diluted sample was added to 5 ml of Selenite broth (Oxoid, England) for enrichment and incubated at 42℃ for 24–48 h. The enrichment samples were then subcultured onto Salmonella-Shigella (Oxoid) and Salmonella chromogenic (Oxoid) agars at 37℃ for 24 h. Suspected colonies by their color were further screened using biochemical tests such as Kliger's iron agar, motility indol-urea agar, Lysin iron agar, and *o*-nitrophenyl-ß-D-galactopyranoside. One presumptive colony from each sample chosen by this screen was identified with confirmatory biochemical tests using API 20E (bioMéreux, France) or VITEK II (bioMéreux).

Serotyping

Serotyping of *Salmonella* strains was performed by slide and tube agglutination according to the Kauffmann-White scheme (Popff, 2001). Somatic (O) antigen of each tentative *Salmonella* isolate was identified with antisera of O antigen (Difco, Becton, Dickinson and company Sparks, USA) using slide agglutination. Positively reacting strains were identified as *Salmonella*, and further serogrouping and serotyping were conducted using polivalent and monovalent O and flagella (H) antisera (Difco). The O- and H- reactions of each *Salmonella* isolate were combined and the specific serotype of the isolate was identified.

Antibiotic susceptibility testing

Antibiotic susceptibility of the *Salmonella* isolates collected for 10 years was tested by standard disc diffusion method on Mueller Hinton agar (BBL: Becton-Dickinson) using 16 kinds of commercial discs (BBL). Discs contained the following amounts of antibiotic: ampicillin (AM, 10 μg), amikacin (AN, 30 μg), ampicillin/sulbactam (SAM, 20 μg), cephalothin (CF, 30 μg), cefazolin (CZ, 30 μg), cefepime (FEP, 30 μg), cefotetan (CTT, 30 μg), cefotaxime (CTX, 30 μg), ciprofloxacin (CIP, 5 μg), chloramphenicol (C, 30 μg), gentamycin (GM, 10 μg), imipenem (IPM, 10 μg), nalidixic acid (NA, 30 μg), tetracycline (TE, 30 μg), ticarcillin (TIC, 75 μg), and trimethoprim/sulphamethoxazele (SXT, 25 μg). *Escherichia coli* ATCC 25922 was used as the quality control strain. Inhibitory zones were interpreted according to the guidelines of Clinical Laboratory Standards Institute (CLSI, 2010). In this study, MDR was defined as *Salmonella* resistant to three or more antibiotics.

Results

Antibiotic resistance rates of *Salmonella* spp. isolates

Antibiotic resistance of 596 *Salmonella* isolates from patients with acute gastroenteritis during 2000–2009 in Gwangju area was presented in Table 1. No resistance was observed to imipenem, cefepime, or ciprofloxacin. Only a few *Salmonella* isolates showed resistance to cefotetan (0.2%) and amikcacin (0.7%) only once in 2007 and 2006, respectively.

Table 1. Number of antibiotic resistant *Salmonella* spp. isolated from patients with acute gastroenteritis in Gwangju, Korea during 2000-2009

Class of antibiotics	Anti-microbials	No. of resistant isolates (%) by year									
		2000 (n=66)	2001 (n=48)	2002 (n=46)	2003 (n=43)	2004 (n=57)	2005 (n=31)	2006 (n=58)	2007 (n=76)	2008 (n=63)	2009 (n=108)	Total (n=596)
Penicillins	AM	12(18.2)	20(41.7)	8(17.4)	17(39.5)	10(17.5)	21(67.7)	38(65.5)	33(43.4)	23(36.5)	74(68.5)	256(43.0)
	TIC	11(16.7)	20(41.7)	9(19.6)	15(34.9)	10(17.5)	21(67.7)	37(63.8)	32(42.1)	21(33.3)	74(68.5)	250(41.9)
B-Lactam combination	SAM	2(3.0)	2(4.2)	1(2.2)	1(2.3)	1(1.8)	5(16.1)	12(20.7)	6(7.9)	3(4.8)	11(10.2)	44(7.4)
Cephalosporins	CF	0(0.0)	0(0.0)	1(2.2)	2(4.7)	1(1.8)	2(6.5)	6(10.3)	2(2.6)	0(0.0)	9(8.3)	23(3.9)
	CZ	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.3)	0(0.0)	9(8.3)	10(1.7)
	CTT	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.3)	0(0.0)	0(0.0)	1(0.2)
	CTX	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.3)	0(0.0)	8(7.4)	9(1.5)
	FEP	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Carbapenems	IPM	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Phenicols	C	5(7.6)	13(27.1)	4(8.7)	3(7.0)	3(5.3)	7(22.6)	22(37.9)	32(42.1)	18(28.6)	49(45.4)	156(26.2)
Aminoglycosides	GM	1(1.5)	2(4.2)	3(6.5)	3(7.0)	1(1.8)	2(6.5)	7(12.1)	5(6.6)	5(7.9)	10(9.3)	39(6.5)
	AN	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4(6.9)	0(0.0)	0(0.0)	0(0.0)	4(0.7)
Quinolones	NA	8(12.1)	8(16.7)	3(6.5)	12(27.9)	22(38.6)	5(16.1)	20(34.5)	18(23.7)	22(34.9)	70(64.8)	188(31.5)
	CIP	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Tetracyclines	TE	9(13.6)	36(75.0)	12(26.1)	19(44.2)	22(38.6)	18(58.1)	16(27.6)	35(46.1)	16(25.4)	31(28.7)	214(35.9)
Sulfonamides	SXT	1(1.5)	8(16.7)	1(2.2)	3(7.0)	2(3.5)	2(6.5)	2(3.4)	5(6.6)	2(3.2)	3(2.8)	29(4.9)

* AM, Ampicillin; TIC, Ticarcillin; SAM, Ampicillin/Sulbactam; CF, Cephalothin; CZ, Cefazolin; CTT, Cefotetan; CTX, Cefotaxime; FEP, Cefepime; IPM, Imipenem; C, Chloramphenicol; GM, Gentamicin; AN, Amikacin; NA, Nalidixic acid; CIP, Ciprofloxacin; TE, Tetracyclin; SXT, Trimethoprim/sulfamethoxazole

The most common resistance observed in *Salmonella* isolates was to penicillin group antibiotics (about 42–43%), followed by tetracycline (35.9%), nalidixic acid (31.5%), and chloramphenicol (26.2%). Less than 10% of *Salmonella* isolates showed resistance to cephalothin (3.9%), trimethoprim/sulfamethoxazole (4.9%), gentamicin (6.5%), and ampicillin/sulbactam (7.4%). Resistance to these antibiotics mostly has persisted since 2000, except for resistance to cephalosporins group antibiotics. Among the cephalosporin group antibiotics tested, resistance was observed only to the first generation cephalosporins, cephalotin, in 2002. Resistance to other four cephalosporins group antibiotics was not observed until 2007. Although no significant change was observed in the prevalence of antibiotic resistance during the study period, resistance to penicillin group antibiotics, gentamicin, chloramphenicol, and nalidixic acid tended to be increased during late 2000s, compared to early 2000s.

Antibiotic resistance rates by serotypes of *Salmonella* isolates

All *Salmonella* isolates (n=596) were grouped into three categories including the two most important serotypes, *S*. Enteritidis, *S*. Typhimurium. *Salmonella* isolates not belonging to serotypes *S*. Enteritidis or *S*. Typhimurium were all included in the group of "Others". The prevalence of antibiotic resistance in each group was presented in Table 2. Overall, a similar

Table 2. Number of antibiotic resistant *Salmonella* strains by serotype isolated during 2000–2009 in Gwangju, Korea

Class of antibiotics	Anti-microbials	No. of resistant strains (%)			
		S. Enteritidis (n=286)	*S*. Typhimurium (n=122)	Others (n=188)	Total (n=596)
Penicillins	AM	149(52.1)	64(52.5)	43(22.9)	256(43.0)
	TIC	141(49.3)	59(48.4)	50(26.6)	250(41.9)
B-Lactams combination	SAM	16(5.6)	12(9.8)	16(8.5)	44(7.4)
Cephalosporins	CF	13(4.5)	7(5.7)	3(1.6)	23(3.9)
	CZ	9(3.1)	1(0.8)	0(0.0)	10(1.7)
	CTT	0(0.0)	1(0.8)	0(0.0)	1(0.2)
	CTX	8(2.8)	1(0.8)	0(0.0)	9(1.5)
	FEP	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Carbapenems	IPM	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Phenicols	C	88(30.8)	34(27.9)	34(18.1)	156(26.2)
Aminoglycosides	GM	9(3.1)	23(18.9)	7(3.7)	39(6.5)
	AN	2(0.7)	0(0)	2(1.1)	4(0.7)
Quinolones	NA	122(42.7)	33(27.0)	33(17.6)	188(31.5)
	CIP	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Tetracyclines	TE	45(15.7)	95(77.9)	74(39.4)	214(35.9)
Sulfonamides	SXT	3(1.0)	14(11.5)	12(6.4)	29(4.9)

* AM, Ampicillin; TIC, Ticarcillin; SAM, Ampicillin/Sulbactam; CF, Cephalothin; CZ, Cefazolin; CTT, Cefotetan; CTX, Cefotaxime; FEP, Cefepime; IPM, Imipenem; C, Chloramphenicol; GM, Gentamicin; AN, Amikacin; NA, Nalidixic acid; CIP, Ciprofloxacin; TE, Tetracyclin; SXT, Trimethoprim/sulfamethoxazole

Table 3. Number of multidrug resistant *Salmonella* spp. strains isolated during 2000–2009 in Gwangju, Korea

No. of anti-microbials / year	No. of *Salmonella* isolates resistant to antibiotics by year (%)										
	2000 (n=66)	2001 (n=48)	2002 (n=46)	2003 (n=43)	2004 (n=57)	2005 (n=31)	2006 (n=58)	2007 (n=76)	2008 (n=63)	2009 (n=108)	Total (n=596)
0	43(65.2)	5(10.4)	29(63.0)	14(32.6)	22(38.6)	4(12.9)	0(0.0)	9(11.8)	14(22.2)	15(13.9)	155(26.0)
1	11(16.7)	18(37.5)	8(17.4)	10(23.3)	14(24.6)	3(9.7)	10(17.2)	18(23.7)	20(31.7)	16(14.8)	128(21.4)
2	5(7.6)	8(16.7)	1(2.2)	6(14.0)	9(15.8)	8(25.8)	14(24.1)	23(30.3)	9(14.3)	4(3.7)	87(14.6)
3	3(4.5)	4(8.3)	4(8.7)	6(14.0)	9(15.8)	10(32.2)	21(36.2)	10(13.2)	10(15.9)	16(14.8)	93(15.6)
4	2(3.0)	6(16.7)	1(2.2)	4(9.3)	2(3.5)	1(3.2)	7(12.1)	8(10.5)	7(11.1)	37(34.3)	75(12.6)
5	1(1.5)	3(2.1)	2(4.3)	1(2.3)	1(1.8)	2(6.5)	3(5.2)	6(7.9)	2(3.2)	9(8.3)	30(5.0)
6	1(1.5)	3(6.3)	1(2.2)	0(0.0)	0(0.0)	2(6.5)	0(0.0)	0(0.0)	1(1.6)	2(1.9)	10(1.7)
7	0(0.0)	1(2.1)	0(0.0)	2(4.7)	0(0.0)	0(0.0)	3(5.2)	1(1.3)	0(0.0)	1(0.9)	8(1.3)
8	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(3.2)	0(0.0)	0(0.0)	0(0.0)	8(7.4)	9(1.5)
9	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.3)	0(0.0)	0(0.0)	1(0.2)
>3	7(10.6)	17(35.4)	8(17.4)	13(30.2)	12(21.0)	16(51.6)	34(58.6)	26(34.2)	20(31.7)	73(67.5)	226(37.9)

pattern of antibiotic activities was observed in all three groups: higher rates of resistance were seen to penicillin group antibiotics, chloramphenicol, nalidixic acid, and tetracycline, compared to other antibiotics. Generally, however, resistance to these antibiotics was much higher in isolates belonging to serotype *S*. Enteritidis or *S*. Typhimurium than in those of other serotypes. Compared to other serotypes, *S*. Typhimurium showed higher resistance to all antibiotics while *S*. Enteritidis exhibited lower resistance to some of the antibiotics such as tetracycline and trimethoprim/sulfamethoxazole.

The prevalence of antibiotic resistance differed among the three groups, and the most notable differences were observed in resistance to tetracycline and nalidixic acid: 15.7% and 42.7% of *S*. Enteritidis, 77.9% and 27.0% of *S*. Typhimurium, and 39.4% and 17.6% of serotype Others, respectively. *S*. Typhimurium group showed higher resistance to gentamicin and trimethoprim/sulfamethoxazole, compared to other two groups. In *S*. Enteritidis group, the highest resistance was to ampicillin (52.1%), followed by resistance to ticarcillin (49.3%), nalidixic acid (42.7%), chloramphenicol (30.8%), and tetracycline (15.7%). In *S*. Typhimuirum group, the highest resistance was to tetracyclines (77.9%), penicillins (52.5%), ticarcillin (48.4%), chloramphenicol (27.9%), nalidixic acid (27.0%), gentamicin (18.9%), and trimethoprim/sulfamethoxazole (11.5%).

Multi-drug resistance of *Salmonella* isolates

The number of MDR *Salmonella* isolates from patients with acute diarrhea during 2000–2009 was presented in Table 3. Of 596 isolates, 155 (26.0%) were susceptible to all the antibiotic agents tested in this study. About 21% (128/596), 14.6% (87/596), 15.6% (93/596), and 12.6% (75/596) of the *Salmonella* isolates presented resistance to 1, 2, 3, and 4 antibiotic agents tested, respectively. In addition, about 10% (58/596) of *Salmonella* isolates showed resistance to five or more antibiotic agents tested. In total, 37.9% (226/596) of *Salmonella* isolates were resistant to at least three antibiotic agents tested in this study. Although year-to-year variations were observed, multidrug resistance rates tend to increase in later years compared to earlier years of the 10-year study period.

Multiple drug resistance of *Salmonella* isolates by serotypes

Table 4 presented the rates of multidrug resistance in *Salmonella* serotypes ten most frequently isolated in this study.

Table 4. Number of multidrug resistant *Salmonella* isolates by serotypes 10 most frequently isolated during 2000–2009 in Gwangju, Korea

Serotypes / No. of antibiotics	No. of resistant isolates (%)				
	0	1–2	3–7	8–9	>3
S. Enteritidis (n=286)	59(20.6)	112(39.2)	107(37.4)	8(2.8)	115(40.2)
S. Typhimurium (n=122)	16(13.1)	37(30.3)	67(54.9)	2(1.6)	69(56.6)
S. Braenderup (n=19)	13(68.4)	4(21.1)	2(10.5)	0(0.0)	2(10.5)
S. Montevideo (n=17)	3(17.6)	13(76.5)	1(5.9)	0(0.0)	1(5.9)
S. Paratyphi B (n=17)	3(17.6)	1(5.9)	13(76.5)	0(0.0)	13(76.5)
S. London (n=14)	7(50.0)	7(50.0)	0(0.0)	0(0.0)	0(0.0)
S. Bardo (n=10)	0(0.0)	10(100)	0(0.0)	0(0.0)	0(0.0)
S. Virchow (n=10)	7(70.0)	3(30.0)	0(0.0)	0(0.0)	0(0.0)
S. Infantis (n=9)	6(66.7)	3(33.3)	0(0.0)	0(0.0)	0(0.0)
S. Typhi (n=9)	6(66.7)	2(22.2)	1(11.1)	0(0.0)	1(11.1)
Others (n=83)	35(42.1)	23(27.7)	25(30.1)	0(0.0)	25(30.1)
Total (n=596)	155(26.0)	215(36.1)	216(36.2)	10(1.7)	226(37.9)

Table 5. Antibiotic resistance patterns by serotypes of *Salmonella* spp. isolated during 2000–2009 in Gwangju, Korea (n=596)

No. of Antimicrobials	*S.* Enteritidis (n=286)				*S.* Typhimurium (n=122)				Other serotypes (n=188)			
	No. of resistant isolates (%)	No. of resistance patterns	Most frequent pattern		No. of resistant isolates (%)	No. of resistance patterns	Most frequent pattern		No. of resistant isolates (%)	No. of resistance patterns	Most frequent pattern	
			Type	No. of isolates (%)			Type	No. of isolates (%)			Type	No. of isolates (%)
0	59(20.6)	0	-	0(0.0)	16(13.1)	0	-	-	80(42.5)	0	-	
1	70(24.5)	4	NA	43(61.4)	20(16.4)	5	TE	15(75.0)	38(20.2)	2	TE	16(42.1)
2	42(14.7)	8	AM-TIC	17(40.5)	17(13.9)	5	NA-TE	8(47.0)	28(14.9)	9	C-TE	12(42.9)
3	50(17.5)	10	AM-C-TIC	28(56.0)	32(26.2)	9	AM-TE-TIC	22(68.8)	11(5.8)	8	AM-TE-TIC	4(36.4)
4	51(17.8)	10	AM-C-NA-TIC	35(68.6)	12(9.8)	9	AM-NA-TE-TIC	2(16.7)	12(6.3)	9	AM-C-TE-TIC	2(16.7)
5	4(1.4)	2	AM-SAM-C-NA-TIC	3(75.0)	13(10.7)	8	AM-C-GM-TE-TIC	3(23.0)	12(6.3)	5	AM-SAM-C-TE-TIC	6(50.0)
6	0(0.0)	0	-	-	5(4.1)	5	AM-C-GM-NA-TE-TIC	1(20.0)	6(3.2)	4	AM-C-GM-NA-TE-TIC	1(20.0)
7	2(0.7)	2	AM-AN-SAM-CF-C-GM-TE	1(50.0)	5(4.1)	4	AM-C-GM-NA-TE-TIC-SXT	2(40.0)	1(0.5)	1	AM-SAM-CF-NA-TE-TIC-SXT	1(100.0)
8	8(2.8)	4	AM-CF-CTX-GM-NA-TE-TIC-CZ	4(50.0)	1(0.8)	1	AM-SAM-CF-C-GM-TE-TIC-SXT	1(100.0)	-	-	-	-
9	-	-	-	-	1(0.8)	1	AM-SAM-CF-CTX-C-NA-TE-CZ-CTT	1(100.0)	-	-	-	-

Among the top ten serotypes, *S*. Enteritidis (47.9%, 286/596) and *S*. Typhimurium (20.5%, 122/596) accounted for 68.5% of all *Salmonella* isolates. The number of isolates belonging to other serotypes was all less than 20 (>4%). Of 596 *Salmonella* isolates, 226 (37.9%) showed resistance to more than three antibiotic agents tested in this study. Over 81% (184/226) of the *Salmonella* isolates resistant to at least three antibiotic agents belonged to serotypes *S*. Enteritidis (115/226, 50.8%) and *S*. Typhimurium (69/226, 30.5%). About 57% (69/122) of *S*. Typhimurium and 40% of *S*. Enteritidis (115/286) were resistant to at least three antibiotics tested in this study. Ten (1.7%) isolates showed resistance to more than eight antibiotic agents tested and all of them belonged to *S*. Enteritidis (8/286, 2.8%) and *S*. Typhimurium (2/122, 1.6%). In contrast, almost no multidrug (≥3) resistance was observed among isolates belonged to serotypes other than *S*. Enteritidis and *S*. Typhimurium, except for *S*. Paratyphi B. About 77% (13/17) of *S*. Paratyphi B isolates were resistant to more than three antibiotic agents tested. Percentage of pan-susceptible isolates varied substantially by serotypes (range: 0%–70%): All isolates of *S*. Bardo showed resistance to at least one antibiotic agent, while 70% of the *S*. Virchow isolates were susceptible to all the antibiotic agents tested in this study.

Antibiotic resistance patterns by serotypes

All *Salmonella* serotypes were categorized into three groups, *S*. Enteritidis, *S*. Typhimurium, and Others and the most common type of antibiotic resistance patterns of each group was presented in Table 5. A total of 89 resistance patterns were observed among the 596 *Salmonella* isolates, and the most common resistance pattern of MDR isolates was ampicillin-chloramphenicol-nalidixic acid-ticarcillin (36/227, 15.8%). The most frequently observed resistance patterns differed among serotypes: ampicillin-chloramphenicol-nalidixic acid-ticarcillin resistance in *S*. Enteritidis (35/286, 12.2%) and ampicillin-tetracycline-ticarcillin in *S*. Typhimurium (22/122, 18.0%). Almost half (42.5%) of the *Salmonella* isolates belonging to serotypes other than *S*. Enteritidis and *S*. Typhimurium were pan-susceptible.

Discussion

The most common resistance observed in this study was to penicillin and ticarcillin (>42.0%), followed by tetracycline (35.9%), nalidixic acid (31.5%), and chloramphenicol (26.2%). *Salmonella* isolates from diarrheic patients in Seoul, Korea during 1996–2001 also showed high rate of tetracycline resistance (32.7%) but much lower resistance to ampicillin (12.4%), chlroramphenicol (7.5%), and nalidixic acid (5.8%) (Park *et al*., 2002). In general, *Salmonella* isolated in this study presented higher rates of antibiotic resistance, compared to those reported by Park *et al*. (2002). Difference in time period of the two studies (from 1996 to 2001 and 2000 to 2009) and regional differences may be the reason for the discrepancy of resistance between these two studies. Most of the *Salmonella* isolates were sensitive to ciprofloxacin, cephalothin and other cephalosporins, amikacin, and gentamicin. Similar result was reported from Korea (Park *et al*., 2002) and other countries including Malawi (Gordon *et al*., 2008) and Italy (Monno *et al*., 2007). In general, resistance to nalidixic acid, tetracycline,

ampicillin, chloramphenicol, and Sulfamethoxazole-trimethoprime seems to be relatively common in *Salmonella* isolates from other parts of the world such as Italy (Monno *et al*., 2007) and Brazil (Castro *et al*., 2002; Fernandes *et al*., 2003). It may reflect the frequent use of those drugs to treat *Salmonella* infections (Hur *et al*., 2011; Álvarez-Fernández *et al*., 2012).

Resistance profiles differ among *Salmonella* species and more frequent resistance of *S*. Typhimuium compared to other serotypes including *S*. Enteritidis have been reported by many researchers worldwide (Park *et al*., 2002; Busani *et al*., 2004). We also observed in this study that resistance was much higher in serotype *S*. Typhimurium, compared to other serotypes, although the pattern of antibiotic activities was somewhat similar in all serotypes. In particular, *S*. Typhimurium showed higher resistance to antibiotics tested in this study than *S*. Enteritidis. This result agrees with studies in Korea (Park *et al*., 2002) and other countries as well (Cruchaga *et al*., 2001; Erdem *et al*., 2005; Monno *et al*., 2007).

The resistance of *S*. Enteritidis to ampicillin, ticarcillin, and chloramphenicol in this study were relatively similar to the results of national survey conducted in 2009 and 2010 (KCDC, 2010, 2011), while nalidixic resistance of this study (42.7%) was about half of the results of national survey (86.6% and 82.4%). Park *et al*. (2002) reported much lower resistance to those antibiotics in 298 *S*. Enteritidis isolates, compared to our results: 34.7%, 13.5%, 23.2%, 7.7%, and 5.4% to tetracycline, ampicillin, ticarcillin, chloramphenicol, and nalidixic acid, respectively. Another study conducted in Incheon, Korea reported that the highest rate of resistance observed in *S*. Enteritidis isolates were against nalidixic acid (54.8%), followed by ampicillin (40%), ticarcillin (38.3%), chloramphenicol (20.9%), and tetracycline (17.4%) (Hwang *et al*., 2009). Ampicillin, ticarcillin, and chloramphenicol resistance rates of the study were lower than those of our study, while resistance to nalidixic acid was higher than our result. Resistance of *S*. Enteritidis to antibiotics such as ampicillin, chloramphenicol, tetracycline, and nalidixic acid has also been reported from other countries such as Brazil (Fernandes *et al*., 2003) and Turkey (Erdem *et al*., 2005), although the prevalence of the studies varied. *S*. Enteritidis isolated from 27,000 cases of human salmonellosis in 2000 in 10 European countries also showed the highest rate of resistance to nalidixic acid, followed by streptomycin and ampicillin (Threlfall *et al*., 2003).

Almost 80% of *S*. Typhimurium isolates showed resistance to tetracycline that was the highest rate of resistance among antibiotics tested in this study. Same result was also observed in other studies in Korea, where tetracycline resistance was the most prevalent among *S*. Typhimurium isolated from diarrheic patients in Seoul during 1996–2001 (Park *et al*., 2002) and 1999–2002 (Oh *et al*., 2005). According to the reports of national survey (KCDC, 2010, 2011), tetracycline resistance was also highly prevalent among *S*. Typhimurium isolates from acute diarrheic patients in 2009 and 2010 (60.4% and 60.3%), although ampicillin resistance was the most prevalent among the isolates (61.4% and 65.5%). Tetracyclines, broad-spectrum agents exhibiting activity against a wide range of Gram-positive and Gram-negative bacteria, are currently used widely for therapy and prophylaxis for human and animal infections, as well as animal growth promoters (Roberts, 1996; Chopra and Roberts, 2001). High resistance to tetracycline among the *S*. Typhimurium isolates may have arisen by the intensive use of the antibiotics.

S. Typhimurium is one of the most important foodborne pathogens worldwide (Karatzas *et al*., 2008), and the emergence of resistant strains to multiple antibiotics poses a serious threats to public health (Lugo-Melchor *et al*., 2010). We found that over half (56.6%) of *S*. Typhimurium isolates had resistance to at least three antibiotics tested, which was higher than that of *S*. Enteritidis (40%). Lo *et al*. (2012) also reported that multidrug resistance was less common in *S*. Enteritidis compared to *S*. Typhimurium. In other study conducted in Korea (Oh *et al*., 2005), 67.4% (33/49) of *S*. Typhimurium isolates from feces of acute diarrheic patients during 1999–2002 showed resistance to more than three antibiotics. The most frequently observed resistance pattern of the isolates in the study was streptomycin-sulfonamide-tetracycline, which differed from that of our study. In this study, about 18% of *S*. Typhimurium isolates showed resistance to ampicillin-tetracycline-ticarcillin simultaneously.

Multidrug-resistant *S*. Typhimurium phage type DT104, which emerged during the last decade as a global health problem, is mostly resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines (Cloeckaert *and Schwarz,* 2001). Although we did not examine streptomycin resistance, resistant rates of *S*. Typhimurium isolates in this study varied widely among the other four antibiotics: 77.9%, 52.5%, 27.9%, and 11.5% against tetracycline, ampicillin, chloramphenicol, and sulfonamide, respectively. It was reported that the epidemic of DT104 has now become worldwide with a considerable number of outbreaks since 1996 in the USA (Glynn *et al*., 1998). According to the reports of KCDC (2010, 2011), DT104 has been identified since 2007 but the prevalence seemed to be very low so far.

Increasing occurrence of antibiotic resistance in *Salmonella* is a major public health problem. Also, patterns of resistance in Salmonella are constantly changing (Parry and Threlfall, 2008). In this study, we also found that multidrug resistance rates tend to increase in later years compared to earlier years of the

10-year study period, although there were year-to-year variations.

In conclusion, this study revealed that the most common resistance observed in *salmonella* isolates was to penicillin antibiotics, chloramphenicol, nalidxic acid, and tetracycline, while resistance to ciprofloxacin, cephalosporins, amikacin, and gentamicin was rare.

적 요

지난 2000년부터 2009년까지 10년간 광주광역시 지역의 급성설사환자로부터 분리한 총 596개의 살모넬라균주에 대하여 총 16종의 항생제에 대한 감수성 검사를 실시한 결과, 내성빈도가 가장 높았던 약제는 ampicillin (43%), tetracycline (35.9%), nalidixic acid (31.5%), 그리고 chloramphenicol (26.2%) 순으로 나타났다. 혈청형 간에 항생제 내성률에 차이가 있었는데 *S*. Enteritidis와 *S*. Typhimurium이 각각 가장 높은 내성률을 보인 약제는 ampicillin (51.1%)과 tetracycline (77.9%)이었다. 총 89가지 내성패턴이 관찰되었으며, 26% (155/596)의 살모넬라 분리주는 검사한 16가지 약제에 모두 감수성을 보였다. 약 21% (127/596) 및 15% (87/596)의 분리주는 각각 한 개 및 두 개의 약제에 내성을 나타냈다. 그 외의 살모넬라 분리주(227/596, 38%)는 세가지 이상의 약제에 내성을 나타냈다(다제내성균). 다제내성균이 가장 높은 비율로 나타난 혈청형은 *S*. Paratyphi B (76.5%), *S*. Typhimurium (58.2%), 그리고 *S*. Enteritidis (40.2%)의 순이었다. 가장 흔한 다제내성패턴은 ampicillin-chloramphenicol-nalidixic acid-ticarcillin (36/227, 15.8%)이었으며 이러한 패턴을 보였던 다제내성균의 대부분(35/36, 97.2%)이 *S*. Enteritidis이었다.

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