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Original Article

Serotyping and Antimicrobial Susceptibility of *Salmonella* spp.: Nationwide Multicenter Study in Korea

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SUMMARY: The study aimed to investigate the prevalence of various serotypes and extended-spectrum β-lactamase-producing features of *Salmonella* strains and to determine the antimicrobial susceptibility of 256 *Salmonella* strains other than *Salmonella* serotype Typhi, which were isolated at 12 university hospitals in Korea. We identified 46 serotypes of *Salmonella* spp. Serogroup D was the most common (39.5%), followed by B (32.4%), C (22.7%), E (2.7%), A (2.3%), and G (0.4%). The three most common *Salmonella* serotypes were Enteritidis (36.3%), Typhimurium (16.8%), and Infantis (7.8%). Six strains that belonged to serotype Paratyphi A and nine that belonged to serotype Paratyphi B were also detected. The 256 *Salmonella* strains had a 38.7% rate of resistance to ampicillin, 23.0% to chloramphenicol, 8.2% to cefotaxime, 8.6% to ceftriaxone, and 6.3% to trimethoprim-sulfamethoxazole. The antimicrobial resistance rates of *Salmonella* serogroups B and D were higher than those of the other serogroups. Seven isolates carried $bla_{\text{CTX-M}}$: four CTX-M-15, two CTX-M-14, and one CTX-M-3.

INTRODUCTION

Salmonella is an important pathogen that causes food-borne infection worldwide, and more than 2,500 serotypes have been discovered till date (1). Among these, *Salmonella* serotypes Enteritidis and Typhimurium are most commonly isolated from clinical specimens. However, other specific serotypes are more prevalent in some regions, and we are sometimes faced with outbreaks of unusual serotypes (2–5). The serotyping of *Salmonella* is an essential step for the identification of the causative organism for ensuring appropriate diagnosis and treatment as well as for epidemiologic investigation. However, many studies were conducted either in single institutions (6,7) or many years ago (8), which makes their findings of questionable relevance in Korea today. Recent reports of nationwide multicenter studies in Korea are rare.

Most infections caused by *Salmonella* spp. are controlled by fluid and electrolyte replacement; however, antimicrobial agents are required to treat very young children or immunocompromised patients with invasive infections such as septicemia (9). During the past two decades, ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (TMX) have been the primary antimicrobial agents; however, resistance rates have been increasing (10). Fluoroquinolones or third-generation cephalosporins are now recommended as first-line antimicrobial agents for the management of invasive disease caused by *Salmonella* spp. (11). However, there have also been reports of increased resistance to these agents, including bacterial production of extended-spectrum β-lactamases (ESBLs) (12,13).

This study aimed to investigate the prevalence of various serotypes and ESBL-producing *Salmonella* strains as well as to determine the antimicrobial susceptibility of *Salmonella* spp. other than *Salmonella* serotype

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Typhi, which were isolated throughout Korea.

METHODS

A total of 256 *Salmonella* strains, excluding *S.* Typhi isolates, were recovered from clinical specimens between January and December 2008 at 12 Korean tertiary-care hospitals from Seoul in the north to Jeju in the south. These isolates were identified by conventional biochemical tests (14) and serogrouping according to each laboratory's routine protocols. We categorized the 12 hospitals into 4 regions: North (Seoul and Gyeonggi Province), Southwest (Jeolla Province), Southeast (Gyeongsang Province), and Southernmost (Jeju Island). The participating hospitals and the numbers of isolates were as follows: Ajou University Medical Center (n = 28), Asan Medical Center (n = 26), Ewha Women's University Medical Center (n = 35), and Yonsei University Hospital (n = 42) at Seoul and Gyeonggi Province; Gyeongsang National University Hospital (n = 3), Inje University Busan Paik Hospital (n = 17), Pusan National University Hospital (n = 6), Ulsan University Hospital (n = 22), and Yeungnam University Medical Center (n = 9) at Busan and Gyeongsang Province; Chonnam National University Hospital (n = 22) and Wonkwang University Hospital (n = 18) at Jeolla Province; and Jeju National University Hospital (n = 28) at Jeju Island. These strains were used in a previous study of quinolone resistance in *Salmonella* (15). The serotype was determined by slide agglutination according to the Kauffman-White scheme using O and H antisera (DIFCO, Detroit, Mich., USA) (16).

Antimicrobial susceptibility tests were performed using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI). The antimicrobial agents tested were amoxicillin-clavulanate, ampicillin, cephalothin, chloramphenicol, imipenem, ceftriaxone, cefotaxime, gentamicin, amikacin, tetracycline, and TMX. *Escherichia coli* ATCC 25922 was used as the quality control strain. The results were interpreted according to the criteria recommended by the CLSI (17). We reused the ciprofloxacin susceptibility results from our previous report (15).

All the *Salmonella* isolates were examined for the presence of $bla_{\text{CTX-M}}$ by PCR and direct sequencing using 4 primer sets (Table 1) (18,19). The PCR reaction was performed under the following conditions: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min. The purified PCR products were bidirectionally sequenced with the same primer sets used for amplification using an ABI 3130 genetic analyzer automated DNA sequencer (Applied Biosystems, Foster City, Calif., USA).

RESULTS

A total of 256 *Salmonella* strains were isolated from feces (n = 210), blood (n = 38), urine (n = 3), pus (n = 1), abscess (n = 1), ascites (n = 1), bile (n = 1), and other specimens (n = 1). Serogroup D was the most common, accounting for 39.5% of all isolates (n = 101). Serogroup B ranked second (n = 83; 32.4%), followed by serogroups C (n = 58; 22.7%), E (n = 7; 2.7%), A (n = 6; 2.3%), and G (n = 1; 0.4%).

We identified 46 *Salmonella* serotypes among all the 256 *Salmonella* isolates. The 2 most common were Enteritidis (93; 36.3%) and Typhimurium (43; 16.8%), followed by Infantis (20; 7.8%), Paratyphi B (9; 3.5%), Virchow (8; 3.1%), Paratyphi A (6; 2.3%), and Montevideo (6; 2.3%). The other serotypes accounted for less than 2% each (Table 2). All serogroup A *Salmonella* isolates belonged to *Salmonella* serotype Paratyphi A, while *Salmonella* serotype Paratyphi B accounted for 10.8% of serogroup B *Salmonella* isolates. There was no difference in the prevalence of serogroup or serotype according to the types of specimens (data not shown).

We found regional differences in the distributions of serogroups and serotypes (Table 3). Serogroup B was prevalent at Seoul and Gyeonggi Province, whereas serogroup D was dominant at Busan and Gyeongsang Province. Serogroup C was the most prevalent serogroup at Jeolla Province, and all isolates from Jeju Island belonged to serogroup D. The frequency of *Salmonella* serotypes also showed distinct features according to the region. At Busan and Gyeongsang Province, *S.* Enteritidis accounted for 45.6% (n = 26) of the *Salmonella* isolates, while Typhimurium accounted for 12.3% (n = 7). At Seoul and Gyeonggi Province, the percent of *S.* Enteritidis (n = 28; 21.4%) was similar to that of *S.* Typhimurium (n = 27; 20.6%), and *S.* Infantis was the third most common strain (10.7%). At Jeolla Province, the frequencies of *S.* Enteritidis (27.5%) and *S.* Typhimurium (22.5%) were similar and serotype Virchow was more prevalent than Infantis. At Jeju National University Hospital, all 28 *Salmonella* isolates belonged to serotype Enteritidis.

Antimicrobial resistance of the *Salmonella* isolates is shown in Table 4. The 256 *Salmonella* isolates had a 38.7% rate of resistance to ampicillin, 23.0% to chloramphenicol, 2.0% to ciprofloxacin, 8.2% to cefotax-

Table 1. Primers used for PCR amplification and sequencing of ESBL genes

Primer	Target gene	Nucleotide sequence (5′ to 3′)	Reference
CTX-M-uniF	CTX-M	CVA TGT GCA GYA CCA GTA A	Yong et al. (19)
CTX-M-uniR		ARG TSA CCA GAA YMA GCG G	
CTX-M-1F	CTX-M-1 group	CCG TCA CGC TGT TGT TAG G	Bae et al. (18)
CTX-M-1R		ACG GCT TTC TGC CTT AGG TT	
CTX-M-2F	CTX-M-2 group	CGA CGC TAC CCC TGC TAT T	Bae et al. (18)
CTX-M-2R		CAG AAA CCG TGG GTT ACG AT	
CTX-M-9F	CTX-M-9 group	TGC AAC GGA TGA TGT TCG	Bae et al. (18)
CTX-M-9R		CGG CTG GGT AAA ATA GGT CA	

Table 2. Serogroup and serotype distribution of *Salmonella* isolates

Serogroup A		Serogroup B		Serogroup C	
Serotype	No. (%)	Serotype	No. (%)	Serotype	No. (%)
Paratyphi A	6 (2.3)	Typhimurium	43 (16.8)	Infantis	20 (7.8)
		Paratyphi B	9 (3.5)	Virchow	8 (3.1)
		Stanley	5 (2.0)	Montevideo	6 (2.3)
		II 4, 12, [27]:i:z35	4 (1.6)	Bareilly	4 (1.6)
		II [1], 4, 12, 27:l, v:z39	3 (1.2)	Newport	3 (1.2)
		Derby	3 (1.2)	Rissen	3 (1.2)
		Bradford	2 (0.8)	II 6, 7:g, m, s, t:z39	2 (0.8)
		Lagos	2 (0.8)	Larochelle	2 (0.8)
		Wien	2 (0.8)	Othmarschen	2 (0.8)
		II4, 12:|, z28:[e, n, x]	1 (0.4)	Bardo	1 (0.4)
		Agama	1 (0.4)	Braenderup	1 (0.4)
		Canada	1 (0.4)	Galiema	1 (0.4)
		Coeln	1 (0.4)	Kastrup	1 (0.4)
		Essen	1 (0.4)	Kentucky	1 (0.4)
		Indiana	1 (0.4)	Litchfield	1 (0.4)
		Saintpaul	1 (0.4)	Mbandaka	1 (0.4)
		Schwarzengrund	1 (0.4)	Narashino	1 (0.4)
		Tudu	1 (0.4)		
		Wagenia	1 (0.4)		
Serogroup D		Serogroup E		Serogroup G	
Serotype	No. (%)	Serotype	No. (%)	Serotype	No. (%)
Enteritidis	93 (36.3)	London	5 (2.0)	Kedougou	1 (0.4)
Panama	4 (1.6)	Hayindogo	1 (0.4)		
Hillingdon	2 (0.8)	Lerum	1 (0.4)		
India	1 (0.4)				
Seremban	1 (0.4)				

Table 3. Regional distribution of serogroup and common serotypes of *Salmonella* isolates from Korea

Serogroup and common serotype	No. of isolates			
	Seoul and Gyeonggi Province	Busan and Gyeongsang Province	Jeolla Province	Jeju Island
A (*S.* Paratyphi A)	4	1	1	
B	55	16	12	
S. Typhimurium	(27)	(7)	(9)	
C	32	11	15	
S. Infantis	(14)	(3)	(3)	
S. Virchow		(1)	(7)	
D	35	27	11	28
S. Enteritidis	(28)	(26)	(11)	(28)
E	5	1	1	
G	0	1	0	
Total	131	57	40	28

ime, 8.6% to ceftriaxone, and 6.3% to TMX. The antimicrobial resistance rates of *Salmonella* serogroups B and D were higher than those of the other serogroups. *S.* Paratyphi B had the highest resistance rate, being resistant to most drugs, except TMX. Among the 9 *S.* Paratyphi B strains, 8 (88.9%) were resistant to ampicillin and chloramphenicol and 2 were resistant to third-generation cephalosporin. *S.* Typhimurium had high resistance rates to ampicillin (60.5%), chloramphenicol (39.5%), ciprofloxacin (2.3%), and TMX (11.6%); however, all the isolates were susceptible to third-generation cephalosporin. The resistance rates to third-generation cephalosporin were higher among *S.* Enteritidis than those among Typhimurium. Of the 6 *S.* Paratyphi A strains, 1 strain was resistant to ampicillin, ciprofloxacin, and TMX. There were regional differences in the antimicrobial resistance rates according to the *Salmonella* serotype. The resistance rates of *S.* Typhimurium were higher at Seoul and Gyeonggi Province than those at the other provinces. In contrast, the resistance rates of *S.* Enteritidis were higher at Busan and Gyeongsang Province. With the exception of 1 isolae, all the *S.* Enteritidis isolates from Jeju were susceptible to all antimicrobial agents.

Table 4. Antimicrobial resistance rates by serotype of *Salmonella* isolates at 12 university hospitals in Korea

Serotype	No.	No. (%) of isolates by resistance pattern											
		AMC	AMP	CEP	CHL	CIP	IMI	CTX	CFA	GEN	AMI	TET	TMX
Enteritidis	93	3 (3.2)	37 (39.8)	14 (15.1)	20 (21.5)	1 (1.1)	0 (0)	12 (12.9)	12 (12.9)	12 (12.9)	0 (0)	23 (24.7)	5 (5.4)
Typhimurium	43	2 (4.7)	26 (60.5)	3 (7.0)	17 (39.5)	1 (2.3)	0 (0)	0 (0)	0 (0)	7 (16.3)	0 (0)	33 (76.7)	5 (11.6)
Infantis	20	4 (20.0)	4 (20.0)	4 (20.0)	3 (15.0)	0 (0)	0 (0)	3 (15.0)	3 (15.0)	0 (0)	0 (0)	3 (15.0)	0 (0)
Paratyphi B	9	1 (11.1)	8 (88.9)	2 (22.2)	8 (88.9)	0 (0)	0 (0)	2 (22.2)	2 (22.2)	0 (0)	0 (0)	8 (88.9)	0 (0)
Virchow	8	0 (0)	2 (25.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (25.0)	0 (0)
Paratyphi A	6	0 (0)	1 (16.7)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	1 (16.7)	1 (16.7)
Montevideo	6	1 (16.7)	2 (33.3)	3 (50.0)	0 (0)	0 (0)	0 (0)	1 (16.7)	1 (16.7)	1 (16.7)	0 (0)	3 (50.0)	0 (0)
Others	71	6 (8.5)	19 (26.8)	8 (11.3)	11 (15.5)	2 (2.8)	0 (0)	3 (4.3)	4 (5.6)	1 (1.4)	0 (0)	25 (35.2)	5 (7.0)
All isolates	256	17 (3.3)	99 (38.7)	34 (13.3)	59 (23.0)	5 (2.0)	0 (0)	21 (8.2)	22 (8.6)	22 (8.6)	0 (0)	98 (38.3)	16 (6.3)

AMC, amoxicillin-clavulanate; AMP, ampicillin; CEP, cephalothin; CHL, chloramphenicol; CIP, ciprofloxacin; IMI, imipenem; CTX, cefotaxime; CFA, ceftriaxone; GEN, gentamicin; AMI, amikacin; TET, tetracycline; TMX, trimethoprim-sulfamethoxazole.

Table 5. Serovar distribution and antimicrobial resistance of ESBL-producing *Salmonella* isolates

Sample	Serogroup	Serotype	*Bla* gene	AMP	CTX	CFA	CIP	TMX	CHL
Stool	B	Paratyphi B	CTX-M-14	R	R	R	S	S	R
Stool	C	Montevideo	CTX-M-14	R	R	R	S	S	S
Stool	D	Enteritidis	CTX-M-15	R	R	R	S	S	S
Stool	D	Enteritidis	CTX-M-15	R	R	R	S	S	S
Stool	D	Enteritidis	CTX-M-15	R	R	R	R	R	S
Stool	D	Enteritidis	CTX-M-15	R	R	R	S	S	S
Urine	G	Kedougou	CTX-M-3	R	R	R	S	R	S

Abbreviations are in Table 4.

We identified 7 ESBL-producing *Salmonella* isolates (Table 5). Four isolates carried $bla_{\text{CTX-M-15}}$ and were serotyped as Enteritidis. The CTX-M-14 type was detected in 2 isolates of *S.* Paratyphi B and *S.* Montevideo. With the exception of 1 isolate, all of these were isolated from stool. One isolate from urine harbored CTX-M-3 and was identified as serotype Kedougou.

DISCUSSION

It is thought that *S.* Enteritidis is the most common nontyphoidal *Salmonella* worldwide, except in the United States, where *S.* Typhimurium is the most common (4). In Asia, *S.* Enteritidis is also the most common in Hong Kong, Singapore, and Korea, whereas *Salmonella* serotypes Typhimurium, Weltevreden, and Stanley are the most prevalent in Taiwan, the Philippines, and Thailand (20). In Korea, serogroup D has been the most common among *Salmonella* spp. since 1996 (7,21), whereas serogroup B, including *S.* Typhimurium, was the most prevalent before that year (6). However, these results are from a single institution, and we obtained some different results in our nationwide multicenter study. Overall, serogroup D *Salmonella* isolates were the most common, followed by serogroups B, C, and others. However, when we divided them geographically into 4 regions, i.e., Seoul and Gyeonggi, Busan and Gyeongsang, Jeolla, and Jeju, the most prevalent serogroup was B, D, C, and D, respectively. From these results, we may assume that there are differences in the most prevalent serogroup or serotype, depending on the region in Korea, and that there is a necessity for a nationwide multicenter study. Interestingly, at Seoul and Gyeonggi Province, the numbers of *S.* Typhimurium (n = 27) were similar to those of *S.* Enteritidis (n = 28), although serogroup B (n = 55) was more prevalent than serogroup D (n = 35). This confirms the necessity for serotyping and the finding of a high percentage of group B serotypes other than Typhimurium. In this study, *Salmonella* serogroup C accounted for more than 20%, and various serotypes were identified. *S.* Infantis is the most common, followed by Virchow and Montevideo. In previous studies from Korea (6,7), the incidences of serogroup C were 7.6% and 8.8%, respectively. The increase in serogroup C could have resulted from the introduction of a new serotype or the influx and spread of food from overseas. The isolates of serogroup C exhibited regional differences: 14 of 20 *S.* Infantis were isolated from Seoul and Gyeonggi Province, whereas 7 of 8 Virchow isolates were isolated from Jeolla Province.

Of the 256 *Salmonella* isolates, 118 (46.1%) were susceptible to all the tested antimicrobial agents. The antimicrobial resistance rates to conventional antibiotics such as ampicillin, chloramphenicol, and TMX were higher than those to ciprofloxacin, cefotaxime, and ceftriaxone. The resistance rates to ampicillin and TMX were 28.0% and 4.1%, respectively, between 1997 and 2002; however, these had increased to 40.0% and 6.6%, respectively, in 2008 (7). Among these, *S.* Paratyphi B had the highest resistance rates to most antimicrobial agents, except TMX, although the numbers were too small to analyze statistically. The resistance rates to ampicillin and chloramphenicol were also higher in *S.* Typhimurium. This serotype had the highest resistance rates in the world, and the antimicrobial resistance rates

in the present study were similar to those found in other countries (12,22–24). *S.* Enteritidis also had higher resistance rates to ampicillin, chloramphenicol, cefotaxime, and TMX.

In recent years, fluoroquinolones or third-generation cephalosporins have been the first-line antimicrobial agents recommended for the treatment of invasive *Salmonella* infection (11), and resistance to these antibiotics has emerged. In the present study, the resistance rates to cefotaxime and ceftriaxone were 8.2% and 8.6%, respectively. In particular, *S.* Enteritidis had a higher resistance rate to third-generation cephalosporin, whereas all strains of serotype Typhimurium were susceptible to these agents. Most previous studies conducted between 1997 and 2009 (7,25,26) reported a resistance rate of 3% or less to third-generation cephalosporins, except for the study by Na et al. in 2002 (27) who reported an 11% resistance rate to cefotaxime. However, these strains were isolated from children only and half of the patients were immunosuppressed or had a hematological malignancy. The increased resistance rates to third-generation cephalosporins may be explained by the high prevalence of *Salmonella* resistant to conventional antibiotics, thereby mandating the use of third-generation cephalosporin as the treatment of choice for infection. It would be helpful to provide susceptibility data periodically for understanding the local epidemiology of *Salmonella* isolates and for appropriate management of patients.

With the advent of resistance to third-generation cephalosporin in *Salmonella*, it is not surprising that there are increasing reports on ESBL-producing *Salmonella* (10,28). Most common resistance genes are derivatives of the TEM, SHV, and CTX-M families, and these were detected in *Salmonella* serotypes Enteritidis and Typhimurium. In Korea, a few isolates of TEM-52, CTX-M-14, and CTX-M-15 ESBL-producing *Salmonella* spp. were detected, of which CTX-M-15 was the most common, followed by CTX-M-14 (13,19,29,30). In the present study, 7 CTX-M-producing *Salmonella* isolates were identified. Four isolates belonged to *S.* Enteritidis containing CTX-M-15, and the other 2 isolates belonged to *Salmonella* serotypes Paratyphi B and Montevideo containing CTX-M-14. We detected 1 CTX-M-3 ESBL-producing serotype Kedougou. CTX-M-15 differs from CTX-M-3 only with respect to the amino acid change from glycine to aspartate at position 240 (31). To our knowledge, this is the first report of the CTX-M-3 type in *S.* Kedougou in Korea.

In conclusion, we detected a wide variety of serotypes and susceptibility results among *Salmonella* spp. isolated from clinical specimens in Korea. We verified that CTX-M-type ESBL-producing *Salmonella* strains are not uncommon. Continuous investigation of *Salmonella* serotypes and antimicrobial susceptibility testing are required for the management of patients and for ensuring public health.

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Conflict of interest None to declare.

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