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Prevalence and Mechanisms of Quinolone Resistance Among Selected Nontyphoid *Salmonella* Isolated from Food Animals and Humans in Korea

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Abstract

The aim of this study was to investigate the prevalence and mechanism of quinolone resistance among selected nontyphoid *Salmonella* (NTS) isolates. A total of 1279 NTS isolated from food animals ($n = 692$) and humans ($n = 587$) between 1995 and 2009 were investigated by serotyping, antimicrobial susceptibility testing, screening for plasmid-mediated quinolone resistance (PMQR) genes *qnr*, *aac(6′)-Ib-cr*, and *qepA* and mutations in the quinolone resistance-determining region (QRDR) of *gyrA* and *parC* by PCR, and DNA sequencing. Three hundred thirty (47.7%) of 692 animal isolates and 177 (30.2%) of 587 human isolates were resistant to nalidixic acid. Most animal (94.8%, 313/330) and human (99.4%, 176/177) NTS exhibited decreased ciprofloxacin susceptibility (minimum inhibitory concentration [MIC]: 0.125–2 mg/L). None of them carried *qnr* or *qepA* gene. However, *aac(6′)-Ib* was identified in six animal isolates, of which four carried *aac(6′)-Ib-cr* gene. Based on antimicrobial resistance profile, year of isolation, MIC for quinolones and fluoroquinolones, and isolation frequency of serotype, 114 animal and 83 human isolates were tested for QRDR mutations. All contained a single mutation within the QRDR of *gyrA* at either codon 87 or 83, and 41 of them contained an additional mutation in *parC*. The most prevalent mutation was Asp87-Tyr ($n = 107$), followed by Asp87-Gly ($n = 28$), Asp87-Asn ($n = 26$), Ser83-Tyr ($n = 22$), and Ser83-Phe ($n = 14$). Point mutations in *parC* were observed outside the QRDR, which included 40 isolates with Thr57-Ser substitution and 1 *Salmonella* Typhimurium with a novel Glu51-Lys substitution. In conclusion, a point mutation within the QRDR of *gyrA* was primarily responsible for quinolone resistance and reduced susceptibility to fluoroquinolones in NTS in Korea. To our knowledge, this is the first report of occurrence of *aac(6′)-Ib-cr* gene among NTS in Korea. The spread of NTS carrying *aac(6′)-Ib*-cr is of serious concern and should be carefully monitored.

Introduction

Salmonellosis constitutes an important public health problem in both developing and developed countries, including Korea (Weinberger and Keller, 2005). Approximately 1 million cases of Salmonellosis, causing 19,336 hospitalizations and nearly 400 deaths, occur each year in the United States (Scallan *et al.*, 2011). Nontyphoid *Salmonella* (NTS) strains are the second most common cause of food poisoning in humans in Korea (Lee *et al.*, 2001). Among these, serotypes Enteritidis and Typhimurium are the two most prevalent serotypes causing salmonellosis in humans as well as livestock (Cheong *et al.*, 2007; Lee *et al.*, 2009). Fluorinated quinolones are among the most extensively used antimicrobial agents for treating salmonellosis in both human and veterinary medicine because of their broad-spectrum antimicrobial activity (Angulo *et al.*, 2000; Giraud *et al.*, 2006). Unfortunately, extensive use of fluoroquinolones has led to increasing number of NTS isolates resistant to quinolones and reduced susceptibility to fluoroquinolones in many countries, including the Republic of Korea (Angulo *et al.*, 2000; Choi *et al.*, 2005; Bertrand *et al.*, 2006; Lee *et al.*, 2009). Reduced susceptibility to fluoroquinolones among the *Salmonella* species has been associated with clinical treatment failure in many parts of the world, causing significant therapeutic problems (Aarestrup *et al.*, 2003). Further, Molbak *et al.* (1999) reported quinolone-resistant *Salmonella* infections in humans that were difficult to treat and were associated with transmission from food animals.

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In *Salmonella* species, a single mutation in the quinolone resistance-determining region (QRDR) of *gyrA* can mediate resistance to quinolones such as nalidixic acid and reduced susceptibility to fluoroquinolones. Mutations in the *gyrB* or topoisomerase IV (*parC* and *parE*) genes are considered rare in salmonellae (Giraud *et al.*, 2006). In the recent years, plasmid-mediated quinolone resistance (PMQR) has emerged in *Salmonella* and in other Enterobacteriaceae, and the prevalence of PMQR is increasing (Strahilevitz *et al.*, 2009). Since it was first reported in 1998, three PMQR mechanisms have been described (Martínez-Martínez *et al.*, 1998). The first PMQR mechanism includes *qnr* genes, which encode proteins that directly protect DNA gyrase from quinolone inhibition (Strahilevitz *et al.*, 2009). The second mechanism consists of *aac(6′)-Ib-cr* gene, which encodes a new variant of the common aminoglycoside acetyltransferase that is capable of acetylating the piperazinyl substituent of some fluoroquinolones, thereby reducing their activities (Robicsek *et al.*, 2006). The third mechanism involves *qepA* gene, which encodes an efflux pump belonging to the major facilitator superfamily (Yamane *et al.*, 2007).

Korea is one of the areas with a high incidence of quinolone and/or fluoroquinolone resistance for Enterobacteriaceae (Kim *et al.*, 2009). Recently, PMQR determinants alone or in association with plasmid-mediated extended-spectrum β-lactamase (ESBL) or AmpC β-lactamase are increasingly being reported among clinical isolates of Enterobacteriaceae from humans (Tamang *et al.*, 2008; Kang *et al.*, 2009; Kim *et al.*, 2009). Similarly, resistance to nalidixic acid and ciprofloxacin in NTS has been constantly increasing in Korea in the recent years (Lee *et al.*, 2009). However, only few studies have investigated the molecular basis of quinolone resistance in NTS isolates from Korea, and among them, majority of the studies were reported in *Salmonella* of human origin (Choi *et al.*, 2005). Therefore, in the present study, the prevalence and mechanism of quinolone resistance including the three novel PMQR mechanisms (*qnr*, *aac(6′)-Ib-cr*, and *qepA* genes) were investigated among the nalidixic acid-resistant (Nal^r) NTS isolated from food animals and humans in Korea between 1995 and 2009.

Materials and Methods

Salmonella *strains*

A total of 692 NTS isolates recovered from various samples of food animals submitted to the National Veterinary Research and Quarantine Service, Korea, for diagnostic investigation between 1995 and 2009 or received from Korean Veterinary Antimicrobial Resistance Monitoring System (KVARMS) participating laboratories/centers between 2008 and 2009 were investigated. The samples or KVARMS isolates were received from all the nine provinces of Korea and were of pigs ($n=455$), pork ($n=5$), poultry ($n=54$), chicken meat ($n=171$), and cattle ($n=7$) origin. Around 38% of them were from healthy animals, 37% from sick animals, and 25% from meat. The material was selected to comprise only one isolate per animal. Similarly, 587 nonduplicate human isolates of NTS isolated during the study period and received from the Gwangju Research Institute of Public Health and Environment, Gwangju, Southwest Korea, were also included. These isolates were isolated from clinical samples of suspected patients with gastroenteritis. Identification and serotyping of *Salmonella* isolates was done as previously described (Lim *et al.*, 2009). Among them, NTS isolates resistant to nalidixic acid were further studied in detail to elucidate their mechanism of quinolone resistance.

Antimicrobial susceptibility testing

The antimicrobial susceptibility tests were performed by standard disc diffusion method according to the guidelines of Clinical Laboratory Standards Institute (CLSI) (CLSI, 2010) on Mueller Hinton agar (Becton-Dickinson, Sparks, MD) using commercial discs (Becton-Dickinson, Cockeysville, MD). The MICs of nalidixic acid (Sigma Chemical Co., St. Louis, MO), ciprofloxacin (Sigma Chemical Co.), and enrofloxacin (Sigma Chemical Co.) for all the Nal^r NTS isolates were determined according to the CLSI guidelines (CLSI, 2010). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.

Detection of PMQR determinants

Screening of six PMQR determinants was carried out by two sets of multiplex PCR amplification, one for *qnrA, qnrB, qnrC*, and *qnrS* and the other for *qnrD, aac(6′)-Ib*, and *qepA* genes as previously described (Park *et al.*, 2006; Jacoby *et al.*, 2009; Kim *et al.*, 2009). The primer set for amplification of *qnrD* allele was designed using the *qnrD* nucleotide sequence reported in the GenBank (GenBank accession number: EU692908). These primer sequences are listed in Table 1. All PCR products positive for *aac(6′)-Ib* were further analyzed by digestion with *Bts*CI (New England Biolabs, Ipswich, MA) to identify *aac(6′)-Ib-cr*, which lacks the *Bts*CI restriction site present in the wild-type gene as previously described (Park *et al.*, 2006). Clinical isolates that had been previously confirmed to carry *qnr, aac(6′)-Ib, aac(6′)-Ib-cr*, and *qepA* genes (Tamang *et al.*, 2008; Kang *et al.*, 2009) were used as positive controls.

PCR amplification and sequencing of gyrA *and* parC

PCR amplifications of the QRDR of *gyrA* and *parC* genes in selected isolates were carried out using previously described primers and protocols (Giraud *et al.*, 1999). The isolates were selected representing all serotype and on the basis of year of isolation, livestock farm, antimicrobial resistance profile, MIC for quinolones and fluoroquinolones, and isolation frequency of serotype. Altogether, 197 Nal^r NTS strains isolated from food animals ($n=114$) and humans ($n=83$) were investigated for QRDR mutations. Purified PCR products were sequenced with primers GyrA-1 and ParC-1 (Table 1) for *gyrA* and *parC* fragments, respectively, using an automated ABI Prism 3100 Analyzer (Applied Biosystems, Foster City, CA). The QRDR DNA sequences of *gyrA* and *parC* for each of the isolates tested were compared with the QRDR of *gyrA* and *parC* DNA sequences of *Salmonella enterica* serotype Typhimurium LT2 strain (GenBank accession number: AE006468). Analysis and comparison were performed with BLAST program at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/BLAST) and ExPASY proteomics tools (www.expasy.ch/tools/#similarity).

Results

Three hundred thirty (47.7%) of 692 animal NTS strains and 177 (30.2%) of 587 human NTS strains isolated were resistant

Table 1. Sequence of Oligonucleotide Primers Used in This Study

Primers	*Primer sequences (5′→3′)*	*Target gene*	*Amplicon size (bp)*	*Reference*
QnrA-F	ATTTCTCACGCCAGGATTTG	*qnrA*	574	Jacoby *et al.* (2009)
QnrA-R	TGCCAGGCACAGATCTTGAC			
QnrB-F	CGACCTKAGCGGCACTGAAT	*qnrB*	513	Jacoby *et al.* (2009)
QnrB-R	GAGCAACGAYGCCTGGTAGYTG			
QnrC-F	GGGTTGTACATTTATTGAATCG	*qnrC*	307	Kim *et al.* (2009)
QnrC-R	CACCTACCCATTTATTTTCA			
QnrD-F	TGTGATTTTTCAGGGGTTGA	*qnrD*	520	This study
QnrD-R	CCTGCTCTCCATCCAACTTC			
QnrS-F	ACTGCAAGTTCATTGAACAG	*qnrS*	431	Jacoby *et al.* (2009)
QnrS-R	GATCTAAACCGTCGAGTTCG			
AAC(6′)-Ib-F	TTGCGATGCTCTATGAGTGGCTA	*aac(6′)-Ib*	482	Park *et al.* (2006)
AAC(6′)-Ib-R	CTCGAATGCCTGGCGTGTTT			
QepA-F	AACTGCTTGAGCCCGTAGAT	*qepA*	596	Kim *et al.* (2009)
QepA-R	GTCTACGCCATGGACCTCAC			
GyrA-1	TGTCCGAGATGGCCTGAAGC	*gyrA*	470	Giraud *et al.* (1999)
GyrA-2	CGTTGATGACTTCCGTCAG			
ParC-1	ATGAGCGATATGGCAGAGCG	*parC*	413	Giraud *et al.* (1999)
ParC-2	TGACCGAGTTCGCTTAACAG			

to nalidixic acid. Altogether, 22 different *Salmonella* serotypes were identified among the 330 animal isolates and 177 human isolates that were resistant to nalidixic acid. The various Nal^r NTS serotypes identified in this study are listed in Table 2. Overall, the most frequent serotype was *Salmonella* Typhimurium (45.2%, 229 of 507) followed by *Salmonella* Enteritidis (30.9%, 157 of 507).

The MICs of nalidixic acid and selected fluoroquinolones were determined for 507 Nal^r NTS isolates derived from food animals ($n=330$) and humans ($n=177$). Three hundred eleven (94.2%) of the total 330 animal isolates and 175 (98.9%) of the 177 human isolates exhibited high-level nalidixic acid resistance (MIC $\geq$ 256 mg/L). The MIC_{50}, MIC_{90}, and MIC range for nalidixic acid, ciprofloxacin, and enrofloxacin for Nal^r NTS isolated from food animals and humans were compared and are shown in Table 3. According to the current CLSI criteria, none of the isolates of either animal or human origin was resistant to ciprofloxacin at breakpoint of $\geq$4 mg/L. However, three *S. enterica* serotype Gallinarum strains isolated from livestock showed intermediate ciprofloxacin resistance (MIC 2 mg/L). Similarly, 12.4% (41/330) of the animal isolates and none but two human isolates was resistant to enrofloxacin at the breakpoint MIC of $\geq$2 mg/L (CLSI, 2008).

Multiplex PCR screening using specific primers failed to detect *qnrA, qnrB, qnrC, qnrD*, and *qnrS* as well as *qepA* genes in any of the Nal^r NTS isolates of either animal or human origin. Nevertheless, *aac(6′)-Ib* determinant was identified in six animal isolates, of which four were *aac(6′)-Ib-cr*. Of the four *aac(6′)-Ib-cr*–positive isolates, two were *Salmonella* Typhimurium (one each isolated from diseased pig and cattle) and one each Derby (isolated from pork) and Essen (isolated from healthy cattle) serotypes. Further, all the four *aac(6′)-Ib-cr*–positive isolates exhibited high-level nalidixic acid resistance (MIC 512 mg/L) and decreased susceptibility to ciprofloxacin (MIC 1 mg/L) or enrofloxacin (MIC 1 mg/L).

All the 197 Nal^r NTS isolates sequenced contained a mutation that encoded single amino acid substitution within the QRDR of *gyrA* at either codon 87 (161 strains) or codon 83 (36 strains). Of the 103 isolates for which *parC* sequence analysis was successful, a single mutation was detected in *parC* of 41 isolates, in addition to a mutation in *gyrA* at Asp87 (20 strains) or Ser83 (21 strains). Point mutations within *parC* in these isolates were observed outside the QRDR, which included 40 isolates with Thr57 to serine substitution and 1 *Salmonella* Typhimurium with a novel Glu51 to lysine substitution.

Table 2. Serotype Distribution of Nalidixic Acid-Resistant Nontyphoid *Salmonella* Isolated from Food Animals ($N=330$) and Humans ($N=177$) in This Study

Salmonella *serotype*	*No. of NTS isolated from*		
	Animals (%)	*Humans (%)*	*Total (%)*
S. Typhimurium	201 (60.9)	28 (15.8)	229 (45.2)
S. Enteritidis	49 (14.8)	108 (61.0)	157 (30.9)
S. Gallinarium	8 (2.4)		8 (1.5)
S. Schwarzengrund	3 (0.9)		3 (0.6)
S. Afula	1 (0.3)		1 (0.2)
S. Blockley	1 (0.3)	3 (1.7)	4 (0.8)
S .Derby	1 (0.3)		1 (0.2)
S. Essen	2 (0.6)		2 (0.4)
S. Newport	2 (0.6)	3 (1.7)	5 (1.0)
S. Pullorum	1 (0.3)		1 (0.2)
S. Rissen	2 (0.6)		2 (0.4)
S. Tennessee	1 (0.3)		1 (0.2)
S. Bardo		9 (5.1)	9 (1.8)
S. Champaign		1 (0.6)	1 (0.2)
S. Haardt		1 (0.6)	1 (0.2)
S. Hadar		3 (1.7)	3 (0.6)
S. Heidelberg		1 (0.6)	1 (0.2)
S. Mbandaka		1 (0.6)	1 (0.2)
S. Montevideo		1 (0.6)	1 (0.2)
S. Panama		1 (0.6)	1 (0.2)
S. Stanley		3 (1.7)	3 (0.6)
S.. Virchow		2 (1.1)	2 (0.4)
Not identified	58 (17.6)	12 (6.8)	70 (13.8)
Total	330	177	507

NTS, nontyphoid *Salmonella*.

Table 3. Minimum Inhibitory Concentrations of Nalidixic Acid, Ciprofloxacin, and Enrofloxacin for Nalidixic Acid–Resistant Nontyphoid *Salmonella* of Animal and Human origins

	MIC (mg/L)					
	Animal isolates (n = *330)*			*Human isolates* (n = *177)*		
Salmonella *serotype and antimicrobial agent*	*Range*	MIC_{50}	MIC_{90}	*Range*	MIC_{50}	MIC_{90}
S. Enteritidis (*n* = 157)						
Nalidixic acid	256–1024	512	1024	128–1024	256	512
Ciprofloxacin	0.06–1	0.125	0.25	0.125–0.25	0.125	0.25
Enrofloxacin	0.25–2	0.5	0.5	0.25–1	0.5	0.5
S. Typhimurium (*n* = 229)						
Nalidixic acid	32–1024	1024	>1024	256–1024	512	1024
Ciprofloxacin	0.03–1	0.25	0.5	0.06–0.5	0.5	0.5
Enrofloxacin	0.06–4	1	2	0.25–2	1	1
Other serotypes (*n* = 121)[a]						
Nalidixic acid	32–1024	512	>1024	256–1024	512	1024
Ciprofloxacin	0.03–2	0.25	1	0.125–0.5	0.125	0.25
Enrofloxacin	0.125–2	0.5	1	0.5–1	0.5	0.5

[a]Other serotypes included nontypoid *Salmonella* other than Typhimurium and Enteritidis as well as unidentified serotypes mentioned in Table 2.

MIC, minimum inhibitory concentration; MIC_{50}, MIC at which 50% of the isolates were inhibited; MIC_{90}, MIC at which 90% of the isolates were inhibited.

Further, all the four *aac(6′)-Ib-cr*–positive isolates had single mutation in the QRDR of *gyrA* at codon 87 (Asp87-Gly substitution).

The comparisons of point mutation patterns and MICs for nalidixic acid, ciprofloxacin, or enrofloxacin between the Nal^r NTS isolates of animal and human origins tested in this study are separately shown for Typhimurium and Enteritidis (Table 4) and for serovars other than Typhimurium and Enteritidis (Table 5). Overall, substitution within the *gyrA* at codon 87 or 83 conferred similar level of nalidixic acid resistance (MIC 128–1024 mg/L vs. 256–1024 mg/L) or ciprofloxacin (MIC 0.06–0.5 mg/L vs. 0.125–2 mg/L) irrespective of isolate origin. Further, the number of isolates with amino acid substitution at codon Asp87 was higher among the human (71/83, 85.5%) isolates compared with animal (90/114, 78.9%) isolates.

Discussion

In the present study, the prevalence and molecular basis of quinolone resistance among the NTS strains isolated from food animals (*n* = 692) and humans (*n* = 587) between 1995 and 2009 were investigated. During the study period, 330 (47.7%) of 692 animal NTS isolates and 177 (30.2%) of 587 human NTS isolates examined were resistant to nalidixic acid. In contrast, Hwang *et al.* (2010) found 13.9% of the 36 human isolates examined and 68.7% of the 151 livestock isolates resistant to nalidixic acid. Similarly, Choi *et al.* (2005) reported that 1 (1.8%) of the 55 human NTS strains collected during 1995–1996 was resistant to nalidixic acid compared with 21.8% (45 of 206) of the strains collected during 2000–2002, indicating the increasing trend of nalidixic acid resistance among the NTS isolates in Korea. Also, Lee *et al.* (2009) recently reported similar incidence of nalidixic acid (36.5%) resistance among the human NTS isolates from Korea. However, they reported higher (13.5%) prevalence of ciprofloxacin resistance among them, in contrast to our results in which none of the Nal^r NTS isolates was resistant to ciprofloxacin.

In this study, most of the animal (94.8%, 313 of 330) and human (99.4%, 176 of 177) NTS isolates resistant to nalidixic acid showed decreased ciprofloxacin susceptibility (MIC 0.125–2 mg/L). Recently, a multinational study revealed different rates of reduced ciprofloxacin susceptibility among the human NTS isolates from Taiwan (48.1%), Thailand (46.2%), Korea (36.5%), Singapore (24.5%), Philippines (14.9%), Sri Lanka (8.0%), and Hong Kong (7.1%) (Lee *et al.*, 2009). In addition, we recently found that 20 (1.6%) of 1279 NTS isolates examined were resistant to extended-spectrum cephalosporins because of production of CTX-M type ESBL among the food animals and humans in Korea (unpublished data). Fluoroquinolones are important alternative antimicrobials for treatment of invasive salmonellosis in adults, especially for NTS producing ESBL, but there are several reports of treatment failures for *Salmonella* infections caused by strains with decreased susceptibility to fluoroquinolones (Aarestrup *et al.*, 2003). Within this context, it is of great concern to detect the decreased susceptibility to fluoroquinolones among the NTS from both animal and human origins, which would further exacerbate the complexity of the problem. Thus, more prudent use of antimicrobial agents is recommended in both human and veterinary medicine.

The *aac(6′)-Ib-cr* genes were the only PMQR determinant found among the Nal^r NTS isolates in this study. In contrast, Kim *et al.* (2009) reported increasing prevalence and diversity of PMQR determinants among human clinical Enterobacteriaceae isolates in Korea. The absence of *qnr* or *qepA* and low prevalence of *aac(6′)-Ib-cr* genes may be related to the study design of this work, as nalidixic acid–susceptible NTS isolates were excluded from screening (Gunell *et al.*, 2009). Since the *aac(6′)-Ib-cr* determinant was originally reported in 2003 in a clinical isolate of *E. coli* collected in Shanghai, China, it has appeared in several countries of Asia, North America, and Europe among various enterobacteria (Strahilevitz *et al.*, 2009). In Korea, the gene has been previously detected in human Enterobacteriaceae isolates (Kang *et al.*, 2009; Kim

Table 4. Correlation Between Nalidixic Acid, Ciprofloxacin, or Enrofloxacin Minimum Inhibitory Concentrations and *gyrA* or *parC* Mutations Among Representative Nalidixic Acid–Resistant *Salmonella* spp.

Point mutations within the		*Animal isolates*				*Human isolates*			
			MIC range (MIC_{50})				*MIC range (MIC_{50})*		
gyrA	parC	*No.*	*NAL*	*CIP*	*ENR*	*No.*	*NAL*	*CIP*	*ENR*
Salmonella Enteritidis ($n=52$)									
Ser83→Tyr		1	1024	0.25	0.5				
Ser83→Phe						3	1024	0.25	0.5–1
Asp87→Asn		8	256–512 (512)	0.12–0.25 (0.12)	0.25–0.5 (0.5)	9	256–1024 (512)	0.12–0.25 (0.12)	0.5
Asp87→Gly		6	256–512 (256)	0.12	0.25–0.5 (0.25)	8	128–512 (256)	0.12–0.25 (0.12)	0.25–0.5 (0.5)
Asp87→Tyr						5	512–1024 (512)	0.12–0.25 (0.12)	0.5
Ser83→Phe	Thr57→Ser	1	512	0.5	1				
Ser83→Tyr	Thr57→Ser	10	512->1024 (1024)	0.25–0.5 (0.25)	0.5–1 (0.5)	1	1024	0.25	0.5
Salmonella Typhimurium ($n=97$)									
Ser83→Phe		3	>1024	0.5–1	2				
Ser83→Tyr		3	512–1024	0.25	0.5				
Asp87→Tyr		55	256–1024 (1024)	0.12–1 (0.5)	0.25–2 (1)	21	256–1024 (512)	0.12–0.5 (0.5)	0.5–2 (1)
Asp87→Gly		8	256–1024 (256)	0.12–1 (0.5)	0.25–4 (0.5)	1	256	0.6	0.25
Asp87→Asn		4	512–1024	0.12–1	0.5–1	1	512	0.12	0.5
Asp87→Tyr	Glu51→Lys	1	512	0.5	1				

NAL, nalidixic acid; CIP, ciprofloxacin; ENR, enrofloxacin; No., number of isolates.

Table 5. Distribution of *gyrA* and *parC* Mutations Among the Nontyphoid *Salmonella* Other Than Typhimurium and Enteritidis Serotypes

Origin and Salmonella *serotype*	*No. of isolates*	*Point mutation within the*		*MIC range in mg/L (MIC_{50})*		
		gyrA	parC	*NAL*	*CIP*	*ENR*
Animal *Salmonella* isolates						
S. Afula	1	Asp87→Tyr	wild type	>1024	0.5	1
S. Blockley	1	Ser83→Tyr	Thr57→Ser	1024	0.25	0.5
S. Derby	1	Asp87→Gly	wild type	512	1	1
S. Essen	1	Asp87→Gly	wild type	512	1	1
S. Essen	1	Asp87→Tyr	ND	512	0.125	0.5
S. Gallinarium	2	Ser83→Phe	ND	1024, >1024	1, 2	2
S. Gallinarium	1	Ser83→Tyr	ND	>1024	2	2
S. Newport	2	Asp87→Tyr	Thr57→Ser	256	0.125	0.5
S. Rissen	1	Ser83→Tyr	wild type	512	0.25	0.5
S. Schwarzengrund	2	Asp87→Tyr	wild type	>1024	0.5	1
S. Tennessee	1	Ser83→Phe	Thr57→Ser	512	0.125	0.5
Human *Salmonella* isolates						
S. Bardo	9	Asp87→Tyr	Thr57→Ser	256	0.125	0.5
S. Blockley	3	Ser83→Tyr	Thr57→Ser	1024	0.25	0.5
S. Champaign	1	Ser83→Phe	Thr57→Ser	512	0.25	0.5
S. Haardt	1	Ser83→Tyr	Thr57→Ser	1024	0.25	1
S. Hadar	3	Asp87→Tyr	Thr57→Ser	512	0.125	0.5
S. Heidelberg	1	Asp87→Asn	Thr57→Ser	256	0.125	0.5
S. Mbandaka	1	Asp87→Gly	wild type	256	0.125	0.5
S. Montevideo	1	Ser83→Phe	Thr57→Ser	1024	0.25	0.5
S. Newport	3	Asp87→Tyr	Thr57→Ser	256	0.125	0.5
S. Panama	1	Asp87→Gly	Thr57→Ser	512	0.25	1
S. Stanley	2	Asp87→Tyr	wild type	512	0.25, 0.5	1
S. Virchow	1	Asp87→Tyr	wild type	256	0.125	0.5
S. Virchow	1	Ser83→Phe	wild type	512	0.5	0.5
S. spp.	1	Asp87→Tyr	wild type	512	0.125	0.5
S. spp.	3	Asp87→Asn	wild type	512	0.125	0.5
S. spp.	1	Asp87→Gly	wild type	256	0.125	0.5
S. spp.	1	Ser83→Phe	Thr57→Ser	512	0.25	0.5

ND, not determined because of repeated sequencing failure.

et al., 2009), but to our knowledge it has not been reported from zoonotic bacteria. In this study, the *aac(6′)-Ib-cr* gene was detected among four Nal^r NTS isolates from livestock for the first time in Korea. The low prevalence (0.7%) of *aac(6′)-Ib-cr* observed in this study contrasts with a previous Chinese report in which 35% (18/35) of *S. enterica* isolates with reduced susceptibility to ciprofloxacin-harbored *aac(6′)-Ib-cr* gene (Cui *et al.*, 2009). Further, in China, the *aac(6′)-Ib-cr* gene has been detected in both human and veterinary Enterobacteriaceae isolates, with high frequency among the clinical *E. coli* isolates (Strahilevitz *et al.*, 2009). On the other hand, our findings are consistent with previous data obtained in the United States in which 0.4% (1/283) of human NTS isolates exhibiting reduced susceptibility to ciprofloxacin (≥0.25 mg/L) harbored *aac(6′)-Ib-cr* gene (Sjolund-Karlsson *et al.*, 2009). Nevertheless, emergence of *aac(6′)-Ib-cr* gene among NTS is of serious concern, because the decreased susceptibility to fluoroquinolones owing to *aac(6′)-Ib-cr* genes being plasmid mediated is mobilizable and may spread by horizontal transmission to other susceptible isolates in the community.

For QRDR mutation analysis, the isolates were selected based on history of isolates and the experimental data as described earlier to exclude same or similar clones as much as possible. Overall, the most prevalent mutation in the QRDR of *gyrA* among the isolates tested was Asp87-Tyr ($n=107$), followed by Asp87-Gly ($n=28$), Asp87-Asn ($n=26$), Ser83-Tyr ($n=22$), and Ser83-Phe ($n=14$). These results contrast with those reported by Liebana *et al.* (2002), in which the most prevalent mutation among the 100 veterinary isolates of *S. enterica* tested from farm animals in England and Wales was Asp87-Asn ($n=42$), followed by Ser83-Phe ($n=38$), Ser83-Tyr ($n=12$), Asp87-Tyr ($n=4$), and Asp87-Gly ($n=3$). This discrepancy could be due to geographical variation in the origin of the isolates, variation in the management practices, for instance, variation in the use of a particular fluoroquinolone for selection in United Kingdom and Korea (KFDA, 2009), or adaption of isolates with a particular mutation to local environment. Our results also differ from those of Hwang *et al.* (2010), in which a Ser83-Phe mutation was detected in 64 (76.2%) of 84 NTS isolates tested from humans and livestock in Korea. In addition, they found nine double mutations within the QRDR of *gyrA*, that is, Ser83-Phe and Asp87-Glu ($n=6$) or Tyr ($n=2$) or Arg ($n=6$), but did not detect any mutation in *parC*, in contrast to our findings. This discrepancy may be due to differences in selection criteria, that is, inclusion of isolates resistant to both nalidixic acid and ciprofloxacin for mutation analysis in their study, in contrast to ours.

It is interesting to note that the Thr57 to serine substitution in this study was observed in various serotypes but was never identified among the *Salmonella* Typhimurium strains. Our

findings are in agreement with previous studies that suggest that mutations in *parC* of salmonellae are not as frequent as in *E. coli* and they do not play an important role in quinolone resistance or they may only be required to achieve high-level resistance, which occurs infrequently among *Salmonella* species (Hopkins *et al.*, 2005). Similarly, Gunell *et al.* (2009) detected a Thr57-Ser substitution in *par*C among the NTS strains showing nonclassical quinolone resistance, that is, showing susceptibility or low-level resistance to nalidixic acid but decreased susceptibility to ciprofloxacin. However, they also detected the same Thr57-Ser substitution in all the quinolone-susceptible control strains except *Salmonella* Typhimurium strains and suggested that this substitution is likely to be a polymorphism common in serotypes other than Typhimurium (Gunell *et al.*, 2009). Moreover, Thr57-Ser substitution has also been reported in *E. coli*, but its role in quinolone resistance is unknown (Hopkins *et al.*, 2005). In addition, one *Salmonella* Typhimurium in this study contained a novel Glu51-Lys substitution outside the QRDR of *parC*. Thus, to our knowledge, this study also reports Glu51-Lys substitution in *parC* for the first time, although its role in quinolone resistance has to be yet determined. Further, there was no NTS strain with a *parC* mutation alone, which is in agreement with a previous study done in Korea (Kim *et al.*, 2009).

In conclusion, a point mutation within the QRDR of *gyrA* was primarily responsible for resistance to quinolones and reduced susceptibility to fluoroquinolones among the Nal^r NTS strains isolated from food animals and humans in Korea. To the best of our knowledge, this study is the first report of occurrence of *aac(6′)-Ib-cr* among NTS isolates in Korea. This is also the first time *aac(6′)-Ib-cr* gene was identified among serotypes Derby and Essen. Isolation of *aac(6′)-Ib-cr* from food animals is of serious concern as it could be transmitted to humans via the food chain. As the impact of PMQR is immense, surveillance and careful monitoring of PMQR using a monitoring system as sensitive as possible to detect PMQR determinants among the relevant bacterial strains from food animals and humans should be continuously performed.

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Disclosure Statement

No competing financial interests exist.

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