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Prevalence of Antimicrobial Resistance and Transfer of Tetracycline Resistance Genes in *Escherichia coli* Isolates from Beef Cattle

Seung Won Shin,[a] Min Kyoung Shin,[a] Myunghwan Jung,[a] Kuastros Mekonnen Belaynehe,[a] Han Sang Yoo[a,b]

Department of Infectious Diseases, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea[a]; Institute of Green Bio Science and Technology, Seoul National University, Pyeungchang, Republic of Korea[b]

The aim of this study was to investigate the prevalence and transferability of resistance in tetracycline-resistant *Escherichia coli* isolates recovered from beef cattle in South Korea. A total of 155 *E. coli* isolates were collected from feces in South Korea, and 146 were confirmed to be resistant to tetracycline. The tetracycline resistance gene *tet*(A) (46.5%) was the most prevalent, followed by *tet*(B) (45.1%) and *tet*(C) (5.8%). Strains carrying *tet*(A) plus *tet*(B) and *tet*(B) plus *tet*(C) were detected in two isolates each. In terms of phylogenetic grouping, 101 (65.2%) isolates were classified as phylogenetic group B1, followed in decreasing order by D (17.4%), A (14.2%), and B2 (3.2%). Ninety-one (62.3%) isolates were determined to be multidrug resistant by the disk diffusion method. MIC testing using the principal tetracyclines, namely, tetracycline, chlortetracycline, oxytetracycline, doxycycline, and minocycline, revealed that isolates carrying *tet*(B) had higher MIC values than isolates carrying *tet*(A). Conjugation assays showed that 121 (82.9%) isolates could transfer a tetracycline resistance gene to a recipient via the IncFIB replicon (65.1%). This study suggests that the high prevalence of tetracycline-resistant *E. coli* isolates in beef cattle is due to the transferability of tetracycline resistance genes between *E. coli* populations which have survived the selective pressure caused by the use of antimicrobial agents.

Antimicrobial resistance in humans and animals is considered a problem worldwide. Resistance to antimicrobial agents impedes the effective prevention and treatment of infectious disease, and thus, many governments have planned and implemented national programs for monitoring resistance in humans and animals (1–4). Surveillance data show that the inadequate selection and extensive use of antimicrobials result in the emergence and spread of resistant bacteria, particularly multidrug-resistant bacteria, and increase resistance to newer compounds, such as tetracycline-class antimicrobials (5).

The tetracyclines are one of the most widely used classes of antimicrobial agents in human and veterinary medicine because they have several advantages, which include a broad spectrum of activity, low cost, oral administration, and few side effects (6). After chlortetracycline was introduced into clinical medicine in 1948, many derivatives, such as tetracycline, oxytetracycline, doxycycline, and minocycline, were developed, and today, these derivatives are widely used to treat disease and as growth promoters in the food animal industry. However, the widespread and indiscriminate use of tetracyclines has subjected bacterial populations to selection pressure and increased the prevalence of tetracycline resistance (6, 7).

Tetracycline resistance is generally caused by the acquisition of a tetracycline resistance (*tet*) gene, as these genes are associated with primary resistance mechanisms, which involve active efflux pumps, ribosomal protection, and enzyme inactivation (8). To date, more than 40 different resistance genes have been identified (7). In Gram-negative bacteria, the most important mechanism involves the efflux pump system, which is encoded by tetracycline resistance genes *tet*(A), *tet*(B), *tet*(C), *tet*(D), and tet(G) (6).

Although most *Escherichia coli* strains are considered harmless commensal bacteria of the gastrointestinal tracts of humans and animals, pathogenic strains that can cause several intestinal and extraintestinal infections exist. Surveillance of *E. coli* isolates is also considered to provide an excellent means of monitoring antimicrobial resistance in food and the environment because of the wide range of hosts of *E. coli* and because it easily acquires resistance (9). Thus, the degrees of resistance in commensal and pathogenic *E. coli* strains provide indicators of antimicrobial selection in their environment, and tetracycline-resistant *E. coli* strains could be used for surveillance for tetracycline resistance in humans and animals. Studies have reported tetracycline-resistant *E. coli* strains in various environments (8, 10–13), but only a small number of studies have been conducted in animals.

The aim of this study was to determine the prevalence of tetracycline-resistant *E. coli* isolates in South Korean beef cattle and determine the phenotypes and genotypes of these isolates with a view toward investigating the transferabilities of tetracycline resistance determinants between *E. coli* isolates.

MATERIALS AND METHODS

Bacterial strains. In total, 290 *E. coli* strains were isolated from feces collected from clinically healthy beef cattle during 2011 and 2012 (14). *E. coli* isolates that showed resistance and intermediate resistance to tetracycline were obtained by culture on MacConkey agar plates containing tetracycline at a concentration of 8 μg/ml (the MIC of tetracycline for *E. coli* indicating tetracycline resistance is ≥16 μg/ml) (15). As a result, 155 *E. coli* isolates were selected for analysis. *E. coli* ATCC 25922 and *Pseudomo-*

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Address correspondence to Han Sang Yoo, yoohs@snu.ac.kr.

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TABLE 1 Primers used in this study

Target gene	Primer	Sequence	Amplicon size (bp)	GenBank accession no.	Reference
tet(A)	TetA-F	GCTACATCCTGCTTGCCTTC	210	X61367	16
	TetA-R	CATAGATCGCCGTGAAGAGG			
tet(B)	TetB-F	TTGGTTAGGGGCAAGTTTTG	659	J01830	16
	TetB-R	GTAATGGGCCAATAACACCG			
tet(C)	TetC-F	CTTGAGAGCCTTCAACCCAG	418	J01749	16
	TetC-R	ATGGTCGTCATCTACCTGCC			
tet(D)	TetD-F	AAACCATTACGGCATTCTGC	787	L06798	16
	TetD-R	GACCGGATACACCATCCATC			
tet(G)	TetG-F	GCTCGGTGGTATCTCTGCTC	468	S52437	16
	TetG-R	AGCAACAGAATCGGGAACAC			
chuA	ChuA-F	GACGAACCAACGGTCAGGAT	279	HQ284193	17
	ChuA-R	TGCCGCCAGTACCAAAGACA			
yjaA	Yja-F	TGAAGTGTCAGGAGACGCTG	211	HQ284194	17
	Yja-R	ATGGAGAATGCGTTCCTCAAC			
TspE4C2	TspE4C2-F	GAGTAATGTCGGGGCATTCA	152	HQ284195	17
	TspE4C2-R	CGCGCCAACAAAGTATTACG			

nas aeruginosa ATCC 27853 were used as quality control organisms in antimicrobial susceptibility tests and MIC tests.

Antimicrobial susceptibility testing. The *E. coli* isolates were tested for susceptibility by the disk diffusion method in accordance with the guidelines issued by the Clinical and Laboratory Standards Institute (CLSI) (15). The antimicrobial disks (Oxoid, Basingstoke, United Kingdom) used in this study included ampicillin (10 μg), streptomycin (25 μg), gentamicin (10 μg), chloramphenicol (C, 30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), and tetracycline (30 μg) disks.

Detection of tetracycline resistance genes. All 155 tetracycline-resistant isolates were tested by multiplex PCR for the presence of the *tet*(A), *tet*(B), *tet*(C), *tet*(D), and *tet*(G) genes, as described previously (16). Bacterial DNA for PCR was obtained by suspending colonies of bacteria grown on tryptic soy broth (TSB) in 500 μl of ultrapure water and boiling at 100°C for 10 min. The oligonucleotide primers used in this study are shown in Table 1. The PCRs included a negative and a positive control, and reactions were run in duplicate to confirm the results. Sequence alignments were performed by use of a search of the GenBank database via the National Center for Biotechnology Information website with the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST).

Phylogenetic grouping. The phylogenetic tree described by Clermont et al. was used to classify all *E. coli* isolates into one of four phylogenetic groups, that is, groups A, B1, B2, and D (17). Triplex PCR was used to determine the phylogenetic groupings by targeting two genes (*chuA* and *yjaA*) and an anonymous DNA fragment (TspE4.C2) (17). The result of phylogenetic typing was used to compare the pattern of antimicrobial resistance and the *tet* gene distributions among the *E. coli* isolates tested in this study.

Determination of MICs of principal tetracyclines. To investigate the phenotypic characteristics of tetracycline-resistant isolates, the MIC values of the principal tetracycline antibiotics, tetracycline, chlortetracycline, oxytetracycline, doxycycline, and minocycline, were determined using the broth dilution method (15). All antimicrobials used in this study were tested in 2-fold dilutions from 1 to 2,048 μg/ml. MIC tests were conducted in triplicate for each sample.

Conjugation assay and plasmid replicon typing. To determine the transferability of tetracycline resistance, conjugation assays were conducted on tetracycline-resistant isolates using the broth mating method. *E. coli* J53 Az^r was used as the recipient strain, and tetracycline-resistant isolates served as the donors (18). Eight-hour cultures of recipient and donor cells grown in Luria-Bertani (LB) broth at 37°C were mixed with each other at a ratio of 1:1, and the mixture was incubated for 20 h. To identify resistance carried by plasmids, 100-μl aliquots of these mixtures were spread onto tryptic soy agar (TSA) plates containing tetracycline (8 μg/ml) and sodium azide (200 μg/ml) and incubated at 37°C for 20 h. PCR was used to confirm that the transconjugants carried the *tet* gene of their donors. Multiplex PCR was conducted on all donors and transconjugants to type the plasmid replicons, as described previously (19).

TABLE 2 Resistances of 146 tetracycline-resistant *E. coli* isolates in different phylogenetic groups to other antimicrobials

	No. (%) of strains showing antimicrobial resistance[a]						
Phylogenetic group	AMP	GN	STR	C	SXT	NA	CIP
Total	66 (45.3)	8 (5.5)	120 (82.2)	42 (28.8)	37 (25.3)	48 (32.8)	15 (10.3)
A	14 (9.6)	3 (2.1)	15 (10.3)	7 (4.8)	7 (4.8)	6 (4.1)	5 (3.4)
B1	43 (29.5)	4 (2.7)	75 (51.4)	33 (22.6)	26 (17.8)	24 (16.4)	10 (6.8)
B2			5 (3.4)				
D	9 (6.2)	1 (0.7)	25 (17.1)	2 (1.4)	4 (2.7)	18 (12.3)	

[a] AMP, ampicillin; GN, gentamicin; STR, streptomycin; C, chloramphenicol; SXT, sulfamethoxazole-trimethoprim; NA, nalidixic acid; CIP, ciprofloxacin.

TABLE 3 Distributions of tetracycline resistance genes in *E. coli* isolates in the four identified phylogenetic groups

Phylogenetic group	No. (%) isolates with the following tetracycline resistance gene(s):					
	Total	*tet*(A)	*tet*(B)	*tet*(C)	*tet*(A) plus *tet*(B)	*tet*(B) plus *tet*(C)
Total	155 (100)	72 (46.5)	70 (45.1)	9 (5.8)	2 (1.3)	2 (1.3)
A	22 (14.2)	6 (3.9)	9 (5.8)	5 (3.2)		2 (1.3)
B1	101 (65.2)	41 (26.5)	54 (34.8)	4 (2.6)	2 (1.3)	
B2	5 (3.2)		5 (3.2)			
D	27 (17.4)	25 (16.1)	2 (1.3)			

Statistical analysis. Data were analyzed using IBM SPSS Statistics, version 21, software (SPSS Inc., Chicago, IL). The distributions of the *tet* genes were analyzed using the chi-square test. To compare the different *tet* genes and MIC values, survival analysis was carried out using the Kaplan-Meier method, and the curves so obtained were compared using the log-rank test. *P* values of <0.05 were considered statistically significant.

RESULTS

Antimicrobial resistance profile. Among 155 *E. coli* isolates, 146 (94.2%) isolates were resistant to tetracycline, as determined using the disk diffusion method. The tetracycline-resistant isolates detected in this study showed concurrent resistance to streptomycin (82.2%), ampicillin (45.3%), nalidixic acid (32.8%), chloramphenicol (28.8%), trimethoprim-sulfamethoxazole (25.3%), ciprofloxacin (10.3%), and gentamicin (5.5%) (Table 2). Of these 146 tetracycline-resistant *E. coli* isolates, 91 (62.3%) were multidrug resistant. The most frequent combination of multidrug resistance was tetracycline-streptomycin-ampicillin, which was detected in 20 (13.7%) isolates. Five (3.4%) isolates in phylogenetic group B2 showed resistance to streptomycin; resistance to no other antimicrobial was found (Table 2).

Phylogenetic classification. Of the 155 *E. coli* isolates, 101 (65.2%) isolates were classified as phylogenetic group B1; 27 (17.4%) were classified as group D, which is associated with pathogenic bacteria; 22 (14.2%) were classified as group A; and 5 (3.2%) were classified as group B2, the phylogenetic lineage associated with virulent extraintestinal strains (Table 3).

Prevalence of tetracycline resistance determinants. All 155 isolates carried at least one of the *tet* genes examined. PCR detection of single *tet* determinants showed that 142 (91.6%) isolates carried *tet*(A) or *tet*(B) only: 72 (46.5%) harbored *tet*(A) only, and 70 (45.1%) isolates harbored *tet*(B) only. *tet*(C) was detected in 11 (7.1%) isolates. Four (2.6%) isolates contained two *tet* genes:

TABLE 4 MICs of tetracycline antimicrobials for *E. coli* isolates with different tetracycline resistance genes

Antimicrobial	Gene profile	No. of strains	Avg MIC (μg/ml)	No. of isolates for which the MIC (μg/ml) was:											
				1	2	4	8	16	32	64	128	256	512	1,024	2,048
Tetracycline	*tet*(A)	72	200.0							3	27	42			
	tet(B)	70	245.9							1	4	65			
	tet(C)	9	23.1					5	4						
	tet(A) and *tet*(B)	2	256.0									2			
	tet(B) and *tet*(C)	2	256.0									2			
Chlortetracycline	*tet*(A)	72	1,365.3											48	24
	tet(B)	70	1,682.3											25	45
	tet(C)	9	170.7								6	3			
	tet(A) and *tet*(B)	2	1,536											1	1
	tet(B) and *tet*(C)	2	1,536											1	1
Oxytetracycline	*tet*(A)	72	384.0									36	36		
	tet(B)	70	479.1									9	61		
	tet(C)	9	49.8						4	5					
	tet(A) and *tet*(B)	2	512.0										2		
	tet(B) and *tet*(C)	2	384.0									1	1		
Doxycycline	*tet*(A)	72	17.3		1		7	54	10						
	tet(B)	70	42.5					4	41	25					
	tet(C)	9	5.8		6	2			1						
	tet(A) and *tet*(B)	2	32.0						2						
	tet(B) and *tet*(C)	2	32.0						2						
Minocycline	*tet*(A)	72	3.3		39	28	4	1							
	tet(B)	70	13.7		1	6	29	24	10						
	tet(C)	9	1.1	8	1										
	tet(A) and *tet*(B)	2	6.0			1	1								
	tet(B) and *tet*(C)	2	8.0				2								

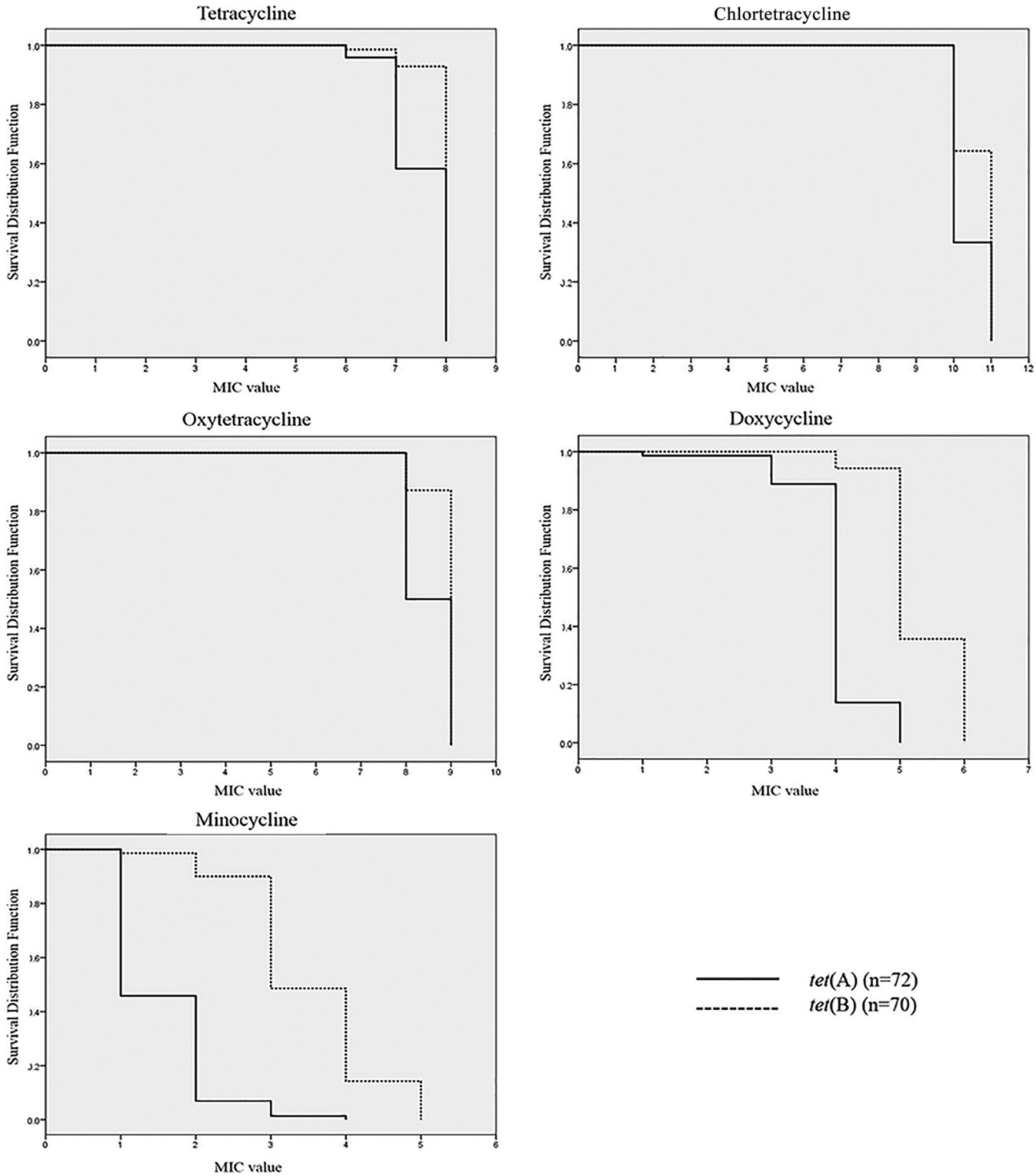

FIG 1 Survival curves (obtained by the Kaplan-Meier method) of *E. coli* isolates harboring *tet*(A) or *tet*(B) for resistance to the tetracycline family of antimicrobials. The survival rates of the *E. coli* isolates are compared with the MIC values of the five tetracyclines (tetracycline, chlortetracycline, oxytetracycline, doxycycline, and minocycline). Full and dotted lines, survival rates of *tet*(A)-carrying and *tet*(B)-carrying strains, respectively. [a], the MIC values of the five tetracyclines were log transformed (base 2).

TABLE 5 Characterization and transferability of resistance in *E. coli* isolates classified into phylogenetic groups B2 and D

Strain	Phylogenetic group	Resistance phenotype[a]	Resistance gene	MIC[b] (μg/ml)					Plasmid replicon type[c]	Transconjugants		
				TET	OXY	CTC	DOX	MIN		Transferability	*tet* gene	Plasmid replicon type[c]
60	B2	TE, S	*tet*(B)	256	512	1,024	32	8	FIB, Y, I1, Frep	+	*tet*(B)	FIB, I1, Frep
61	B2	TE, S	*tet*(B)	256	512	1,024	32	16	FIB, Y, I1, Frep	−		
62	B2	TE, S	*tet*(B)	256	512	1,024	32	8	FIB, Y, I1	+	*tet*(B)	FIB, I1
64	B2	TE, S	*tet*(B)	256	512	1,024	32	8	FIB, Y, I1	+	*tet*(B)	FIB, I1
68	B2	TE, S	*tet*(A)	256	512	1,024	32	8	FIB, Y, I1, Frep	−		
90	D	TE, S, AMP	*tet*(A)	256	512	2,048	32	4	P, FIA, FIB, Frep	+	*tet*(A)	FIA, FIB, Frep
106	D	TE, S	*tet*(B)	256	512	2,048	32	16	Frep	+	*tet*(B)	Frep
123	D	TE, NA	*tet*(A)	256	512	1,024	16	2	FIB, Frep	+	*tet*(A)	FIB, Frep
124	D	TE, GN, SXT, C, S, NA, AMP	*tet*(B)	256	512	2,048	64	8	FIA, FIB, Frep	+	*tet*(B)	FIB, Frep
127	D	TE, S, AMP	*tet*(A)	256	512	2,048	32	4	P, I1	+	*tet*(A)	I1
128	D	TE, AMP	*tet*(A)	256	512	2,048	16	4	FIB, I1	+	*tet*(A)	Frep, I1
133	D	TE, S, NA	*tet*(A)	256	512	1,024	2	2	FIB, Frep	+	*tet*(A)	Frep
135	D	TE, S, NA	*tet*(A)	256	512	1,024	8	2	FIB	+	*tet*(A)	FIB
136	D	TE, S, NA	*tet*(A)	256	512	1,024	16	2	FIB, Frep	+	*tet*(A)	FIB
147	D	TE, S, NA	*tet*(A)	256	512	1,024	16	2	FIB	+	*tet*(A)	FIB
148	D	TE, S, NA	*tet*(A)	256	512	1,024	8	2	FIB, Frep	+	*tet*(A)	FIB, Frep
152	D	TE, S, NA	*tet*(A)	256	512	1,024	16	2	FIB, Frep	+	*tet*(A)	FIB, Frep
153	D	TE, S, NA	*tet*(A)	256	512	1,024	16	2	FIB, Frep	+	*tet*(A)	FIB, Frep
156	D	TE, S, NA	*tet*(A)	256	512	1,024	16	2	FIB, Frep	+	*tet*(A)	FIB
162	D	TE, S, NA	*tet*(A)	128	256	1,024	32	2	FIB, Frep	+	*tet*(A)	FIB, Frep
163	D	TE, S, NA	*tet*(A)	128	256	1,024	16	2	FIB, Frep	+	*tet*(A)	FIB, Frep
164	D	TE, S, NA	*tet*(A)	128	256	1,024	16	2	FIB, Frep	+	*tet*(A)	FIB, Frep
167	D	TE, S, NA	*tet*(A)	128	256	1,024	16	2	FIB, Frep	+	*tet*(A)	FIB
172	D	TE, S, NA	*tet*(A)	128	256	1,024	16	2	FIB, Frep	+	*tet*(A)	FIB
173	D	TE, S, NA	*tet*(A)	128	256	1,024	16	2	FIB, Frep	+	*tet*(A)	FIB, Frep
174	D	TE, S, NA	*tet*(A)	128	256	1,024	16	2	FIB, Frep	+	*tet*(A)	FIB, Frep
175	D	TE, S, NA	*tet*(A)	256	512	1,024	8	2	FIB, Frep	+	*tet*(A)	FIB
177	D	TE, S, AMP	*tet*(A)	64	256	2,048	16	4	P, FIA, FIB, Frep	+	*tet*(A)	FIB, Frep
178	D	TE, S, AMP	*tet*(A)	64	256	2,048	16	4	P, FIA, FIB, Frep	+	*tet*(A)	FIB, Frep
192	D	TE, SXT, C, S, AMP	*tet*(A)	256	512	1,024	16	2	P, FIB, Frep	+	*tet*(A)	FIB, Frep
194	D	TE, SXT, S, AMP	*tet*(A)	128	256	1,024	16	2	Frep	+	*tet*(A)	Frep
198	D	TE, SXT, S, AMP	*tet*(A)	128	256	1,024	32	2	Frep	+	*tet*(A)	Frep

[a] TE, tetracycline; S, streptomycin; GN, gentamicin; SXT, sulfamethoxazole-trimethoprim; C, chloramphenicol; NA, nalidixic acid; AMP, ampicillin.
[b] TET, tetracycline; OXY, oxytetracycline; CTC, chlortetracycline; DOX, doxycycline; MIN, minocycline.
[c] FIB, IncFIB replicon; I1, IncI1 replicon; P, IncP replicon, FIA, IncFIA replicon; Y, IncY replicon.

tet(A) plus *tet*(B) in two (1.3%) isolates and *tet*(B) plus *tet*(C) in two (1.3%) isolates. *tet*(D) and *tet*(G) were not detected. The distributions of *tet*(A) and *tet*(B) in the phylogenetic groups were not significantly different (chi-square test, $P > 0.05$) (Table 3).

MIC values of tetracycline-class antimicrobials. The MIC distributions of tetracycline, chlortetracycline, oxytetracycline, doxycycline, and minocycline for each group of isolates containing the same *tet* genes are shown in Table 4. The MIC values of all tetracyclines for isolates susceptible by the disk diffusion method were higher than the breakpoint (MIC ≥ 16 μg/ml). The MIC of chlortetracycline (range, 1,024 to 2,048 μg/ml) was much higher than the MICs of the four other tetracyclines. Resistance to minocycline (MIC ≥ 16 μg/ml) was observed for 35 (22.6%) isolates, and the genomes of 34 of these isolates encoded only the *tet*(B) resistance determinant. In fact, the average MICs for isolates containing the *tet*(B) gene were higher than those for isolates harboring the *tet*(A) gene (Fig. 1). Furthermore, the differences in the MICs between isolates containing *tet*(A) or *tet*(B) were greater for doxycycline and minocycline than the other three tetracyclines (Fig. 1).

Conjugative transfer of plasmid-mediated tetracycline resistance genes. Of the 146 tetracycline-resistant isolates, 121 (82.9%) isolates were found to transfer the *tet* gene to the recipient strain in conjugation assays. Transfer frequencies ranged from 1.26×10^{-8} to 9.26×10^{-6}. For 121 isolates possessing *tet*(A) or *tet*(B), the transconjugants possessed the same *tet* gene as their donors. Interestingly, for isolates containing *tet*(A) plus *tet*(B) or *tet*(B) plus *tet*(C), the transconjugants carried only the *tet*(B) gene. Plasmid replicon typing revealed that the most frequent replicon in the

transconjugants was IncFIB, which was found in 95 (65.1%) isolates, and this was followed by Frep (45.2%), IncI1 (25.3%), IncP (24.7%), IncFIA (19.2%), and IncY (17.1%). The results of the conjugation assay with *E. coli* isolates included in phylogenetic groups B2 and D are shown in Table 5. The tetracycline resistance gene was successfully transferred for all except two isolates in these phylogenetic groups. IncFIB was the most frequent plasmid replicon detected in transconjugants of these groups (Table 5).

DISCUSSION

In the present study, all tetracycline-resistant isolates carried either *tet*(A) or *tet*(B), suggesting that these genes are important for the development of tetracycline resistance. Actually, *tet*(A) and/or *tet*(B), encoding efflux mechanisms, has been reported to be the most common tetracycline resistance determinant in *E. coli* isolates from humans and animals in many countries (12, 13, 20–22). Previous studies conducted in cattle disagree: some have reported that the *tet*(A) determinant is dominant in *E. coli* isolates recovered from cattle (23–25), whereas others found *tet*(B) to be dominant (26–28). In the present study, the prevalences of *tet*(A) and *tet*(B) were almost equal at 46.5% and 45.1%, respectively, which is consistent with other reports that showed a similar distribution pattern for the *tet* gene in *E. coli* isolates recovered from animals (23, 29). The degree of resistance to tetracycline is associated with the presence of *tet*(B) (10). In the present study, MIC testing showed that *E. coli* isolates carrying only *tet*(B) appeared to have higher MIC values for tetracycline, chlortetracycline, oxytetracycline, doxycycline, and minocycline, which concurs with previous reports (10, 13, 30). Furthermore, we found that the MIC values for isolates carrying *tet*(B) were significantly higher for doxycycline and minocycline. These results are consistent with those of a previous study, in which *tet*(B) was found to confer resistance to expanded-spectrum tetracyclines, including minocycline and doxycycline (31).

In a previous study, *tet*(C) was frequently identified in *E. coli* isolates recovered from a commercial beef processing plant (32). However, we found *tet*(C) in only nine strains isolated from beef cattle, and those isolates showed susceptibility, but with low MIC values, to tetracycline, which concurs with the findings of previous studies (8, 33). Interestingly, the prevalences of *tet*(C) in *E. coli* isolates recovered from animals was reported to be higher than the prevalences of *tet*(C) in *E. coli* isolates recovered from meat and meat products (8), which suggests that some processing stages may reduce tetracycline resistance in *E. coli*.

Several studies have described *E. coli* isolates carrying more than two *tet* genes (11, 34, 35). In South Korea, 40% of *E. coli* strains isolated from cows and pigs in slaughterhouses were found to have two different *tet* genes (36), and in the present study, four *E. coli* isolates were found to carry more than two *tet* genes. Although the prevalence of isolates containing both *tet*(A) and *tet*(B) in the present study was lower than that reported in previous studies (11, 34), we found two isolates harboring *tet*(B) and *tet*(C), which is the first report of this combination in *E. coli* strains isolated from beef cattle in South Korea. However, this conflicts with the findings of a previous study, in which *tet*(C) was always found with *tet*(A) (37). Our study also showed that two isolates that carried more than one *tet* gene did not have higher MIC values than isolates that harbored one *tet* gene. This phenomenon was described in a previous study, in which it was proposed that the acquisition of more than one *tet* gene is caused by strong selective pressure rather than a selective advantage (35).

The long-term use of tetracycline confers resistance to other antimicrobial agents by *E. coli*. This phenomenon, called coselection, could be the result of *tet* genes being located on the same mobile genetic elements, such as plasmids, transposons, or integrons, as other resistance genes (38). In the present study, many isolates were resistant to tetracycline and other antimicrobials, and 62.3% of tetracycline-resistant isolates exhibited multidrug resistance. Thus, coselection has important implications, as it means that tetracycline resistance has contributed much to the increased prevalence of multidrug resistance in *E. coli*.

Phylogenetic groups B2 and D are associated with pathogenicity, whereas strains of groups A and B1 are classified as nonpathogenic commensal strains (17, 39). In the present study, most isolates were classified as group B1 (65.2%). This is consistent with the results of other studies that found that bovine *E. coli* isolates most frequently belong to group A and/or B1 (25, 40). Twenty-seven isolates (17.4%) were classified as group D, even though they were cultured from clinically healthy cattle in this study.

Conjugative transfer is the most common mechanism for the delivery of antimicrobial resistance between Gram-negative isolates because plasmid conjugation can occur at a high frequency and transfer resistance genes (41). In the present study, most tetracycline-resistant isolates (82.9%) exhibited conjugative transfer, which means that most *tet* genes are carried and transferred by conjugative plasmids. Therefore, we presume that the horizontal transfer of *tet* genes provides an effective mechanism for the widespread distribution of tetracycline resistance in bacterial populations and explains the high prevalence of tetracycline-resistant *E. coli* isolates.

In South Korea, although the use of tetracyclines as feed additives was entirely banned in July 2011, in 2013, about 40% of bovine *E. coli* isolates were found to be resistant to tetracycline (42). Accordingly, we propose that the high prevalence of tetracycline resistance in *E. coli* is probably due to the horizontal transfer of *tet* determinants from *E. coli* isolates carrying *tet* genes which have survived selective pressure caused by the use of tetracycline derivatives. We hope that these findings can be utilized as basic data for epidemiologic studies and studies to assess the risk of tetracycline resistance.

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