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# Molecular Detection of Florfenicol and Chloramphenicol Resistance among *Escherichia coli* Isolates from Healthy Pigs During 2003 to 2007

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## ABSTRACT

In order to elucidate the possible genetic determinants of resistance to florfenicol and chloramphenicol in porcine *Escherichia coli* in Taiwan, 600 fecal samples of healthy pigs from 50 different farms were collected from 2003 to 2007. The florfenicol resistance in the isolated *E. coli* strains doubled from 39.2% in 2003 to 78.3% in 2007. A total of 351 florfenicol-resistant *E. coli* isolates were isolated from nursery pigs (61.5%), grower-finisher pigs (62.5%), and sows (51.5%). The prevalence of resistance genes, *floR*, *cmlA*, *cat-1*, *cat-2* and *cat-3*, was 82.9, 61.3, 10.8, 3.7, and 0%, respectively. Of the 351 florfenicol-resistant isolates, 184 (52.4%) were positive for both *floR* and *cmlA*. Furthermore, the results of efflux inhibitor studies with Phe-Arg- $\beta$ -naphthylamide showed a 4- to 64-fold decrease in the florfenicol MIC levels. The FloR efflux pump may play a role in phenicol resistance among porcine *E. coli* isolates in Taiwan. More detailed studies are required to focus on the public health concerns about the spread of antimicrobial resistance from animal food products to humans through the food chain.

Key words: florfenicol, chloramphenicol, *Escherichia coli*, resistance, efflux pump

## INTRODUCTION

Chloramphenicol is a broad-spectrum antibiotic that was used extensively in veterinary medicine until concerns over its toxicity emerged<sup>(1)</sup>. Resistance to chloramphenicol may be mediated enzymatically through the chemical inactivation of the drug. Different types of chloramphenicol acetyltransferases (CATs) are responsible for most enzymatic resistance to chloramphenicol<sup>(2-4)</sup>. CATs are only able to inactivate chloramphenicol and thiamphenicol<sup>(2,3)</sup>. Because of the existing adverse effects and a high prevalence of resistance to chloramphenicol, the newly developed fluorinated derivative of chloramphenicol, florfenicol, has been used as an alternative agent for the control of bacterial respiratory tract infections in cattle, pigs, poultry, and other animal species<sup>(5-10)</sup>. After florfenicol replaced chloramphenicol in most countries,

the prevalence of florfenicol resistance in *E. coli* and other bacteria from animals has increased significantly in the past decade<sup>(6,11-17)</sup>. Active efflux pumps (*cmlA* and *floR*) are important for intrinsic and acquired antibiotic resistance. Over-expression of efflux pumps affecting florfenicol or chloramphenicol is becoming increasingly common in *E. coli*<sup>(6,17,18)</sup>. The *floR* gene confers resistance to both chloramphenicol and florfenicol, but the *cmlA* gene only mediates resistance to chloramphenicol<sup>(15,17-20)</sup>. These two genes are located either in the chromosomal DNA or on plasmids in *E. coli*<sup>(6,12,13,21)</sup>. Recently, organization of the *floR* gene on plasmid pMBSF1 in porcine *E. coli* has been reported by Blickwede and Schwarz<sup>(6)</sup>. By comparing the locations, the detailed sequences and the pulsed field gel electrophoresis (PFGE) patterns with other bacterial species or *E. coli* isolates<sup>(6,14,16,17,21,22)</sup>, the presence of a mobile *floR*-carrying element, a putative new integron, was identified<sup>(6)</sup>. Efflux pump inhibitors have been investigated with a view to improving and potentiating

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ing the activity of exported antibiotics. Phe-Arg- $\beta$ -naphthylamide (PA $\beta$ N) has been described as a broad-spectrum efflux pump inhibitor in *E. coli*<sup>(23)</sup>. In this study, the effects of PA $\beta$ N on the active efflux systems were tested for possible additive effects on resistance to phenicols.

Currently, very little information is available regarding the prevalence of phenicol-resistant *E. coli* in domestic animals, particularly in healthy pigs. The aim of this study was thus to determine the prevalence of resistance to florfenicol and chloramphenicol, and of efflux pump systems present in porcine *E. coli* isolates.

## MATERIALS AND METHODS

### I. Bacterial Isolation and Culture Conditions

We evaluated fecal samples from 50 swine farms in Taiwan from 2003 to 2007. At each farm, 12 fecal samples were collected directly from the rectum of the individual animal. The rectal samples from pigs were collected in three different production stages in each farm: nursery pigs ( $n = 4$ ), grower-finisher pigs ( $n = 4$ ), and sows ( $n = 4$ ). The sampling number for different stages was based on the average total number of pigs in each farm. Each year, 120 samples (from 10 pig farms) were collected. A total of 600 samples were obtained from 50 different farms. Fecal samples were collected directly from the rectums of the animals using aseptic cotton swabs and buffered peptone water (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). After overnight incubation at 35°C, the broth was inoculated onto MacConkey agar plates (Becton Dickinson) containing 8 mg/L of florfenicol (Sigma Aldrich, St Louis, MO, USA). For individual rectum sample, two colonies with typical *E. coli* morphologic characters were selected at random and subjected to standard biochemical tests (gram stain, oxidase, triple sugar iron (TSI), indole production, citrate fermentation, methyl red, ornithine decarboxylase fermentation, and urea agar) for identification<sup>(24)</sup>. Furthermore, all *E. coli* isolates were confirmed biochemically by using the API 20E system (BioMérieux, Marcy-l'Étoile, France). If the two colonies had the same results, only one of the colonies was used for further analysis.

### II. Antimicrobial Susceptibility Testing

The minimal inhibitory concentrations (MICs) for florfenicol-resistant *E. coli* strains were determined in Mueller-Hinton agar by the standard twofold dilution method in accordance with the guidelines of the CLSI (Clinical and Laboratory Standards Institute; formerly NCCLS)<sup>(25)</sup>. Eleven antimicrobial agents were tested. Ampicillin, chloramphenicol, colistin, florfenicol, gentamicin, nalidixic acid, and oxytetracycline were purchased from Sigma, enrofloxacin and ciprofloxacin from Fluka Chemie (Buchs, Switzerland), amoxicillin-clavulanic acid

from GlaxoSmithKline Biologicals (Stevenage, UK), and ceftiofur (Excenel RTU) from Pfizer Animal Health (Karlruhe, Germany). The solvents and diluents used for stock and standard solutions followed the CLSI guidelines<sup>(25)</sup>. The antimicrobial agents to be tested were selected according to the following criteria: substances commonly used in pig farms (all listed antimicrobial agents, except for amoxicillin-clavulanic acid, ciprofloxacin, and chloramphenicol), substances used exclusively for veterinary clinical therapy (florfenicol, ceftiofur, and enrofloxacin), substances used exclusively for human medicine (ciprofloxacin), and substances used for both human and veterinary medical purposes (ampicillin, and colistin). Based on their spectra of activity and clinical usage, these agents may induce different levels of resistance in bacteria. Reference strains of *E. coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls for MIC determination on each plate. Bacteria were incubated in the testing medium with various antimicrobials and concentrations at 37°C for 16-18 hr. The percentage of isolates showing resistance to each antimicrobial agent was determined by measuring the MIC and comparing it to the resistance breakpoint established by CLSI<sup>(25)</sup>. For the MIC breakpoint values which were not provided by CLSI, the resistance breakpoints for bovine respiratory disease pathogens were used<sup>(25)</sup>. These included ceftiofur (susceptible:  $\leq 2$  mg/L; resistant:  $\geq 8$  mg/L) and florfenicol (susceptible:  $\leq 2$  mg/L; resistant:  $\geq 8$  mg/L). According to the MIC breakpoints of the BSAC (British Society for Antimicrobial Chemotherapy) for *Acinetobacter* spp. and *Enterobacteriaceae*, resistance to colistin was defined as MIC  $> 4$  mg/L<sup>(26)</sup>.

### III. Detection of Florfenicol and Chloramphenicol Resistance Genes

Chromosomal DNA was prepared using a Wizard genomic DNA purification kit (Promega, Madison, WI, USA). The chloramphenicol resistance genes (*cat-1*, *cat-2*, and *cat-3*) and efflux pump genes (*floR* and *cmlA*) were amplified by polymerase chain reaction (PCR). The primers used are listed in Table 1 and PCR was performed as previously described<sup>(21,22)</sup>. The PCR was performed on a GeneAmp PCR System 9700 (Applied Biosystems, Inc., Foster City, CA, USA). The PCR products were purified using a PCR purification kit (Qiagen, Valencia, CA, USA) and confirmed by nucleotide sequencing.

### IV. The Effects of PA $\beta$ N on the Active Efflux Systems

In order to investigate the inhibitory ability of PA $\beta$ N (Sigma) on bacterial multiple component efflux systems, 44 isolates were selected at random from each resistance genotype. These included four *cmlA*-carrying isolates, twelve *floR*-carrying isolates, twelve *floR*- and *cmlA*-carrying isolates, three *cmlA*-, *floR*-, and *cat-1*-carrying isolates, two *cmlA*- and *cat-1*-carrying isolates,

**Table 1.** Primers used in this study

Target genes	Sequence (5'-3')	Annealing temp (°C)	Products size (bp)	References
<i>cat-1-F</i>	AGTTGCTCAATGTACCTATAACC	50	547	22
<i>cat-1-R</i>	TTGTAATTCATTAAGCATTCTGCC			
<i>cat-2-F</i>	ACACTTTGCCCTTTATCGTC	50	543	22
<i>cat-2-R</i>	TGAAAGCCATCACATACTGC			
<i>cat-3-F</i>	TTCGCCGTGAGCATTTTG	50	286	22
<i>cat-3-R</i>	TCGGATGAGTATGGGCAAC			
<i>floR-F</i>	CGCCGTCATTCCTCACCTTC	50	215	22
<i>floR-R</i>	GATCACGGGCCACGCTGTGTC			
<i>cmlA-F</i>	CCGCCACGGTGTGTTGTTATC	40	698	21
<i>cmlA-R</i>	CACCTTGCCTGCCCATCATTAG			

**Table 2.** Antimicrobial susceptibility profiles and percentage of resistance of 351 pig florfenicol-resistant *E. coli* strains

Antimicrobial agents (mg/L)	Florfenicol-resistant <i>E. coli</i> strains (n = 351)			Breakpoint	
	MIC <sub>50</sub>	MIC <sub>90</sub>	R% <sup>a</sup>	S <sup>b</sup>	R <sup>b</sup>
Amoxicillin-clavulanic acid	16/8	128/64	37.8	≤ 8/4	≥ 32/16
Ampicillin	> 1024	> 1024	93.9	≤ 8	≥ 32
Ceftiofur	1	16	26.3	≤ 2	≥ 8
Colistin	2	8	45.9	–	> 4
Gentamicin	64	> 1024	61.0	≤ 4	≥ 16
Oxytetracycline	> 1024	> 1024	99.4	≤ 4	≥ 16
Nalidixic acid	> 1024	> 1024	96.2	≤ 8	≥ 32
Ciprofloxacin	2	128	55.2	≤ 1	≥ 4
Enrofloxacin	8	256	61.7	≤ 0.5	≥ 2
Florfenicol	512	> 1024	100	≤ 2	≥ 8
Chloramphenicol	512	1024	100	≤ 8	≥ 32

<sup>a</sup> R%: percentage of resistance

<sup>b</sup> S: susceptibility, R: resistant.

eight *floR*- and *cat-1*-carrying isolates, and three *floR*- and *cat-2*-carrying isolates. The MICs of florfenicol and chloramphenicol were determined in the presence or absence of PAβN at 80 mg/L<sup>(27)</sup>. The reference strain *E. coli* ATCC25922 was used as a control. A 4-fold or 8-fold reduction in the MIC value after the addition of PAβN was considered as a positive effect<sup>(23)</sup>.

#### V. Statistical Analysis

The statistical tests used were the chi-square test and Fisher's exact test, using the Mixed Procedure in SAS (version 8.2; SAS Institute, Inc., Cary, NC, USA).

The correlations between MICs of florfenicol/chloramphenicol and resistance genes (*floR* and *cmlA*) were performed using the Pearson correlation coefficient. For all comparisons, a value of  $p < 0.05$  was considered to be of statistically significant difference.

## RESULTS

### I. Florfenicol-Resistant *E. coli* Strains and Antimicrobial Susceptibility Testing

A total of 351 florfenicol-resistant *E. coli* strains

**Table 3.** Prevalence of *cmlA*, *floR*, *cat-1*, and *cat-2* genes in florfenicol-resistant *E. coli* isolates

Group	Resistance genotype	No. of isolates positive for resistance gene/No. tested (%)	MIC (mg/L) <sup>a</sup>	
			Florfenicol	Chloramphenicol
1	<i>cmlA</i> only <sup>b</sup>	29/351 (8.3)	8-512	32-512
2	<i>floR</i> only <sup>b</sup>	69/351 (19.7)	32- > 1024	32- > 1024
3	<i>cmlA</i> + <i>floR</i> <sup>c</sup>	173/351 (49.3)	32- > 1024	32- > 1024
4	<i>cmlA</i> + <i>floR</i> + <i>cat-1</i>	11/351 (3.1)	32-1024	256- > 1024
5	<i>cmlA</i> + <i>cat-1</i>	2/351 (0.6)	16-256	128-256
6	<i>floR</i> + <i>cat-1</i>	25/351 (7.1)	32- >1024	256-1024
7	<i>floR</i> + <i>cat-2</i>	13/351 (3.7)	32-512	256- > 1024
8	N.I. <sup>d</sup>	29/351 (8.3)	16-512	32-1024

<sup>a</sup> Determined by agar-dilution methods according to CLSI standards and guidelines.

<sup>b</sup> Statistically significant differences ( $p < 0.001$ ) between *cmlA* only (Group 1) and *floR* only (Group 2).

<sup>c</sup> Statistically significant differences ( $p < 0.001$ ) between *cmlA* & *floR* (Group 3) and *cmlA* only (Group 1) and *floR* only (Group 2).

<sup>d</sup> Not identified: the isolates showed florfenicol resistance but didn't carry any of these four genes.

were isolated from 600 samples from porcine rectal swabs of nursery pigs (61.5%; 123/200), grower-finisher pigs (62.5%; 125/200), and sows (51.5%; 103/200) between 2003 and 2007. For the florfenicol-resistant *E. coli* isolates ( $n = 351$ ), the MIC of chloramphenicol ranged from 32 to > 1024 mg/L and the MIC of florfenicol ranged from 8 to > 1024 mg/L. Our results also showed that most isolates (70.4%; 247/351) had high level of resistance to both phenicols (MICs 512- > 1024 mg/L; Table 2). The annual percentage of florfenicol-resistant *E. coli* increased continuously throughout the sampling period (2003-2007), from 39.2% (47/120) to 45.8% (55/120), 60% (72/120), 69.2% (83/120), and 78.3% (94/120), respectively. The MICs at which 50% and 90% of the isolates were inhibited (MIC<sub>50</sub> and MIC<sub>90</sub>) and the antimicrobial susceptibility profiles against the different antimicrobial agents are summarized in Table 2. The resistance of florfenicol-resistant *E. coli* strains to chloramphenicol, oxytetracycline, nalidixic acid, ampicillin, enrofloxacin, gentamicin, and ciprofloxacin was 100%, 99.4%, 96.2%, 93.9%, 61.7%, 61.0%, and 55.2%, respectively. These isolates also showed a moderate level of resistance to colistin (45.9%), amoxicillin-clavulanic acid (37.8%), and ceftiofur (26.3%).

## II. Detection of Florfenicol and Chloramphenicol Resistance Genes

The total DNA of the 351 florfenicol-resistant *E. coli* isolates was extracted as the DNA template for PCR detection of *floR*, *cmlA*, *cat-1*, *cat-2*, and *cat-3* genes. These results are presented in Table 3. A total of 291 *floR*-positive *E. coli* strains were detected from nursery pigs (72.4%; 89/123), grower-finisher pigs (92.8%; 116/125), and sows (83.5%; 86/103). The frequency of

**Table 4.** Correlations between minimum inhibitory concentration (MIC) of florfenicol/chloramphenicol and resistance genes

Antibiotic	<i>floR</i>		<i>cmlA</i>	
	r	p value	r	p value
Florfenicol	0.8643	0.002	-0.2961	0.4392
Chloramphenicol	0.7845	0.036	0.7969	0.032

detection of the *floR* (or *cat-1*) gene was greater than that of the *cmlA* (or *cat-2*) gene. Our results also showed that 184 *E. coli* isolates (52.4%) were carrying both the *floR* and *cmlA* genes. However, the *cat-1* and *cat-2* genes did not exist concurrently in any of the tested isolates. No *cat-3* gene was detected in any of the isolates screened. Within the sampling period, the annual percentage of the *floR* resistance gene increased over time. In the years 2003 thru 2007, the annual percentage of *floR*-positive *E. coli* isolates was 33%, 38%, 48%, 55.8%, and 69.2%, respectively. The percentage of florfenicol-resistance *E. coli* isolates between 2003 and 2007 with the *cmlA* gene was 23.3%, 24.2%, 30.8%, 48.3%, and 52.5%, respectively. However, the percentage of the *cat-1* and *cat-2* genes decreased over time. Between 2003 and 2007, the percentage of florfenicol-resistance *E. coli* isolates with the *cat-1*(*cat-2*) gene was 9.2% (6.7%), 7.5% (1.7%), 5.8% (0.8%), 5.8% (1.7%), and 3.3% (0%), respectively. The Pearson correlation test indicated that florfenicol/chloramphenicol resistance was correlated with the *floR* gene ( $r = 0.78 - 0.86$ ;  $p < 0.05$ ). However, the chloramphenicol efflux gene (*cmlA*) did not confer resistance to florfenicol ( $r = -0.2961$ ;  $p = 0.4392$ ; Table 4).

### III. The Effects of PA $\beta$ N on the Active Efflux Systems

The MIC values of florfenicol in the absence and in the presence of the efflux inhibitor PA $\beta$ N are listed in Table 5. Forty-four randomly preselected isolates and *E. coli* ATCC 25922 could be subdivided into seven groups. Group 1 and group 5 isolates carried the *cmlA* gene plus PA $\beta$ N in the medium, with only a 2-fold decrease in the MIC of florfenicol but a 4- to 8-fold decrease in the MIC of chloramphenicol. Group 2, group 6, and group 7 isolates carried the *floR* gene, with a 4- to 32-fold decrease in the MIC of florfenicol and a 4- to 8-fold decrease in the MIC of chloramphenicol. Group 3 and group 4 isolates carried *floR* and *cmlA* genes, with a 4- to 64-fold decrease in the MIC of florfenicol and a 4- to 16-fold decrease in the MIC of chloramphenicol. Group 4, group 5, group 6, and group 7 isolates also carried *cat-1* or *cat-2*. However, these two *cat* genes did not have any significant effect on the MICs of the two phenicols.

## DISCUSSIONS

In Taiwan, chloramphenicol has not been used for any purpose in food-producing animals since 2002. After chloramphenicol was banned, florfenicol was used to replace chloramphenicol in clinical therapy. The annual

percentage of resistance to florfenicol in the isolated *E. coli* strains doubled from 39.2% in 2003 to 78.3% in 2007. This elevation of resistance is correlated with the parallel increase in the annual percentage of isolates carrying the *floR* gene (from 33% to 69.2%; Table 4). This is the most important reason to explain the increase of florfenicol resistance over time. Additionally, the florfenicol-resistant isolates contained the *cmlA* gene and chloramphenicol resistance in our samples was unexpectedly high. Our susceptibility data have demonstrated the persistence of florfenicol resistance in porcine *E. coli* in combination with a high percentage of resistance to chloramphenicol (Table 2). Previous studies have shown that the continuous use of florfenicol could have increased the selective pressure for both florfenicol and chloramphenicol resistance<sup>(6,15,17)</sup> and the existence of *floR* could contribute to enhance the ability to efflux chloramphenicol<sup>(15,17-20)</sup>.

According to the farm medical records, no farm has used florfenicol in the grower-finisher pigs and sows prior to sampling. However, the higher percentage of *floR* observed in these pigs may be due to the dissemination of *floR* via high molecular weight plasmids and/or other mobile genetic elements (transposons, integrons)<sup>(6,17)</sup>. The location of the resistance gene on a mobile element is an important prerequisite for fast and efficient distribution among bacteria of the same or different genera and species, as well as for the resistant genes transfer between

**Table 5.** MICs determined in the absence or presence of PA $\beta$ N and the two phenicol resistance genes detected in the *E. coli* isolates

Strains	florfenicol	F+I <sup>a</sup>	Relative fold	chloramphenicol	C+I <sup>b</sup>	Relative fold
<b>Group 1 <i>cmlA</i> only</b>						
93-14	16	8	2	128	16	8
93-89	32	16	2	64	8	8
96-78	512	256	2	128	32	4
96-109	512	256	2	512	64	8
<b>Group 2 <i>floR</i> only</b>						
92-91	1024	256	4	1024	256	4
92-111	32	4	8	256	32	8
93-26	256	32	8	1024	256	4
93-27	32	2	16	512	64	8
93-62	32	8	4	32	8	4
94-28	512	32	16	512	128	4
94-30	512	128	4	512	128	4
94-2367	512	128	4	256	64	4
95-32	1024	256	4	1024	256	4
95-93	512	128	4	1024	256	4
96-73	512	128	4	32	8	4



Table 5. Continued.

Strains	florfenicol	F+I <sup>a</sup>	Relative fold	chloramphenicol	C+I <sup>b</sup>	Relative fold
96-94	1024	128	8	1024	256	4
<b>Group 3 <i>cmIA</i> + <i>floR</i></b>						
92-23	512	128	4	512	32	16
92-36	32	0.5	64	128	16	8
93-129	512	128	4	1024	128	8
93-2389	512	64	8	1024	256	4
94-36	256	32	8	512	32	16
94-52	512	64	8	32	8	4
95-37	256	32	8	64	8	4
95-50	512	128	4	512	128	4
95-128	512	64	8	1024	64	16
96-65	32	2	16	16	4	4
96-131	> 1024	64	> 16	> 1024	256	> 4
96-165	512	128	4	32	8	4
<b>Group 4 <i>cmIA</i> + <i>floR</i> + <i>cat-1</i><sup>c</sup></b>						
95-77	32	4	8	256	32	8
95-133	32	4	8	1024	128	8
96-118	32	2	16	1024	256	4
<b>Group 5 <i>cmIA</i> + <i>cat-1</i><sup>c</sup></b>						
93-34	16	8	2	128	32	4
94-49	32	16	2	256	32	8
<b>Group 6 <i>floR</i> + <i>cat-1</i><sup>c</sup></b>						
92-42	> 1024	256	> 4	1024	128	8
93-116	256	32	8	512	128	4
94-14	32	1	32	512	128	4
94-44	> 1024	256	> 4	1024	256	4
94-123	1024	256	4	512	128	4
95-31	512	64	8	512	128	4
95-42	> 1024	256	> 4	1024	128	8
96-58	32	2	16	256	64	4
<b>Group 7 <i>floR</i> + <i>cat-2</i><sup>c</sup></b>						
92-121	512	64	8	1024	256	4
93-55	512	64	8	> 1024	256	> 4
95-142	32	4	8	256	64	4
Reference strain						
<i>E. coli</i> ATCC 25922	1	1	-	4	4	-

<sup>a</sup>F+I: florfenicol+PAβN<sup>b</sup>CHL+I: chloramphenicol+PAβN<sup>c</sup>The isolates carried the *cat-1* or *cat-2* gene which showed no significant effect on the MICs of the two phenicols.

human and animal bacteria<sup>(6)</sup>. The mechanism of resistance in these isolates is the subject of further study.

In the present study, the number of *floR*-carrying isolates (291/351;  $p < 0.001$ ) was more than the number of *cmlA*-carrying isolates (215/351) and the number of *cat-1* or *cat-2* carrying isolates (51/351; Table 3). Most of the isolates (184/351) carried both *cmlA* and *floR* (group 3: 173 strains and group 4: 11 strains) and only 29 carried the single *cmlA* gene (group 1). After analyzing the distribution of group 1 isolates, it was found that most of them were collected in the first and second years (data not shown). Interestingly, 60 isolates with florfenicol MICs  $> 8$  mg/L were negative for the *floR* gene (group 1, group 5, and group 8; Table 3). The results of Southern blot hybridization also indicated that no plasmid or chromosomal resistance determinants (*floR* gene) were found for these isolates. Whether some nonspecific mechanisms, such as overproduction of the AcrAB-TolC multidrug efflux system, were responsible for the elevated MICs for florfenicol among these *floR*-negative *E. coli* isolates remains unknown<sup>(2)</sup>.

After comparing the florfenicol and chloramphenicol MIC values as shown in Table 3, we arrived at the following conclusions. First, group 2 (*floR* only) had higher florfenicol and chloramphenicol MIC values than group 1 (*cmlA* only). Second, the isolates containing *floR* and *cmlA* genes (group 3) or only the *floR* gene (group 2) had no significant difference on the MIC values. Third, the chloramphenicol acetyltransferases (*cat-1* and *cat-2*) were not the predominant contributors for chloramphenicol resistance, which is consistent with previous studies<sup>(20)</sup>. There was no additive effect on MIC values (Table 3) by two different resistance mechanisms, i.e., *floR* and *cat* genes (Group 6 and 7). Fourth, the MIC values increased significantly as long as the isolates were carrying *floR* since FloR can efflux both florfenicol and chloramphenicol out of the bacterial cell.

Active extrusion of florfenicol via efflux pumps is an important mechanism for the resistance of gram-negative bacteria. In this study, the MICs for both florfenicol and chloramphenicol of 44 randomly selected isolates are shown in Table 5. The effects of PA $\beta$ N on the active efflux systems were tested. On the basis of fold decrease of florfenicol resistance in the presence of PA $\beta$ N in each group, our data indicated that group 2 (only *floR* gene), group 3 (*floR* and *cmlA* genes), group 4 (*floR*, *cmlA*, and *cat-1* genes), group 6 (*floR* and *cat-1* genes), and group 7 isolates (*floR* and *cat-2* genes) showed a 4- to 64-fold decrease in the florfenicol MIC levels. Group 1 (only *cmlA*) and group 5 (*cmlA* and *cat-1* genes) had little or no change in the MIC values for florfenicol. Once again, these results confirm the *floR* was the predominant contributor for the florfenicol resistance.

In summary, we describe the prevalence of resistance of *E. coli* to florfenicol and chloramphenicol and their relationship to *floR* or *cmlA* gene. When a stronger efflux system was present, the *cat* genes showed much

less or no effect on the MICs of the two phenicols. When PA $\beta$ N was present, the *floR* gene was inhibited and the MICs were altered accordingly. More detailed studies are required to focus on the importance of these genetic relationships and to rationalize the use of antimicrobial agents in both humans and other animals.

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