Original Article

Association between virulence profile and fluoroquinolone resistance in *Escherichia coli* isolated from dogs and cats in China

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Abstract

Introduction: *Escherichia coli* is not only a commensal organism in humans and animals, but also a causative agent of diarrhea and extraintestinal infections. Information about the relationship between population structure, virulence gene profiles, and fluoroquinolone resistance of *E. coli* in dogs and cats in China is limited.

Methodology: A total of 174 pathogenic and commensal *E. coli* isolates were evaluated in terms of phylogenetic group, virulence gene profile, sequence types (STs), and fluoroquinolone susceptibility.

Results: A total of 46.6% of isolates belonged to phylogenetic group B2. Isolates displayed high resistance to tetracycline (82.2%), amoxicillin/clavulanic acid (73.6%), gentamicin (62.1%), and enrofloxacin (60.9%). *fimH* (81.6%) was the most prevalent virulence gene, and 83.9% of isolates contained one or more investigated virulence genes. The majority of the investigated virulence genes were more prevalent in fluoroquinolone-susceptible isolates and pathogenic isolates. Multilocus sequence typing (MLST) showed that *E. coli* isolates analyzed were assigned to 65 STs. Among of them, pathogenic-resistant and pathogenic-susceptible isolates had 44 and 10 STs, respectively, while there were 8 and 3 STs in the commensal resistant and susceptible isolates, respectively.

Conclusions: Phylogenetic group B2 was the dominant group, accounting for 46.6% of the isolates. Pathogenic isolates and fluoroquinolone-susceptible isolates possessed more virulence genes. Pathogenic isolates and fluoroquinolone-resistant isolates exhibited high population diversity, and pandemic clone ST131 appeared in 9.8% of isolates.

Key words: *Escherichia coli*; dogs; cats; virulence genes; fluoroquinolone resistance; MLST.

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Introduction

Escherichia coli are a very diverse species of bacteria found naturally in humans and animals in terms of gene content, phenotype, and virulence [1]. The fluoroquinolones are the most commonly used class of antibacterials in the treatment of several infectious caused by *E. coli*, although effective treatment of *E. coli* infections is impaired by the swift emergence of fluoroquinolone resistance. Usually, *E. coli* isolates can be classified into commensal, intestinal pathogenic, and extraintestinal pathogenic *E. coli* (ExPEC) groups, based on different virulence genes [2]. ExPEC usually possesses diverse virulence genes responsible for pathogenesis outside the gastrointestinal tract, and is also distinguished from commensal *E. coli* by the presence of a broad spectrum of virulence genes [3-5], while commensal isolates can also develop into intestinal or ExPEC isolates by acquiring virulence genes [2-7].

Previous studies have shown that virulence genes of *E. coli* isolates are strongly associated with antimicrobial resistance, whereas other studies have reported that antimicrobial resistance and virulence genes are weakly linked [8,9]. Moreover, previous data also suggested that fluoroquinolone-resistant ExPEC isolates harbored fewer virulence genes than did fluoroquinolone-susceptible isolates from humans [10,11].

Until now, knowledge about phylogenetic group, virulence gene profiles, ST types, and fluoroquinolone susceptibility in *E. coli* isolates from dogs and cats in Shaanxi province of China has been limited. The present study aimed to investigate and compare virulence gene frequencies based on fluoroquinolone susceptibility and source of isolates, and tried to clarify the association between virulence gene profiles, phylogenetic groups, ST types, and fluoroquinolone resistance.

Methodology

Bacterial isolates

From March 2012 to October 2014, a total of 174 *E. coli* (143 from dogs, 31 from cats) were isolated from urine and blood (n = 137, including 115 dog and 22 cat isolates) with evidence of infection, and from feces of healthy animals (n = 37, including 28 dog and 9 cat isolates) in small-animal hospitals in Shaanxi province in China. All isolates were identified by conventional microbiological procedures and biochemical tests as described previously [9], with minor modifications. Briefly, the urine, fecal swabs, or blood samples were streaked out on MacConkey agar plates (Qingdao Hope Bio-Technology Co., Ltd., Qingdao, China) and incubated at 37°C for 18 to 24 hours. The putative *E. coli* isolates on MacConkey agar (bright pink with a dimple) per sample were transferred to eosin methylene blue agar (Qingdao Hope Bio-Technology Co., Ltd., Qingdao, China) for further purification and were incubated at 37°C for 18 to 24 hours. The suspect *E. coli* isolates on eosin methylene blue agar (green colonies with a metallic sheen) were then taken for biochemical tests (indole, methyl red, oxidase, citrate, and triple sugar iron). After identification and confirmation, the isolates were stored in tryptic soy broth (Difco, Sparks, USA) containing 30% glycerol at -80°C for further analysis.

Antimicrobial susceptibility testing

All isolates underwent susceptibility testing to a panel of eleven antimicrobial agents, representing five drug classes, including fluoroquinolones (enrofloxacin, ciprofloxacin, marbofloxacin, and pradofloxacin), β-lactams (amoxicillin/clavulanic acid and ceftiofur), sulfonamides (trimethoprim/sulfamethoxazole), tetracycline (tetracycline and vibramycin), and aminoglycosides (gentamicin and amikacin). Each isolate was categorized in terms of its resistance phenotype as fluoroquinolone susceptible and fluoroquinolone resistant, the latter including non-multidrug resistant (SDR, resistant to one or two classes of antibacterial agents) and multidrug resistant (MDR, resistant to three or more classes of antibacterial agents). The minimum inhibitory concentration (MIC) for each isolate was determined by a standardized microdilution method using Mueller-Hinton broth (Huyu Biotech Co., Ltd., Shanghai, China) following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [12]. For pradofloxacin, the clinical breakpoints for *E. coli* were ≤ 0.25 µg/mL (susceptible) and ≥ 2 µg/mL (resistant), based on the European Union registration [13]. All MIC determinations were performed in triplicate, and *E. coli* ATCC 25922 was used for quality control. In addition, the *E. coli* isolates with reduced susceptibility to oxyimino-cephalosporins were examined for extended-spectrum β-lactamase (ESBL) production using Sensititre ESBL confirmatory MIC plates (ESB1F, Thermo Scientific, Xi'an, China) as described by Aly *et al*. [14].

Table 1. The oligonucleotide primers used in this study.

Target	Accession	Primer sequence (5'-3')	Fragment
fimH	AJ225176	TGCAGAACGGATAAGCCGTGG/ GCAGTCACCTGCCCTCCGGTA	508
papA	X61239	ATGGCAGTGGTGTCTTTTGGTG/ CGTCCCACCATACGTGCTCTTC	717
papG	X61239	CTGTAATTACGGAAGTGATTTCTG/ ACTATCCGGCTCCGGATAAACCAT	1070
papG I	X61239	TCGTGCTCAGGTCCGGAATTT/ TCCAGAAATAGCTCATGTAACCCG	479
papG II	M20181	GGGATGAGCGGGCCTTTGAT/ CGGGCCCCCAAGTAACTCG	190
papG III	X61238	GGCCTGCAATGGATTTACCTGG/ CCACCAAATGACCATGCCAGAC	258
sfa/focDE	Unpublished	CTCCGGAGAACTGGGTGCATCTTAC/CGGAGGAGTAATTACAAACCTGGCA	410
focG	DQ301498	CAGCACAGGCAGTGGATACGA/GAATGTCGCCTGCCCATTGCT	364
hlyA	M10133	AACAAGGATAAGCACTGTTCTGGCT/ACCATATAAGCGGTCATTCCCGTCA	1177
hlyD	AM690759	CTCCGGTACGTGAAAAGGAC/ GCCCTGATTACTGAAGCCTG	904
cnfI	U42629	ATCTTATACTGGATGGGATCATCTTGG/ GCAGAACGACGTTCTTCATAAGTAT	974
kpsMT II	X53819	GCGCATTTGCTGATACTGTTG/ CATCCAGACGATAAGCATGAGCA	272
kpsMT K1	M57382	TAGCAAACGTTCTATTGGTGC/ CATCCAGACGATAAGCATGAGCA	153
fyuA	Z38064	TGATTAACCCCGCGACGGGAA/ CGCAGTAGGCACGATGTTGTA	880
iroN	CP001671	AAGTCAAAGCAGGGGTTGCCCG/ GACGCCGACATTAAGACGCAG	667
ireA	AF320691	GATGACTCAGCCACGGGTAA/ CCAGGACTCACCTCACGAAT	254
iutA	X05874	GGCTGGACATCATGGGAACTGG/ CGTCGGGAACGGGTAGAATCG	302
ibeA	L42624	AGGCAGGTGTGCGCCGCGTAC/ TGGTGCTCCGGCAAACCATGC	170
traT	J01769	GGTGTGGTGCGATGAGCACAG/ CACGGTTCAGCCATCCCTGAG	290
cvaC	X57525	CACACACAAACGGGAGCTGTT/ CTTCCCGCAGCATAGTTCCAT	680

Phylogenetic typing and virulence genotyping

All investigated *E. coli* isolates were assigned to seven phylogenetic groups (A, B1, B2, D, C, E, and F) by polymerase chain reaction (PCR) as recently described by Clermont *et al*. [15]. Twenty virulence genes, representing six categories: adhesins (*fimH*, *papA*, *papG*, *papG* allele I, *papG* allele II, *papG* allele III, *sfa*/*focDE*, and *focG*), toxins (*hlyA*, *hlyD*, and *cnf1*), capsule synthesis (*kpsMT* II and *kpsMT* K1), siderophores (*fyuA*, *iroN*, *ireA*, and *iutA*), invasin (*ibeA*), and miscellaneous genes (*traT* and *cvaC*) (Table 1) were screened based on a previous study [16]. All oligonucleotide primers were designed using Lasergene software package (DNASTAR, Madison, USA), and were synthesized by Shanghai Sangon Biotech Co., Ltd. (Shanghai, China).

Genomic DNA was extracted from bacterial preparations using the PreMan Ultra Preparation Reagent (Applied Biosystems, Foster City, USA), according to the manufacturer's instructions. The PCR procedure was as follows: initial denaturation at 94°C for 5 minutes, followed by 30 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 58–60°C for 30 seconds, and extension at 72°C for 3 minutes, followed by a final extension step at 72°C for 10 minutes. The PCR products were evident after migration by gel electrophoresis on 2% agarose gel.

Multilocus sequence typing (MLST)

MLST was performed using seven conserved housekeeping genes of *E. coli* (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*). A detailed scheme of the MLST procedure, including the PCR conditions, primers, allelic type, and sequence type assignment methods, is available at MLST databases at the at the Warwick University website (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli).

Statistical analysis

Significance was determined by Pearson's Chi-squared test with Yates continuity correction using R software (version 3.0.1). The threshold for statistical significance was a p value < 0.05.

Results

Antimicrobial susceptibility testing

Among the 174 *E. coli* isolates surveyed, 60.9% of isolates (n = 106, including 17 SDR and 89 MDR) were resistant to enrofloxacin, 21.3% of isolates (n = 37) remained susceptible to fluoroquinolones but expressed resistance to other antibacterial agents, and only 31 (17.8%) isolates were fully susceptible to the antibacterial agents tested. Additionally, the investigated isolates were highly resistant to tetracycline (82.2%), amoxicillin/clavulanic acid (73.6%), trimethoprim-sulfamethoxazole (68.4%), and gentamicin (62.1%). The resistance rates to other antibacterials ranged from 19.4% to 54.6%. It is noteworthy that 80.6% of isolates remained susceptible to pradofloxacin, a new fluoroquinolone that has not been approved in China.

Phylogenetic groups

All 174 isolates were categorized; the results are shown in Table 2. Phylogenetic group B2 was the dominant group, accounting for 46.6% of isolates. A total of 35 isolates (20.1%) belonged to group B1, 24 (13.8%) to group D, 18 (10.3%) to group A, 7 (4.0%) to group C, 6 (3.4%) to group E, and 3 (1.7%) to group F. Furthermore, the isolates falling into phylogenetic group B2 accounted for 58.8% of fluoroquinolone-resistant and 38.7% of fluoroquinolone-susceptible isolates, respectively. A more detailed analysis revealed that the isolates from different sources exhibited different structural characteristics; the distribution of phylogenetic groups among the isolates from urine and blood were as follows: group B2 (51.0%), D (16.7%), B1 (15.6%), A (10.4%), C (3.1%), E (3.12%); and group B2 (53.7%), D (12.2%), B1 (12.2%), C (7.3%), A (4.9%), F (4.9%), E (2.4%), respectively. The fecal isolates revealed a different distribution: phylogenetic group B1 (40.5%), B2 (27.0%), A (16.2%), D (8.1%), E (5.4%), F (2.7%) (Table 3).

Virulence genotyping

The frequencies of 20 virulence genes tested are summarized in both Table 2 and Table 3 based on fluoroquinolone susceptibility and the source of the isolates, respectively. Overall, 146 (83.9%) isolates possessed at least one investigated virulence gene. Among them, 22 (15.1%) isolates harbored one virulence gene, 31 (21.2%) isolates contained 2 to 5 virulence genes, 65 (44.5%) isolates contained 6 to 10 virulence genes, 23 (15.8%) isolates contained 11 to 15 virulence genes, and 5 (3.2%) isolates contained 16 to 17 virulence genes. No isolates harbored more than 17 of the 20 virulence genes tested. The overall prevalence of the virulence genes ranged from 1.1% (*papG* allele I) to 81.6% (*fimH*). The *fimH* gene was the most prevalent virulence gene among adhesins, and the siderophores ranged from 14.4% (*ireA*) to 73% (*fyuA*). Of the toxins studied, *hlyD* (58.6%) was more prevalent than *cnf1* (46.6%) and *hlyA* (36.8%), and *cnf1* and *hlyA* predominantly appeared in the group B2 isolates (p <

0.05). Additionally, capsule synthesis ranged from 23% (*kpsMT* K1) to 31% (*kpsMT* II), and invasion gene *ibeA* appeared in 11.5% of isolates.

With respect to the link between fluoroquinolone susceptibility and virulence gene profiles, the prevalence of 20 virulence genes tested ranged from 1.5% (*papG* allele I and *cvaC*) to 91.1% (*fimH*) among fluoroquinolone-susceptible isolates, and the majority of the investigated virulence genes showed grater prevalence in fluoroquinolone-susceptible isolates in respect to fluoroquinolone-resistant isolates, with the exception of *ireA* and *iutA* (Table 2). Moreover, an inverse relationship was found between the frequencies of virulence genes and the degree of resistant phenotype

(*i.e.*, fluoroquinolone-resistant MDR isolates harbored fewer virulence genes than did SDR isolates). In addition, a more detailed analysis revealed the association between resistance to trimethoprim-sulfamethoxazole and the presence of *hlyD*, and the association between resistance to gentamicin and *iroN*. More associations between resistance and specific virulence genes could not be found, as most of the virulence genes were detected in different combinations. Likewise, with respect to the link between virulence gene profiles and sources of the isolates, the fecal isolates (commensal isolates) were associated with significantly decreased virulence gene frequencies compared with the isolates from urine and

Table 2. The prevalence of virulence genes among 174 *E. coli* isolates from dogs and cats.

Virulence genes	Total (n = 174)	Fluoroquinolone susceptible (n = 68)	Fluoroquinolone resistant (n = 106)	P value Fluoroquinolone susceptible *vs* fluoroquinolone resistant
Adhesins				
fimH	142 (81.6%)	62 (91.1%)	80 (75.5%)	0.0080
papA	87 (50%)	47 (69.1%)	40 (37.7%)	0.0044
papG	67 (38.5%)	36 (52.9%)	31 (29.2%)	0.0012
papG allele I	1 (0.6%)	1 (1.5%)	0 (0)	0.0205
papG allele II	6 (3.4%)	5 (7.4%)	1 (1.0%)	0.0038
papG allele III	41 (23.6%)	24 (35.3%)	17 (16.0%)	0.0053
sfa/focDE	104 (59.8%)	55 (80.9%)	49 (43.4%)	0.0004
focG	78 (44.8%)	36 (52.9%)	42 (39.6%)	0.0074
Toxins				
hlyA	64 (36.8%)	35 (51.5%)	29 (27.4%)	0.0021
hlyD	102 (58.6%)	60 (88.2%)	42 (39.6%)	0.0015
cnf1	81 (46.6%)	49 (72.1%)	32 (30.2%)	0.0001
Capsule synthesis				
kpsMT II	54 (31%)	24 (35.3%)	30 (28.3%)	0.0460
kpsMT K1	40 (23%)	18 (26.5%)	22 (20.8%)	0.0705
Siderophores				
fyuA	124 (73%)	60 (88.2%)	64 (60.4%)	0.0010
iroN	106 (60.9%)	52 (76.5%)	54 (50.9%)	0.0013
ireA	25 (14.4%)	8 (11.8%)	17 (16.0%)	0.2879
iutA	118 (67.8%)	37 (54.4%)	81 (76.4%)	0.0012
Invasin				
ibeA	20 (11.5%)	8 (11.8%)	12 (11.3%)	0.5734
Others				
traT	66 (37.9%)	35 (51.5%)	31 (29.2%)	< 0.0001
cvaC	1 (0.6%)	1 (1.5%)	0 (0)	0.0205
Phylogenetic group				
A	18 (10.3%)	6 (8.8%)	12 (11.3%)	0.1879
B1	35 (20.1%)	11 (16.2%)	24 (22.6%)	0.0460
B2	81 (46.6%)	40 (58.8%)	41 (38.7%)	0.0012
D	24 (13.8%)	7 (10.3%)	17 (16.0%)	0.0365
C	7 (4.0%)	3 (4.4%)	4 (3.8%)	0.2954
E	6 (3.4%)	4 (5.9%)	2 (1.9%)	0.0309
F	3 (1.7)	2 (2.9%)	1 (0.9%)	0.0318

The 20 virulence factors analysed were *fimH*: mannose-specific adhesin oftype 1fimbriae; *papA*: P; fimbriae structural subunit; *papG*: p fimbriae adhesin (and alleles I, II, and III); *sfa/focDE*: S and F1C; fimbriae; *focG*: F1C fimbriae; *hlyD*, *hlyA*: α-haemolysin; *cnf1*: cytotoxic necrotizing factor type 1; *kpsM*; II: group 2 capsule in addition to specifically targeting K1 gene; *fyuA*: ferric yersiniabactin receptor; *iroN*: almochelin receptor; *ireA*: iron-responsive element gene; *iutA*: aerobactin receptor; *ibeA*: invasion of brain endothelium; *traT*: serum-resistance associated; and *cvaC*: colicin V.

blood (pathogenic isolates) (p < 0.01), while no significant differences emerged between the clinical urinary and blood isolates for the majority of studied virulence genes (Table 3).

Moreover, 16 ESBL-producing *E. coli* isolates from both pathogenic (87.5%; 14/16) and commensal (12.5%; 2/16) isolates belonged to phylogenetic groups B2 (10/16), group D (4/16), and group A (2/16), respectively. It is noteworthy that *hlyA*, *hlyD*, and *iutA* were more prevalent in ESBL-producing isolates than in non-ESBL-producing isolates (p < 0.05).

MLST

MLST analysis revealed that 174 *E. coli* isolates analyzed were assigned to 65 STs, including 3 novel STs, which were due to new combinations of previously known alleles, and the isolates of the same STs generally showed similar drug resistance profiles. More highly diverse populations were found in pathogenic isolates and fluoroquinolone-resistant isolates, as they were represented by 54 STs and 48 STs, respectively. ST69 (18.4%; 32/174) was the predominant ST, followed by ST648 (13.2%; 23/174), ST73 (10.9%; 19/174), ST405 (10.3%; 18/174), ST12 (10.3%; 18/174), and ST131 (9.8%; 17/174). Other sequence types, such as ST23, ST38, ST10, ST167, ST641, ST127, ST1294, ST354, ST4002, and ST3388 were also detected in the present study. The link between STs and the sources of the isolates revealed that ST73 and ST12 accounted for nearly 50% of the commensal isolates; clonal groups ST648, ST131, and ST69 were the most frequent STs in the urine isolates, while ST69

Table 3. Prevalence of virulence genes among *E. coli* isolates from the feces (n = 37), urine (n = 72), and blood (n = 37) of dogs and cats.

Virulence genes	Source of *E. coli* isolates			P value		
	Feces (n = 37)	Urine (n = 96)	Blood (n = 41)	Feces *vs* Urine	Feces *vs* Blood	Urine *vs* Blood
Adhesins						
fimH	16 (42.2%)	92 (95.8%)	34 (82.9%)	0.0003	0.0005	0.2879
papA	9 (24.3%)	56 (57.3%)	22 (53.7%)	< 0.0001	< 0.0001	0.0376
papG	4 (10.8%)	45 (46.9%)	17 (41.5%)	< 0.0001	< 0.0001	0.3739
papG allele I	0 (0)	1 (1.0%)	0 (0)	0.0236	1	0.0236
papG allele II	0 (0)	4 (4.2%)	2 (4.9%)	< 0.0001	< 0.0001	0.2746
papG allele III	3 (8.1%)	27 (28.1%)	11 (26.8%)	< 0.0001	0.0002	0.2355
sfa/focDE	12 (32.4%)	66 (68.8%)	26 (63.4%)	< 0.0001	< 0.0001	0.0721
focG	7 (18.9%)	51 (53.1%)	20 (48.8%)	< 0.0001	< 0.0001	0.2962
Toxins						
hlyA	3 (8.1%)	45 (46.9%)	16 (39.0%)	< 0.0001	< 0.0001	0.5522
hlyD	12 (32.4%)	59 (61.5%)	31 (75.6%)	0.0001	< 0.0001	0.2946
cnf1	13 (35.1%)	50 (52.1%)	18 (43.9%)	0.0004	0.0006	0.3827
Capsule synthesis						
kpsMT II	3 (8.1%)	38 (39.6%)	13 (31.7%)	0.0002	0.0002	0.3401
kpsMT K1	2 (5.4%)	26 (27.1%)	12 (29.3%)	0.0006	0.0006	0.9483
Siderophores						
fyuA	15 (27.0%)	87 (90.6%)	22 (53.7%)	0.0001	0.0018	0.0079
iroN	13 (32.4%)	66 (68.8%)	27 (65.9%)	0.0005	0.0009	0.1786
ireA	1 (2.7%)	17 (17.7%)	7 (17.1%)	0.0003	0.0004	0.6520
iutA	18 (48.6%)	71 (74.0%)	29 (7.3%)	0.0003	0.0005	0.5152
Invasin						
ibeA	2 (5.4%)	15 (15.6%)	3 (8.1%)	0.0016	0.0756	0.0090
Others						
traT	6 (16.2%)	47 (49.0%)	13 (31.7%)	0.0012	0.0211	0.0057
cvaC	0 (0)	1 (1.0%)	0 (0)	0.0236	1	0.0236
Phylogenetic group						
A	6 (16.2%)	10 (10.4%)	2 (4.9%)	0.0070	0.0101	0.8119
B1	15 (40.5%)	15 (15.6%)	5 (12.2%)	0.0030	0.0045	0.1429
B2	10 (27.0%)	49 (51.0%)	22 (53.7%)	0.0007	0.0009	0.5412
D	3 (8.1%)	16 (16.7%)	5 (12.2%)	0.1785	0.3757	0.5304
C	0 (0)	3 (3.1%)	3 (7.3%)	0.6164	0.0147	0.0198
E	2 (5.4%)	3 (3.1%)	1 (2.4%)	0.5734	0.4472	0.9140
F	1 (2.7%)	0 (0)	2 (4.9%)	0.0201	0.0273	0.0042

and ST73 were the predominant clonal groups in the isolates from blood. Additionally, 56.3% (9/16) of ESBL-producing *E. coli* isolates were assigned into ST131 clone. Moreover, ST12, ST131, ST73, and ST69 isolates harbored more virulence genes compared with other isolates (p < 0.05).

Discussion

In China, several studies focused on virulence gene frequencies in *E. coli* isolates from food-producing animals [9,17-19] but not from companion animals. The present study explored virulence gene frequencies and the association between virulence gene profiles, phylogenetic group, ST type, and fluoroquinolone resistance among *E. coli* from dogs and cats in China. Our study revealed high resistance to tetracycline, ampicillin, amoxicillin/clavulanic acid, trimethoprim-sulfamethoxazole, gentamicin, and enrofloxacin, and showed a similar pattern found in another study among *E. coli* isolates from companion animals in China [20]. The resistance rate to fluoroquinolones (60.9%) was similar to that found in previous studies among food-producing animals and humans in China [21,22], while it was significantly greater than the fluoroquinolone resistance rates (about 10%) in companion animals in the United States and Portugal [23,24]. Increased fluoroquinolone resistance can limit the treatment of *E. coli* infection in companion animals, and in turn may limit the treatment options in humans, who may contract *E. coli* infections from their pets. The possible reasons for the high antibacterial resistance rates include the inappropriate use of antimicrobials in small-animal clinics and cross-resistance among the antimicrobials of the same class or across different antimicrobial categories.

Usually, commensal *E. coli* isolates derive from phylogenetic groups A and B1, while most ExPEC isolates usually belong to the B2 and D groups and harbor more virulence genes, which allows them to induce diseases in both healthy and compromised hosts [25-28]. Furthermore, it has been reported that antimicrobial-susceptible and -resistant ExPEC isolates are fundamentally different bacterial populations [19,29,30]. Our data certified that both fluoroquinolone-susceptible and resistant isolates mainly belonged to phylogenetic group B2, moreover, the fluoroquinolone-susceptible isolates were significantly associated with group B2 ones (58.8% *vs* 38.7% of fluoroquinolone-resistant isolates, p < 0.01). One possible reason is that some isolates grouped into group D with triplex PCR were classified into group E based on the new quadruplex method. Another possible

reason is that the isolates came from different sources, patients, and geographic locations.

One focus of this study was to clarify the association between fluoroquinolone resistance and virulence gene frequencies, although it is very complex. Overall, the majority of the virulence genes tested were more prevalent in fluoroquinolone-susceptible isolates than in their resistant counterparts, with the exception of *ireA* and *iutA*, which is consistent with a previous study that reported that *ireA* and *iutA* were significantly more prevalent in fluoroquinolone-resistant isolates [10]. Additionally, all investigated virulence genes were more prevalent in pathogenic isolates than in commensal isolates, consistent with previous studies [2,31]. The *fimH* gene (81.6%) was the most frequent virulence gene, particularly as it appeared in 95.8% of uropathogenic isolates, which is in accordance with a previous study that reported that the *fimH* gene played an important role in the initiation of colonization and infection, particularly in urinary tract infections [24,32]. *papG* allele I (0.6%) and *papG* allele II (3.4%) were rarely found compared with the *papG* allele III (23.6%), indicating that they have little importance in the pathogenesis of *E. coli*. Furthermore, as more and more people keep dogs and cats in China, the occurrence of resistant bacteria is gradually increasing with the widespread use of antimicrobial agents. It is necessary to further study the resistance of *E. coli* that can be potentially transmitted from pet animals to humans, and then from humans to food animals, as there is a strong association between resistance and virulence genes.

Previous studies indicated that MDR isolates exhibited significantly reduced virulence traits compared with those from susceptible isolates [26,28,29]; this was also confirmed by the present study in fluoroquinolone-resistant MDR isolates. Moreover, there is an inverse relationship between the frequencies of virulence genes and the degree of MDR phenotype, videlicet, the fluoroquinolone-resistant MDR isolates bearing less virulence genes showed resistance to a greater range of drug classes. However, a negative correlation between antimicrobial resistance and the occurrence of virulence genes was reported in a previous study [33]. The possible reasons were that the correlations between antimicrobial resistance and virulence genes may vary depending on animal origin, antimicrobials, virulence gene types, and even the geographical origin of the isolates being investigated.

MLST was developed as a scalable typing system to determine the diversity and phylogenetic relationships of the isolates. Our results revealed that

the pathogenic isolates had a richer ST diversity than did the commensal isolates, with the corresponding ratio of ST types to number of isolates of 39.4% (54/137) and 29.7% (11/37), respectively. Similarly, fluoroquinolone-resistant isolates had a richer ST diversity than did their susceptible counterparts. Some STs, such as ST10, ST38, ST69, and ST131 identified in this study have also been reported in human and other animals according to data from the MLST database, which provided further evidence that *E. coli* from companion animals are probable sources of human infections. Moreover, it is noteworthy that 17 ST131 isolates associated with fluoroquinolone-resistant MDR were identified in pathogenic isolates. Ongoing study of ST131 isolates in the future is necessary, as they are now rapidly and globally disseminated.

Conclusions

In summary, our results revealed a clear association between fluoroquinolone resistance, virulence gene profile, phylogenetic group, ST type, and source of *E. coli* isolates. Overall, pathogenic isolates and fluoroquinolone-susceptible isolates possessed more virulence genes. There is an inverse relationship between the prevalence of virulence genes and the resistant phenotype, and the greater the resistance to more classes of antibiotics, the lower the frequency of virulence genes. The MLST analyses certified that a highly diverse population was found in pathogenic isolates and fluoroquinolone-resistant isolates. Our results suggest that fluoroquinolone-resistant *E. coli* isolates are common among dogs and cats in China, and also suggest high virulence gene frequencies and high population diversity in *E. coli* among dogs and cats in China.

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