Occurrence and molecular epidemiology of *fosA3*-bearing *Escherichia coli* from ducks in Shandong province of China

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ABSTRACT The plasmid-borne fosfomycin resistance gene *fosA3* has been identified in *Escherichia coli* (***E. coli***) from various animals but has rarely been reported in ducks. In this study, we investigated the *fosA3* prevalence and molecular characteristics of *fosA3*-harboring *E. coli* strains from ducks in Shandong province of China. In 416 *E. coli* isolates, 91 (21.88%) were identified as *fosA3*-bearing strains, and the fosfomycin-resistant phenotype of 88 of the 91 *fosA3*-harboring strains was successfully transferred to the recipient strains. Seven different genetic structures surrounding the *fosA3* gene were detected and 2 new contexts were discovered among the *fosA3*-carrying *E. coli*. Twenty *fosA3*-harboring isolates and their trans-conjugants were randomly selected for pulsed-field gel electrophoresis (**PFGE**) typing and S1-nuclease PFGE, respectively. The PFGE patterns revealed that the 20 randomly selected *fosA3*-bearing isolates were not a result of clonal dissemination. S1-PFGE showed that 15 of the 20 randomly selected trans-conjugants carried a single plasmid, and these 15 plasmids that harbored *fosA3* (55−190 kb) were distributed into the following replicon types: IncF (n = 11), IncI1 (n = 1), IncN (n = 1), untypable (n = 1), and W-FIC (n = 1). Additionally, as vectors for *fosA3* in *E. coli*, F-: A1:B6, N/ST1, IncI1/ST2, W-FIC, and one untypable plasmid had never been reported before. These observations highlighted the importance of ducks as a reservoir for multidrug-resistant *fosA3*-carrying *E. coli*.

Key words: *Escherichia coli*, *fosA3*, genetic environment, ducks, plasmids

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INTRODUCTION

The members of Enterobacteriaceae, particularly extraintestinal pathogenic *Escherichia coli* (**ExPEC**), are some of the most important zoonotic pathogens. ExPEC causes a wide range of infections in mammals and birds (Mellata, 2013). ExPEC isolates from human and avian pathogenic *E. coli* (**APEC**) strains may share similar virulence genes and capacities to cause disease (Johnson et al., 2008). Previous studies have suggested that poultry is a reservoir of ExPEC (Ewers et al., 2007; Johnson et al., 2008; Kobayashi et al., 2011; Manges and Johnson, 2012; Mellata, 2013). Multidrug-resistant (**MDR**) ExPEC isolates pose a zoonotic risk because they can spread from poultry to humans through food or the environment (Johnson et al., 2008; Manges and Johnson, 2012).

Because of the recent emergence of MDR and pandrug-resistant pathogens, fosfomycin, an older antibiotic agent, has been reintroduced as a first-line drug against uncomplicated urinary tract infections almost all over the world. However, the widespread dissemination of *fosA*-like genes (*fosA1*−*fosA10*) is seriously threatening the clinical effectiveness of fosfomycin (Wachino et al., 2010; Yang et al., 2019; Huang et al., 2020). *fosA3* is the most prevalent plasmid-mediated fosfomycin resistance gene (Yang et al., 2019). Presently, *fosA3* is widely distributed among *E. coli* strains isolated from Asian countries, the United States, and Europe (Wachino et al., 2010; Hou et al., 2012; Lee et al., 2012; Alrowais et al., 2015; Benzerara et al., 2017; Wang et al., 2017; Sorlozano-Puerto et al., 2020). Therefore, the possible global dissemination of *fosA3*-bearing *E. coli* isolates has become a serious public health concern (Zurfluh et al., 2020). Conjugative plasmids, particularly IncF and IncN plasmids seem to play a predominant role in the rapid dissemination of the *fosA3* gene (Yang et al., 2014; Villa et al., 2015; Huang et al., 2020; Zurfluh et al., 2020).

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Although the use of fosfomycin is forbidden in animals raised for food in China, an increasing number of studies have reported *fosA3*-bearing *E. coli* isolates recovered from pets, wildlife, pigs, chickens, and cows in China (Hou et al., 2012; Ho et al., 2013a; Wang et al., 2017). To date, there are few reports on the occurrence of plasmid-mediated fosfomycin resistance gene *fosA3* among *E. coli* strains from ducks. In this study, we investigated the prevalence of mobile fosfomycin resistance gene *fosA3* and the molecular characteristics of *fosA3*-harboring *E. coli* strains from ducks in Shandong province of China.

MATERIALS AND METHODS

Bacterial Isolates

E. coli isolates were recovered from fecal swab samples of healthy ducks and different tissues and organs (heart, lung, spleen, liver, and kidney) of diseased ducks from 23 farms in Shandong from September 2014 to February 2018. The animal experiments were performed in accordance with the guidelines issued by the Shandong Agricultural University Animal Care and Use Committee (Approval Number: # SDAUA-2018-045). All samples were placed in LB broth and shaken sufficiently at 37°C for 24 h. A loop of the broth was seeded onto MacConkey agar plates containing 128 μg/mL fosfomycin and 25 μg/mL glucose-6-phosphate (**G-6-P**) and incubated for 24 h at 37°C. From one sample, only a single isolate could be obtained. Pink and round colonies were picked and identified using MALDI-TOF MS (Shimadzu, Japan) and 16S rDNA analysis.

Antimicrobial Susceptibility Testing

The minimum inhibitory concentrations (**MICs**) of 9 antibiotics (fosfomycin, tetracycline, gentamicin, imipenem, ciprofloxacin, cefotaxime, colistin, amikacin, and florfenicol) were tested by the agar dilution method or broth microdilution method for the *E. coli* isolates selected from MacConkey agar plates using the recommended break points (CLSI, 2018). The MICs of fosfomycin were determined by the agar dilution method on Mueller-Hinton agar supplemented with 25 μg/mL G-6-P. *E. coli* ATCC 25922 was used as the control strain. All fosfomycin-resistant *E. coli* underwent polymerase chain reaction (**PCR**) assays to detect the *fosA3* gene (Hou et al., 2012).

Molecular Detection

The existence of *floR,* extended-spectrum β-lactamase (**ESBL**), *rmtB, mcr-1*, and *tet*(A) was also determined by PCR and sequencing (Maynard et al., 2003; Stürenburg et al., 2004; Chen et al., 2007; Liu et al., 2016). The virulence genes, namely *sfa/foc* (S/F1C fimbriae), *papA*, and/or *papC* (P fimbriae), *afa/dra* (Dr-binding adhesins), *kpsMT* II (group 2 capsules synthesis), and *iut*A (aerobactin system), were screened using previously published primers (Johnson and Stell, 2000). The isolate was defined as ExPEC if it carried 2 or more of these 5 virulence factors (Johnson et al., 2003). The surrounding regions of the *fosA3* gene were determined by PCR mapping using previously published primers (Hou et al., 2012; Sato et al., 2013).

Conjugation Experiments

Conjugation experiments were performed using filter mating with azide-resistant *E. coli* J53 as the recipient (Borgia et al., 2012). Trans conjugants were selected on MacConkey agar (200 μg/mL sodium azide, 256 μg/mL fosfomycin, and 25 μg/mL G-6-P) and confirmed by the enterobacterial repetitive intergenic consensus (**ERIC**)-PCR method (Versalovic et al., 1991). Resistance genes [*fosA3, floR,* β-Lactamase, *rmtB, mcr-1*, and *tet* (A)], virulence genes (*sfa/foc, papA*, and/or *papC, afa/dra, kpsMT* II, and *iut*A), and the MICs of trans conjugants were also detected as described above.

Multilocus Sequence Typing and Phylogenetic Characterization

Multilocus sequence typing (**MLST**) was used to genotype the 91 *fosA3*-positive *E. coli* isolates as previously described (Tartof et al., 2005). Triplex PCR was used to perform the phylogenetic classification as previously described (Clermont et al., 2000).

Pulsed-Field Gel Electrophoresis and Plasmid Analysis

Twenty *fosA3*-positive isolates were randomly selected for pulsed-field gel electrophoresis (**PFGE**) and plasmid analysis. Using the CHEF-MAPPER System (Bio-Rad Laboratories, CA), XbaI-PFGE was performed as described previously (Gautom, 1997). A phylogenetic analysis of PFGE patterns was performed using the PyElph software version 1.4 (Pavel and Vasile, 2012). The UPGMA method was used for clustering.

Plasmids carried by trans-conjugants of the randomly selected *fosA3*-harboring strains were assessed using S1 nuclease PFGE (**S1-PFGE**) (Gautom, 1997). Trans-conjugants with a single plasmid were used for the plasmid analysis by PCR-based replicon typing (**PBRT**) (Carattoli et al., 2005). The IncFII, IncN, and IncI1 plasmids were further typed as described previously (García-Fernández et al., 2008, 2011; Villa et al., 2010).

RESULTS

Detection of Antimicrobial Resistance Patterns and Resistance Genes

In this study, 416 *E. coli* strains were isolated from healthy (n = 120) and diseased (n = 296) ducks between September 2014 and February 2018. A total of 91

(21.88%) strains were resistant to fosfomycin, all of which harbored the resistance gene *fosA3*. The percentage of *fosA3*-positive *E. coli* strains (22.64%, 67/296) isolated from diseased ducks was slightly higher than the percentage (20.00%, 24/120) of *fosA3*-positive *E. coli* strains isolated from healthy ducks. These *fosA3*-harboring *E. coli* isolates were also resistant to other antibiotics including tetracycline (100%, 91/91), cefotaxime (98.90%, 90/91), florfenicol (98.90%, 90/91), ciprofloxacin (95.60%, 87/91), gentamicin (87.91%, 80/91), amikacin (25.27%, 23/91), and colistin (12.09%, 11/91). All 91 *fosA3*-harboring *E. coli* strains were MDR strains (resistant to agents from at least 3 classes), and 72 (79.12%) strains were resistant to more than 5 antibiotics. Fortunately, all 91 *fosA3*-bearing *E. coli* were sensitive to imipenem. PCR amplification and sequencing confirmed that 76 of the 91 strains harbored $bla_{\text{CTX-M}}$, and $bla_{\text{CTX-M-55}}$ was the most prevalent variant (40.79%, 31/76), followed by $bla_{\text{CTX-M-14}}$ (22.37%, 17/76), $bla_{\text{CTX-M-64}}$ (11.84%, 9/76), $bla_{\text{CTX-M-24}}$ (7.89%, 6/76), $bla_{\text{CTX-M-65}}$ (6.58%, 5/76), $bla_{\text{CTX-M-123}}$ (6.58%, 5/76), and $bla_{\text{CTX-M-224}}$ (3.95%, 3/76). Among the 91 *fosA3*-carrying isolates, 72 (79.12%), 72 (79.12%), 61 (67.03%), 4 (4.40%), and 3 (3.30%) isolates harbored the *tet* (A), *floR*, $bla_{\text{TEM-1}}$, *rmtB*, and *mcr-1* genes, respectively.

The conjugation experiment and ERIC-PCR indicated that the *fosA3* gene was successfully transferred from 88 (96.70%, 88/91) donors to the recipient *E. coli* J53 at conjugation frequencies of 4.45×10^{-7} to 3.64×10^{-1} (trans-conjugants/recipients). A total of 71 trans-conjugants of *fosA3*-harboring *E. coli* were MDR, while the recipient strain *E. coli* J53 was sensitive to the abovementioned 9 agents. Furthermore, the 88 trans-conjugants showed resistance to cefotaxime (89.77%, 79/88), tetracycline (81.82%, 72/88), florfenicol (72.73%, 64/88), ciprofloxacin (36.36%, 32/88), gentamicin (35.23%, 31/88), amikacin (12.5%, 11/88), and colistin (3.41%, 3/88). Among the trans-conjugants, $bla_{\text{CTX-M}}$ (71.59%, 63/88), *tetA* (69.32%, 61/88), *floR* (59.09%, 52/88), *rmtB* (4.55%, 4/88) were co-transferred with *fosA3*. The $bla_{\text{CTX-M}}$ variants included $bla_{\text{CTX-M-55}}$ (39.68%, 25/63), $bla_{\text{CTX-M-14}}$ (23.81%, 15/63), $bla_{\text{CTX-M-64}}$ (11.11%, 7/63), $bla_{\text{CTX-M-65}}$ (7.94%, 5/63), $bla_{\text{CTX-M-123}}$ (7.94%, 5/63), $bla_{\text{CTX-M-24}}$ (6.35%, 4/63), and $bla_{\text{CTX-M-224}}$ (3.17%, 2/63).

Phylogenetic Groups and Virulence Genes

Among the 91 *fosA3*-bearing *E. coli* isolates, 50 (54.95%), 26 (28.57%), and 15 (16.48%) belonged to groups A, D, and B1, respectively, according to the phylogenetic group analysis (Table 1). A total of 46 isolates carried virulence genes, including 8 isolates that carried 2 or more virulence genes and were classified as ExPEC (Table 1). Seven of the 8 ExPEC strains were isolated from diseased ducks and one was isolated from a healthy duck. None of the other virulence genes were found in trans-conjugants except for *iutA*. The eight ExPEC strains belonged to phylogroups A (5 isolates) and D (3 isolates; Table 1).

Strain Typing

According to the MLST analysis, the 91 *fosA3*-harboring isolates belonged to 58 STs, and ST69 (12/91) was the most prevalent type. The PFGE analysis revealed a high genetic diversity of the 20 randomly selected *fosA3*-bearing *E. coli* isolates (Figure 1).

Genetic Environments of fosA3

This study identified 7 different genetic contexts associated with the *fosA3* gene (Table 2, Figure 2). The *fosA3* gene was flanked by 2 copies of IS*26* in 76 isolates. Two novel genetic contexts (types I and II) were first identified in this study (accession no. MW221981 and MW221982). Type I structure IS*26*-*fosA3*-*orf1*-*orf2*-*orf3*-IS*26*-hp-intI1-IS*26*-$bla_{\text{TEM-1}}$-hp-hp-$bla_{\text{CTX-M-55}}$-hp-IS*26* was highly similar to part of plasmid pCTX-M-55_05-237 (accession no. CP026576.2) whereas type II structure IS*26*-hp-$bla_{\text{CTX-M-55}}$-hp-hp-$bla_{\text{TEM-1}}$-IS*26*-*fosA3*-*orf1*-*orf2*-*orf3*-IS*26*-hp-intI1-IS*26* showed high similarity to part of plasmid pHNBC6-3 (accession no. MK079570.1). The type I structure was found in 17 *E. coli* isolates whereas the type II structure was identified in 11 isolates.

Plasmid Analysis

According to the S1-PFGE analysis, 15 of the 20 trans-conjugants of randomly selected isolates carried a single plasmid (Figure 3). The plasmids were 55 to 190 kb in size and were classified as the IncFII (n = 11), IncN (n = 1), IncI1 (n = 1), W-FIC (n = 1), and untypable (n = 1) replicon types (Table 3). According to replicon sequencing, the IncF replicons were divided into 5 subtypes: F16:A-:B- (n = 4), F33:A-:B- (n = 2), F18:A-:B- (n = 2), F24:A-:B- (n = 2), and F-:A1:B6 (n = 1). The other 2 plasmids belonged to IncN/ST1 and IncI1/ST2, respectively (Table 3).

Table 1. Distribution of virulence factors (Vfs) and phylogenetic groups among the 91 *fosA3*-harboring *Escherichia coli* isolates in this study.

Prevalence of Vfs	Phylogenetic group			Total no. (%) (*n* = 91)
	A	B1	D	
iutA	15	6	8	29 (31.87)
papC	3	0	3	6 (6.59)
sfa	0	0	2	2 (2.20)
*KpMT*II	0	1	0	1 (1.10)
iutA + *papC**	4	0	1	5 (5.49)
iutA + *sfa**	0	0	1	1 (1.10)
papC+*sfa**	1	0	0	1 (1.10)
iutA + *KpMT*II + *papC**	0	0	1	1 (1.10)
Total no. of isolates with Vfs	23	7	16	46 (50.55)
No of isolates without Vfs	27	8	10	45 (49.45)

*The ExPEC strains.

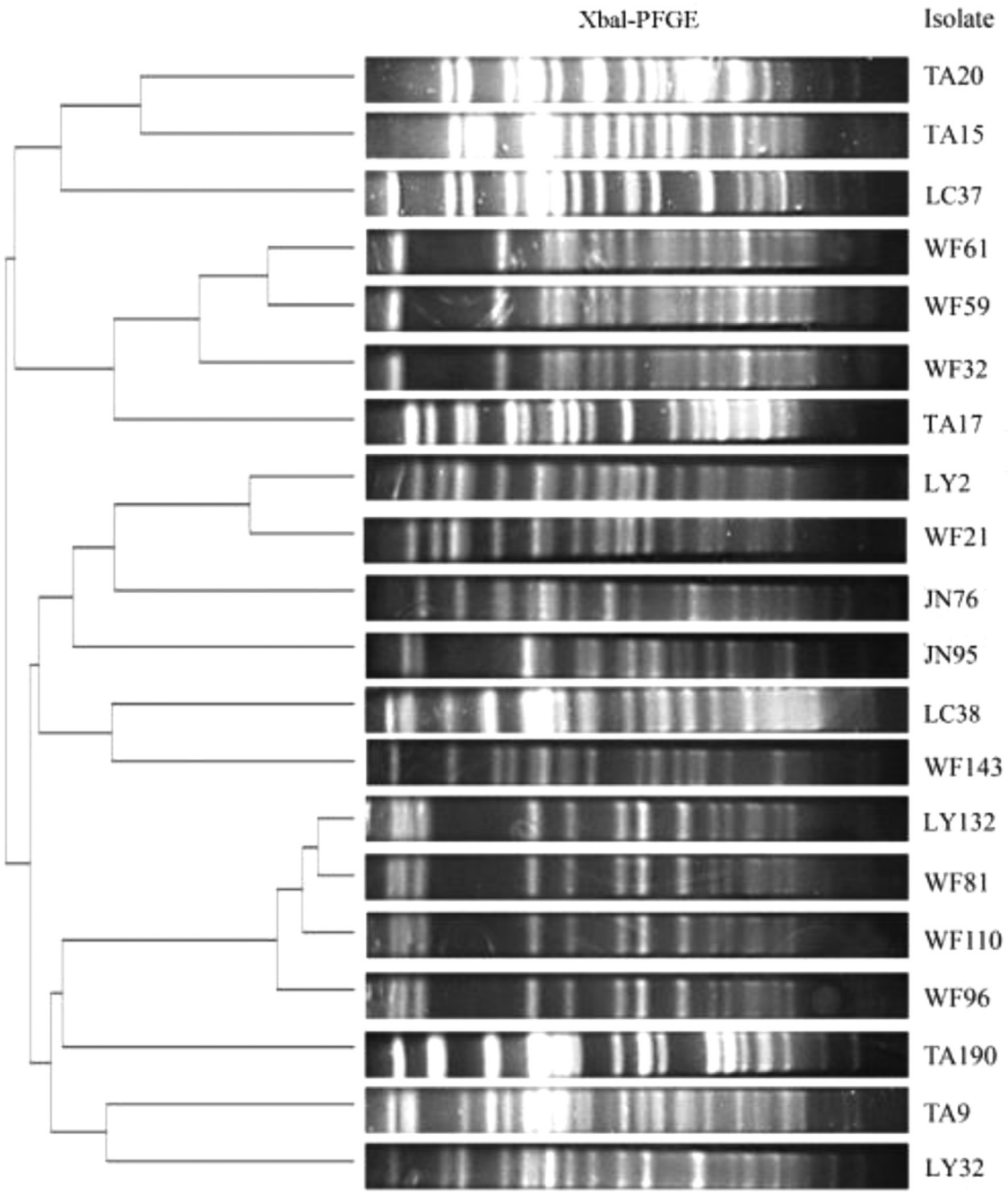

Figure 1. XbaI-PFGE dendrograms showing the genetic relationships of the 20 *fosA3*- positive *Escherichia coli* strains isolated in this study.

DISCUSSION

Food animals are considered reservoirs of drug-resistant bacteria, and resistance genes can be transmitted to humans through the food chain, which is an important mechanism of drug-resistant bacteria spread to humans (Hu et al., 2016; Cao et al., 2020). Although many *E. coli* isolates are common harmless bacteria, a particular subset can cause diverse diseases in humans and animals (Stromberg et al., 2017). China is the world's largest duck producer and an important exporter of this animal. Therefore, monitoring the antimicrobial resistance of *E. coli* in ducks and studying the transmission mechanisms of resistance genes are of great importance for guiding the rational use of antibiotics in production. Thus, this study investigated the occurrence of *fosA3*-positive *E. coli* in ducks.

Using fosfomycin in veterinary medicine has been forbidden in China since 2005. However, this study revealed an unexpectedly high prevalence of *fosA3* (21.88%) among *E. coli* isolates from ducks; this rate was much higher than the positive rate of the *fosA3* gene among humans (0.32−3.0%), pets, wild animals, pigs, cattle, and other food animals in China (0.75−10.54%) reported previously (Hou et al., 2012; Lee et al., 2012; Ho et al., 2013a, b; Wang et al., 2017). Therefore, this study investigated the potential dissemination mechanism of the *fosA3* gene behind this phenomenon.

Table 2. Molecular characteristics of the 20 *fosA3*-positive *Escherichia coli* strains isolated from ducks in this study.

Isolate	MLST	Group	Context of fosA3	Conjugation efficiency	Virulence genes	Other resistance genes	Resistant pattern
JN76	ST949	D	III	9.90×10^{-7}	*iutA*	$bla_{\text{CTX-M-123}}$, $bla_{\text{TEM-1}}$, *tet*(A), *floR*	FOS/CTX/FFC/TET/GEN/CIP/AK
JN95	ST69	B1	V	2.98×10^{-6}	*kpsMT* II	$bla_{\text{CTX-M-14}}$	FOS/CTX/FFC/TET/GEN/CIP/AK
LC37	ST2973	A	IV	1.70×10^{-5}	/	$bla_{\text{CTX-M-24}}$, *mcr-1*	FOS/CTX/FFC/TET/GEN/CL
LC38	ST164	A	VII	1.13×10^{-2}	/	$bla_{\text{CTX-M-55}}$, *tet*(A), *floR*	FOS/CTX/FFC/TET/GEN
LY2	ST1642	A	IV	5.34×10^{-7}	*iutA*	$bla_{\text{CTX-M-64}}$, $bla_{\text{TEM-1}}$, *tet*(A), *floR*	FOS/CTX/FFC/TET/GEN/CIP
LY32	ST69	D	III	4.45×10^{-7}	*iutA*	$bla_{\text{CTX-M-55}}$, $bla_{\text{TEM-1}}$, *floR*	FOS/CTX/FFC/TET/GEN/CIP/AK
LY132	ST398	A	I	1.88×10^{-4}	*papC*	$bla_{\text{CTX-M-55}}$, $bla_{\text{TEM-1}}$, *tet*(A), *floR*	FOS/CTX/FFC/TET/GEN/CIP
TA9	ST457	A	VII	1.65×10^{-5}	*iutA, papC*	*floR, mcr-1*	FOS/FFC/TET/CIP/CL
TA17	ST410	B1	IV	2.41×10^{-5}	*iutA*	*rmtB, mcr-1*	FOS/CTX/FFC/TET/CIP/AK/CL
TA20	ST2973	A	II	2.16×10^{-3}	*iutA*	$bla_{\text{CTX-M-55}}$, $bla_{\text{TEM-1}}$, *floR, mcr-1*	FOS /CTX/FFC/TET/CIP/CL
TA15	ST69	A	I	3.64×10^{-1}	*iutA*	*mcr-1,* $bla_{\text{TEM-1}}$	FOS/CTX/CIP/TET/AK/CL
TA190	ST50	A	V	1.23×10^{-4}	/	$bla_{\text{CTX-M-14}}$, *tet*(A), *floR*	FOS/CTX/FFC/TET/GEN/CIP/AK
WF21	ST2179	A	III	1.36×10^{-3}	*iutA, papC*	$bla_{\text{CTX-M-224}}$, $bla_{\text{TEM-1}}$, *tet*(A), *floR*	FOS/CTX/FFC/TET/GEN/CIP
WF32	ST69	D	II	6.61×10^{-4}	*iutA*	$bla_{\text{CTX-M-55}}$, $bla_{\text{TEM-1}}$, *tet*(A), *floR*	FOS/CTX/FFC/TET/GEN/CIP
WF59	ST69	D	II	5.53×10^{-5}	*iutA*	$bla_{\text{CTX-M-55}}$, $bla_{\text{TEM-1}}$, *tet*(A), *floR*	FOS/CTX/FFC/TET/GEN/CIP
WF61	ST69	D	IV	2.17×10^{-5}	*iutA*	$bla_{\text{CTX-M-55}}$, *tet*(A), *floR*	FOS/CTX/FFC/TET/GEN/CIP
WF81	ST398	A	III	2.98×10^{-6}	*papC, sfa*	$bla_{\text{CTX-M-64}}$, $bla_{\text{TEM-1}}$, *tet*(A), *floR*	FOS/CTX/FFC/TET /GEN/CIP/AK
WF96	ST398	A	I	8.79×10^{-7}	/	$bla_{\text{CTX-M-55}}$, $bla_{\text{TEM-1}}$, *tet*(A), *floR*	FOS/CTX/FFC/TET/GEN/CIP
WF110	ST398	A	VI	1.77×10^{-5}	/	$bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1}}$	FOS/CTX/FFC/TET/GEN/CIP
WF143	ST3944	D	VI	1.56×10^{-4}	*sfa*	$bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1}}$, *tet*(A), *floR*	FOS/CTX/FFC/TET/GEN/CIP/AK

Abbreviations: AK, amikacin; CIP, ciprofloxacin; CL, colistin; CTX, cefotaxime; FFC, florfenicol; FOS, fosfomycin; GEN, gentamicin; TET, tetracycline; .

In the present study, all *fosA3*-bearing *E. coli* strains were MDR and displayed high levels of resistance to cefotaxime, florfenicol, and tetracycline. The high occurrence of drug resistance among *fosA3*-containing *E. coli* may be associated with the overuse of these drugs in ducks on these farms. Among the 91 *fosA3*-bearing *E. coli*, 76 harbored $bla_{\text{CTX-M}}$, and $bla_{\text{CTX-M-55}}$ was the most prevalent $bla_{\text{CTX-M}}$ genotype. Furthermore, *fosA3* was co-localized with *tet*(A), *floR*, and *mcr-1* on the same *E. coli* strain in this study, highlighting the possibility that pan-drug-resistant bacteria are emerging (Yang et al., 2014; Wang et al., 2017; Liu et al., 2020). The coexistence with other resistance genes on the same strain is likely to accelerate the dissemination of *fosA3* by co-selection because tetracycline, cephalosporins, aminoglycosides, and florfenicol are used extensively in animal husbandry in China (Deng et al., 2013; Yang et al., 2014). This may be why a high frequency of *fosA3* was found in the *E. coli* isolates of ducks. The trans-conjugants harbored multiple resistance genes, which indicate that under certain selective pressures, the *fosA3*-bearing plasmids are very easily transferred to recipients J53 from *fosA3*-containing *E. coli*, leading to the spread of *fosA3* and other resistance genes, which creates some difficulties with clinical treatment.

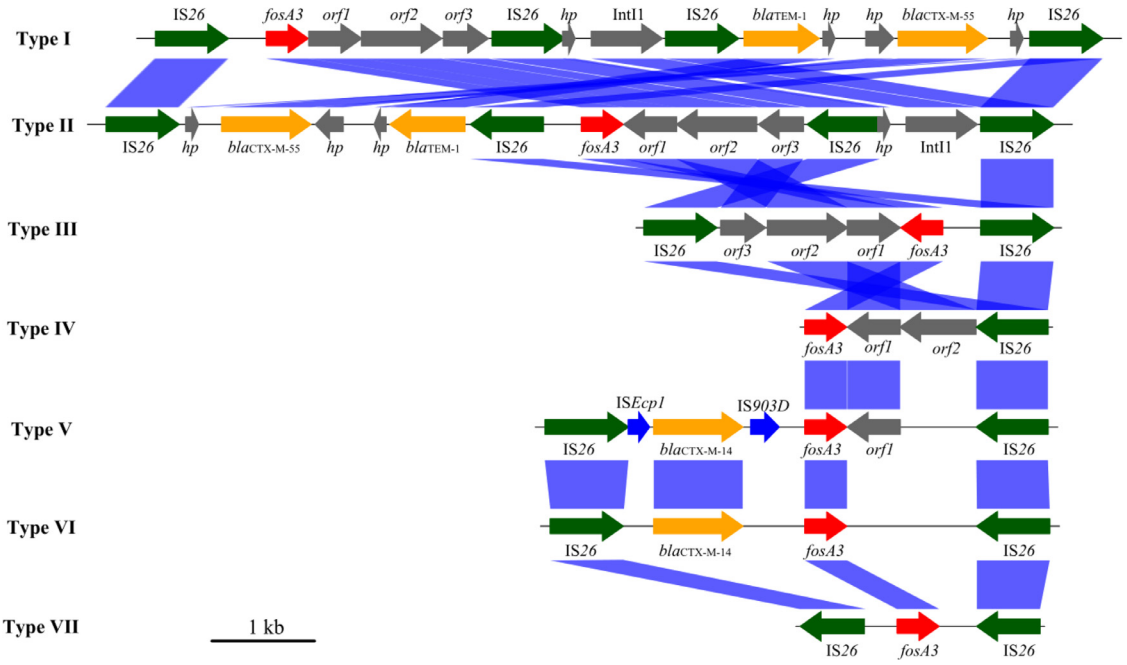

Figure 2. Schematic representation of the genetic environments of *fosA3*. Blue areas indicate regions with 100% nucleotide sequence identity, the position and orientation of the depicted genes shown by the arrowhead.

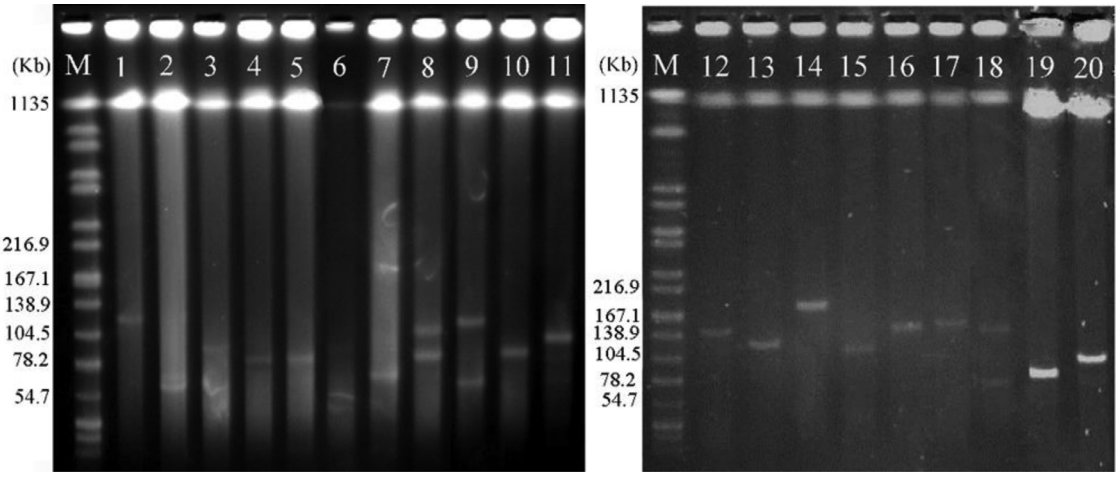

Figure 3. Identification of *fosA3* gene-carrying plasmids by S1-PFGE. Lanes 1-20: TA190, TA17, LC37, LC38, TA9, WF143, TA15, LY2, LY132, TA20, WF110, WF21, WF32, WF59, WF61, WF81, LY32, WF96, JN76, JN95. Lane M, *Salmonella* serovar Braenderup H9812.

APEC strains can cause colibacillosis in poultry, which leads to significant economic damage in the poultry industry every year. Certain groups of APEC strains possess zoonotic potential (Adiri et al., 2003; Mokady et al., 2005; Rodriguez-Siek et al., 2005; Ron et al., 2006). The current study identified 8 APEC strains, and the *iutA* gene was the most commonly detected gene in MDR strains, which was consistent with a prior study indicating that *iutA* was a marker for antimicrobial resistance and that the carriage of *iutA* allowed bacteria to easily colonize the human gut (Karami et al., 2017). Previous studies have shown that APEC strains and their plasmids might be transferred from poultry to humans (Levy et al., 1976). Fortunately, only 13 *iutA* were co−transferred with *fosA3* from *fosA3*-harboring *E. coli* to the recipient J53. The presence of *fosA3*−bearing APEC strains in ducks may pose serious risks to human public health. To our best knowledge, this is the first report on *fosA3*-harboring ExPEC identified in China. The 8 ExPEC strains belonged to phylogroups A (5 isolates) and D (3 isolates). Similar results were found in previous studies indicating that the majority of ExPEC strains belonged to groups A and D (Ewers et al., 2007; Johnson et al., 2008; Kobayashi et al., 2011). *FosA3*-harboring *E. coli* contain various virulence and resistance genes, which allow these strains to easily adapt to the environment, thus accelerating their further transmission to humans (Losada et al., 2016).

Previous studies have reported that *fosA3*-harboring *E. coli* isolates have been found in animals in Guangdong, Henan, Guangxi, Jilin, Liaoning, and Anhui provinces of China (Yang et al., 2014; Wang et al., 2017). In this study, the 91 *fosA3*-harboring *E. coli* strains were isolated from ducks in Shandong, which suggests that *fosA3*-carrying *E. coli* isolates are widespread among animals in China. The PFGE analysis revealed a high genetic diversity of 20 randomly selected *fosA3*-bearing *E. coli* isolates, and almost none of the strains were clonally related. Fifty-eight STs were detected, and ST69 (12/91) was the most prevalent type among the 91 *fosA3*-bearing isolates. ST69 is a dominant ExPEC lineage that causes urinary tract infections worldwide, and it has been detected in humans and food of animal origin

Table 3. Characterization of some plasmids carrying *fosA3* of transconjugants.

				fosA3-carrying plasmids	
plasmid	Co-transfer of other resistance genes	Co-transfer of virulence genes	Resistant pattern	Size (kb)	Replicon type
pTA190	$bla_{\text{CTX-M-14}}$, *floR*, *tet*(A)	/	FOS/CTX/TET/FFC	~120	W, FIC
pTA17	*rmtB*, *mcr-1*	*iutA*	FOS/FFC/AK/ CL	~ 65	F33:A-:B-
pLC37	*mcr-1*	/	FOS/CL	~90	F33:A-:B-
pLC38	$bla_{\text{CTX-M-55}}$, *tet*(A), *floR*	/	FOS/CTX/TET/FFC	~78	F16:A-:B-
pTA9	*floR*	*iutA*	FOS/FFC/CIP	~78	F16:A-:B-
pTA15	$bla_{\text{TEM-1}}$	/	FOS/CTX	~55	F16:A-:B-
pTA20	$bla_{\text{CTX-M-55}}$, *floR*,	/	FOS/CTX/TET/FFC	~78	F18:A-:B-
pWF21	$bla_{\text{CTX-M-224}}$, $bla_{\text{TEM-1}}$, *tet*(A)	/	FOS/CTX/TET	~140	F16:A-:B-
pWF32	$bla_{\text{CTX-M-55}}$, $bla_{\text{TEM-1}}$, *tet*(A)	*iutA*	FOS/CTX/TET	~120	F-:A1:B6
pWF59	$bla_{\text{CTX-M-55}}$, $bla_{\text{TEM-1}}$, *tet*(A)	*iutA*	FOS/CTX/TET	~190	N/ST1
pWF61	$bla_{\text{CTX-M-55}}$, *tet*(A)	/	FOS/CTX/TET	~110	untypable
pWF81	$bla_{\text{CTX-M-64}}$,$bla_{\text{TEM-1}}$, *tet*(A), *floR*	/	FOS/CTX/TET/FFC	~140	F24:A-:B-
pLY32	$bla_{\text{CTX-M-55}}$, $bla_{\text{TEM-1}}$, *tet*(A), *floR*	/	FOS/CTX/TET/FFC	~140	F24:A-:B-
pJN76	$bla_{\text{CTX-M-123}}$	*iutA*	FOS/CTX	~90	F18:A-:B-
pJN95	$bla_{\text{CTX-M-14}}$	/	FOS/CTX/GEN	~105	IncI1/ST2

Abbreviations: AK, amikacin; CL, colistin; CIP, ciprofloxacin; CTX, cefotaxime; FFC, florfenicol; FOS, fosfomycin; GEN, gentamicin; TET, tetracycline; .

(Riley, 2014; Giacobbe et al., 2015; Hammad et al., 2019). The strong linkage of *fosA3* ST69 isolates found in this study warrants further attention to this type of *E. coli*. In this study, ST410 was assigned to two *fosA3*-containing *E. coli* strains that were previously found to harbor *mcr-1* in isolates recovered from food and human samples worldwide (Falgenhauer et al., 2016; Rocha et al., 2017). Based on the PFGE and MLST results, the high positive rate of *fosA3* was not due to clonal dissemination. Furthermore, this work identified 7 genetic environments of *fosA3* among the *fosA3*-containing *E. coli* strains. In 6 structures (types I, II, III, V, VI, and VII), *fosA3* was flanked by IS*26* elements. He and colleagues found that an IS*26*-flanked composite transposon may form a circular intermediate that could accelerate the spread of *fosA3* (Harmer and Hall., 2016).

Conjugative plasmids including IncFII, IncI1, and IncN play important roles in the spread of *fosA3* (Sun et al., 2012; Yang et al., 2014; Wang et al., 2017). In this study, conjugative plasmids of incompatibility groups IncFII, IncN, and IncI1 were found, and 11 plasmids belonged to IncFII replicons. Among the 11 IncFII plasmids, 4 F16:A-:B- plasmids were the predominant replicons identified in Shandong. These data indicate that F16:A-:B- plasmids play a particular role in spreading the *fosA3* gene in Shandong. In our study, two F33:A-:B- plasmids carrying *fosA3* were identified in 15 *E. coli* isolates. Similar plasmids harboring *fosA3* were also found in *E. coli* isolated from poultry in different geographical locations of China (Yang et al., 2014; Yang et al., 2016; Wang et al., 2017). These findings indicate that F33:A-:B- plasmids drive the spread of *fosA3* in poultry farms in China (Yang et al., 2014). Since IncFII plasmids are efficient tools for spreading resistance genes in *Enterobacteriaceae*, the coexistence of the *fosA3* gene with other resistance determinants in these plasmids is worrisome (Carattoli, 2011). In a previous study, an IncI1/ST2 plasmid with no *fosA3* was found in one isolate from a human in Australia (Tagg et al., 2014). Interestingly, this study identified one *fosA3*-positive IncI1/ST2 plasmid. Previous studies have shown that IS*26* is an important vehicle in spreading antimicrobial resistance genes in gram-negative bacilli (Harmer et al., 2014; He et al., 2015). The insertion IS*26* elements surrounding *fosA3* might play a critical role in the transfer of *fosA3* between these plasmids. IncN plasmids harboring *fosA3* have been identified in *E. coli* in China and in *Klebsiella pneumonia* in Korea (Lee et al., 2012; Wang et al., 2017). This replicon-type plasmid was also found in duck farms in this study. Additionally, as vectors for *fosA3* in *E. coli*, F-:A1:B6, N/ST1, IncI1/ST2, W-FIC, and untypable plasmids had never been reported before. These observations indicate that the prevalence of *fosA3* is a result of the effective horizontal transfer of plasmids.

Conjugation plays a key role in the dissemination of resistance genes (Mazel and Davies, 1999; de la Cruz and Davies, 2000). Several recent studies have reported the occurrence of horizontal gene transfer from pathogens to the microbiota in the human gut (Karami et al., 2007; Goren et al., 2010; Crémet et al., 2012). Resistance genes could be transferred to the gut microbiota through conjugative plasmids (Aviv et al., 2016). In this study, *fosA3* was successfully transferred from donors to the recipient *E. coli* J53 at a high success rate of conjugation (96.70%). This result indicated that the *fosA3* gene could be horizontally transferred to distinct bacteria in vivo by bacterial conjugation under appropriate physiologically relevant conditions.

In summary, this study revealed a high positive rate of *fosA3* among *E. coli* obtained from ducks in China. Based on the PFGE and MLST results, the high positive rate of *fosA3* was not due to clonal dissemination but rather was caused by the horizontal transfer of multiple conjugative plasmids. The high positive rate of *fosA3* between healthy and diseased ducks is worrisome and indicates that *fosA3* is widely distributed in ducks in Shandong province of China. Through the food chain, the *fosA3* gene is potentially transferred to the gut flora of humans, thus increasing the risk for human public health. Therefore, restricting the use of antibiotics in animal husbandry, particularly in poultry in China, may help prevent the dissemination of *fosA3* among *E. coli*.

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DISCLOSURES

We confirm that there are no know conflicts and interests associated with this publication and it can't be influenced on its outcome.

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