Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2018.008

RESEARCH ARTICLE

Topoisomerase Mutations are Associated with High-Level Ciprofloxacin Resistance in *Staphylococcus saprophyticus*, *Enterococcus faecalis* and *Escherichia coli* Isolated from Ducks

Guo Ji, Qiwei Chen, Xiaowei Gong, Fuying Zheng*, Shengdou Li and Yongsheng Liu*

State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, No. 1 Xujiaping, Yanchangbao, Lanzhou 730046, China
*Corresponding author: zfycaas@126.com; liuyongsheng@caas.cn

ARTICLE HISTORY (17-281)

Received: August 08, 2017
Revised: October 06, 2017
Accepted: October 28, 2017
Published online: January 10, 2018

Key words:
Ciprofloxacin
DNA gyrase
Mutation
Topoisomerase IV

ABSTRACT

This study investigated the effects of ciprofloxacin on specie and resistance of the bacteria isolated from the ducks reared in ciprofloxacin-present and ciprofloxacin-free duck population. Bacteria were isolated from the carcass swab samples collected from the slaughtered ducks. The result showed that the dominant bacteria isolated from the ducks were *Macrococcus caseolyticus* (*M. caseolyticus*), *Staphylocuccus saprophyticus* (*S. saprophyticus*), *Enterococcus faecalis* (*E. faecalis*) and *Escherichia coli* (*E. coli*). The minimum inhibitory concentrations (MICs) of ciprofloxacin against all isolates were measured, and the genes of topoisomerase II (DNA gyrase) and topoisomerase IV of *S. saprophyticus*, *E. faecalis* and *E. coli* were amplified and sequenced, respectively. The results showed that the site-directed mutations of DNA gyrase and topoisomerase IV of the above three bacteria caused the strains to generate high-level resistance to ciprofloxacin (MIC≥32 μg/ml). No plasmid-mediated quinolone resistance (PMQR) genes were detected. These results suggested that ciprofloxacin could affect bacterial species in the duck and induce bacterial resistance. Site-directed mutations of DNA gyrase and topoisomerase IV of *S. saprophyticus*, *E. faecalis* and *E. coli* were closely related to high-level ciprofloxacin resistance.

To Cite This Article: Ji G, Chen Q, Gong X, Zheng F, Li S and Liu Y, 2018. Topoisomerase mutations are associated with high-level ciprofloxacin resistance in *Staphylococcus saprophyticus*, *Enterococcus faecalis* and *Escherichia coli* isolated from ducks. Pak Vet J, 38(1): 39-45. http://dx.doi.org/10.29261/pakvetj/2018.008

INTRODUCTION

In recent years, the duck industry has made considerable progress and duck-derived foods are commonly consumed in daily life. So, understanding the types of bacteria carried by duck's carcass samples is important to human health.

Ciprofloxacin is an effective broad-spectrum quinolone agent. It has strong antibacterial activity toward gram-negative bacteria and *Staphylococcus* (Casas *et al*., 2016; Hur *et al*., 2016). Thus, ciprofloxacin is widely used as a prophylactic and therapeutic drug to suppress bacterial infection in animal husbandry (Tu *et al*., 2015). On the contrary, more and more bacteria acquire high resistance to ciprofloxacin, which decreases animal welfare and brings risk to animal food and human health (Lin *et al*., 2015).

Current studies have shown that there are three mechanisms mediating bacterial resistance to quinolone. Firstly, the target enzyme-mediated resistance mechanism (Karczmarczyk *et al*., 2011). The target sites of quinolone antibiotics are located in DNA gyrase (topoisomerase II) and topoisomerase IV. Specific-site mutations weaken the interaction of quinolone with the target enzymes, which is the most important mechanism of resistance in clinical strains. Secondly, plasmid-mediated quinolone resistance (PMQR) refers to the extrachromosomal elements that encode proteins which interferes with the interaction between quinolone and enzymes, altering the metabolism of drugs or increasing the efflux of quinolone (Rodríguez-Martínez *et al*., 2016; Yanat *et al*., 2016). Thirdly, chromosome-mediated drug resistance refers to low expression of porins or overexpression of extracellular pumps, decreasing the intracellular concentration of quinolone (Buffet-Bataillon *et al*., 2016).

This study was carried out to investigate the effect of ciprofloxacin on specie and resistance of the bacteria isolated from the ducks reared in the conventional and ciprofloxacin-free duck population. This work also elucidates the DNA gyrase, topoisomerase IV and PMQR resistance mechanisms of the bacteria to ciprofloxacin.

MATERIALS AND METHODS

Sample collection: 60 ducks (named C group) were reared under the conventional systems and received ciprofloxacin in drink water for therapeutic purpose. Each duck had a daily intake of 0.3 mg of ciprofloxacin. Ciprofloxacin was added for 3 days, followed by just water without ciprofloxacin for 7 days, persisting five cycles to slaughter. The other 60 ducks (named CF group) were free to ciprofloxacin. The ducks were slaughtered according to the procedure requested by farm-slaughter. Then, one carcass swab sample from each duck was collected. Thus, 60 samples were collected from each group. The samples were stored in sterile cups and transported to the laboratory on ice and processed for isolation of bacteria on the same day upon arrival at the laboratory.

Isolation and identification of bacteria: Tryptic soy broth (TSB) is a general medium, in which general bacteria can grow. Therefore, TSB is used to isolate bacteria from the carcass swab samples. Samples were enriched in 20 ml TSB containing 5% calf serum (TSBS) and incubated at 37°C for 48 h with 5% carbon dioxide. A loopful of the enriched sample was streaked onto tryptone soy agar plates containing 5% calf serum (TSAS) and incubated for 20-24 h at 37°C with 5% carbon dioxide. The isolated colonies with different morphology were picked out from each plate, and each colony was inoculated into 2 ml of TSBS, and shaken with 200 rpm at 37°C until the OD_{600} value was 0.8-1.0.

As described in the literature (Moreno *et al*., 2002), the 16S rRNA fragment of each isolate was amplified with the primers 27F (5'-AGAGTTTGATCCTGGCTC AG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') and sequenced. The obtained sequences were aligned in the GenBank library to determine bacterial species.

Antibiotic susceptibility test: The MICs of ciprofloxacin against all the isolates were determined by broth microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (USA, 2015). TSBS was used as the basal medium. *E. coli* ATCC 25922 was used as the quality control strain in each MIC test. The dilution ranges of ciprofloxacin were 0.125-128 μg/ml. The breakpoints (μg/ml) for susceptibility (≤1), intermediate (2) and resistance (≥4) of ciprofloxacin were defined by the CLSI.

DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) amino acid mutation detection: The bacteria, the rates of separation and resistance to ciprofloxacin having significant differences between two groups, were selected. Primers were designed to amplify the *gyrA* and *gyrB* genes of DNA gyrase and the *parC* and *parE* genes of topoisomerase IV from the selected strains. The specific DNA fragments were sequenced by Takara Biotechnology Co., Ltd. (Dalian, China), using the corresponding primers. The relationships between resistance to ciprofloxacin and amino acid mutations of *gyrA, gyrB, parC, and parE* genes were analyzed.

Detection of plasmid-mediated quinolone resistance (PMQR) genes: 9 pairs of primer were synthesized to screen PMQR genes. Primer sequences and amplification conditions were described in reference (Sun *et al*., 2012).

Statistical analysis: The data derived from the CF and C groups were analyzed using SPSS 20.0 version software. The *t-test* was used to compare the differences of bacterial numbers and resistance between the two groups. A value of $P<0.05$ was considered to be statistically significant, and remarkable significance was considered on the basis of $P<0.01$.

RESULTS

Isolation and identification of bacteria: a total of 136 strains including 11 species were isolated from the carcass of the ducks from group C. Among these isolates, 90 strains were *Macrococcus caseolyticus* (*M. caseolyticus*) with the highest percentage of 66.18% (90/136), followed by 15 strains of *E. faecalis* with a percentage of 11.03% (15/136), 12 strains of *E. coli* with a percentage of 8.82% (12/136) and 4 strains of *S. saprophyticus* with a percentage of 2.94% (4/136). On the other hand, 121 isolates including 11 species were obtained from the samples of the ducks from group CF. 61 strains of *M. caseolyticus* were isolated with the highest percentage of 50.41% (61/121), followed by 27 strains of *S. saprophyticus* with a percentage of 22.31% (27/121), 11 strains of *E. faecalis* with a percentage of 9.09% (11/121) and 3 stains of *E. coli* with a percentage of 2.48% (3/121). Details of the bacteria were shown in Table 1.

The numbers of *M. caseolyticus, S. saprophyticus, E. faecalis* and *E. coli* were sufficient statistical testing in groups C and CF. By *t-test* analysis, the separation rate and resistance rate of *M. caseolyticus* had no differences ($P>0.05$) in two groups. The separation rates of *S. saprophyticus* and *E. coli* were significantly different in groups C and CF ($P<0.01$). The separation rates of *E. faecalis* in groups C and CF had no differences ($P>0.05$), but the numbers of ciprofloxacin-resistant strains of *E. faecalis* from two groups were significantly different ($P<0.01$).

Susceptibility test: The results of susceptibility tests were shown in Table 1. All strains of *M. caseolyticus* were highly resistant to ciprofloxacin (MICs ≥32 μg/ml). Among 30 isolates of *S. saprophyticus*, MICs of ciprofloxacin against 16 strains were equal to or higher than 32 μg/ml, and these isolates were highly resistant to ciprofloxacin. The MICs of ciprofloxacin against 12 strains were ≤2 μg/ml, and other two isolates had the MICs of 8 μg/ml and 16 μg/ml, respectively. For 26 strains of *E. faecalis*, nine were highly resistant to ciprofloxacin with MICs >64 μg/ml. In addition, the MICs of ciprofloxacin against 14 strains were ≤2 μg/ml. The MICs deriving from other three isolates of *E. faecalis* were 4 μg/ml, 16 μg/ml and 32 μg/ml, respectively. Among 15 strains of *E. coli*, five strains had MICs of more than 64 μg/ml, four isolates had MICs of 32 μg/ml, and the other six strains had MICs of ≤4 μg/ml.

Table 1: Susceptibility testing of ciprofloxacin against the isolates

Groups	Susceptibility testing	MC	SS	EF	KO	EH	EC	ECA	AB	LL	PM	KP	BS	LC	SSC
C	R	90	4	10	4	1	8	0	1	1	1	1	0	0	0
	I	0	0	2	0	0	0	0	0	2	0	0	0	0	0
	S	0	0	3	0	2	4	2	0	0	0	0	0	0	0
	Total	90	4	15	4	3	12	2	1	3	1	1	0	0	0
	Resistance (%)	100	100	66.67	100	33.33	66.67	0	100	33.33	100	100	0	0	0
	Isolates (%)	66.18	2.94	11.03	2.94	2.21	8.82	1.47	0.74	2.21	0.74	0.74	0	0	0
CF	R	61	15	2	2	0	3	1	1	0	0	0	1	2	1
	I	0	2	9	0	0	0	0	0	4	0	0	0	0	1
	S	0	10	0	0	3	0	1	0	0	0	0	1	0	1
	Total	61	27	11	2	3	3	2	1	4	0	0	2	2	3
	Resistance (%)	100	55.56	18.18	100	0	100	50	100	0	0	0	50	100	33.3
	Isolates (%)	50.41	22.31	9.09	1.65	2.48	2.48	1.65	0.83	3.31	0	0	1.65	1.65	2.48

Note: MC, *Macrococcus caseolyticus*; SS, *Staphylocuccus saprophyticus*; EF, *Enterococcus faecalis*; KO, *Klebsiella oxytoca*; EH, *Enterococcus hirae*; EC, *Escherichia coli*; ECA, *Enterococcus casseliflavus*; AB, *Acinetobacter baumannii*; LL, *Lactococcus bactis*; PM, *Proteus mirabilis*; KP, *Klebsiella pneumoniae*; SSC, *Staphylococcus sciuri*; BS, *Bacillus subtilis*; LC, *Lactobacillus curvatus*. R, I and S represented resistance, intermediate and susceptibility; A total of 136 and 121 isolates were obtained from C and CF groups, respectively.

Table 2: Oligonucleotides used for PCR and DNA sequence determination

Isolates	Accession numbers	Genes	locus_tags	Primers	Oligonucleotide sequence (5' to 3')	Amplified Region	Sequenced region	Amino acid region
E. coli	AE014075.1	GyrA	c2773	EC-gyrA-F	gaactcacccttccagatcca	-248 to 734	1 to 687	1 to 229
				EC-gyrA-R	gagcgcggatatacacctt			
		GyrB	c4621	EC-gyrB-F	ggacaaagaaggctacagca	891 to 1770	925 to 1737	309 to 579
				EC-gyrB-R	cgtcgcgttgtactcaga ta			
		parC	c3760	EC-parC-F	atgagcgatatggcagagcgc	1 to 2259	49 to 2208	17 to 736
				EC-parC-R	ttactcttcgctatcaccgct			
		parE	c3776	EC-parE-F	atgacgcaaacttataacgct	1 to 1893	49 to 1845	17 to 615
				EC-parE-R	tcagacctcaatctctgccat			
E. faecalis	NZ_KE136402.1	GyrA	DI50_RS05295	EF-gyrA-F	gaccagcgaaatgaaaga	42 to 2416	91 to 2367	31 to 789
				EF-gyrA-R	cagccattgttaccacttt			
		GyrB	DI50_RS05300	EF-gyrB-F	atgacagaagaagaaaaaaac	1 to 1950	49 to 1899	17 to 633
				EF-gyrB-R	ttaaatatccaagtttttcac			
		parC	DI50_RS13515	EF-parC-F	accagatattcgagatgg	99 to -2365	160 to 2307	54 to 769
				EF-parC-R	atgttcgttcactgattgg			
		parE	DI50_RS13510	EF-parE-F	ttggctaaaaaaattaacaat	1 to 2058	49 to 2013	17 to 671
				EF-parE-R	ctactctatgtcaaacaaact			
S. saprophyticus	CP014113.1	GyrA	AL528_02495	SS-gyrA-F	cagtacatcgacgcatattg	131 to 2593	181 to 2508	61 to 836
				SS-gyrA-R	gtgcatcgtcctctatcaca			
		GyrB	AL528_09940	SS-gyrB-F	gtatgctatgtattctagtg	138 to 2265	211 to 2199	71 to 733
				SS-gyrB-R	cgtgtgctcaggttgaatta			
		parC	AL528_09945	SS-parC-F	ataattattcggatgattcca	20 to 1983	76 to 1929	26 to 643
				SS-parC-R	attaggatgttcctgttctaa			
		parE	AL528_02490	SS-parE-F	gtgtcagatgtgaacaacacg	1 to 1926	49 to 1875	17 to 625
				SS-parE-R	ttagaaatctaagttggcata			

Note: A minus sign refers to nucleotides upstream of the start codon.

DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) amino acid mutation detection: Based on the results of *t-test*, *S. saprophyticus*, *E. faecalis* and *E. coli* were selected to further study specific-site amino acid mutations of DNA gyrase and topoisomerase IV. The detailed information about the reference sequences, primer sequences, and the results of amplification and sequencing were shown in Table 2.

Among 30 strains of *S. saprophyticus*, the amino acids of *gyrA*, *gyrB*, *parC* and *parE* genes of 16 strains with MICs ≥32 μg/ml showed regular site-directed mutations, compared with those of the other 14 isolates with MICs ≤16 μg/ml. The mutations were as follows: *gyrA* gene: S-84 to L, L-490 to I (13 of the 16 strains had mutations, and the mutation rate was 81.25%). In addition, for the 9 isolates with MICs ≤16 μg/ml, amino acid 567 of the *gyrA* gene was I, and it was V in all the other strains. *gyrB* gene: S-80 to L, C-96 to R, L-733 to Y (11 of the 16 strains had mutations, and the mutation rate was 68.75%). *parC* gene: G-140 to S, A-550 to P. *parE* gene: S-179 to P, E-239 to D, Q-242 to E, S-254 to T, N-307 to S (14 of the 16 strains had mutations, and the mutation rate was 87.5%). Specific-site mutations of the amino acids of each strain were shown in Fig. 1.

For the 26 strains of *E. faecalis*, the amino acids of *gyrA* and *parC* genes of nine isolates with MICs >64 μg/ml showed regular specific-sited mutations, compared with those of the other 17 stains with MICs ≤32 μg/ml, and the specific mutation sites were as follows: *gyrA* gene: S-84 to Y, V-311 to M, V-731 to I. *parC* gene: S-82 to I. The amino acids of *gyrB* and *parE* genes did not change. Specific mutations of the amino acid of *gyrA* and *parC* genes of each strain were shown in Fig. 2.

Among 15 strains of *E. coli*, the amino acids of *gyrA* and *parC* genes of nine isolates with MICs >64 μg/ml occurred regular specific-sited mutations, compared with those of the other 10 strains with MICs ≤32 μg/ml. The mutations were as follows: *gyrA* gene: S-83 to L, D-87 to N. *parC* gene: S-80 to I (four of five strains occurred mutation, and mutation rate was 80%), L-440 to R. (three of five strains had mutations, and mutation rate was 60%). The amino acid sequences of the *gyrB* and *parE* genes did not change. Specific mutations in the amino acid sequence of each strain were shown in Fig. 3.

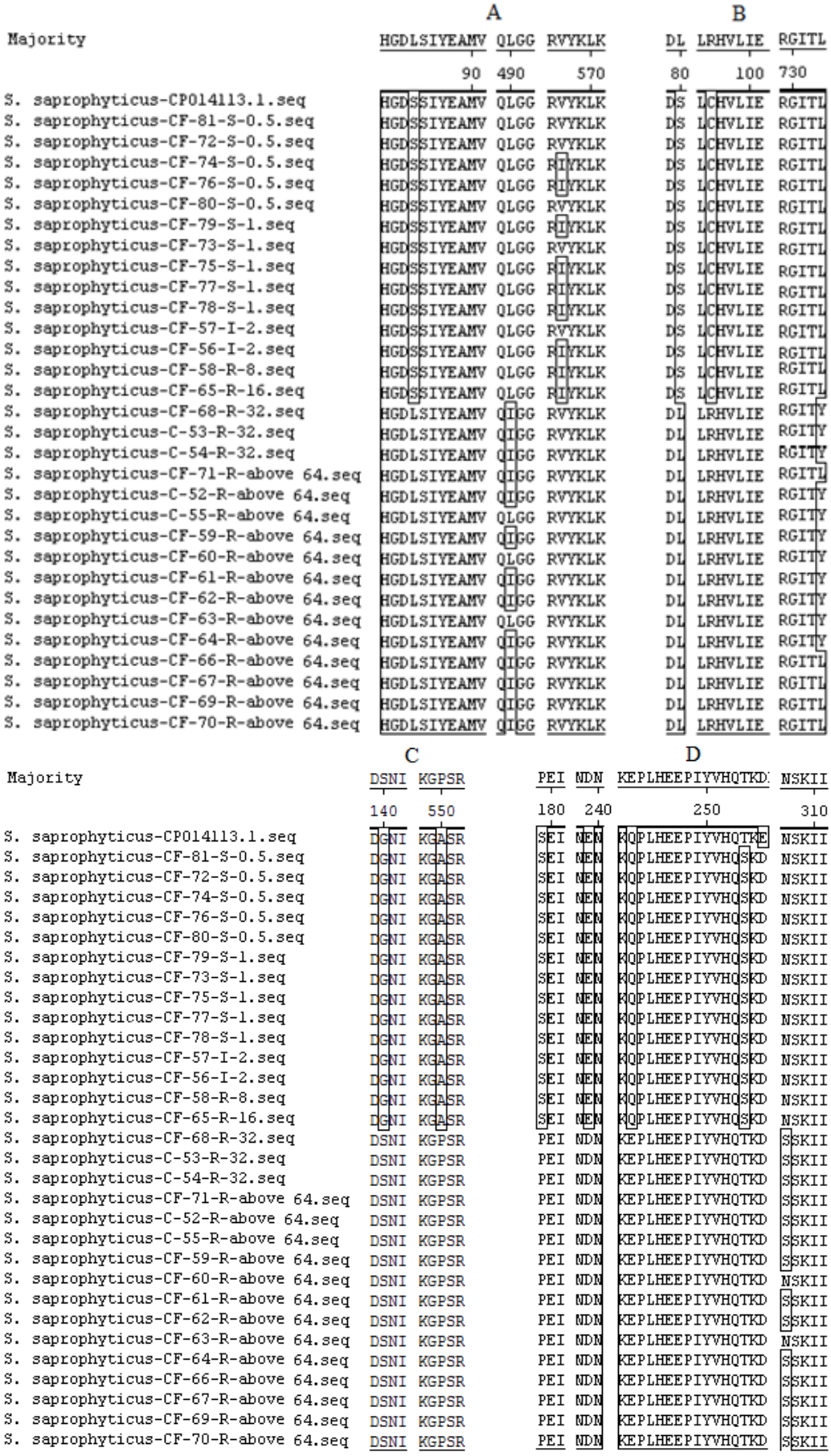

Fig. 1: The specific-site mutations in *gyrA* gene (A), *gyrB* gene (B), *parC* gene (C) and *parE* gene (D) of *S. saprophyticus*. S. saprophyticus-CP014113.1 was reference sequence. CF and C showed that the isolates came from the CF and C groups, respectively. The numbers from 52 to 81 were strain numbers. The letters S, I and R represented that the strains showed susceptibility, intermediate and resistance to ciprofloxacin, respectively. The final numbers from 0.5 to 64 represented the MICs of ciprofloxacin against the strains.

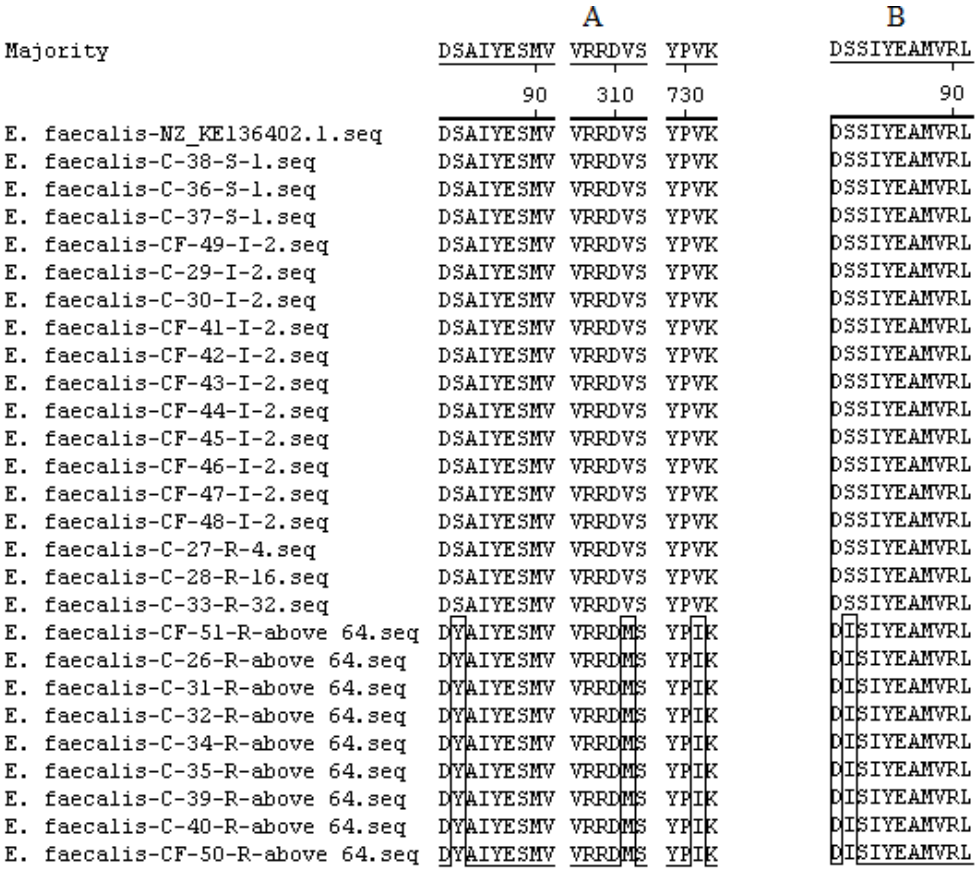

Fig. 2: The specific-site mutations in *gyrA* gene (A) and *parC* gene (B) of *E. faecalis*. E. faecalis-NZ_KE136402.1 was reference sequence. The numbers from 26 to 51 were strain numbers. The final numbers from 1 to 64 represented the MICs of ciprofloxacin against *E. faecalis*. The letters C, CF, S, I and R had the same meanings as those in Fig. 1.

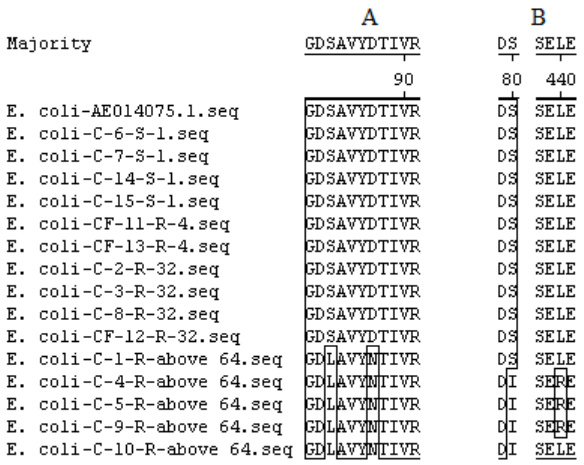

Fig. 3: The specific-site mutations in *gyrA* gene (A) and *parC* gene (B) of *E. coli*. E. coli-AE014075.1 was reference sequence. The numbers from 1 to 15 were strain numbers. The final numbers from 1 to 64 represented the MICs of ciprofloxacin against *E. coli*. The letters C, CF, S, I and R had the same meanings as those in Fig. 1.

Detection of PMQR genes: No PMQR genes were detected in any of the isolates of *M. caseolyticus*, *S. saprophyticus*, *E. faecalis* and *E. coli*.

DISCUSSION

Antibiotics are widely used in animal husbandry, resulting in the occurrence and spread of multi-drug-resistant bacteria, which is a threat to human health. Ciprofloxacin as the representative of the third-generation quinolone has good bactericidal effect, which is widely used in animal husbandry (Sun *et al*., 2012; Dahshan *et al*., 2015; Sierra-Arguello *et al*., 2016). Whether the application of ciprofloxacin will affect the bacterial species and lead to drug-resistant strains in carcass of healthy duck, it has never been reported. Therefore, the present study set out to isolate and identify bacteria from the carcass of slaughtered healthy ducks with presence and absence of ciprofloxacin, carry out drug sensitivity test of ciprofloxacin on the isolates, and study the mechanism of ciprofloxacin resistance in these bacteria.

The percentage of *M. caseolyticus* isolated from each group was more than 50%, and all isolates were highly resistant to ciprofloxacin. The results showed that *M. caseolyticus* was a common bacterium in healthy ducks, and it had natural resistance to ciprofloxacin. *M. caseolyticus* can be isolated from animal skin and animal foods, such as milk, raw beef, chicken, ham and Cantonese-style sausage (Wu *et al*., 2011). This study reported for the first time that *M. caseolyticus* could be isolated from carcass of healthy ducks, enriching the source of the host. At present, although there is no case of human, the bacterium could cause diseases of fish (Lan *et al*., 2015) and dog with rhinitis symptoms (Gómez-Sanz *et al*., 2015). The bacterium reflected high resistance to penicillin (Tsubakishita *et al*., 2010; Wang *et al*., 2012). This study found the high-level resistant strains of *M. caseolyticus* to ciprofloxacin (MICs ≥32 μg/ml),

suggesting that ciprofloxacin was ineffective in the treatment of clinical animals caused by this bacterium.

By *t-test* analysis, the isolation rate of *S. saprophyticus* in group C was remarkable significantly low compared with that in group CF, which indicated that ciprofloxacin could effectively inhibit the growth of *S. saprophyticus.* However, the isolation rate of *E. coli* in group C was significantly high than that in group CF, which showed that ciprofloxacin could not inhibit the growth of *E. coli*. In addition, the isolation rates of *E. faecalis* in groups C and CF had no difference, but the resistant rates of *E. faecalis* isolated in two groups had remarkable significant difference, which indicated that ciprofloxacin could not effectively inhibit *E. faecalis* but accelerate the strains to acquire resistance.

In recent years, *S. saprophyticus* has gained recognition as an important uropathogen, which is the second most common cause of uncomplicated urinary tract infections (UTIs) in young females (behind only *E. coli*). Ciprofloxacin usually was used to treat UTIs in clinical, but subinhibitory concentrations of ciprofloxacin increased *S. saprophyticus* adherence and virulence (Erdeljan *et al*., 2012). At present, no resistance mechanism of *S. saprophyticus* against ciprofloxacin was reported. This work discovered that the *S. saprophyticus* strains with MICs ≥32 μg/ml occurred specific-site mutations in the amino acid sequences of *gyrA, gyrB, parC* and *parE* genes compared with those of *S. saprophyticus* with MICs ≤16 μg/ml, indicating that the amino acid mutations of DNA gyrase and topoisomerase IV could lead to high-level ciprofloxacin resistance in *S. saprophyticus*, which has never been reported.

Studies have shown that in clinical isolates of *E. faecalis* resistant to ciprofloxacin, 28.5% of the strains have site-directed mutation of amino acid 83 in the *gyrA* gene, and 85.7% of the strains have a mutation at amino acid 80 in the *parC* gene (Rathnayake *et al*., 2012). For some *E. faecalis* strains resistant to quinolone, amino acids 84, 87 and 88 of the gyrA gene and amino acids 80 and 85 of the parC gene were substituted (Grohs *et al*., 2003; Oyamada *et al*., 2006). In the present study, apart from the conventional substitution at position 84, the novel mutations at amino acids 311 and 731 in the *gyrA* gene and the amino acid 82 in the *parC* gene were detected among nine strains of *E. faecalis* with MICs >64 μg/ml, which indicated that these mutations were closely related to the high-level ciprofloxacin resistance in *E. faecalis*.

E. coli resistant to quinolone, the amino acids 83 and 87 in the *gyrA* gene and the amino acid 80 in the *parC* gene mainly mutated (Komp Lindgren *et al*., 2003). In some strains isolated from patient treated with quinolone, the amino acid 84 in the *parC* gene and the amino acids 444, 458 and 529 in the *parE* gene mutated (Komp Lindgren *et al*., 2003). In addition to the above mutations, for some strains derived from food-producing animals with resistance to ciprofloxacin, the amino acid 492 in the *gyrB* gene, the amino acid 84 in the *parC* gene, and the amino acids at positions 355, 416, 458, and 460 in the *parE* gene mutated (Karczmarczyk *et al*., 2011). In this study, substitutions at amino acids 83 and 87 of the *gyrA* gene were detected in five strains of *E. coli* with MICs >64 μg/ml, and substitution at amino acid 80 of the *parC* gene of four strains was observed, which was consistent with the previous report. In addition, mutation at amino acid 440 in the *parC* gene of three *E. coli* strains were detected, which had not been reported.

Plasmid-mediated quinolone resistance genes were not founded in this study, indicating that this mechanism did not mediate resistance to ciprofloxacin in the strains isolated in this trial.

Conclusions: This study discovered that ciprofloxacin had effects on the specie and resistance of the bacteria isolated from the ducks reared under conventional and ciprofloxacin-free duck system. Site-directed mutations of DNA gyrase and topoisomerase IV of *S. saprophyticus*, *E. faecalis* and *E. coli* were closely related to high-level ciprofloxacin resistance. Some amino acid mutations were detected for the first time.

Acknowledgements: This work was financially supported by grants from National Natural Science Foundation of China (31572532 and 31201935), Science and Technology Plan Project of Gansu Province (17YF1WA170) and Bacterial Disease in Grazing animal Team.

Authors contribution: FZ and YL conceived and designed the study. GJ performed the tests, analyzed and interpreted the data, and wrote the manuscript. QC, XG and SL substantially contributed to collection of samples, isolation of strains and revision of the manuscript. All authors read and approved the final manuscript.

REFERENCES

Buffet-Bataillon S, Tattevin V, Maillard JY, *et al*., 2016. Efflux pump induction by quaternary ammonium compounds and fluoroquinolone resistance in bacteria. Future Microbiol 11:81-92.

Casas MR, Camargo CH, Soares FB, *et al*., 2016. Presence of plasmid-mediated quinolone resistance determinants and mutations in gyrase and topoisomerase in *Salmonella enterica* isolates with resistance and reduced susceptibility to ciprofloxacin. Diagn Microbiol Infect Dis 85:85-9.

Dahshan H, Abd-Elall AM, Megahed AM, *et al*., 2015. Veterinary antibiotice resistance, residues, and ecological risks in environmental samples obtained from poultry farms, Egypt. Environ Monit Assess 187:2.

Erdeljan P, MacDonald KW, Gonear LW, *et al*., 2012. Effects of subinhibitory concentrations of ciprofloxacin on *Staphylococcus saprophyticus* adherence and virulence in urinary tract infections. J Endourol 26:32-7.

Gómez-Sanz E, Schwendener S, Thomann A, *et al*., 2015. First staphylococcal cassette chromosome *mec* Containing a *mecB* carrying gene complex independent of transposon Tn*6045* in a *Macrococcus caseolyticus* isolate from a canine infection. Antimicrob Agents Chemother 59:4577-83.

Grohs P, Houssaye S, Aubert A, *et al*., 2003. *In vitro* activities of garenoxacin (BMS-284756) against *Streptococcus pneumoniae*, viridans group streptococci, and *Enterococcus faecalis* compared to those of six other quinolones. Antimicrob Agents Chemother 47:3542-7.

Hur J, Lee A, Hong J, *et al*., 2016. *Staphylococcu saprophyticus* bacteremina originating from urinary tract infections: A case report and literature review. Infect Chemother 48:136-9.

Komp Lindgren P, Karlsson A, Hughes D, 2003. Mutation rate and evolution of fluoroquinolone resistance in *Escherichia coli* isolates from patients with urinary tract Infections. Antimicrob Agents Chemother 47:3222-32.

Karczmarczyk M, Martins M, Quinn T, *et al*., 2011. Mechanisms of fluoroquinolone resistance in *Escherichia coli* isolates from food-producing animals. Appl Environ Microb 77:7113-20.

Lan SH, Wang L, Deng JJ, *et al*., 2015. Identification and drug sensitivity of Megasphaera from yellow catfish pelteobagrus fulvidraco. Fish Sci 34:778-82.

Lin D, Chen K, Wai-Chi Chan E, *et al*., 2015. Increasing prevalence of ciprofloxacin-resistant food-borne *Salmonella* strains harboring multiplie PMQR eliments but not target gene mutations. Sci Rep 5:14754.

Moreno C, Romero J, Espejo RT, *et al*., 2002. Polymorphism in repeated 16S rRNA genes is a common property of type strains and environmental isolates of the genus *Vibrio*. Microbiology 148:1233-9.

Oyamada Y, Ito H, Inouse M, *et al*., 2006. Topoisomerase mutations and efflux are associated with fluoroquinolone resistance *in Enterococcus faecalis*. J Med Microbiol 55:1395-401.

Performance standards for antimicrobial susceptibility testing, 2015. Twenty–Fifth Informational Supplement, M100-S25. Clinical and Laboratory Standards Institute, USA.

Rathnayake IU, Hargreaves M, Huygens F, 2012. Antibiotic resistance and virulence traits in clinical and environmental *Enterococcus faecalis* and *Enterococcus faecium* isolates. Syst Appl Microbiol 35:326-33.

Rodríguez-Martínez JM, Machuca J, Cano ME, *et al*., 2016. Plasmid-mediated quinolone resistance: Two decades on. Drug Resist Updat 29:13-29.

Sierra-Arguello YM, Perdoncini G, Morgan RB, *et al*., 2016. Fluoroquinolone and maerolide resistance in Campylobacter jejuni isolated from broiler slaughterhouses in southern Brazil. Avian Pathol 45:66-72.

Sun N, Liu JH, Yang F, *et al*., 2012. Molecular characterization of the antimicrobial resistance of *Riemerella anatipestifer* isolated from ducks. Vet Microbiol 158:376-83.

Tsubakishita S, Kuwahara-Arai K, Baba T, *et al*., 2010. Staphylococcal cassette chromosome *mec*-like element in *Macrococcus caseolyticus*. Antimicrob Agents Chemother 54:1469-75.

Tu LT, Hoang NV, Cuong NV, *et al*., 2015. High levels of contamination and antimicrobial-resistant non-typhoidal *Salmonella* serovars on pig and poultry farms in the Mekong Delta of Vietnam. Epidemiol Infect 143:3074-86.

Wang Y, Wang Y, Schwarz S, *et al*., 2012. Detection of the staphylococcal multiresistance gene *cfr* in *Macrococcus caseolyticus* and *Jeotgalicoccus pinnipedialis*. J Antimicrob Chemoth 67:1824-7.

Wu YT, Zhao MM, Sun WZ, *et al*., 2011. Analysis of volatile compounds of cantonese sausage fermented by *Macrococcus caseolyticus* separated from cantonese sausage. Sci Technol Food Industry 32:206-9.

Yanat B, Rodríguez-Martínez JM and Touati A, 2017. Plasmid-mediated quinolone resistance in Enterobacteriaceas: a systematic review with a focus on Mediterranean countries. Eur J Clin Microbiol Infect Dis 36:421-35.