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Characteristics of Fluoroquinolone-Resistant of Avian Pathogenic *Escherichia coli* Isolates in China

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Abstract: A total of 121 Avian Pathogenic *Escherichia coli* (APEC) isolates recovered from diagnosed cases of avian colibacillosis from China during 2009 and 2011 were serotyped and examined for susceptibility to nalidixic acid and 6 fluoroquinolones. About 23 different serotypes were determined by agglutination using antisera and 078 (28/121), O143 (21/121) and O2 (15/121) were predominant serotypes. APEC isolates displayed resistance to nalidixic acid (95.9%), norfloxacin (95.0%), ciprofloxacin (94.2%), enrofloxacin (86.8%), levofloxacin (75.2%), lomefloxacin (63.6%) and ofoxacin (61.2%), respectively. Single gyrA changes (mainly Ser 83 to Leu) correlated with nalidixic acid MICs ≥32 μg mL^{-1}. The Asp87 changes (mainly Asp87 to Asn) in gyrA were associated with higher ciprofloxacin MICs. ParC alterations comprised amino acid changes Ser 80 to Ile, Ser 80 to Arg, Glu84 to Gly, Glu84 to Lys. The fluoroquinolone-resistant strains for which nalidixic acid MICs were >256 μg mL^{-1} had both gyrA sand parC QRDR point mutations. The fluoroquinolone-resistant isolates had common point mutations in gyrA (Ser 83 to Leu, Asp87 to Asn) and parC (Ser80 to Ile). These strains for which ciprofloxacin MICs were >8 μg mL^{-1} had double gyrA mutations, accompanying with ParC alterations. Seven recent isolates carried *qnrS* gene and three carried *aac(6')-Ib-cr* gene. This suggests that the widespread mutations at position 83, 87 of gyrA and position 80 of parC were crucial for resistance to fluoroquinolone and showed significant relation to the high-level fluoroquinolone resistance in APEC.

Key words: Fluoroquinolone, resistant, avian pathogenic *Escherichia coli*, nalicliuic acid, China

INTRODUCTION

Avian Pathogenic *Escherichia coli* (APEC) belongs to the extra-intestinal pathogenic group of *E. coli*. These bacteria cause several severe disease syndromes in farmed birds such as peritonitis, enteritis, air sac disease, pericarditis, perihepatitis, salpingitis, synovitis, panophthalmitis in poultry (Gross, 1994). APEC has been known to cause disease among chickens and other fowls which usually results in large economic losses for the poultry industry (Antao *et al*., 2009), its strains belong predominantly to three serogroups, O1, O2 and O78 in China.

Research on APEC has increased greatly over the years where the susceptibility of the pathogen to those antimicrobial agents commonly used for treatment such as flouroquinolones is decreasing (Gomis *et al*., 2003). APEC are mostly associated with infection of extraintestinal tissues in chickens, turkeys, ducks and other avian species. The most important disease syndrome associated with APEC begins as a respiratory tract infection and may be aerosacculitis or the air sac disease (Dho-Moulin and Fairbrother, 1999). This inevitably results in severe systemic infection leading to death of the animal infected. APEC have recently been classified under the category of Extraintestinal Pathogenic *E. coli* (ExPEC), a group which includes human pathogens like the Uropathogenic *E. coli* (UPEC), Newborn Meningitic *E. coli* (NMEC) and animal pathogens which in turn suggest APEC have the possibility of zoonotic potential (Antao *et al*., 2009; Ewers *et al*., 2007; Johnson *et al*., 2003; Kariyawasam *et al*., 2007).

Fluoroquinolones are considered the drug of choice for the treatment of *E. coli* infections of human (Noguera *et al*., 2011; Obeng *et al*., 2012; Yasufuku *et al*., 2011) and the recently observed increase in the number of Fluoroquinolone-resistant strains is an alarming public health concern (Karaca *et al*., 2005). Fluoroquinolones are also widely used in farm animals, mainly in poultry and Fluoroquinolone-resistant *E. coli* strains are frequently isolated from healthy and diseased birds in China and other country (McPeake *et al*., 2005;

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Zhao *et al*., 2005). Therefore, resistance to fluoroquinolones in *E. coli* has increased due to their large use. Fluoroquinolones resistance involves three main mechanisms: target mutations, reduced antibiotic intracellular accumulation by lowering outer membrane permeability or increasing efflux activity (Swick *et al*., 2011) and target protection mediated by the qnr protein (Martinez-Martinez *et al*., 1998). Among them, clinical resistance to quinolones in *E. coli* is mostly associated with mutations in genes that encode subunits of the quinolone target DNA gyrase and topoisomerase IV (Yoshida *et al*., 1988). More recently, plasmid-mediated mechanisms were reported such as those due to *qnr* genes encoding pentapeptide repeat proteins, aac(6')-Ib-cr encoding a modified acetyltransferase (Warburg *et al*., 2009) and qepA encoding an active efflux pump. *qnr* and *aac(6')-Ib-cr* genes confer reduced quinolone susceptibility, facilitating the selection of chromosomal mutations that confer high-level resistance (George *et al*., 2006; Liu *et al*., 2012a, b; Vasilaki *et al*., 2008). Fluoroquinolone resistance in *E coli* appeared to be a stepwise phenomenon with MIC increasing as the number of point mutations in gyrA increased but high-level resistance and multidrug resistance associated with fluoroquinolone resistance reflected overexpression of the AcrAB efflux pump (Shaheen *et al*., 2011). In fluoroquinolone-resistant isolates, DNA gyrase, the primary target in Gram-negative bacteria, commonly presents substitutions at amino acid position Ser83 and/or Asp87 of the gyrA subunit while substitutions at residues Ser80 and Glu84 are commonly identified alterations in the parC subunit of the topoisomerase IV (Karczmarczyk *et al*., 2011; Li *et al*., 1998).

MATERIALS AND METHODS

Isolation and identification of avian *E. coli* strains: A total of 121 (31 isolated in 2009, 28 in 2010, 33 in 2011 and 29 in 2012) avian *E. coli* strains were isolated from poultry outbreaks of APEC infections occurring during 2009-2012 in free-range poultry farm or intensive farming units in eastern China. Bacteria from diseased animals were isolated from necropsy specimens and cultured on 5% sheep blood and MacConkey agar. The isolates were serotyped at the Key Laboratory of Animal Infectious Diseases of Ministry of Agriculture located at Yangzhou University. Serotyping was performed by agglutination using antisera for 181 somatic O types (Orskov and Orskov, 1990). *E. coli* strains were stored in tryptone soy broth (Oxoid, Hampshire, UK) with 15% glycerol at -70°C.

Fluoroquinolones susceptibility testing: For nalidixic acid, the susceptibility determination was performed by a Broth Microdilution Method and according to the breakpoints defined by the Clinical and Laboratory Standards Institute (CLSI), resistance was defined as isolates having a Minimal Inhibitory Concentration (MIC) ≥ 32 μg mL^{-1}.

The MICs of ciprofloxacin, ofloxacin, norfloxacin (Sigma Chemical, MO, USA), levofloxacin, enrofloxacin and lomefloxacin (Sangon, Shanghai, China) were determined against 121 APEC isolates by the Agar Dilution Method according to CLSI guidelines using Mueller-Hinton agar as a culture medium. The range of concentrations evaluated was 0.5-512 μg mL^{-1} for all the fluoroquinolones tested. *Escherichia coli* ATCC 25922 was included as control strain.

Mutational analysis of QRDRs in gyrA, gyrB and parC: A total of 24 strains of fluoroquinolone-resistant APEC, together with 4 strains of APEC that were susceptible to fluoroquinolones (ciprofloxacin and levofloxacin), 4 susceptible to nalidixic acid were grown in Luria Bertani (LB) medium. Their genomic DNA was extracted and used as a template. The QRDRs of gyrA, gyrB and parC were amplified by PCR using earlier published primers (Everett *et al*., 1996; Sorlozano *et al*., 2007; Vila *et al*., 1996; Zhao *et al*., 2005). The PCR was performed in a 25 μL reaction mixture that contained 2 μL DNA template, 4 pmol (each) primer, 0.2 mM (each) deoxynucleoside triphosphate, 2.5 μL 10x PCR buffer with 15 mM $MgCl_2$ and 1 U Taq DNA Polymerase (Promega, USA). The reaction was carried out in the PTC-0200G DNA Engine Thermal Cycler (Bio Rad) with the following schedule: preheating at 94°C for 2 min followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 59°C for 30 sec and extension at 72°C for 50 sec with a final extension at 72°C for 7 min. The PCR products were electrophoresed in 2% agarose gels stained with ethidium bromide and certificated under UV transillumination. The PCR products were purified using the MinElute PCR Purification kit (QIAGEN, Valencia, CA, USA) and sequenced by the Applied Biosystems 3730 DNA analyzer at Sangon biotech (Shanghai) Co., Ltd. Pairwise alignments of DNA sequences were carried out using the BLAST server of the National Center for Biotechnology Information.

RESULTS

Serotyping: About 121 APEC strains were serotyped with antisera specific for 181 somatic O types. Serotype O78 was the most frequently observed (23.1%, 28/121) followed by O143 (17.4%, 21/121), O2 (12.4%, 15/121), O127 (8.3%, 10/121), O1 (7.4%, 9/121), O18 (5.8%, 7/121), O15 (4.1%, 5/121), O11 (3.3%, 4/121), O109 (3%, 3/121), O4,

Table 1: Serotype identification of avian pathogenic *Escherichia coli* from the Eastern China

Serotypes	No. of strains (%)	Serotype	No. of strains (%)
O78	28 (23.1)	O8	2 (1.7)
O143	21 (17.4)	O6	2 (1.7)
O2	15 (12.4)	O17	2 (1.7)
O127	10 (8.30)	O9	2 (1.7)
O1	9 (7.400)	O88	1 (0.8)
O18	7 (5.800)	O96	1 (0.8)
O15	5 (4.100)	O152	1 (0.8)
O11	4 (3.300)	O20	1 (0.8)
O109	3 (2.500)	O21	1 (0.8)
O4	2 (1.700)	O27	1 (0.8)
O22	2 (1.700)	O160	1 (0.8)

O22, O8, O6, O17, O9 (1.7%, 2/121) and O88, O96, O152, O20, O21, O27, O160 (0.8%, 1/121). Information on the APEC strains examined in this study is given in Table 1.

Fluoroquinolones susceptibility: During the 4 years study period, resistance to nalidixic acid, norfloxacin, ciprofloxacin, enrofloxacin, levofloxacin, lomefloxacin and ofoxacin was found in APEC at rates of 95.9, 95.0, 94.2, 86.8, 75.2, 63.6 and 61.2%, respectively (Table 2). Resistance to nalidixic acid was detected in 100% of the APEC isolates since 2010. Percentages of resistance to antibiotics are presented by year in Fig. 1. Among APEC isolates consistently high rates of norfloxacin and ciprofloxacin resistance were detected during the study period ranging from 80.6% in 2009 to 100% in 2012 and from 83.9% in 2009 to 100% in 2012. Furthermore, resistance rates to fluoroquinolone tested remained high (>60%) during the survey and had an increasing trend.

The >50% of APEC isolates exhibited resistance to all six fluoroquinolones tested. MIC50 and MIC90 values were higher for the APEC isolates as compared to the data reported in other country (Lo *et al*., 2011). At a concentration of 8 μg mL^{-1} or higher, >50% of the *E. coli* isolates were inhibited by levofloxacin, enrofloxacin, lomefloxacin, ofoxacin and norfloxacin. The MIC of nalidixic acid of the inherently ciprofloxacin-resistant strain (e.g., D2 strain) was also determined and an even higher level of resistance was observed (MIC ≥512 μg mL^{-1}). In strains that gained high-level resistance to ciprofloxacin (MIC ≥8 μg mL^{-1}) the nalidixic acid resistance also increased (MIC ≥512 μg mL^{-1}) (Table 3). Two of the veterinary fluoroquinolones, norfloxacin and enrofloxacin, possessed identical MIC50 and MIC90 values of 16 and 64 μg mL^{-1}, respectively. The greatest MIC90 for the fluoroquinolones was observed with ciprofloxacin (128 μg mL^{-1}) whereas levofloxacin and lomefloxacin exhibited the lowest MIC90, 16 μg mL^{-1} (Table 2).

Mutations in the QRDRs of fluoroquinolone-resistant APEC isolates: Researchers selected 28 isolates for molecular characterization of *gyrA*, *gyrB*, *parC*, *qnrS* and *aac(6')-Ib-cr* genes. Of these, 22 out of 28 isolates with ciprofloxacin MIC ≥4 μg mL^{-1} and 6 out of 28 isolates with MIC ≤2 μg mL^{-1} were successfully sequenced. As shown in Table 3, 28 isolates contained at least one mutation in gyrA, 22 isolates contained double mutations in gyrA, 21 isolates contained at least one mutation in parC and 21 isolates contained at least one mutation in parC and double mutations in gyrA. Among isolates containing mutations in both parC and gyrA all were resistant to ciprofloxacin with MIC ≥8 μg mL^{-1} (Table 3).

An amino acid replacement in the QRDR of gyrA (Ser-83→Leu) was predicted for all the nalidixic acid-resistant isolates. About 23 isolates with nalidixic acid MIC ≥128 μg mL^{-1} and resistant to ciprofloxacin possessed double point mutations in gyrA, Ser-83→Leu and Asp-87→Asn. It was predicted the replacements of Ser-83→Leu and Asp-87→Asn in gyrA of ciprofloxacin-resistant isolates was the principal replacement (23 of 23 isolates; 100%) while other substitutions were rare. In gyrB, researchers found changes of Asp-96 to Glu in 1 isolate, Ala-97 to Pro in 1 isolate and to Phe in 1 isolate and Ser-491 to Asn in 1 isolate.

Table 2: *In vitro* activities of avian pathogenic *E. coli* recovered from diseased poultry (n = 121)

	MIC (μg mL^{-1})				Susceptibility[a] (n = 121)	
Antibiotic	Resistant breakpoint	Range	MIC50[b]	MIC90[c]	S%	R%
Nalidixic acid	≥32	32 to >512	128	>512	0	95.9
Norfloxacin	≥16	1-128	16	64	3	95.0
Ciprofloxacin	≥4	0.5-128	8	64	0	94.2
Enrofloxacin	≥8	≤0.5-64	16	64	11	86.8
Levofloxacin	≥8	≤0.5-16	8	16	20	75.2
Lomefloxacin	≥8	≤0.5-32	8	16	31	63.6
Ofoxacin	≥8	≤0.5-32	8	32	27	61.2

[a]S: Susceptible; R: Resistant, MICs determined via broth Micro-Dilution Methods in accordance CLSI standards (CLSI in 2007); [b]The MIC at which 50% of the isolates are inhibited; [c]The MIC at which 90% of the isolates are inhibited

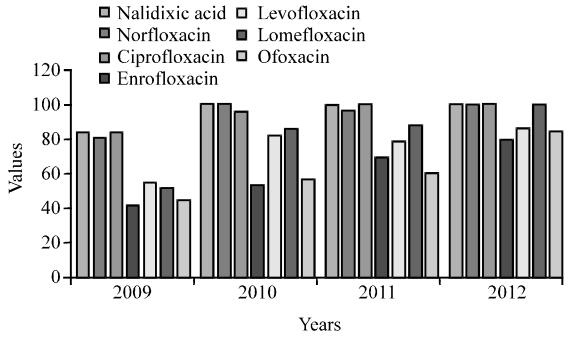

Fig. 1: Trend of selected APEC isolates resistant to quinolone in China from 2009-2012

Table 3: Characteristics fo the fluoroquinolone-resistant isolates

						Mutations in the QRDRs						
			MIC (μg mL^{-1})			gyrA			parC			
Strains	Serotype	Years	NAL[a]	CIP	NOR	83	87	gyrB	80	84	qnrS	aac(6')-Ib-cr
C1	O2	2009	>512	8.0	16	S[b]→L	D→N	_	S→I	_	_	_
C5	O2	2010	>512	16.0	16	S→L	D→N	_	S→I	_	_	_
D2	O2	2011	>512	64.0	128	S→L	D→N	_	S→I	_	+	_
C10	O2	2012	>512	8.0	128	S→L	D→N	D96E	S→I	_	+	+
G1	O78	2009	>512	16.0	128	S→L	D→N	_	S→I	_	_	_
C12	O78	2010	>512	32.0	128	S→L	D→N	_	S→I	_	+	_
C3	O78	2011	>512	32.0	128	S→L	D→N	_	S→I	_	+	_
C8	O78	2012	>512	64.0	32	S→L	D→N	_	S→I	_	+	_
D13	O143	2009	512	8.0	128	S→L	D→N	_	S→I	E→G	_	_
C11	O143	2010	>512	16.0	128	S→L	D→N	_	S→I	_	_	_
C20	O143	2011	>512	8.0	64	S→T	D→N	_	S→I	_	_	_
C9-3	O143	2012	512	8.0	64	S→L	D→N	_	S→I	_	+	_
D8-1	O127	2009	512	8.0	64	S→L	D→N	A97P	S→I	_	_	_
C28	O127	2011	>512	16.0	32	S→L	D→N	_	S→I	_	_	_
C30	O1	2009	>512	16.0	32	S→L	D→N	_	S→I	_	_	_
D7-4	O1	2010	>512	64.0	64	S→L	D→N	_	S→I	E→K	_	+
G3b	O1	2011	256	8.0	64	S→L	D→N	_	S→R	_	_	_
C21-2	O1	2012	512	64.0	128	S→L	D→N	_	S→I	_	+	+
D9d	O15	2009	512	16.0	32	S→L	D→N	_	S→I	_	_	_
C91	O18	2012	>512	64.0	16	S→L	D→N	S491N	S→I	_	_	_
C33	O11	2010	>512	64.0	8	S→L	D→N	_	S→I	_	_	_
C99	O11	2012	256	4.0	8	S→L	_	_	S→I	_	_	_
D3a	O109	2009	128	4.0	8	S→L	D→N	_	_	E→G	_	_
C4f	O4	2010	64	1.0	4	S→L	_	_	_	_	_	_
C41	O22	2012	64	0.5	2	S→L	_		_	_	_	_
G56	O6	2011	32	1.0	8	S→L	_	_	_	_	_	_
D17	O8	2009	32	1.0	4	S→L	_	_	_	_	_	_
C73	O9	2012	32	1.0	2	S→L	_	_	_	_	_	_
G95c	O20	2010	32	0.5	1	S→L					_	_

[a]NAL: Nalidixic Acid; CIP: Ciprofloxacin; NOR: Norfloxacin. [b]S: Ser; L: Leu; D: Asp; N: Asn; Y: Tyr; P: Pro; A: Ala; I: Ile; R: Arg; K: Lys; G: Gly

The amino acid sequences in the QRDR of parC showed a high frequency of replacement of Ser-80 to Ile (22 isolates) or Arg (1 isolates) while other replacements were rare. It is notable that all of the isolates with mutation in parC had a mutation in gyrA. Thus, it was confirmed that alterations in parC occurred at a second step in strains already having a single alteration in gyrA in *E. coli*. Moreover, strains with two alterations (Ser-83→Leu in gyrA plus Ser-80→Ile in parC) were most frequently identified in clinical isolates (22 isolates). All nalidixic acid-resistant isolates for which MICs of nalidixic acid were ≥512 μg mL^{-1} and MIC of ciprofloxacin ≥4 μg mL^{-1} had substitutions in both QRDRs of gyrA and parC. The ParC alterations were seen in these isolates only in the presence of gyrA changes. Among ciprofloxacin-resistant strains it was found that seven recent isolates carried *qnrS* gene and three carried *aac(6')-Ib-cr* gene.

DISCUSSION

Avian pathogenic *E. coli* is an economically important pathogen of chickens worldwide and is responsible for increased mortalities in poultry flocks. Worldwide, including China, Korea, Japan, avian pathogenic *E. coli* strains commonly belong to certain serogroups, O78, O2 and O1 and to a restricted number of clones (Dho-Moulin and Fairbrother, 1999; Jeong *et al*., 2012; Wang *et al*., 2010). The frequencies of these serogroups among APEC isolates vary according to location and host. In this study, serotyping revealed that about 43% of the strains belonged to these three serogroups. However, >50% of APEC isolates (O143, 21/121) could not be assigned to the serogroups indicating that a great variety of *E. coli* serogroups can be widespread in China.

The prevalence of fluoroquinolone resistance among APEC increased following the introduction of norfloxacin, ciprofloxacin and enrofloxacin into the veterinary use since 1990s. Resistance in *E. coli* to the fluoroquinolone has caused their use to decline in the treatment of colibacillosis (Boyd *et al*., 2008). Fluoroquinolone use and resistance to fluoroquinolones are increasing. In the study, in the past 10 years, resistance to fluoroquinolone such as ofoxacin and lomefloxacin was present in more than half of *E. coli* isolates, representing about 40% increase in resistance since 2009 and continuing the concerning trend noted during that period.

The present study demonstrates that high-level fluoroquinolone resistance is a multifactorial process and that the most resistant isolates (nalidixic acid MICs ≥256 μg mL^{-1}) usually possess at least two mutations within target genes and the isolates (ciprofloxacin MICs ≥8 μg mL^{-1}) have at least two mutations within target genes and show enhanced efflux (Everett *et al*., 1996). Everett *et al*. (1996) inferred that that high-level resistant strains containing multiple mutations are unlikely selected in a single step within a host organism.

Those which acquire the right combination of mutations before becoming nonviable are able to resume growth. Studies are in progress to examine whether antibiotic resistance mutations can arise via such a process. The fact is that the low selective pressure allow single mutants and continued selective pressure or a new antibiotic challenge then favors those progeny containing further mutations (Alessiani *et al*., 2009; Aparicio *et al*., 1999). The relationship between ciprofloxacin MICs and alterations to the QRDR has been reported in other studies on clinical isolates of *E. coli*. The study of Liu *et al*. (2012a, b) demonstrated that the number of mutations in QRDRs of gyrA and/or parC was significantly associated with the MICs for quinolones ($p<0.01$). The resistance rates of ciprofloxacin, enrofloxacin and nalidixic acid in PMQR-positive isolates were significantly higher than those in PMQR-negative isolates, respectively ($p<0.05$). And the prevalence of oqxAB had significant Spearman correlation coefficients in relation to the MICs of all the four tested quinolones ($p<0.01$) (Liu *et al*., 2012a, b).

The occurrence of gyrA mutations at positions corresponding to amino acid residues 83 and 87 and similarly, the occurrence of parC mutations corresponding to residues 80 and 84 are in agreement with data reported in other studies on quinolone-resistant clinical isolates (Karczmarczyk *et al*., 2011; Sun *et al*., 2012). In particular, Ser83 to Leu and Asp87 to Asn substitutions in gyrA were commonly reported by other research and ubiquitous in the isolates studied here. In this study, an amino acid replacement in the QRDR of gyrA (Ser-83→Leu) was predicted for all the nalidixic acid-resistant isolates. When MICs of nalidixic acid of >256 mug mL^{-1} and MICs of ciprofloxacin ranging from 4 to >32 mug mL^{-1}, several mutations were identified in genes coding for quinolone target enzymes (3-5 mutations per strain). All isolates harbored gyrA amino acid substitutions at positions 83 and 87 (Karczmarczyk *et al*., 2011). About 23 isolates studied here with nalidixic acid MIC ≥128 μg mL^{-1} and resistant to ciprofloxacin, possessed double point mutations in gyrA, Ser-83→Leu and Asp-87→Asn. It was predicted the replacements of Ser-83→Leu and Asp-87→Asn in gyrA of ciprofloxacin-resistant isolates was the principal replacement (23 of 23 isolates; 100%) while other substitutions were rare. Polymorphisms within the gyrB sequence have rarely been reported in quinolone-resistant isolates. Substitutions at residue positions 389, 426, 447 and 464 were earlier described (Couzinet *et al*., 2005; Moon *et al*., 2010; Ouabdesselam *et al*., 1995).

In one study, the Ser492-to-Asn substitution identified in gyrB of two isolates is localized outside the putative QRDR. In gyrB, researchers found changes of Asp-96 to Glu in 1 isolate, Ala-97 to Pro in 1 isolate and to Phe in 1 isolate and Ser-491 to Asn in 1 isolate. It is difficult to conclude the correlation between the mutation of gyrB with MIC values for ciprofloxacin and the various mutation simultaneously possessed in gyrA and parC. The amino acid sequences in the QRDR of parC showed a high frequency of replacement of Ser-80 to Ile (22 isolates) or Arg (1 isolates) while other replacements were rare. Moon *et al*. (2010) detected a new mutation in topoisomerase IV genes and found all ciprofloxacin-resistant *E. coli* isolates carried double mutations in gyrA and at least a single mutation in parC some isolates also carried a single mutation in parE. The most common mutations were S83L and D87N in gyrA, S80I in parC and S458A in parE which accounted for 25% of isolates. Double mutations in parC and a combination of single mutations in parC and parE significantly increased the MIC values of fluoroquinolones (Moon *et al*., 2010).

Plasmid-mediated *qnr* and *aac(6')-Ib-cr* genes confer reduced quinolone susceptibility, facilitating the selection of chromosomal mutations that confer high-level resistance (Jouini *et al*., 2010; Shin *et al*., 2009; Warburg *et al*., 2009). Qnr and aac(6')-Ib-cr have therefore been hypothesized as potential contributors to the increase in prevalence of quinolone resistance among gram-negative bacteria. Among ciprofloxacin-resistant strains studied, it was found that seven isolates carried *qnrS* gene and three carried *aac(6')-Ib-cr* gene.

CONCLUSION

Thus, it was concluded that the *qnrS* and *aac(6')-Ib-cr* genes were rare in APEC and showed little significant relation to the high-level quinolone in APEC.

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