Detection of Plasmid-Mediated Quinolone and β-lactam Resistant Genes in Escherichia Coli Isolates from Diseased Poultry in Egypt

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Abstract

Escherichia coli bacteria are normal inhabitant in the intestine of man, animals and poultry. A total of 20 isolates of E. coli, isolated from diseased chickens belonged to different O-serogroups and showed resistance to one or more antibiotics, were investigated for antimicrobial resistance against β-lactam and quinolone groups of antibiotics and tested for detection of plasmid mediated quinolone resistant genes. The result was resistance of 60% of the isolates to ampicillin and amoxicillin (β-lactam group) and 95%, 85%, 80%, 45% and 20% to enrofloxacin, norfloxacin, nalidixic acid, danofloxacine and ciprofloxacin (quinolone group), respectively. All the selected isolates showed multi-resistance when they were tested against 8 antibiotic groups. Among the twenty E. coli isolates tested, 6 (30%) isolates were positive for the qnr genes, where 5 (25%) isolates were positive for qnrS gene; one of them was positive for qnrB gene and only one isolate carried qnrA gene. All the E. coli isolates were negative for aac (6')-Ib-cr gene. On the other hand, the qepA gene was the most commonly found in the PMQR genes, as it was detected in 14 (70%) E. coli isolates. All qnr positive isolates carried the qepA gene. The β-lactam resistant gene (blaTEM) was detected in 16 (80%) of the isolates; while the blaSHV gene was detected only in one isolate. There was a high correlation between the presence of the qepA gene and resistance to ENR, NA, and NOR, as well as between and blaTEM gene and resistance to AML and AMP.

INTRODUCTION

Escherichia coli is one of the most common microorganisms, which affect both animals and humans worldwide by a wide spectrum of diseases ranging from opportunistic wound infection to severe systemic infections (Gyles and Fairbrother, 2010). Avian colibacillosis refers to any localized or systemic infection caused entirely or partly by avian pathogenic Escherichia coli (APEC), including colisepticemia, coligranuloma (Hjarre’s disease), air sac disease (chronic respiratory disease, CRD), coliform cellulitis (inflammatory process), swollen-head syndrome, coliform peritonitis, coliform salpingitis, coliform osteomyelitis/synovitis (turkey osteomyelitis complex), coliform panophthalmitis, and coliform omphalitis/yolk sac infection (Barnes, 2000). It is the most frequently reported disease in surveys of poultry diseases or condemnations at processing and responsible for significant economic losses to the poultry industry (Yogaratnam, 1995). Escherichia coli acquire antimicrobial resistance faster than other bacteria. Thus, changes in the resistance of this species may serve as a good indicator of resistance in potentially pathogenic bacteria (Von Baum and Marre, 2005). It has been established that the introduction and worldwide use of fluoroquinolones in the treatment of enterobacterial infections has led to the occurrence of (fluoro)quinolone resistance, both in the human-and veterinary
The extended use of (fluoro) quinolones to treat poultry infections lead to the increase of quinolone resistance among *E. coli* strains in poultry industry (Kojima *et al.*, 2009). In the last two decades, however, the plasmid-mediated resistances to (fluoro) quinolones (PMQR) are raising concerns as transferable mechanisms in human and in veterinary enterobacterial isolates (Poirel, 2012). Plasmid mediated bacterial resistance is determined by *qnr* (formerly named *qnr* (*qnr*B, *qnr*S, *qnr*C, *qnr*D), which are variants of *qnr* gene, and encodes *Qnr*B, *Qnr*S, *Qnr*C and *Qnr*D proteins respectively), *aac* (6′)-Ib-cr (encodes an aminoglycoside acetyltransferase for enzymatic inactivation of quinolones) and *qep*A (encodes a new quinolone efflux pump protein, *Qep*A) genes located on plasmids (Poirel, 2005).

Prior to the 1980s, the most common problems associated with β-lactamases of Enterobacteriaceae were caused by plasmid-mediated β-lactamases, such as TEM-1, TEM-2 and SHV-1. The development of hydrolysis-resistant β-lactams and the use of combinations of β-lactams and β-lactamase inhibitors are the two main strategies to circumvent this problem. Introduction of these derivatives in therapy was quickly followed by the emergence of numerous β-lactamases. These enzymes, capable of hydrolyzing oxyimino β-lactams, were isolated in the mid-1980s and are variants of the well-established TEM and SHV penicillinases (Thomson and Moland, 2000).

The aim of the present work was to evaluate the multiple resistant *E. coli* in poultry and detection of the plasmid mediated quinolone and β-lactam resistance genes among *E. coli* isolated from different poultry farms in Egypt.

### MATERIALS AND METHODS

**Bacterial isolates**

A total of 20 isolates of *E. coli* isolated from diseased chickens were taken from the collection centre of the Central Laboratory for Veterinary Quality Control on Poultry Production, Dokki. The *E. coli* isolates were recovered from different organs (liver, lung, yolk sac and bone marrow) of chickens that suffered from colisepticaemia. The isolates belonged to different O-serogroups and showed resistance to one or more antibiotics.

**Antimicrobial susceptibility test:**

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (Difco), according to the Clinical and Laboratory Standards Institute guidelines (Anonymous 2006). A total of 9 different antibacterial sensitivity discs were used for confirmation of resistance of the isolates to one or more antibiotics. The antimicrobial agents tested and their corresponding concentrations were as follows: gentamycin (GM) (10 µg/disk), tetracycline (TE) (30 µg/disk), streptomycin (S) (10 µg/disk), doxycycline (DO) (30 µg/disk), CL (colistin sulphate) (10 µg/disk), chloramphenicol (C) (30 µg/disk), trimethoprim-sulphamethoxazole (SX) (23.75 µg/disk), neomycin (N) (30 µg/disk). Moreover, Seven different antibacterial sensitivity discs of the β-lactam and Quinolone antibiotics were used in the present investigation: ampicillin (AMP) (10 µg/disk), amoxicillin (AML) (10 µg/disk), norofloxacin (NOR) (10 µg/disk), enrofloxacin (ENR) (5 µg/disk), danofoflaxcin (DFX) (5 µg/disk), nalidixic acid (NA) (30 µg/disk), ciprofloxacin (CIP) (5 µg/disk), and After incubating the inoculated plates aerobically at 37 °C for 18 to 24 h, the susceptibility of the *E. coli* isolates to each antimicrobial agent was measured and the results were interpreted in accordance with criteria provided by CLSI (Anonymous 2006). *E. coli* NCIMB-50034- ATCC43894, was used as quality control organisms in antimicrobial susceptibility determination.

**DNA extraction and PCR amplification**

*E. coli* were subcultured overnight in Luria Bertani broth (Merck, Germany) and genomic *E. coli* isolates DNA was extracted using a Genomic DNA purification kit QIAamp® DNA Mini Kit (Cat. No. 51304 Qiagen). according to the manufacturer’s instructions.

**Oligonucleotide primers and PCR assay:**

Oligonucleotide primers sequences encoding for quinolone resistant genes:  

<table>
<thead>
<tr>
<th>Gene</th>
<th>F</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>5′-AGGATGACTGTGCTTTTGT-3′</td>
<td>5′-ATTTGCTGTCTCGTGTC-3′</td>
</tr>
<tr>
<td><em>bla</em>&lt;sub&gt;TEM&lt;/sub&gt;</td>
<td>5′-ATCAGCATAAAACCAGC-3′</td>
<td>5′-CCCCGAAGAAGGGTTTC-3′</td>
</tr>
<tr>
<td><em>aac</em>(6′)-Ib-cr</td>
<td>5′-CTGCAGTACTGCTGTCATG-3′</td>
<td>5′-ACCACATCCGTCAACTGCA-3′</td>
</tr>
<tr>
<td><em>qnr</em>B</td>
<td>5′-ATTCTCAGCCAGATTTG-3′</td>
<td>5′-TTAACATACCTGCTGTC-3′</td>
</tr>
<tr>
<td><em>qnr</em>S</td>
<td>5′-GATCGCAGAAGTATTG-3′</td>
<td>5′-AACGATACTGCACCCAT-3′</td>
</tr>
<tr>
<td><em>aac</em>(6′)-Ib-cr</td>
<td>5′-CTGCAGTACTGCTGTCATG-3′</td>
<td>5′-ACCACATCCGTCAACTGCA-3′</td>
</tr>
<tr>
<td><em>qnr</em>B</td>
<td>5′-ATTCTCAGCCAGATTTG-3′</td>
<td>5′-TTAACATACCTGCTGTC-3′</td>
</tr>
<tr>
<td><em>qnr</em>S</td>
<td>5′-GATCGCAGAAGTATTG-3′</td>
<td>5′-AACGATACTGCACCCAT-3′</td>
</tr>
</tbody>
</table>

The preparation of PCR Master Mix for cPCR was according to EmeraldAmp 2X PCR Master Mix kit as follow, PCR reactions were performed in a total volume of 25µl, including 12.5 µl MASTER MIX, 1 µm primers ,5 µl (40–
260 ng/µl) of DNA and nuclease water up to 25 µl. Amplification reactions were carried out as follows: Three min at 95 °C, 35 cycles each consisting of 1 min at 94 °C, 90 s at ~55 °C (differ according each primer Tm) and 1 min at 72 °C, followed by a final extension step of 10 min at 72 °C. Amplified samples were analysed by electrophoresis in 1.5% agarose gel and stained by ethidium bromide. A molecular weight marker with 100 bp increments (100 – 600 bp DNA ladder, Fermentas) was used as a size standard. Strains of E. coli NCIMB-50034- ATCC43894 was used as positive controls.

RESULTS

Confirmation of E. coli isolates
All recovered isolates showed the typical colony characteristic of E. coli, where they appeared as rose pink colonies on MacConkey’s agar medium. On EMB medium they gave the characteristic metallic sheen appearance. All isolates showed the typical biochemical reactions (Quinn et al., 2002). The serotyping confirmed the following serogroups: O26, O55, O86, O91, O103, O111, O114, O118, O119, O142, O145, and O158.

Results of antibiotic resistance:

![Fig. 1: Results of antibiotic sensitivity of the isolated E. coli strains from different poultry to 16 antibiotics](image)

As shown in Fig. 1, it is clear that the highest rate of resistance was shown against the quinolone group of antibiotics, where 80-95 % of the tested isolates were resistant, followed by the 2 β-lactam antibiotics and doxycycline, where 60% of the isolates were resistant. The number of resistant isolates against the remaining antibiotics decreased, and only one isolate was resistant to colistin-sulphate and chloramphenicol, while all isolates were sensitive to gentamycin. It is evident that the highest resistance was recorded against enrofloxacin (95%), followed by norfloxacin (85%), nalidixic acid (80%), danofloxacin (45%) then the least resistance was observed against ciprofloxacin (20%).
The results shown in Fig. 2 demonstrate the number of antibiotics to which the tested *E. coli* showed resistance. It is evident that none of the isolates was resistant to less than 4 antibiotics, while 40% of the isolates were resistant to 8 or 9 antibiotics, while 20% of the isolates were resistant to 10 or 11 antibiotics.

**Multiple resistances**

Multiple resistances, i.e. to 3 or more antibiotics, when the isolates were tested against 9 antibiotics, was detected in 17 isolates (85%), while the three remaining isolates were non-multiple resistant. On the other hand, 60% of the isolates showed resistance to ≥ 4 antibiotics and 15% of the isolates were resistant to 5 antibiotics (Table 1).

<table>
<thead>
<tr>
<th>Number of antibiotics</th>
<th>Percentages of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 3</td>
<td>15%</td>
</tr>
<tr>
<td>≥ 3</td>
<td>85%</td>
</tr>
<tr>
<td>≥ 4</td>
<td>60%</td>
</tr>
<tr>
<td>≥ 5</td>
<td>15%</td>
</tr>
</tbody>
</table>

When multiple resistance was calculated in relation to the groups, i.e. to ≥ antibiotic groups, 14 isolates were considered multiple resistant, while the six remaining isolates were non-multiple resistant. On the other hand, 70% of the isolates showed resistance to ≥ 3 antibiotic groups and 15% of the isolates were resistant to 4 antibiotic groups (Fig. 3).
Fig. 3. Multiple resistance in *E. coli* isolates by testing 9 antibiotics in 6 groups.

When the number of antibiotics used for testing was increased to 16 antibiotics in 8 groups, the non-multiple resistant *E. coli* isolates disappeared and all tested strains (100%) showed resistance to ≥ 3 antibiotic group and over 90% of the isolates were resistant to ≥ 4 antibiotic groups (Fig. 4).

Detection of plasmid-mediated resistance genes

Detection of plasmid-mediated quinolone resistance genes:

Among the twenty *E. coli* isolates tested, 6 (30%) isolates were positive for the *qnr* genes, where 5 (25%) isolates were positive for *qnrS* gene; one of them was positive for *qnrB* gene and only one isolate carried *qnrA* gene. All the *E. coli* isolates were negative for *aac (6’)-Ib-cr* gene.

On the other hand, the *qepA* gene was the most commonly found in the PMQR genes, as it was detected in 14 (70%) *E. coli* isolates. All *qnr* positive isolates carried the *qepA* gene (Fig. 5).
Fig. 5. Agarose gel electrophoresis of products obtained by PCR for *E. coli* strains to detect gene *qepA*.

Detection of β-lactam resistance genes:

Fig. 6. Agarose gel electrophoresis of products obtained by PCR for *E. coli* strains to detect gene *bla<sub>TEM</sub>.*

*Fig. 6* shows the high prevalence of the β-lactam resistant gene (*bla<sub>TEM</sub>* gene), which was detected in 16 (80%) of the isolates; while the *bla<sub>SHV</sub>* gene was detected only in one isolate.

**The correlation between the antibiotic resistance and the serogrouping of *E. coli* isolates:**

It is clear from the Table 2, that there was no correlation between the serogroups and the quinolone resistance in the tested *E. coli* isolates. This was clear in the five isolates of serogroup O91, which showed different patterns of sensitivity, the two isolates of serogroup O26, the two isolates of serogroup O86 and the two isolates of serogroup O103. Only the two isolates of serogroup O158 were similar in their resistance profile.

**The correlation between the antibiotic resistance and the resistance genes detected in the *E. coli* isolates:**

As shown in Table (2), the correlation was 100% between the the *qepA* gene and the antibiotic ENR, as all 14 *E. coli* isolates positive for the gene were resistant to this antibiotic. On the other hand, the correlation between the detection of this gene and the antibiotic resistance was 85% in case of NA and NOR and 57% in case of DFX.
The qepA gene could not be detected in 6 *E. coli* isolates, though one of the isolates was resistant to all 5 quinolone antibiotics; 2 isolates were resistant to NA, ENR and NOR, one isolate to NA and ENR, one to ENR and NOR and one isolate was resistant only to NOR.

In case β-lactam antibiotics, of the 12 resistant isolates there were 11 positive isolates for the blaTEM gene (91%). On the other hand, only 11 of the 16 isolates positive for the blaTEM gene were resistant to both amoxicillin and ampicillin (69%) and 4 of the 6 isolates negative for the gene (67%) were sensitive to both antibiotics.
Table (2): the relation between the antibiotic resistance patterns and the presence of resistant genes.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>O-group</th>
<th>AML</th>
<th>AMP</th>
<th>NA</th>
<th>DFX</th>
<th>ENR</th>
<th>NOR</th>
<th>CIP</th>
<th>Antibiotic resistant genes detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>O91</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>R</td>
<td>S</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>O91</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>+</td>
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<tr>
<td>3</td>
<td>O91</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>+</td>
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<tr>
<td>4</td>
<td>O91</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
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<td>R</td>
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<tr>
<td>5</td>
<td>O91</td>
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<td>R</td>
<td>R</td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>+</td>
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<tr>
<td>7</td>
<td>O158</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>+</td>
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<tr>
<td>8</td>
<td>O142</td>
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<td>R</td>
<td>R</td>
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<td>R</td>
<td>R</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>O145</td>
<td>R</td>
<td>R</td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>O26</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
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<td>+</td>
</tr>
<tr>
<td>11</td>
<td>O26</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
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<td>S</td>
<td>+</td>
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<tr>
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<tr>
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<tr>
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<td>15</td>
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<td>R</td>
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<td>S</td>
<td>+</td>
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<tr>
<td>17</td>
<td>O114</td>
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<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>O118</td>
<td>S</td>
<td>S</td>
<td>R</td>
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<td>R</td>
<td>S</td>
<td>+</td>
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</tr>
<tr>
<td>19</td>
<td>O119</td>
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<td>R</td>
<td>R</td>
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<td>R</td>
<td>S</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>O55</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>+</td>
</tr>
<tr>
<td>Number &amp; percent of drug resistant</td>
<td>12/20</td>
<td>12/20</td>
<td>16/20</td>
<td>9/20</td>
<td>19/20</td>
<td>17/20</td>
<td>4/20</td>
<td>16/20 (80%)</td>
<td>1/20 (5%)</td>
</tr>
</tbody>
</table>

- Number of drug resistant isolates: 20
- Percentage of drug resistant isolates: 60%
- Percentage of qnr genes positive isolates: 30%
DISCUSSION

Escherichia coli bacteria are a normal inhabitant of the gastrointestinal tract of humans and animals; however, some strains are known to be pathogenic. These strains induce colibacillosis in chicken, which is an important cause of economic losses for the poultry industry (Amara et al., 1995).

Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis (Freed et al., 1993). Antibiotic usage is considered the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Witte, 1998).

In the present study, we investigated the antibiotic sensitivity of the collected E. coli isolates against 8 groups of antibiotics, with particular emphasis on quinolone and β-lactam group. The quinolone group included nalidixic acid, which represents the first generation of quinolones and four fluoroquinolones (danofloxacin, enrofloxacin, norfloxacin, and ciprofloxacin). The β-lactam group included ampicillin and amoxicillin. The selection of the antibiotic groups for this study was according to the history of using one or more member of these groups for treatment of the diseased chickens which the isolates were taken from it (Roshdy et al., 2012). These antibiotic groups were commonly used in treatment of colibacillosis (Yue et al., 2008).

The result of the antimicrobial resistance test against the tested antibiotics was ampicillin (60%), amoxicillin (60%), nalidixic acid (80%), danofloxacin (45%), enrofloxacin (95%), norfloxacin (85%), ciprofloxacin (20%).

Variable antibiotic resistance rates to ampicillin and amoxicillin were reported by several authors (EL-Ged et al., 1985, Torky et al., 1995, Ibrahim, 1997 and Miranda et al., 2008). The rates varied from 100% (Tabatabaei et al., 2010) to 99.3% and 65.1% (Jiang et al., 2011), 63% (Ponce-Rivas et al., 2012), 42% (Khan et al., 2005), 20.6% (Miles et al., 2006) and 15.8% (Knezevic, and Petrovic, 2008).

The striking finding in the present work was the wide spread resistance to quinolones and fluoroquinolones, where it reached to 85-95% of the tested isolates. Similar results were reported in Iran (Salehi and Bonab, 2006). The identification of quinolone-resistant E. coli was reported also in several countries (Chen et al., 2011). The high prevalence of quinolone resistant E. coli among broiler chickens is probably due to over use of such antibiotics. Variable quinolone resistance to nalidixic acid was 80.2%, enrofloxacin 64.2%, norfloxacin 46.3%, Ciprofloxacin 37.5% (Chen et al., 2011), while it was 47.3% to enrofloxacin, 43.4% to norfloxacin and 44.4% to Ciprofloxacin (Jiang et al., 2011). Resistance to ampicillin was 84.0%, nalidixic acid 92.5%, norfloxacin 55.6%, ciprofloxacin 57.8%, enrofloxacin 68.4% (Lei et al., 2010).

In this study, the selected isolates, when tested against 6 antibiotic groups, showed resistance to 2 or more antibiotic groups. Multiple resistances, i.e. to 3 or more antibiotic groups, were determined in 14 isolates, while the six remaining isolates were non-multiple resistant. On the other hand, 70% of the isolates showed resistance to ≥ 3 antibiotic groups as reported by (Zhao et al., 2005 and Wang et al., 2010) and 15% of the isolates were resistant to 4 antibiotic groups.

When the number of antibiotics used for testing was increased to 8 antibiotic groups, the non-multiple resistant E. coli isolates disappeared and all tested strains (100%) showed resistance to ≥ 3 antibiotic groups. On the other hand, most of the multidrug resistance incidences were lower than the incidence of this study. Variable multidrug resistance incidences (92% down to 58%) were reported (Zhao et al., and Wang et al., 2005, Jiang et al., 2011 and Ponce-Rivas et al., 2012).

Among 20 avian Escherichia coli isolates, 6 (30%) isolates were positive for the qnr gene, including one isolate that carried qnrA gene and five isolates were carried qnrS gene one of them carried qnrB gene. 14 (70%) isolates were positive for qepA gene While aac(6’)-Ib-cr gene not detected.

On the other hand, it was reported that 14 (6%) isolates were positive for the qnr gene, including one for qnrB, 13 for qnrS, but no qnrA was identified in this population (Zhao et al., 2005). Detection of the aac(6’)-Ib gene showed only one qnrS-positive isolate. While it was found that the prevalence of qnr genes, aa(6’)-Ib-cr, and qepA were 9.8%, 11.7% and 0.75%, respectively. Among the qnr determinants, qnrA-, qnrB-, and qnrS-type genes were detected in 4 (0.75%), 21 (3.9%), and 27 (5.1%) of the examined isolates, respectively.
The \textit{qnrS} gene, but not \textit{qnrA}, \textit{qnrB}, and \textit{qepA} were detected in 6/300 chicken isolates (2\%) and only one \textit{qnrS}-positive isolate carried the \textit{aac(6')-Ib-cr} variant that mediates FQ acetylation (Kuo \textit{et al.}, 2005). As far as \textit{qnrA} was concerned, only one or two positive clinical isolates were detected in most prevalence reports (Xu \textit{et al.}, 2007). In those studies isolates of \textit{E. coli} were selected by different criteria such as resistant to ciprofloxacin or producing ESBLs to be screened. While the first report of \textit{qnrA}-positive strains in China was the six \textit{E. coli} collected from human clinical cases in Shanghai, China (Wang \textit{et al.}, 2004). In the present study, the β-lactam resistant gene \textit{bla}\textsubscript{TEM} detected in 16 out of the 20 isolates tested, while the gene \textit{bla}\textsubscript{SHV} was detected in only one isolate no (9).

The β-lactamase genes were detected, SHV-1 (2.4\%) and TEM 20 (48.7\%) (Domínguez \textit{et al.}, 2002) Three positive \textit{bla}\textsubscript{TEM} PCR result from five Escherichia coli isolates, and a positive \textit{bla}\textsubscript{SHV} PCR result was obtained for an additional isolate (Brinas \textit{et al.}, 2003), which were lower than the result of this study. The \textit{qepA} gene is a new plasmid-mediated gene responsible for reduced FQ susceptibility from Escherichia coli C316, which was isolated in 2002 from the urine of an inpatient in Japan (Yamane \textit{et al.}, 2007) and \textit{qepA} was also reported from \textit{E. coli} isolated in a Belgian hospital (Périchon \textit{et al.}, 2007). \textit{qepA} encodes an efflux pump belonging to the major facilitator subfamily (MSF). The MICs of norfloxacin, enrofloxacin, and ciprofloxacin were 32 to 64 fold higher for the experimental strains expressing \textit{QepA} compared with the host strain (Yamane \textit{et al.}, 2007). The MICs of ampicillin, erythromycin, kanamycin, tetracycline, and chemical substances such as carbonyl cyanide m-chlorophenylhydrazine, acriflavine, rhodamine 6G, crystal violet, and sodium dodecyl sulfate were not affected, however, indicating that FQs are the specific substrates of \textit{QepA}. Moreover, a norfloxacin accumulation assay with or without carbonyl cyanide m-chlorophenylhydrazine, an efflux pump inhibitor, showed that \textit{QepA} is an FQ-specific MSF-type efflux pump (Yamane \textit{et al.}, 2007).

**REFERENCES:**

