Journal of Advanced Veterinary Research

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# Risk Assessment and Quinolones Plasmid-mediated Resistance Genes in Intensive Broiler Farms

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**ARTICLE INFO**

**Original Research**

**Received:**
07 March 2021

**Accepted:**
21 March 2021

*Keywords:*

*qnrA*, *qnrB*, *gyrA*, Broiler-intensive farms, Plasmid-mediated resistant determinates, Enrofloxacin

**ABSTRACT**

Quinolones are frequently applied in intensive production and their risks are creation of residues and development of bacterial resistance, which is one of the major challenges to human health. Muscle, liver and kidney as well as, intestinal contents were collected from intensive broiler farms in Upper Egypt. Samples were analyzed for quinolones residues (norfloxacin, ciprofloxacin, enrofloxacin and danofloxacin) using HPLC. Intestinal contents were investigated for two plasmid-mediated resistance genes; *qnrA*, *qnrB* and one chromosomal gene; *gyrA*. All liver and kidney samples were contaminated with enrofloxacin and the highest level was found in the liver that exceeded the maximum residue limit, which contribute to health hazards for adult and children. QnrB and *gyrA* genes could be detected in 25% and 8% of the samples, respectively. This study represents the first record about existence of *qnrB* and *gyrA* in broiler intestinal content. These results denoted that some broilers' edibles sold in Egypt contain high level of quinolones. Litter from intensive farms maybe considered as a critical source of resistant genes giving a chance of horizontal transfer of resistant determinants. Consequently, it is necessary to examine broiler products and by-products for the presence of residual quinolones and resistance genes.

*J. Adv. Vet. Res. (2021), 11 (2), 110-118*

## Introduction

Antibiotics are frequently applied in intensive poultry production; however, the fundamental risks of improper use include buildup of residues, development of bacterial resistance and environmental contamination. Quinolones is one of the most frequently used antibiotics in food-producing animals (Kirbiš, 2007). They are synthetic antibacterial substances with broad spectrum of activity and widely used in animals as well as humans to treat bacterial infections (Zhao *et al*., 2007). The first-generation quinolones have good antibacterial activity against Gram negative bacteria; however, their antibacterial effect is no longer reliable as resistant bacteria have developed (Ito, 2005). The second and third generation includes fluoroquinolones as danofloxacin, enrofloxacin and norfloxacin, which have a broad antibacterial range and they are effective against Gram -positive, Gram- negative bacteria, mycoplasma, mycobacteria and rickettsia (Kumar *et al*., 2008). Fluoroquinolones are intensively used in poultry production and exert bactericidal action by inhibiting the catalytic activity of DNA gyrase (Sharma *et al*., 2009). They exhibit rapid absorption with wide tissues dissemination and excreted through urine and bile with their residues are situated in the liver and kidney (Goetting *et al*., 2011). Fluoroquinolones can enter cells easily and used to treat intracellular organisms (Lyczak *et al*., 2002). Enrofloxacin is the major product orally applicable to poultry and ciprofloxacin is its metabolite that appears in different foodstuffs after use (Hopkins *et al*., 2005; Kirbiš *et al*., 2005).

Ingestion of broiler edibles containing quinolone residues may harm human health, causing hypersensitivity, aplasia of bone marrow, intestinal microflora imbalance, drug interactions and cancer induction (Kowalski *et al*., 2005; WHO, 2009). The usage of those antimicrobials in livestock production may affect human health throughout selection of resistant microorganisms followed by transmission to humans and dissemination into the environment (Castanon, 2007). The Maximum Residue Limits (MRLs) of quinolones in food products of animal origin were established by the European Union to protect the consumer from exposure to risk levels (European Commission, 2010).

The emergence of antibiotic resistant microorganisms has become one of the main challenges of human health care in the world (Bush *et al*., 2011). In the 21st century, the spread of antibiotic resistance has been considered as one of the great-

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est serious threats to public health (WHO, 2014). The widespread usage of fluoroquinolones in human and veterinary medicine has led to a major increase in antibacterial resistance with important consequences for public health (Christodoulou *et al*., 2007). The FDA has banned the use of enrofloxacin in poultry because of the emergence of *Campylobacter* resistance to quinolones, which may result in ineffective treatment of human diseases (FDA, 2005). Resistance to fluoroquinolones is chiefly produced by mutations in the chromosomal genetic factor that code for the target enzymes (DNA gyrase and/or DNA topoisomerase Iv). Plasmid-mediated quinolone resistance (PMQR) mechanisms involves *qnr* genes that determine target protection proteins of the pentapeptide family. Five *qnr* genes (*qnrA*, *qnrB*, *qnrS, qnrC and qnrD*) can be transferred horizontally. The proposed mechanism for the transfer of the resistance genes through food chain were identified by Poirel *et al*. (2012). Moreover, the gain of the antibiotic resistance through horizontal gene transfer may allow the rapid development and spreading of antibiotic resistance, resulting in replacing the antibiotic susceptible bacteria with resistant one, with the lack of any antibiotic selective pressure (Enne *et al*., 2004; Luo *et al*., 2005). In the last two decades, PMQR are raising concerns as transferable mechanisms in human and veterinary enterobacterial isolates (Poirel *et al*., 2012). The prevalence of antibiotic-resistance determinants among clinical pathogens and environmental bacteria is a worldwide threat to human. A significant relationship has been noticed between the veterinary use of enrofloxacin and diminished susceptibility to ciprofloxacin in salmonella isolated from human (Threlfall *et al*., 1997).

In Egypt as well as other African countries the agricultural sectors consume a large percentage of antibiotics in animal farming with no obvious regulation monitoring for antibiotic residue in feedstuffs (Darwish *et al*., 2013). Additionally, in Egypt, there is a great lack of accessible information about antibiotic residues in poultry-derived foods (very few studies evaluating the quinolones' residue in Egyptian poultry farms). Therefore, intensive broiler farm samples were targeted for quinolones residues levels and resistant genes in Upper Egypt, to cover the gap and lack in information about their risk that might pose a danger to bird and human health.

## Materials and methods

*Quinolones' residue in broiler edibles*

Sample collection and preparation

Broiler farms, with a history of quinolones application, were distributed across four provinces (Qena, Sohag, Assiut and El-Minya), which represents intensive broiler production in Upper Egypt. Two hundred and four samples (muscle, liver and kidney) were collected from 12 farms that had a size ranged from 3,000 to 20,000 birds/house. Samples were transferred in an ice box to the Animal Hygiene laboratory and stored at −20 °C and analyzed within 2 months.

The samples were prepared as described by Dutko *et al*. (2006). In 50 ml polypropylene centrifuge tube, 3 mL acetonitrile and 0.25 mL conc. ammonium hydroxide were added to one gram of homogenized tissue sample (pooled 3 samples of each type) with WiseMixTM Homogenizer HG15D (DAIHAN Scientific, Co., Ltd, Seoul, Korea), then subsequently vortex for 15 sec., after that the uppermost layer was discarded, then nitrogen evaporation at 50ºC was done to reach approximately 200 µL. Phosphate buffer 0.1M (2 ml) was added to each tube, vortexed 15 sec and filtered with 0.2 µm nylon syringe filter and 20 µl was injected onto the HPLC system. Four members of quinolones group norfloxacin (NOR), ciprofloxacin (CIP), enrofloxacin (ENR) and danofloxacin (DAN) were chosen as target compounds based on their frequent usage in intensive broiler production.

Chemicals and standards

Chemicals used in the current study were of Analytical Reagent Grade. Fluoroquinolones antibiotic standards including norfloxacin (NOR>99.6), ciprofloxacin (CIP >84.9 %) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. Enrofloxacin (ENR >99.9 %) and Danofloxacin (DAN) were obtained from Sigma-Aldrich Chemical Company, USA. Acetonitrile (75-05-8, Bishop Meadow Road, Leicestershire, Fisher Scientific UK Ltd), concentrated ammonium hydroxide, monobasic sodium phosphate monohydrate and dibasic sodium phosphate heptahydrate (Fisher Scientific, 7558-79-4, Fair Lawn, NJ). Stock solution of quinolones' standards were prepared every 2 months by dissolving 10 mg of each antibiotic into 10 ml of sodium hydroxide solution (0.03 mol $l^{-1}$) and stored at 4°C in dark place. Working solution was freshly diluted from the stock solution upon analysis.

Chromatogram conditions and quantification

The residue level of the four selected quinolones antibiotics (DAN, ENR, CIP, NOR) were measured using high-performance liquid chromatography (HPLC) at the Analytical Chemistry Unit, Faculty of Science, Assiut University, Egypt. Table 1 shows the conditions followed during the analysis.

Table 1. HPLC conditions for measuring quinolones antibiotics (DAN, ENR, CIP, NOR) residue.

| Project | Parameter |
|---|---|
| HPLC | Agilent Technologies 1200 Series, G1315D DAD |
| Detector | DAD at 275 nm |
| Column | ZorbaxXDB C18 (4.6 × 150mm, 5 Micron) |
| Temperature | 25 °C |
| Flow rate | 1.5 ml min−1 |
| Mobile phase | Solvent A: 82.0 % (Citrate buffer pH4.5)<br>Solvent B: 10.0 % (MeOH)<br>Solvent C: 8.0 % (ACN) |
| Gradient elution | Time Solv.ASolv.BSolv.C<br>\|--------\|-------\|-------\|-------<br>0.00 0.0 10.0 8.0<br>12.00 0.0 10.0 8.0<br>29.00 0.0 10.0 50.0<br>30.00 0.0 10.0 8.0 |

Quality control

Negative control and positive control (spiked with the individual standards for recovery) samples were analyzed with each batch of samples. Detection (LOD), quantification (LOQ) limit and experimental repeatability and reproducibility were applied strictly during the development of this method. The concentration of each antibiotic in the solution was quantified to represent the internal standard using Calibration curves with 4 points; 0.001, 0.1, 0.2 and 2 µg/µl for NOR, CIP and 0.001, 0.5, 1, 5 µg/µl for ENR and DAN. The correlation coefficients were linear and R2 were above 0.98 %. The LOD and LOQ were determined for each antibiotic at the ratio of signal to noise (S/N) to be 3 and 10, respectively (Jacobsen and Halling-Sørensen, 2006). Recoveries were calculated after subsequent injection of residue-free tissue samples with 0.5 and 2 mg $kg^{-1}$ and were analyzed in four replicates. Estimated average recovery were 102 %, 87%, 99% and 92% for NOR, CIP,

ENR and DAN, respectively. The LODs for poultry muscle, liver and kidney samples were 0.001 mg $l^{-1}$. Any sample contains higher concentration than the calibration range was diluted to fall within the range. Reliability of instruments was checked by analyzing a known standard concentration every 10 samples.

*Health hazard from broilers' edibles consumption*

According to European Commission (2010) MRL for ENR is assessed as the sum of ENR and CIP. In broilers, MRL is 100, 200 and 300 µg/kg for muscle, liver and kidney, respectively. The limits for DAN are 200 for muscle and 400µg/kg for liver and kidney. Therefore, the assessed levels in the collected samples were compared with the established MRL.

Acceptable Daily Intake (ADI) of ENR is appraised to be either 2 or 6.2 µg/kg body weight according to FAO/WHO (2004) and EMEA (1998), respectively. The estimated risk assessment from consumption of broilers' edibles contained quinolones' residue was presented by the Estimated Daily Intake (EDI) (Juan *et al*. 2010) with a little modification according to the 66th meeting of JECFA (2006) that recommended the use of the median of antibiotic residue rather than mean value.
EDI= CXFIR/BW

FIR, food ingestion rate (kg/day); BW is the average body weight (70 kg for adults and 12 kg for kids)

EDI were compared with the ADI and the level of achievement percentage was calculated (Vragovic *et al*., 2011):
Level of achievement= EDIX100/ADI

The risk was settled to be negligible when the level of achievement was less than 1% of the ADI, considerable or distinctive if it was 1-5% or greater than 5% of the ADI, respectively.

*Quinolones resistance genes*

Intestinal content sampling

The whole intestine was taken from eight to eleven chicks with age 35-42 days from twelve broiler farms representing Upper Egypt. Samples were maintained at -20 °C in sterile plastic bags until analysis. Before extraction, the intestinal content from each segment was squeezed, then 2-3 g of the whole content was taken in a sterilized tube. Pooled sample was prepared by mixing equal volumes of intestinal content (about 1g each) to get a representative sample of approximately 8-10 g for each farm.

DNA extraction of the intestinal content samples

The homogenized intestinal content was used for DNA extraction and purification using QIAamp DNA Mini Kit (Qiagen) according to the manufacture instruction. DNA purity in the obtained extract was assessed using GeneQuant®1300 Spectrophotometer (Bioscience, Sweden) at absorbance from 230 nm to 320 nm. The A260/A280 and A260/A230 ratio were calculated and ratios between 1.8 and 2.0 and 1.8: 2.2, respectively were accepted for DNA purity (Wilfinger *et al*., 2006).

PCR technique

Two plasmid-mediated quinolones resistance genes; *qnrA*, *qnrB* and one chromosomal gene; *gyrA* were investigated in the collected intestinal samples according to Cattoir *et al*. (2007a,b) and Dutta *et al*. (2005). Based on a sequence alignment of *qnrA* and *qnrB* genes, pairs of primers were used to amplify fragments with size of 580 and 264, respectively (Table 2). PCR was done in Molecular Biology Unit (Assiut University, Egypt). Total DNA (4 uL) was subjected to multiplex PCR in a 20 uL reaction mixture containing 10 ul Green GoTaq master mix (Promega), 1 ul from each primer and distilled deionized water sufficient for a final volume of 20 uL. Amplification was carried out with the following thermal cycling profile: an initial denaturation at 95°C for 5 min; 40 cycles consisting of 1 min at 95°C, annealing for 1 min at 50°C, and 1 min at 72°C; and a final extension step for 10 min at 72°C. For detection of *gyrA* gene, PCR amplification was done in a reaction of 20 uL containing 5 uL of DNA extract, 1 uL of both the forward and reverse primers, 10 uL of Green GoTaq master mix and distilled water was added to reach 20 uL. The PCR thermal conditions included an initial denaturation at 95°C for 5 min; 40 cycles consisting of 1 min at 95°C, annealing for 1 min at 50°C, and 1 min at 72°C; and a final extension step for 10 min at 72°C. Aliquots (5 uL) of the obtained PCR products were analyzed with electrophoresis on a 1.5 % TAE agarose gel (Bioline, Cat. No. Bio-41026) containing ethidium bromide (0.5 ug/ml final concentration). Gel with the produced amplicons stained with ethidium bromide, was visualized under a high-performance UV transilluminator and analyzed with a gel documentation system (Bio Doc analyzer, Biometra).

*Statistical analyses*

Statistical analyses of the obtained data about quinolones' residue in the broiler samples were performed using SPSS version 16.0 for Windows. The obtained data are shown as means and standard error. The antibiotics levels were compared by One-way of variance (ANOVA) and when $P<0.05$, the difference in mean values was accepted as significant statistically. When the difference was significant the means were separated by Duncan's post hoc test. Differences with $P<0.05$ were considered statistically significant.

## Results

*Quinolones' residue in broiler edibles*

Figure 1 displayed the typical chromatogram of quinolones. The mean residue concentrations in broilers' tissue are presented in Fig. 2. The highest DAN mean concentration was identified in the liver, while the lowest was in the muscle (significant difference $P<0.05$) (Fig. 2a). DAN residue

Table 2. Oligonucleotides sequences of qnrA, qnrB and gyrA primers.

| Target gene | Primer sequence (5′-3′) | Amplicon size (bp) | Annealing temperature (°C) |
|---|---|---|---|
| *qnrA* | F: AGAGGATTTCTCACGCCAGG<br>R: TGCCAGGCACAGATCTTGAC | 580 | 50°C |
| *qnrB* | F: GGMATHGAAATTCGCCACTG<br>R: TTTGCYGYYCGCCAGTCGAA | 264 | 50°C |
| *gyrA* | F: TACACCGGTCAACATTGAGG<br>R: TTAATGATTGCCGCCGTCGG | 648 | 50°C |

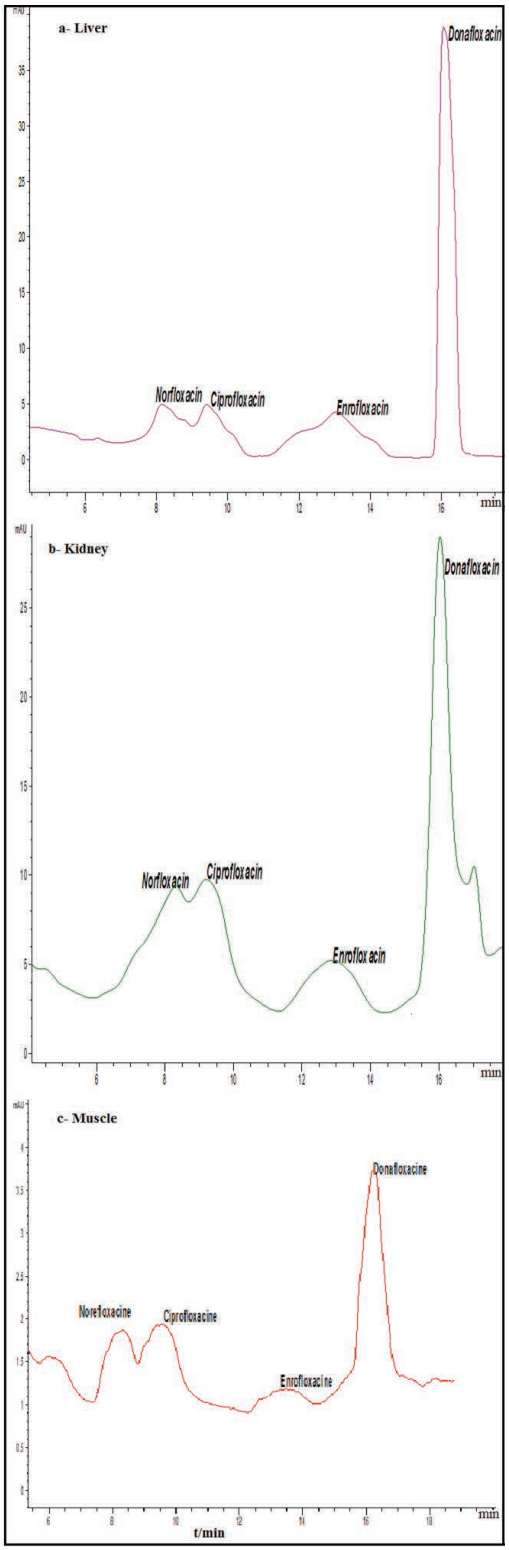


Fig.1. Representatives HPLC Chromatogram of liver (a), kidney (b) and broiler muscle sample (c) spiked with a standard mixture of NOR, CIP, DAN and ENR at 0.01, 0.025, 0.05 and 0.05mgL-1, respectively. RT: retention time in minute; AU: absorbance units.

was detected in all liver samples, while it was detected in 42 and 24% of kidney and muscle samples, respectively. NOR was detected in 86, 83 and 47% of kidney, liver and muscle samples, respectively. It was found a significant difference ($P<0.05$) between level of NOR in kidney and that of the muscle with non- significant difference between level in the liver and kidney (Fig. 2b).

The data in Fig. 2c shows the level of ENR residue. The highest level was recorded in the liver followed by that of the kidney and the smallest was in the muscle samples with significant difference between that in the liver and muscle ($p< 0.05$). Moreover, all the studied liver and kidney samples were contaminated with ENR, while only 59% of muscle samples contained ENR residue.

Data accessible in Fig. 2d, represent the mean concentration of CIP in the surveyed samples. As for DAN and ENR, the highest CIP level was recorded in the liver followed by that in the kidney and the smallest was found in the muscle with significant difference ($p<0.05$). CIP residue was detected in all liver samples, and in 43 and 18% of the investigated kidney and muscle samples, respectively. Finally, it was observed that the highest quinolones' residues were recorded in the liver specially for ENR, DAN and CIP with the highest residue level (1.3 ug/g) recorded for ENR.

*Health risk assessment*

Estimating the frequency of samples containing quinolones residues higher than the MRL revealed that none of the examined muscle samples were higher than the MRL of both DAN and sum of CIP and ENR. Meanwhile, liver and kidney samples exceeded the limits. The analyzed liver samples disclosed that 94 and 89% higher than MRL for DAN and CIP and ENR, respectively. All kidney samples were higher the MRL for CIP and ENR, while only 43% of the samples were higher than MRL for DAN.

The total dietary exposure of ENR levels estimated in the present study were compared with ADI to assess the potential health risk encountered by consumers. We assumed that the average daily consumption for adults (70 kg average body weight) was 120, 50 and 50 g, while that of 2-year-old child (12 kg average body weight) was 60, 25 and 25 g for muscle, liver and kidney, respectively. The level of achievement for ENR in muscle was >1% of ADI according to FAO/WHO guidelines, and a considerable health risk was proven. However, the level of achievement for liver and kidney was >5% of ADI with both guidelines which reveal a typical distinctive health hazards for the adult consumers. Additionally, the level of achievement for 2-year-old child was >5% of ADI according to both guidelines which reflect a distinctive risk was recognized.

*Quinolones resistance genes*

The intestinal contents collected from intensive broiler farms were examined for three quinolones resistance genes. Some of the tested samples possessed either *qnrB* or *gyrA*, while *qnrA* could not be detected. The plasmid-mediated genes *qnrB* was detected in 3 (25%) of the samples. However, *gyrA* was detected in only one (8%) of the samples. Fig. 3A and B, displays the obtained PCR products, which confirmed the presence of the detected genes.

## Discussion

Almost 80% of food-producing animals get medication that may result in presence of residues in their products (Darwish *et al*., 2013). Furthermore, the magnified density of broilers under intensive systems needs a forceful approach to disease control that may lead to dense use of antibiotics (Jinap *et al*., 2010). According to FDA, around 87% of antibiotics used in livestock animals are for treatment, control or prevention of infectious diseases and 13% as food supplement (Donoghue, 2003; Mahgoub *et al*., 2006). The extensive use of antibiotics and lack of sufficient control of administration will cause the increased risk of residue in animal tissues and product (Lemus, 2008).

In this study, it was seen that the liver exhibited the highest

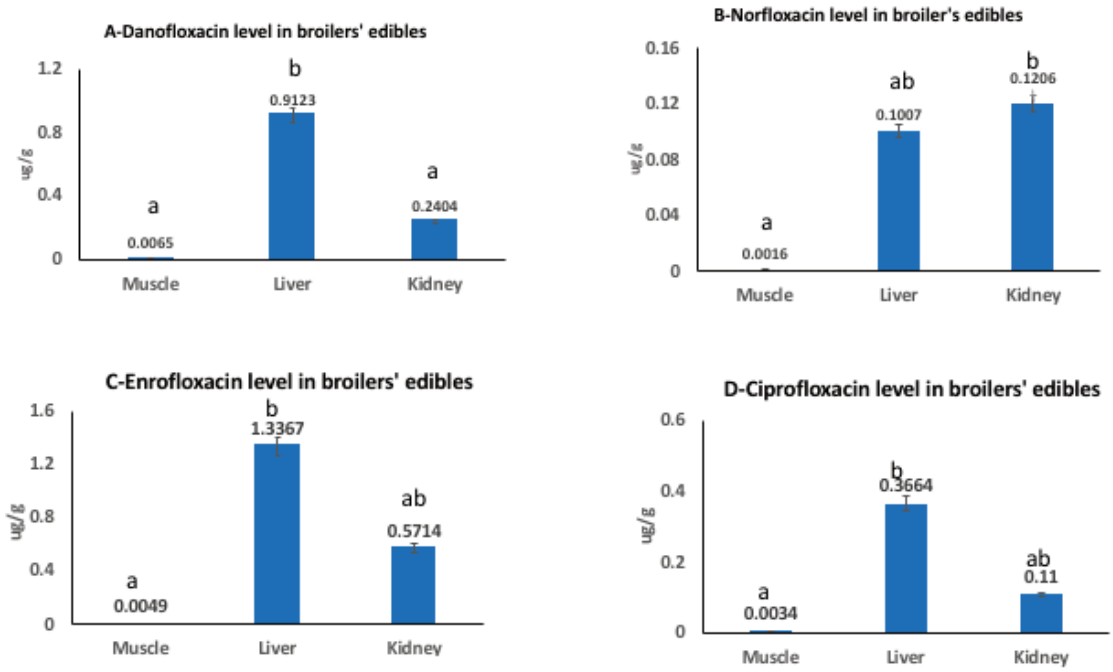


Fig. 2. Quinolones' residues (ug $g^{-1}$) (a) DAN, (b) NOR, (c) ENR and (d) CIP. aandb: means with different letters are significantly different ($P < 0.05$). The highest-level of DAN, ENR and CIP was recorded in liver followed by that in kidney and the smallest was found in muscle with significant differences. Kidney samples exhibited the highest NOR level with a significant difference ($P<0.05$) between the level in kidney and muscle but with non- significant difference between the level in kidney and liver. It was seen that the highest quinolones' residues were recorded for ENR in liver samples (1.3 ug/g).

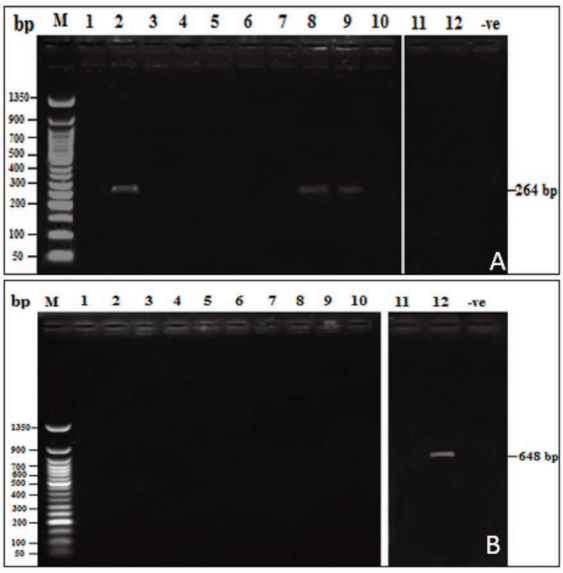


Fig. 3. Agarose gel electrophoreses of PCR for the presence of quinolones resistance genes *qnrB* (A) and *gyrA* (B) in broilers' intestinal content.

residue levels with the highest amount recorded for ENR that could be detected in all inspected liver and kidney samples.

ENR has a wide range of activity specially against enterobacteria, including *Campylobacter* spp., *Enterobacter* spp., and *Serratia* spp. It also active against *Chlamydia* spp. and *Mycobacterium* spp. ENR and CIP are indicated for treatment of *E. coli* and avian mycoplasma (Ito, 2005; Elkholy *et al* 2009). Therefore, high ENR residues may be expected in broilers' edibles specially those reared under intensive system. The recommended dose of ENR and CIP is 10 mg/kg with good absorption and high bioavailability. A withdrawal period of 4 to 10 days was satisfactory (Jelena *et al*. 2006). Quinolones residues in broilers' edibles have been reported by some studies. Hussein and Khalil (2013) analyzed broiler fillet collected from El-Sharkia Governorate, Egypt and confirmed that 14.3% of samples having ENR with level 0.22- 0.42 µg/g. Hasanen *et al*. (2016) detected CIP residues in chicken tissues collected from abattoir in Giza and Qalyiobya Governorates, Egypt with the highest level in kidney then liver and the lowest in muscles and average concentration in kidney, liver, breast and thigh muscle were 316, 211.1, 131.7 and 92.11µg/kg, respectively. Amjad *et al*. (2005) reported that CIP and ENR residue levels were 250 and 1430, 146 and 2143 and 87 and 709 ug/g from liver, kidney and muscle of broiler chicken, respectively. Sahu *et al*. (2014) detected ENR and CIP in 20 and 14.3% of chicken samples in the range of 3.37 to 131.75 µg/kg and 3.55 to 64.59 µg/kg, respectively, with the highest level found in liver. Aslam *et al*. (2016) revealed that 52% meat and 78.7% of liver broiler's samples were positive for ENR with mean residual concentrations 208 and 527 µg/kg, respectively.

Ramatla *et al*. (2017) detected CIP in 72 and 56% of liver and muscle chicken samples with mean concentration of 135.8

and 178.6 µg/kg, respectively. Mashak *et al*. (2017) found that 59.2% of chicken meat samples were contaminated with quinolone residues and the mean levels found to be 37.86 µg/kg. Moghadam *et al*. (2018) found that 100% of the investigated chicken meat samples contained fluoroquinolone with mean levels 72.59 µg/kg, in addition 11.43% of samples exceeded MRL. Sattar *et al*. (2014) confirmed the presence of CIP residues in 44, 42, 34 and 30% in liver, kidneys, thigh muscles and breast muscles from poultry tissues, respectively, while the corresponding rate of ENR were 40, 34, 22 and 18%.

The lipophilic antimicrobial has a great tendency to penetrate many body tissues and owing to long half-lives resulting in residues (Shareef *et al*., 2009). Furthermore, these residues may be formed if right withdrawal period has not been considered. The ENR residue and its metabolite could enter food supply and change ecology of intestinal flora (Chen *et al*., 2011). On the contrary, ENR could not be detected by Weiss *et al*. (2007) from chicken meat in Italy. In addition, ENR, NOR, CIP and DAN residues could not be detected in chicken liver taken from markets in Antakya (Metli *et al*., 2015). Marni *et al*. (2011) reported that DAN could not be detected in chicken muscle collected in Malaysia, while ENR, CIP and NOR were detected in 89, 27 and 3% of the inspected samples, respectively.

The differences among fluoroquinolones residue levels reported by various researchers could be attributed to different methods of analysis, dose and rate of antibiotic use at broiler farms or due to the concern of the appropriate withdrawal period before marketing. The high levels of fluoroquinolones residue detected in the current study may reflect that these substances specially ENR were heavily used in intensive broiler farms in Upper Egypt. Also, suggested that withdrawal period may be insufficiently considered. Therefore, keeping flocks until elapsing of correct withdrawal period is the key factor in determining human exposure to antibiotic residues (Reyes-Herrera *et al*., 2005).

In the current study, residue levels in liver and kidney samples exceeded the guideline limits. Liver revealed 94 and 89 % higher than MRL for DAN and the sum of ENR and CIP, respectively. All kidney samples were higher than MRL for ENR and CIP, while only 43% were higher than MRL for Dan. These results revealed that quinolones residues represent a serious problem for the Egyptian consumers and there is a need of extensive effort to monitor residues in intensive poultry farms. However, these results differ from that reported by Tavakoli *et al*. (2015) and Mashak *et al*. (2017) for absence of chicken meat samples that showed residue level above MRL. The frequency of samples higher than MRL observed in the current study were more or less similar to that reported by Amjad *et al*. (2005), Aslam *et al*. (2016) and Sultan (2014). In the other hand, Nizamlıoglu and Aydın (2012) and Moghadam *et al*. (2018) reported lower rate of chicken samples above MRL.

Risk assessment of antimicrobial substances includes determination of the degree of human exposure. Total dietary exposure levels of ENR estimated in the present study were compared with ADI, which represent a considerable health risk for adult. However, according to the level in liver and kidney, a typical distinctive health hazards was ascertained due to consumption of broiler' edibles contaminated with the assessed ENR level. Therefore, this study indicated that some broilers' edibles as liver and meat sold in Egypt contains high level of quinolones, which contribute a great health hazard to the consumers. Hence, it is necessary to regularly monitor consumed broiler products for the presence of residual quinolones. Furthermore, the obtained results attract some attention to the fact that it is necessary to give information to poultry breeders about withdrawal period. Moreover, it appears that organizations, which are liable for food quality control must do more supervisions and assessments.

Chicken edibles spoiled with antibiotic residues may constitute public health hazards including development of antibiotic resistant bacteria, allergic reactions or imbalance of digestive tract microflora (Mumtaz *et al*., 2000; Gruchalla and Pirmohamed, 2006). CIP residues are well-known to cause toxicity, hinder cytochrome mediated metabolism and increased drug concentration in systemic circulation owing to reduced renal clearance (Khan *et al*., 2015). Additionally, presence of residual amounts could cause serious difficulties for food processors in food fermentation (Toldrá and Reig, 2006). Consequently, routine quality guarantee of food stuff about antibiotic residues is necessary (Farahmand *et al*., 2007).

Under intensive system of poultry production, antibiotics are applied on a regular basis to control the adverse effects of intensification leading to emergence of resistance in some of commensal microbes (Silbergeld *et al*., 2008). A significantly higher incidence of quinolone resistant *E. coli* and salmonella since insertion of quinolones in veterinary medication in the early 1990s was confirmed (Hopkins *et al*., 2005). The intestinal contents from intensive broiler farms were investigated for the presence of quinolones resistance determinants. QnrB and *gyrA* genes were detected in 25 and 8% of the samples, respectively, while *qnrA* could not be detected. According to our best knowledge, there is no data available about the investigation of quinolones-resistant determinants (*qnrA*, *qnrB* and *gyrA*) in intestinal contents or litter from broiler farms. However, one research conducted by Mu *et al*. (2015) reported the presence of *qnrS* in fecal samples collected from chicken farms in Northern China. So, this study is the first to reveal the existence of *qnrB* and *gyrA* determinants in broiler intestinal contents.

However, there are some studies confirmed the existence of quinolones-resistant genes in various bacterial species as *Enterobacteri*aceae isolated from poultry farms. Mart´ın *et al*. (2005) revealed *Salmonella* strains from broiler and laying hens farms in Chile exhibited mutations at *gyrA* gene. Karczmarczyk *et al*. (2010) reported that one isolate of 9 *Salmonell*ae isolated from chicken in Colombia was positive for *qnrB*. Kim *et al*. (2013) detected *qnrB* in 2 strains of 185 *Salmonella*e isolated from poultry in Korea, while *qnrA* could not be detected.

In Nigeria, a commensal *E. coli* strain of chicken origin, possessing *qnrB* genes was detected (Fortini *et al*., 2011). While Liu *et al*. (2012) reported that *qnrA*, *qnrB*, *qnrS*, *qnrC* and *qnrD* were detected in 45.9% of *E. coli* isolates from diseased chickens' cases. Also, Mostafa *et al*. (2014) found 6 of 20 *E. coli* isolates from diseased chickens were positive for *qnr* genes, where 5 isolates were positive for *qnrS* (one of them exhibited also *qnrB*) and one isolate approved *qnrA*. Ponce-Rivas *et al*. (2012) described the prevalence of *qnrA*, *qnrB*, and *qnrS* in *E. coli* isolated from chicken litter and found that *qnrA* and *qnrS* were the prevalent genes. Chen *et al*. (2012) revealed that *qnrA* could not be detected in *E. coli* isolates from chicken, while *qnrB* was detected in 1.3% of the isolates.

DNA gyrase found only in bacteria, is an excellent target for quinolones (mainly in Gram- negative bacteria) because it is not present in eukaryotic cells, while in Gram-positives the target is the topoisomerase IV. The possession of quinolone resistance may be linked to chromosomal mutations in genes encoding the target protein or mutations causing reduced drug accumulation and plasmid-located genes that protect the DNA gyrase and Topo IV from the inhibition of quinolones (target protection) (Tran *et al*., 2005; Vila, 2005). Quinolones plasmid-mediated resistance genes are being transferable horizontally and rapid dissemination of these genes has been stated (Hopkins *et al*., 2008, Minarini *et al*., 2008; Cerquetti *et al*., 2009; Cui *et al*., 2009). Horizontal transfer is the most ef-

fective means in acquisition and widespread dispersion of resistance even between animal and human pathogens (Ochman *et al*., 2000; Ruiz, 2003). Furthermore, location of resistance genes on mobile genetic elements provides the persistence of resistance with the lack of antimicrobial pressure (Sandvang and Aarestrup, 2000). Moreover, horizontal gene transfer allows a bacterial population to develop resistance at a significantly higher rate than that provided by mutations (Alonso *et al*., 2005; Luo *et al*., 2005). Horizontal allocation of resistance genes between bacteria of diverse genera and species occurs readily and commonly in natural systems as soil and groundwater (Chee-Sanford *et al*., 2001; Onan and LaPara, 2003). In this study, the existence of plasmid-mediated resistance gene *qnrB* could be confirmed, which reflects that litter from intensive broiler farms might be considered as an important reservoir of antibiotic- resistant genes giving a chance of horizontal transfer of resistant determinant from either commensal or pathogenic bacteria into antibiotic-sensitive bacterial species.

Litter application to farmlands is a usual practice, however, this contributes to occurrence and persistence PMQR in the environment and changes the environmental bacterial communities with possible impact of resistance genes to soil bacteria and pathogens (Joy *et al*., 2013; Frey *et al*., 2015). PMQR genes might spread from manure bacteria to indigenous soil community, for example, oqxAB harbored by plasmids in *E. coli* (Zhao *et al*., 2010) and *Salmonella* Typhimurium strains (Li *et al*., 2013). As well as elevated loads of resistant genes have been found in soil that was treated with manure and resistance determinants have been detected in harvested crops (Knapp *et al*., 2009; Wang *et al*., 2015). The resistant bacteria and genes could be transferred from environment to humans (Iversen *et al*., 2004; Rodrı´guez *et al*., 2006). Transfer of *qnrS*1 plasmids can occur between *Salmonella* and *E. coli* of animal and human origin, with pigs being one of the potential reservoirs (Szmolka *et al*., 2011).

High prevalence of fluoroquinolone resistance in human and animal *E. coli* isolates have also been reported in China, presumably due to the abuse of quinolones in food animals (Xiao *et al*., 2008). Szmolka and Nagy (2013) supporting the evidence for inter-specific exchange of resistance determinants between commensal *E. coli* and zoonotic bacteria known to colonize humans. So, the obtained results provided a confirmation that broiler production environments may be important reservoirs of antibiotic resistance genes as PMQR genes. This is of concern as fluoroquinolones were listed by the World Health Organization as critically important antimicrobials for human health (Collignon *et al*., 2009). Therefore, it seems very important to pay more attention to the spread of resistance through such production systems and regulation of antimicrobial usage seems crucial.

## Conclusion

The current study has conveyed the first incidence of *qnrB* in broiler litter from the intensive poultry production. The existence of *qnr* genes may be a serious public health concern because they can rapidly increase fluoroquinolone resistance in various bacterial species through the transfer of plasmids harboring *qnr* genes. To protect the public health, continuous monitoring and surveillance systems are necessary to prevent the spread of PMQR genes that can be transmitted from poultry environment to humans. However, a widespread study required in Egypt to detect and evaluate the antibiotics used in broiler chicken to take possible steps to guard human and environment from antibiotics residue hazards as well as the presence and dissemination of resistance genes.

## Acknowledgement

The authors would like to thank Research Fund for Scientific Research, Assiut University for partially support HPLC analysis.

## Conflict of interest

The authors have no conflict of interest to declare.

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