Genetic Analysis of Fluoroquinolone Resistant Genes in *Mycoplasma gallisepticum* Field Isolates

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**ABSTRACT**

*Mycoplasma gallisepticum* (MG) causes economic losses to poultry industry all over the world. This study aimed to investigate the emergence of fluoroquinolone (FQ) resistance in MG field isolates using sequence analysis of genes encoding the subunits of the drug’s target enzymes. The results showed that two MG isolates were resistant to fluoroquinolones. Amino acid mutations were recorded in *gyrA* at codons 59, 73, 157 and 174, *gyrB* at codons 415 in addition to Glu at 416 and *ParC* at codons 92 and 167. No mutations were observed in *gyrE*. Two-dimensional analyses illustrated that mutations in the resistant genes; *gyrA*, *gyrB*, and *ParC* were associated with alterations in the predicted protein structures of quinolone-resistance determining regions (QRDRs). These mutations were accompanied by elevation of minimum inhibitory concentration (MIC) profiles to FQ. It was concluded that the mutations in genes encoding DNA gyrase enzyme were the important causes for the appearance of FQ resistance MG isolates.

**Keywords**: *Mycoplasma gallisepticum*; MIC; Antimicrobial resistant genes; Mutation; Two-dimensional analyses.

**Abbreviations**:

MG: *Mycoplasma gallisepticum*

FQ: fluoroquinolone

QRDRs: quinolone-resistance determining regions

MIC: minimum inhibitory concentration

NCCLS: National Committee for Clinical Laboratory Standards

**INTRODUCTION**

Mycoplasmas are characterized by lack of cell wall, small size, and small genome bacteria (Ferguson-Noel, 2013). Within poultry industry, the power of *Mycoplasma gallisepticum* to infect both of the respiratory and reproductive tracts of poultry has made it a pathogen of highly economic concern (Raviv and Ley, 2013). Avian mycoplasmosis is diagnosed through using three main approaches: isolation and identification, determination of antibodies, and molecular detection of the organism’s nucleic acid by PCR (Kleven, 1998). The gold standard method for direct detection of the organism is culture it, but pathogenic avian mycoplasmas are slow growing, relatively fastidious.
organisms, and might require up to 3 weeks for detectable growth (Kelven, 2003). PCR represents a rapid and sensitive than culture methods. The 16SrRNA PCR method is commonly used for confirmation of mycoplasma infection in chickens.

Antimicrobial agent plays a very important role in treating disease caused by M. gallisepticum (Hannan et al. 1997; Ley and Yoder, 1997). MG is known to be sensitive to antimicrobials that act on targets other than the bacterial cell wall, such as tetracyclines, macrolides, and quinolones (Umar et al. 2017).

In MG, the major target for quinolones is DNA gyrase (Reinhardt et al. 2002 a). This enzyme belongs to type II topoisomerase family, which facilitates DNA unwinding at replication forks, while the main action of topoisomerase IV, is to remove the interlinking of daughter chromosomes at the completion of a round of DNA replication and allows their segregation into daughter cell (Ruiz, 2003; Ambrozic et al. 2007).

Recently, the investigators have to return to the studies of mycoplasmas’ proteome architecture as their proteins are orchestrated to provide cell survival. The cells of pathogenic mycoplasmas are characterized by the high adaptive potential to evade a host immune surveillance, acquire antimicrobial resistance, and to disseminate to new hosts (Butenko et al. 2017). Protein structure comparison is fundamental in structural biology and bioinformatics. The overlap of two-dimensional (2D) maps of two structures can be easily calculated, providing a sensitive measure of protein structure similarity. Also, two-dimensional maps contain sufficient information to restore the 3D representation (Pietal et al. 2007). Chernova et al. (2016) mentioned that the 2D Bioinformatic identification of protein-coding DNA gyrase and topoisomerase IV targeted by fluoroquinolones antibiotics important for understanding the resistance of MG isolates for treatment.

So, our study aimed for identification of fluoroquinolone-resistant Mycoplasma gallisepticum isolated from broiler flocks and studying the sequential and proteomic mutation that occurred in gyrA, gyrB, ParC, and ParE genes and its predicted protein.

MATERIALS AND METHODS

Samples

One hundred samples were collected from broiler flocks, with a history of respiratory manifestations from different localities in (El-Sharkia Governorate) and not respond to treatment. Samples included 56 tissue samples (air sacs, tracheas, and lungs) and 44 choanal cleft swabs from diseased chicken.

Isolation and identification of Mycoplasma species

Mycoplasmas were isolated on PPLO broth and agar medium, as previously mentioned (Sabry and Ahmed, 1975). Mycoplasmas were differentiated from
Acholeplasma using digitonin test (Freundt et al. 1973). Biochemical characterization of Mycoplasma isolates was performed using glucose fermentation and arginine deamination (Erno and Stipkovits, 1973).

**In vitro antimicrobial susceptibility testing**

Antimicrobial susceptibility of ten *M. gallisepticum* field isolates were tested against fluoroquinolones such as enrofloxacin (ENFX), ciprofloxacin (CPFX), difloxacin (DIFX), gatifloxacin (GFLX), levofloxacin (LVFX) and ofloxacin (OFLX) (Oxoid, UK) using broth microdilution method (Hannan, 2000). Antimicrobial concentrations ranged from 0.016 to 16 μg/ml. MIC results were interpreted according to National Committee for Clinical Laboratory Standards Institute (NCCLS, 2002); and Clinical & Laboratory, Standards, Institute (CLSI, 2011). MIC50 and MIC90 were calculated using an orderly array method (Hamilton-Miller, 1991).

**Conventional polymerase chain reaction (PCR)**

**DNA extraction of Mycoplasma isolates**

DNA extraction was performed for ten mycoplasmas recovered from broilers with respiratory manifestations according to Ley and Yoder (1997).

**PCR amplification and cycling protocol**

PCR was done for detection *Mycoplasma* isolates using 16S rRNA and mgc2 primers. In addition, gyrA, gyrB, ParC and parE amplified with genes specific primers designed on the basis of the genomic sequence of *M. gallisepticum* strain R (accession no. AE015450) (Papazisi et al. 2003). The selected primers from the published papers are shown in (Table 1).

The amplified PCR product was electrophoresed on a 1.5% agarose gel in Tris-acetate-EDTA buffer (Ewers et al. 2005). And 100-bp DNA ladder (Invitrogen, Carlsbad, CA) was used as a molecular weight marker.

**Sequence analysis**

DNA sequencing of gyrA, gyrB, ParC, and parE genes was conducted in both directions. The original sequence was trimmed to remove vague nucleotide sequences usually exist at the beginning of the sequencing reaction. Partial DNA sequences were submitted to the Gene Bank database and obtained accession numbers; KM076644 and KJ754683 for gyrA, KJ486460, and KJ754684 for gyrB, KJ778874 and KJ754685 for ParC and KM076645 and KM076646 for parE. Identification of homologies between nucleotide and amino acid sequences of the studied *Mycoplasma gallisepticum* DNA sequences and others published in GenBank were done using BLAST 2.2 search program (National Center for Biotechnology Information “NCBI” http://www.ncbi.nlm.nih.gov/). Comparisons of the obtained nucleotide sequences to *E. coli* sequences that published in GenBank were done using the BioEdit sequence alignment editor (Hall, 1999) and MegAlign, DNASTAR, Lasergene®, Version 7.1.0, USA. The phylogenetic trees were constructed using MegAlign for tree reconstruction of
sequences by Neighbor-joining method based on ClustalW (Thompson et al. 1994). Sequence divergence and identity percentages were calculated by MegAlign. The structural character of GyrA, GyrB, ParC and ParE proteins sequences were identified by Protean (DNASTAR, Lasergene®, Version 7.1.0. USA).

**Two-dimensional protein analysis of the QRDRs:**

The deduced amino acid sequences of GyrA, GyrB and ParC proteins were analyzed using the PROTEIN subroutine in the DNASTAR software package (DNASTAR, Lasergene®, Version 7.1.0. USA). This subroutine uses the algorithm for predicting antigenicity (Jameson and Wolf, 1988).

**RESULTS AND DISCUSSION**

**Mycoplasma isolation rate.**

*Mycoplasma*, belonging to the class Mollicutes, is a small free-living highly fastidious and slow growing micro-organism, (Nicholas and Ayling, 2003). One important feature of *M. gallisepticum* infection is that it can persist in the bird during all life, even in the presence of the humoral antibodies (Stipkovits et al. 2011).

In the present investigation, a recovery rate of MG isolation [(10/100)(10%)] obtained from respiratory organs of broilers suffered from respiratory manifestation in El-Sharkia Governorate. This result agreed with that recorded by several authors (Ulgen and Kahraman, 1993; Dardeer, 1997; Mohamed, 1997; Saif-Edin, 1997; Sharaf, 2000; Mohammed, 2001). The present finding revealed that all examined mycoplasma isolates had 16S rRNA, with a characteristic band at 185 bp (Fig.1a), as mentioned by Lauerman (1998). Further confirmation was made using mgc2 primers for detection of the MG isolates (Fig.1b).

**MICs of fluoroquinolone against *M. gallisepticum* isolates.**

Among ten MG isolates, only two isolates were resistant to used fluoroquinolones, as mentioned before by Reinhardt et al. (2002 b); Jian et al. (2012). The MIC values for fluoroquinolones of tested MG isolates ranged from 0.063 to 8ug/ml as listed in Table (2). These results were consistent with that reported by Gerchman et al. (2008).

MIC50 and MIC90 values were lower for the human-use fluoroquinolones as compared to the veterinary-use agents. The MIC50 for both enrofloxacin and difloxacin was 0.25 μg/ml (Table 3). These findings were lower than those recorded by Gerchman et al. (2008). Also, the MIC90 for enrofloxacin was 0.5 μg/ml which was lower in comparison with previously mentioned (Catania et al. 2019). Gatifloxacin (GFLX) was the most effective fluoroquinolone against all MG isolates, that agreed with previously recorded by Andriole, (2005).
PCR amplification and DNA sequence analysis of quinolone-resistant determining region (QRDR) of MG isolates.

From all tested MG strains, 484-bp covering the entire QRDR of gyrA (Fig. 2a), a 580-bp fragment covering the region of gyrB (Fig.2b), 463-bp PCR products encompassing the regions of ParC and 440-bp fragment covering the region parE (Fig.2c and Fig.2d respectively) were obtained.

Genetic analysis of gyrA

Solid-phase sequencing of the amplified DNA revealed amino acid replacement at codon 59 (Histidine CAT→ Tyrosine TAT) and 157 (Isoleucine ATT→Valine GTT) in both analyzed isolates. Additionally, replacement at codon 73 (Valine GTT→ Alanine GCG) in SAAS.1 isolate and 174 (Isoleucine ATT→ Leucine TTG) in SAAS.12 isolate. Amino acids replacement at codons 157 and 174 were the first reported (Fig. 3a). Reinhardt et al. (2002 a; b) stated that His59→Tyr, Gly81→Ala, and Iso103→ Arg substitutions as favored mutational spots in GyrA subunit. (Jian et al. 2012) found that a new mutation position at 136 involved in high-level fluoroquinolones resistance MG. Lysnyansky et al. (2008) observed that isolates with genotype changes at position 59 in gyrA remained sensitive to enrofloxacin but in this work fluoroquinolone resistant SAAS.12 isolate with MIC 4-8 ug/ml for fluoroquinolone antibiotics had amino acid substitutions at position 59 (His/Tyr) and this may attribute to the presence of other additional sites of mutation. A large number of mutations in gyrA and the presence of alterations in ParC play a vital role in developing resistance to ciprofloxacin in MG isolates (Al-Agamy et al. 2012). Interestingly, four silent mutations were recorded in both fluoroquinolone-resistant M. gallisepticum isolates at nucleotides; none of these resulted in amino acid substitutions.

Genetic analysis of gyrB

Sequencing of the amplified DNA for gyrB of both resistant isolates (SAAS.1 (MG EGY2014), SAAS.12) revealed amino acid replacement in the QRDR of gyrB at codon 415 (Glycine GGG → Methionine AUG) and codon 416 insertion Glutamic acid (GAA) (E. coli numbering). While isolate SAAS.1 (MG EGY2014) had another replacement at codon 325 (serine TCA→ leucine CTC) as the first report that may contribute to elevation of MIC profiles. And at 437 (aspartate GAC → asparagine AAT) (E. coli numbering) (Fig.4a), a similar result was detected previously by Lysnyansky et al. (2008). Heddle and Maxwell (2002) found that mutations in gyrB have been associated with quinolone resistance. Yu et al. (2004); Hopkins et al. (2005); Jacoby (2005) reported that the mutation frequency in gyrB is much lower compared to those for gyrA and ParC.
Genetic analysis of *ParC*

A nucleotide sequence of the amplified DNA revealed amino acid replacement in the QRDR of *ParC* of MG SAAS.1 isolate at codon 92 (Serine AGT→Thrreonine ACC) and outside QRDR of *ParC* at codon 167 (Glycine GGT→Valine GTT) (according to *E. coli* numbering) as showed in Figure (5a) and both replacement positions were first reported. These findings were contrary to that reported by Reinhardt *et al.* (2002 a; b; Lysnyansky *et al.* (2008); Jian *et al.* (2012); Lysnyansky *et al.* (2012).

Genetic analysis of *parE*

In our study, sequence analysis of the *parE* gene of both analyzed MG fluoroquinolones resistant isolates revealed no amino acid replacement. Similar results were obtained in other investigations (Lysnyansky *et al.* 2008; Jian *et al.* 2012; Lysnyansky *et al.* 2012).

In this investigation, MG SAAS.12 isolate had no amino acid substitution in *ParE* or *ParC* and recorded a high level of resistance. These results disagreed with other observations (Fitzgibbon *et al.* 1998). Heisig (1996) found that mutations in *ParC* or *parE* were observed only in mutants bearing at least one mutation in *gyrA* or *gyrB* and exhibiting an increase in the MIC of enrofloxacin. (Jian *et al.* 2012) stated that topoisomerase IV is a primary target of quinolones action in *M. hominis*, but DNA gyrase is a primary target of quinolone action in MG.

The obtained data explained why SAAS.12 had high MIC values of fluoroquinolones tested with only amino acid substitution in *gyrA* and *ParC*. (Jian *et al.* 2012) noted that two MG isolates exhibited no mutation in any target genes for fluoroquinolones or just one mutation in *ParC* but had high resistance to fluoroquinolones. (Reinhardt *et al.* 2002 a; Reinhardt *et al.* 2002 b) stated that mutations elsewhere in the topoisomerase genes or modifications in drug efflux systems may contribute to the resistant phenotype of these mutants.

(Bébéar *et al.* 1998; Bebear *et al.* 1999; Kenny *et al.* 1999; Bebear *et al.* 2000) mentioned that development of highly quinolones resistant mutant strains of *M. gallisepticum* required mutations in both topoisomerases, like for most bacteria.

Two-dimensional protein analysis of the QRDRs:

The prediction protein of GyrA showed amino acid substitution at positions 59, 73 and 157 (numbering according to *E. coli*) consequence by changes in antigenicity index of GyrA protein structures in SAAS 1 and at position 59 and 157 in SAAS 12 of examined MG isolates (Fig. 3b). In GyrB prediction protein amino acid substitution at position 415 and amino acid insertion at position 416 (numbering according to *E. coli*) lead to changes in antigenicity index of GyrB protein structures in both examined MG isolates (Fig.4b). In the case of ParC prediction protein, amino acid substitution at position 92 (numbering according
to *E. coli*) followed by a change in antigenicity index of ParC protein structure in SAAS.1 examined MG isolates (Fig.5b).

The two dimensional protein analysis of both fluoroquinolones drug target enzymes in two MG resistant isolates, illustrated some amino acids substitution in 2D prediction proteins of both GyrA, GyrB, and ParC in addition to insertion of one amino acid at codon 416 in ParC prediction protein. These findings were consistent with previously observed by Chernova et al. (2016).

**Conclusion**

Finally, we can conclude that fluoroquinolones resistant *Mycoplasma gallisepticum* isolates arise mainly from an alteration in genes encoding DNA gyrase enzyme specially gyrA gene. The presences of mutation in *ParC* lead to elevation resistance to fluoroquinolones.

**Declaration**

**Authors’ contribution.** All/both authors contributed equally to this work.

**Acknowledgements.** Preliminary results were presented as an Abstract at 10th Scientific Conference of the Egyptian Veterinary Poultry Association, Egypt, 4 -7 December 2017."

**Competing interests.** The authors declare that they have no competing interests.

**Consent to publish.** Not applicable
**Fig. 1a.** Agarose gel electrophoresis of MG PCR product (16S rRNA gene). *Lane (1) M:* 100 bp DNA ladder "Marker", *Lane (2) control negative,*  *Lane (11) control positive MG reference strain (PG31).*  *Lanes (3-10):* positive MG isolates.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Description</th>
<th>Band Size</th>
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<tbody>
<tr>
<td>1</td>
<td>100 bp DNA ladder &quot;Marker&quot;</td>
<td>100 bp</td>
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<tr>
<td>2</td>
<td>Control negative</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Control positive MG reference strain (PG31)</td>
<td>100 bp</td>
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<tr>
<td>3-10</td>
<td>Positive MG isolates</td>
<td></td>
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</table>

**Fig. 1b.** Agarose gel electrophoresis of MG PCR product (*mgc2* gene). *Lane (1) M:* 100 bp DNA ladder "Marker". *Lane (2):* control positive MG reference strain (PG31). *Lanes (3-5):* positive MG isolates.  *Lane (6):* control negative

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<th>Lane</th>
<th>Description</th>
<th>Band Size</th>
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<td>100 bp</td>
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<tr>
<td>2</td>
<td>Control positive MG reference strain (PG31)</td>
<td>100 bp</td>
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<tr>
<td>3-5</td>
<td>Positive MG isolates</td>
<td></td>
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<tr>
<td>6</td>
<td>Control negative</td>
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**Fig. 2a.** Agarose gel electrophoresis of MG PCR product (*gyrA* gene). *Lane (1) M:* 100 bp DNA ladder "Marker". *Lanes (2,3,4):* positive MG isolates. *Lane (5):* control negative. *Lane (6):* control positive MG reference strain (PG31).

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<th>Description</th>
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<td>100 bp</td>
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<tr>
<td>2-4</td>
<td>Positive MG isolates</td>
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</tr>
<tr>
<td>5</td>
<td>Control negative</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Control positive MG reference strain (PG31)</td>
<td>100 bp</td>
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**Fig. 2b.** Agarose gel electrophoresis of MG PCR product (*gyrB* gene). *Lane (1) M:* 100 bp DNA ladder "Marker". *Lanes (2,3,4):* positive MG isolates. *Lane (5):* control positive MG reference strain (PG31). *Lane (6):* control negative

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<th>Lane</th>
<th>Description</th>
<th>Band Size</th>
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</thead>
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<td>100 bp DNA ladder &quot;Marker&quot;</td>
<td>100 bp</td>
</tr>
<tr>
<td>2-4</td>
<td>Positive MG isolates</td>
<td></td>
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<tr>
<td>5</td>
<td>Control positive MG reference strain (PG31)</td>
<td>100 bp</td>
</tr>
<tr>
<td>6</td>
<td>Control negative</td>
<td></td>
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</tbody>
</table>
**Fig. 2c.** Agarose gel electrophoresis of MG PCR product (*ParC* gene). Lane (1) M: 100 bp DNA ladder "Marker", Lanes (2,3,4) positive MG isolates. Lane (5) control positive MG reference strain (PG31).

**Fig. 2d.** Agarose gel electrophoresis of MG PCR product (*parE* gene). Lane (1) M: 100 bp DNA ladder "Marker", Lanes (2,3,5) positive MG isolates. Lane (4): control negative. Lane (6) control positive MG reference strain (PG31).

**Fig. 3a.** Amino acids sequence similarities for *M. gallisepticum* gyrA of the fluoroquinolones resistant MG isolates under study and the reference *M. gallisepticum* (S6) strains. Numbers refer to the amino acid positions in the *E. coli* gyrA sequence. The Tyr-59, Ala-73, Val-157, and Leu-174 in which mutations associated with fluoroquinolones resistance are founded.
Fig. 3b. The secondary structures protein analysis (antigenicity index) for Gyra (amino acids numbering according to *E. coli* numbering) H: Histidine, Y: Tyrosine, V: Valine, A: Alanine, I: Isoleucine.

Fig. 4a. Amino acids sequence similarities for *M. gallisepticum* gyrB of the fluoroquinolones resistant isolates under study and the reference *M. gallisepticum* (S6) strains. Numbers refer to the amino acid positions in the *E. coli* gyrB sequence. The Leu-325, Met-415, Glu insertion-416 and Asn-437 in which mutations associated with fluoroquinolone resistance are founded.
strains. Numbers refer to the amino acid positions in the E.coli PcoA sequence. The Thr-92 and Val-167 in MG SAAS 1 which mutations associated with fluoroquinolone resistance are founded and no mutation in MG SAAS 12.

Fig. 4b. The secondary structure protein analysis (antigridity index) for GyrB amino acids numbering according to E.coli numbering. G: Glycine, M: Methionine. E: Glutamic acid.
**Table (1): Oligonucleotide primers used for detection 16S rRNA, mgc2, and quinolone-resistance genes.**

<table>
<thead>
<tr>
<th>Primer Designation</th>
<th>Sequence (5’-3’)</th>
<th>Amplified Product Size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16s RNA F</td>
<td>GAGCTAATCTGTAAAGTTGGTC</td>
<td>185 bp</td>
<td>(Lauerman, 1998)</td>
</tr>
<tr>
<td>16S RNA R</td>
<td>GCTTCCTTGCGGTTAGCAAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mgc2-F</td>
<td>GCT TTG TGT TCT CGG GTG CTA</td>
<td>824 bp</td>
<td>(Ferguson et al. 2005)</td>
</tr>
<tr>
<td>mgc2-R</td>
<td>CGG TGG AAA ACC AGC TCT TG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gyrA-F</td>
<td>GAGCTAGAAAACATCATTCATGG</td>
<td>484 bp</td>
<td>(Lysnyansky et al. 2008)</td>
</tr>
<tr>
<td>gyrA-R</td>
<td>CCTACAGCAATACCACCTGAAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gyrB-F</td>
<td>CTGACGGTAGATTAGCAAGAG</td>
<td>580-bp</td>
<td>(Lysnyansky et al. 2008)</td>
</tr>
<tr>
<td>gyrB-R</td>
<td>GACATCAGCATCGGTTCATGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>parE-F</td>
<td>CTGAAGGTGGAAAGTCTGTA</td>
<td>440 bp</td>
<td>(Lysnyansky et al. 2012)</td>
</tr>
<tr>
<td>parE-R</td>
<td>GTTGCCAATCCCAACACCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ParC-F</td>
<td>GATCTTTTAGATGATATCATGTCAC</td>
<td>463 bp</td>
<td>(Lysnyansky et al. 2008)</td>
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<tr>
<td>ParC-R</td>
<td>CCAGTTGAACCATTAACGAGT</td>
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</table>
Table (2): MIC range, MIC\textsubscript{50}, and MIC\textsubscript{90} of fluoroquinolones against *M. gallisepticum* isolates.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Mic (µg/ml)</th>
<th>Range\textsuperscript{a}</th>
<th>MIC\textsubscript{50}\textsuperscript{b}*</th>
<th>MIC\textsubscript{90}\textsuperscript{c}*</th>
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</thead>
<tbody>
<tr>
<td>Enrofloxacin</td>
<td></td>
<td>0.063–4</td>
<td>0.25</td>
<td>0.5</td>
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<tr>
<td>Difloxacin</td>
<td></td>
<td>0.125–8</td>
<td>0.25</td>
<td>1</td>
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<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>0.063–4</td>
<td>0.125</td>
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<td>Gatifloxacin</td>
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<td>0.063–8</td>
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</tr>
<tr>
<td>Levofloxacin</td>
<td></td>
<td>0.063–8</td>
<td>0.25</td>
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\textsuperscript{a} Range of the MIC values.
\textsuperscript{b} The MIC at which 50% of the isolates are inhibited.
\textsuperscript{c} The MIC at which 90% of the isolates are inhibited.

*Both b and c were calculated by an orderly array method.
Table 3

Mutations in topoisomerase genes of two analyzed *M. gallisepticum* strains and their antimicrobial susceptibilities to fluoroquinolones

<table>
<thead>
<tr>
<th>MG isolates</th>
<th>MIC (μg/ml)</th>
<th>Mutations positions in QRDRs*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ENF</td>
<td>DI</td>
</tr>
<tr>
<td>SAAS.1</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>SAAS.12</td>
<td>4</td>
<td>8</td>
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</table>

MIC: minimum inhibitory concentration; ENFX: enrofloxacin; DIFX: difloxacin; CPFX: ciprofloxacin; GFLX: gatifloxacin; OFLX: ofloxacin; LVFX: levofloxacin.

*DNA sequences were analyzed and translated to amino acid sequences and numbering according to *E. coli* amino acid sequences numbering.

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