Original Paper

Detection of mutations involved in fluoroquinolone resistance in *Mycoplasma gallisepticum* positive field samples from broiler chicken flocks in Ecuador

CU-ID: 2248/v44e03

Detección de mutaciones implicadas en la resistencia a las fluoroquinolonas en muestras de campo positivas a *Mycoplasma gallisepticum* procedentes de pollos de engorde en Ecuador

Laura De la Cruz[1*], Adrián A. Díaz-Sánchez[2,3], Rosa E. Hernández-Fillor[2], Dany Naranjo-Feliciano[2], Evelyn Lobo-Rivero[2], Ivette Espinosa-Castaño[2]

[1]Departamento de Veterinaria, Facultad de Ciencias Veterinarias, Universidad Técnica de Manabí, 13132, Ecuador.
[2]Centro Nacional de Sanidad Agropecuaria (CENSA), Carretera de Tapaste y Autopista Nacional, Apartado postal 10, CP. 32700, San José de las Lajas, Mayabeque, Cuba.
[3]Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, Saskatchewan, S7N 5E2, Canada.

ABSTRACT: The aim of this study was to determine the occurrence of mutations in the quinolone resistance-determining regions (QRDRs) of the genes *gyr*A and *par*C in *M. gallisepticum* positive field samples from broiler flocks in Ecuador. DNA was extracted from 24 *M. gallisepticum* PCR-positive samples from 22 commercial broiler flocks. The genes *gyr*A and *par*C were amplified by PCR. PCR products were sequenced by Sanger technology to analyze the genetic characteristics. To identify the mutations involved in fluoroquinolone resistance (FQR), the sequences obtained were processed and analyzed using the tools Geneious R11, BLASTn, MAFFT, ExPASy MBWS, and BioEdit. All samples had mutations in both *gyrA* and *parC* genes, resulting in changes at amino acid positions Ser-83→Ile and Ile-157→Val in GyrA, and Ser-80→Trp in ParC. In addition, a change at position His-59→Tyr in GyrA was also found in one sample. The results showed that alterations in both genes have been commonly linked to FQR in mutants of other Mycoplasma species, including *M. gallisepticum*. This is the first study on *M. gallisepticum* positive samples from chickens in Ecuador which revealed the occurrence of mutations resulting in amino acid changes previously linked to FQR.

Key words: *M. gallisepticum*, antibiotic-resistance, *gyrA*, *parC*, poultry, QRDR-mutations.

RESUMEN: El objetivo de este estudio fue investigar la ocurrencia de mutaciones en las regiones determinantes de resistencia a quinolonas (QRDR de sus siglas en Inglés) de los genes *gyrA* y *parC* en muestras de campo positivas para *M. gallisepticum,* procedentes de parvadas de pollos de Ecuador. Se extrajo el ADN de 24 muestras positivas por PCR a *M. gallisepticum* de 22 parvadas de pollos de engorde de crianza comercial. Los genes gyrA y parC se amplificaron por PCR. Para analizar las características genéticas, los productos de PCR se secuenciaron por tecnología Sanger. Para identificar mutaciones involucradas en resistencia a fluoroquinolonas (FQR), las secuencias obtenidas se procesaron y analizaron utilizando las herramientas Geneious R11, BLASTn, MAFFT, ExPASy MBWS y BioEdit. Todas las muestras presentaron mutaciones, tanto en los genes gyrA como parC, lo que resultó en cambios en las posiciones de aminoácidos Ser-83→Ile e Ile-157→Val en GyrA, y Ser-80→Trp en ParC. Además, también se encontró en una muestra un cambio en la posición His-59→Tyr en GyrA. Los resultados mostraron que las alteraciones en ambos genes se han relacionado comúnmente con FQR en mutantes de otras especies de *Mycoplasma*, incluido *M. gallisepticum*. Este es el primer estudio en muestras positivas de *M. gallisepticum* de pollos en Ecuador que reveló la aparición de mutaciones que resultaron en cambios en aminoácidos, previamente vinculados a FQR.

Palabras clave: *M. gallisepticum*, resistencia a antibióticos, *gyrA*, *parC*, aves de corral, mutaciones QRDR.

INTRODUCTION

Poultry production is one of the main food industries worldwide because of its contribution to feeding rapidly growing human populations, low production costs and the absence of cultural and religious restrictions on its consumption (1). In Ecuador, poultry production represents one of the most important industries and it is a source of income for small farmers (2). *Mycoplasma gallisepticum* causes a chronic respiratory disease in chickens (3), which can lead to their slaughter. It also causes a decrease in weight gain, meat and egg production efficiency (3, 4, 5). Fluoroquinolones (FQs) are broad-spectrum antibiotics that are widely used for the treatment of different diseases in animals, including *M. gallisepticum* (6). The emergence of resistance to FQs is primarily due to point mutations resulting in amino acid substitutions within the quinolone resistance-determining regions (QRDRs) of the DNA gyrase subunits GyrA and GyrB and/or topoisomerase IV ParC and ParE subunits in bacterial species, including mycoplasmas (7,8). The central mechanism involves alterations of the GyrA subunit and/or the ParC subunit, whereas alterations in GyrB and ParE play a complementary role (7).

*Correspondence to: Ivette Espinosa-Castaño, E-mail: espinosa@censa.edu.cu, ivetteespinosa763@gmail.com
Received: 16/02/2022
Accepted: 06/04/2022

Enrofloxacin, oxytetracycline and tylosin resulted in the highest number of resistant isolates of *M. gallisepticum* in most geographic distributions (9). Surveillance of antimicrobial resistance in clinical strains of *M. gallisepticum* is essential for determining subsequent treatment guidelines. However, isolation of *M. gallisepticum* in culture remains a labor-intensive and time-consuming task (10). Mycoplasmas are slow-growing microorganisms with complex requirements. Consequently, standard procedures used for susceptibility testing of classical bacteria, such as disk diffusion method or minimum inhibitory concentration (MIC), are not routinely recommended but only performed by specialized laboratories (9,11). Therefore, to assess potential resistance to fluoroquinolones, a molecular approach that does not involve culture and *in vitro* antimicrobial susceptibility testing will be applied. The aim of this study was to determine the occurrence of mutations in the quinolone resistance-determining regions (QRDRs) of the genes *gyr*A and *par*C in *M. gallisepticum* positive field samples from broiler flocks in Ecuador.

MATERIALS AND METHODS

The study included a total of 24 *M. gallisepticum* PCR-positive field samples collected during 2018 and 2019 from 22 commercially reared broiler flocks located in different areas of Manabí province, Ecuador. Bacterial DNA extracts used as templates were prepared by the heat boiling method described by Hernández *et al*. (12). The samples were previously evaluated and confirmed as positive by PCR for *M. gallisepticum*, as described by De la Cruz *et al*. (13). QRDRs were amplified using gene-specific primers designed from the genomic sequence of *M. gallisepticum* strain R (accession no. AE015450), which included *gyr*A-F5'-GAGCTAGAAACATCATTCATGG-3' and *gyr*A-R 5'-CCTACAGCAATACCACTT GAA-3' for the *gyr*A gene, and *par*C-F 5'-GATCTTGATGATATATCGTCAC-3' and *par*C-F 5'-CCAGTTGAACCATTAACGAGT-3' for the *par*C gene (14). PCR reactions were performed in a total volume of 50 µl containing 1× GoTaq® Green Master Mix (Promega®, Madison, WI, USA), 800 nM of each primer; and 100 ng/5µL of positive mycoplasma sample were used as DNA template. All PCR amplifications were performed on an Eppendorf Mastercycler Gradient Thermal Cycler (Eppendorf, Hamburg, Germany). Nuclease-free water was used as a negative control. DNA from *M. gallisepticum* strain K3254, code 6/85, was used as a positive control. Amplification products were submitted to electrophoresis on 1.5 % agarose gels. Gels were stained with ethidium bromide (0.5 mg/mL). A 100 bp DNA ladder (Promega®, Madison, WI, USA) was used as molecular weight marker.

To analyze the genetic characteristics of the QRDR, PCR products from six *M. gallisepticum*-positive samples (AVMG1 to AVMG6) were selected according to their origin in the northern, southern and central region of Manabí. Amplicons were purified using the QIAquick Gel Extraction kit (Qiagen, Santa Clarita, CA, USA), according to the manufacturer's instructions, and sent for direct sequencing at the Macrogen facility (Macrogen Inc. Company, South Korea). Raw sequences were assembled and edited using Geneious R11 v11.0.3 software (Biomatters Limited, Auckland, New Zealand). The sequences obtained were identified by checking, using the BLASTn search program (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). Nucleotide sequences were aligned with MAFFT v.7 configured for maximum accuracy (MAFFT with default settings) (15). Theoretical translation of nucleotide sequences to amino acid sequences was carried out on the ExPASy molecular biology web server (http://www.expasy.org). Protein sequences were aligned using ClustalW, included in the BioEdit v.7.0.0 package (Tom Hall Ibis Biosciences, USA).Sequences were sent to the GenBank database with accession numbers MK210575-79 for *gyr*A and MK210580-83 for *par*C. For sequencing analysis, substitutions were noted as follows: Xxx##Yyy, where Xxx represents the wild-type amino acid, ## the codon number, and Yyy the substituted amino acid. *E. coli* gene sequence numbering system was used (16).

RESULTS AND DISCUSSION

Mycoplasma spp. are not affected by common antibiotics that target cell wall synthesis; therefore, other antimicrobials such as tetracycline, macrolides and fluoroquinolone are required (1,9, 14). The main objective of this study was to determine the occurrence of mutations related to acquired resistance to FQ in the *gyr*A and *par*C genes corresponding to the DNA gyrase and topoisomerase IV subunits in *MG*-positive samples from poultry farms in Ecuador. Both enzymes are essential for bacterial DNA replication, thus these genes are found in all strains and are targets for resistance mutations to FQ (17). Hence, as expected, *gyr*A and *par*C genes were amplified from a total of twenty-four field samples, and the amplicons corresponded to the expected size of 484 bp and 463 bp for *gyr*A and *par*C genes, respectively.

Mycoplasmas are slow-growing microorganisms with complex requirements. Consequently, standard procedures used for susceptibility testing of classical bacteria, such as disk diffusion method or minimum inhibitory concentration (MIC), are not routinely recommended but only performed by specialized laboratories (6, 9). This study was limited by the lack of *MG* strains to assess microbial susceptibility by phenotypic testing, which would allow defining the MIC of FQ and corroborating the effects of resistance

Table 1. Amino acid changes in GyrA and ParC QRDR fragments in *Mycoplasma gallisepticum* positive samples from chickens in Ecuador. / *Cambios de aminoácidos en los fragmentos QRDR de GyrA y ParC en muestras positivas de Mycoplasma gallisepticum de pollos en Ecuador.*

Isolates	Country	Year	GyrA[a,c]			ParC[b,c]		
			59	83	87	157	45	80
S6	USA	< 1968	(His, H) CAT	(Ser, S) AGT	(Glu, E) GAA	Ile (I) ATT	Val (V) GTT	Ser (S) TCA
SYR-2	Israel	2015	(His, H)	(Ser, S)	(Lys, K)	Val (V)	Ile (I)	Leu (L)
AVMG1	Ecuador	2019	-	-	-	-	Val (V)	**Trp (W)**
AVMG2	Ecuador	2019	**(Tyr, Y)**	**(Ile, I)**	(Glu, E)	**Val (V)**	Val (V)	**Trp (W)**
AVMG3	Ecuador	2019	(His, H)	**(Ile, I)**	(Glu, E)	**Val (V)**	-	-
AVMG4	Ecuador	2019	(His, H)	**(Ile, I)**	(Glu, E)	**Val (V)**	Val (V)	**Trp (W)**
AVMG5	Ecuador	2019	(His, H)	**(Ile, I)**	(Glu, E)	**Val (V)**	-	-
AVMG6	Ecuador	2019	(His, H)	**(Ile, I)**	(Glu, E)	**Val (V)**	Val (V)	**Trp (W)**

The complete genome of Mycoplasma gallisepticum strain S6 (accession number CP006916) was used as reference strain, where amino acid positions indicated the relative position to the proteins:

[a]846 aa of GyrA DNA gyrase subunit A (Protein ID: AHB99967);

[b]796 aa of ParC DNA topoisomerase IV subunit A (Protein ID: AHB99685);

[c]aa substitutions within QRDRs of *Mycoplasma gallisepticum* positive sample (i.e., AVMG1 to AVMG6) in comparison to the reference strain *Mycoplasma gallisepticum* strain S6 are marked.

mutations. In this context, the screening of key genetic mutations directly from clinical samples by PCR and direct DNA sequencing is a well-established method, which could be very useful in many laboratories due to the fastidious nature of mycoplasmas. However, some difficulties may arise in the interpretation of DNA chromatograms resulting from direct sequencing of polybacterial samples. That is the reason why, although advantageous, it is still a challenge to optimize DNA extraction, PCR and DNA sequencing directly from the polybacterial sample, such as that used in this work (18). For this reason, even when both genes were amplified from all 24 samples, it was not possible to recover enough DNA from all the amplicons that were purified for sequencing and, therefore, only five sequences were obtained for the gene *gyr*A and four for the gene *par*C.

The DNA sequences obtained for *gyr*A with the following accession numbers MK210575-79 were derived from specimens AVMG2, AVMG3, AVMG4, AVMG5, and AVMG6, respectively. The sequences were highly identical to each other (98, 97-100 %) and >99 %, the same as *M. gallisepticum* strain S6 (accession number NC023030.2) (Figure S1). The *gyr*A gene sequences obtained showed eight nucleotide substitutions, most of which were silent mutations (5/8; 62.5 %). Sample AVMG2 (accession number MK210575) revealed seven differences in nucleotide sequence compared to the reference strain *M. gallisepticum* S6 representing two transverse and six transitional changes. Three substitutions resulting in an amino acid change were detected in all samples, which included replacements at positions 83 (Serine ATT→ Isoleucine AGT) and 157 (Isoleucine ATT→ValineGTT), while 59 (Histidine CAT→ Tyrosine TAT) was only found in sample AVMG2 (*E. coli* numbering) (Table 1).

There are some studies on the molecular mechanism of FQ resistance in bacteria that have been used *E. coli* sequence numbering system as a reference. The *E. coli* numbering system used in this study showed that, for *MG*, the start codon was located at the tenth amino acid; therefore, the amino acids at positions 68, 69, 93 and 97 in *MG* according to the *E. coli* numbering system corresponded to positions 58, 59, 83 and 87 in *E. coli*. However, some authors did not specify the numbering system used, resulting in discrepancies regarding the location of the resistance mutations (Figure 1). The use of different numbering systems made difficult to confirm previously described mutations and to clearly identify new mutations (20,21).

Previous studies carried out in other bacteria and mycoplasmas have reported quinolone-resistant hot spots by substitution at positions 59, 83, and 157 of GyrA described in *M. gallisepticum* field samples from Ecuador (18,19). Lysnyansky *et al*. (13) described that genotype changes at position 59 of GyrA favored susceptibility to enrofloxacin, whereas Sahar and Abou-Khadra (20) reported a fluoroquinolone-resistant isolate of *M. gallisepticum* that had an amino acid substitution at that position. This may have also been attributed to the presence of additional mutation sites. However, Ser-83→Ile mutation has been correlated with fluoroquinolone-resistant mutants of *M. gallisepticum*, which showed increased MIC *in vitro* (22). Substitutions at position 83 in GyrA were highly variable (e.g., Ser-83→Leu/Trp/Phe/Tyr/Asn/Arg), where some mutations caused greater increases in resistance than other substitutions in different bacteria and mycoplasmas due to structural differences between amino acids (23, 24,25).

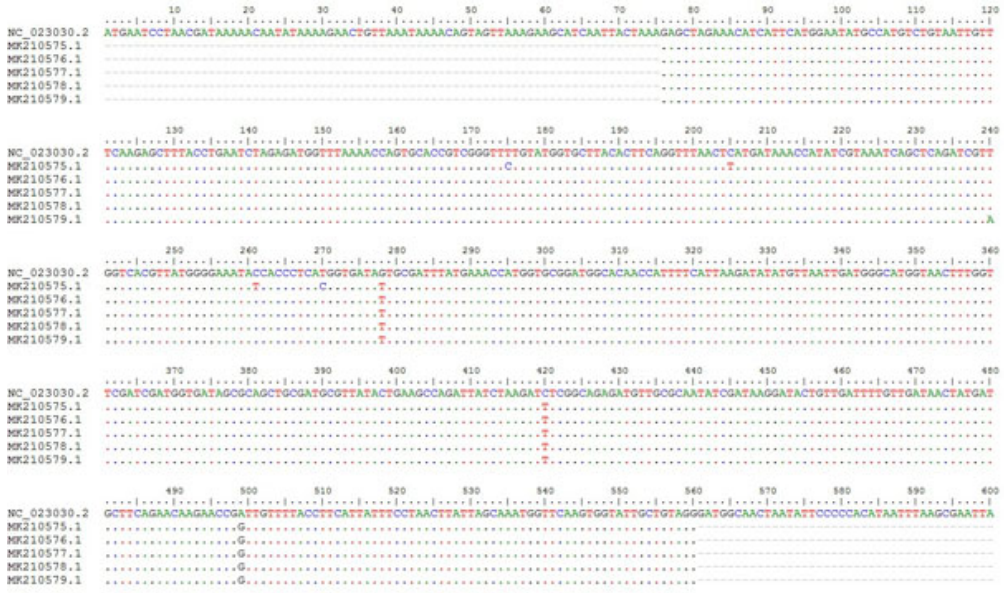

Figure 1. Alignment of the quinolone resistance-determining region (QRDR) of *gyrA* gene of *M. gallisepticum* positive samples with an quinolone-sensitive strain of *M. gallisepticum* (strain S6). / *Alineamiento de la región determinante de la resistencia a las quinolonas (QRDR) del gen gyrA de las muestras positivas de M. gallisepticum con una cepa de M. gallisepticum sensible a las quinolonas (cepa S6).*

Figure 2. Alignment of the quinolone resistance-determining region (QRDR) of *parC* gene of *M. gallisepticum* positive samples with those of other quinolone-sensitive strain of *M. gallisepticum* (strain S6) and quinolone-resistance strains of *M. gallisepticum* (MDE-3, MYZ-8, YDK-4, and SyR-2). / *Alineamiento de la región determinante de la resistencia a las quinolonas (QRDR) del gen parCde las muestras positivas de M. gallisepticum con las de otras cepas de M. gallisepticum sensibles a las quinolonas (cepa S6) y cepas de M. gallisepticum resistentes a las quinolonas (MDE-3, MYZ-8, YDK-4 y SyR-2).*

The analysis of the nucleotide sequences obtained for the *par*C-amplified 463 bp gene fragments was only possible from four *M. gallisepticum* field samples (i.e. AVMG1, AVMG2, AVMG4 and AVMG6) with corresponding accession numbers (MK210580-Mk210583), respectively. The sequences were 100 % identical to each other and 98.35 % identical to the *M. gallisepticum* strain (accession number NC004829.2). The *par*C gene sequences obtained in Ecuador revealed eight nucleotide substitutions compared to the reference strain *M. gallisepticum* S6, resulting in one transverse and seven transient changes (Figure 2). Most of the nucleotide substitutions were silent mutations (7/8; 91.67 %), resulting in amino acid substitution at position 80 (Serine TTA→ Tryptophan TGA) in all samples (*E. coli* numbering) (Table 1). Amino acid changes in *par*C QRDRs played a key role in the development of fluoroquinolone resistance, which were generally detected at positions 80 and 84 (*E. coli* numbering) (19, 20, 25). Serine replacement was the most common in conferring quinolone resistance. Mutations at that residue generally had little effect on the catalytic activity of DNA gyrase and topoisomerase IV (6, 25).Interestingly, the most reported mutation at position 80 of *par*C for *M. gallisepticum* clinical strains was Ser-80→Leu, whereas Ser-80→Trp replacement was only detected in the *Mycoplasma gallisepticum* enrofloxacin-resistant mutant obtained by serial passages *in vitro* (23).

Unlike other bacteria, the horizontal gene transfer (HGT) of mobile genetic elements (MGE) carrying antimicrobial resistant genes (AMR) has been little studied on mycoplasmas. Hence, the main pathway described for the emergence of AMR in *Mycoplasma* spp. is the occurrence, selection, and fixation of chromosomal mutations in target genes, mainly for synthetic antibiotics such as FQ (26). In addition, mycoplasmas are characterized by a high mutation frequency related to their limited amount of genetic information related to the SOS response and DNA repair systems (9).

To summarize, the results revealed that, in the samples from Ecuador, the following mutations Ser-83→Ile in GyrA and Ser -80→Trp in ParC were found simultaneously. Although the effects of amino acid replacements in DNA gyrase and topoisomerase IV in fluoroquinolone-resistant *M. gallisepticum* isolates are not fully elucidated, some studies have described the association between MIC results by *in vitro* microbial susceptibility testing and mutations in QRDRs (20,22, 23). Amino acid change also reveals an important influence on the degree of resistance. For example, MIC values between 0.1-0.025 µg/ml are reported in bacterial strains harboring no mutations (14). An analysis of different results in strains harboring the mutations described here showed that Ser-83→Ile in GyrA was sufficient to reach a level of resistance to enrofloxacin *in vitro* with MIC≥2 µg/ml, whereas the Ser-80→Trp change detected in ParC, had a greater impact on the level of resistance with a 16-fold increase (MIC≥16 µg/ml) (20, 22).

The contribution of mycoplasmas to the global gene flow associated to resistance among different genera of bacteria is low, as the genomic support of resistance is essentially point chromosomal mutations (27). However, the use of antimicrobials to control mycoplasma infections in both animals and humans requires the support of laboratory testing, as these compounds affect other bacterial genera that share the same niches, contributing to the selection of resistant strains. Besides, the use of antimicrobials for their control without the support of laboratory tests also affects other genera of bacteria present in mucous membranes, facilitating the selection of resistant strains.

Up to date, this is the first study carried out in Ecuador focused on the molecular characterization of QRDRs in genes encoding the synthesis of DNA gyrase and topoisomerase IV enzymes, from field samples of *M. gallisepticum* collected from broiler chickens. The alterations found in the genes studied have been identified by other authors in previous studies with an increase in MIC values *in vitro* susceptibility tests using mutants of different *Mycoplasma* spp. species, including *M. gallisepticum* (28, 29).These findings could suggest that *M. gallisepticum* clinical strains with lower susceptibility to fluoroquinolones may have emerged from poultry farms in Manabí province, Ecuador. For this reason, although genotypic methods are advantageous for earlier detection of potential resistance candidates, further studies are needed to culture clinical strains of *M. gallisepticum* and confirm the possible influence of these substitutions on FQ susceptibility in the genes using antimicrobial susceptibility testing.

REFERENCES

1. Nhung NT, Chansiripornchai N, Carrique-Mas, J.J. Antimicrobial Resistance in Bacterial Poultry Pathogens: A Review. Front Vet Sci. 2017;4:126.
2. Medina JL, Lugo K, Vargas J, Morales N, Burgos A, Martínez EP, *et al*. Ecuadorian mainland industrial poultry production is free of H5/H7 Avian influenza virus: National surveillance program in 2016. J. Vet. Med. Sci. 2019;81:1597-1600.
3. Kleven SH. Control of avian mycoplasma infections in commercial poultry. Avian dis. 2008;52:367-374.
4. Ball C, Forrester A, Ganapathy K. Co-circulation of genetically diverse population of vaccine related and unrelated respiratory mycoplasmas and viruses in UK poultry flocks with health or production problems. Vet. Microbiolgy. 2018;225:132-138.
5. Raviv Z, Kleven SH. The development of diagnostic real-time TaqMan PCRs for the four pathogenic avian mycoplasmas. Avian dis. 2009;53:103-107.

6. Gerchman I, Lysnyansky I, Perk S, Levisohn S. *In vitro* susceptibilities to fluoroquinolones in current and archived *Mycoplasma gallisepticum* and Mycoplasma synoviae isolates from meat-type turkeys. Vet Microbiol. 2008;131:266-276.
7. Aldred KJ, Kerns RJ, Osheroff N. Mechanism of quinolone action and resistance. Biochemistry. 2014;53:1565-1574.
8. Beeton ML, Chalker VJ, Kotecha S, Spiller OB. Comparison of full *gyrA, gyrB, parC* and *parE* gene sequences between all Ureaplasma parvum and Ureaplasma urealyticum serovars to separate true fluoroquinolone antibiotic resistance mutations from non-resistance polymorphism. J. Antimicrob. Chemother. 2009;64:529-538.
9. Taiyari H, Faiz NM, Abu J, Zakaria Z. Antimicrobial minimum inhibitory concentration of *Mycoplasma gallisepticum*: a systematic review. J. Appl. Poult. Res. 2021;30:100160.
10. Reinhardt A, Bébéar C, Kobisch M, Kempf I, Gautier-Bouchardon A. Characterization of mutations in DNA gyrase and topoisomerase IV involved in quinolone resistance of *Mycoplasma gallisepticum* mutants obtained *in vitro*. Antimicrob. Agents Chemother. 2002;46:590-593.
11. Gerchman I, Lysnyansky I, Perk S, Levisohn S. *In vitro* susceptibilities to fluoroquinolones in current and archived *Mycoplasma gallisepticum* and *Mycoplasma synoviae* isolates from meat-type turkeys. Vet Microbiol. 2008;131:266-276.
12. Hernández Y, Lobo E, Martínez S, Zamora L. Evaluación de diferentes métodos de extracción de ADN de micoplasmas para su empleo en el diagnóstico por PCR. Rev Salud Anim. 2009;31:108-114.
13. De la Cruz L, Barrera M, Rios L, Corona-González B, Bulnes CA, Díaz-Sánchez AA, *et al*. Unraveling the Global Phylodynamic and Phylogeographic Expansion of *Mycoplasma gallisepticum*: Understanding the Origin and Expansion of This Pathogen in Ecuador. Pathogens. 2020;9(9):E674.
14. Lysnyansky I, Gerchman I, Levisohn S, Mikula I, Feberwee A, Ferguson N, *et al*. Discrepancy between minimal inhibitory concentration to enrofloxacin and mutations present in the quinolone-resistance determining regions of *Mycoplasma gallisepticum* field strains. Vet Microbiology. 2012;160:222-226.
15. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 2013;30:772-780.
16. Al-Agamy, Shibl AM, Radwan HH. Detection of mutations in quinolone-resistant determining regions in clinical isolates of *Escherichia coli* from Saudi Arabia. Afr J. Biotechnol. 2012;11:1054-1058.
17. Correia S, Poeta P, Hébraud M, Capelo JL, Igrejas G. Mechanisms of quinolone action and resistance: where do we stand? J Med Microbiol. 2017;66:551-559.
18. Kommedal Ø, Kvello K, Skjåstad R, Langeland N, Harald G. WikerDirect 16S rRNA Gene Sequencing from Clinical Specimens, with Special Focus on Polybacterial Samples and Interpretation of Mixed DNA Chromatograms Journal of clinical microbiology. 2009:3562-3568.
19. Maruri F, SterlingTR, Kaiga AW, Blackman A, van der Heijden YF. A systematic review of gyrase mutations associated with fluoroquinolone-resistant Mycobacterium tuberculosis and a proposed gyrase numbering system. J Antimicrob Chemother. 2012;67:819-831.
20. Sahar O, Abou-Khadra SH. Genetic Analysis of Fluoroquinolone Resistant Genes in *Mycoplasma gallisepticum* Field Isolates. Egypt. J. Chem. Environ. Health. 2019;5:37-55.
21. Bhatnagar K, Wong A. The mutational landscape of quinolone resistance in *Escherichia coli*. PloS One14. 2019;e0224650.
22. Reinhardt A, Bébéar C, Kobisch M, Kempf I, Gautier-Bouchardon A. Characterization of mutations in DNA gyrase and topoisomerase IV involved in quinolone resistance of *Mycoplasma gallisepticum* mutants obtained *in vitro*. Antimicrob. Agents Chemother. 2002a;46:590-593.
23. Reinhardt A, Kempf I, Kobisch M, Gautier-Bouchardon A. Fluoroquinolone resistance in *Mycoplasma gallisepticum*: DNA gyrase as primary target of enrofloxacin and impact of mutations in topoisomerases on resistance level. J. Antimicrob Chemother. 2002b;50:589-592.
24. Lysnyansky I, Gerchman I, Perk S, Levisohn S. Molecular characterization and typing of enrofloxacin-resistant clinical isolates of *Mycoplasma gallisepticum*. Avian Dis. 2008;52:685-689.
25. Gautier-Bouchardon A. Antimicrobial Resistance in *Mycoplasma* spp. Microbiol SpectrSpectrum. 2018;6(4):ARBA-0030-2018. doi: 10.1128/microbiolspec.ARBA-0030-2018.
26. Faucher M, Nouvel LX, Dordet-Frisoni E, Sagne E, Baranowski E, Hygonenq MC, *et al.* Mycoplasmas under experimental antimicrobial selection: The unpredicted contribution of horizontal chromosomal transfer. PLoS Genet15.2019:e1007910.
27. Pereyre S, Tardy F. Integrating the Human and Animal Sides of Mycoplasmas Resistance to Antimicrobials. Antibiotics. 2021;10:1216. https://doi.org/10.3390/ antibiotics10101216
28. Shimada Y, Deguchi T, Nakane K, Masue T, Yasuda M, Yokoi S, *et al*. Emergence of clinical strains of Mycoplasma genitalium harbouring alterations in ParC associated with fluoroquinolone resistance. Int J Antimicrob Agents 2010; 36: 255-258.

29. Li J, Lu D, Liu Z, Zhang X, Wei F, Liu Y, *et al*. Role of mutations in DNA gyrase and topoisomerase IV in fluoroquinolones-resistance of *Mycoplasma gallisepticum* obtained in vitro and *in vivo*. J Anim Vet Adv. 2012; 11: 2327-2332.

Declaration of conflict of interest: The authors declare that there is no conflict of interest.
Contribution of the authors: Conceptualization: Ivette Espinosa Castaño and Evelyn Lobo Rivero. **Formal analysis:** Adrián A. Díaz-Sánchez, Dany Naranjo Feliciano, Rosa Elena Hernández Fillor and Laura de la Cruz. **Research:** Adrián A. Díaz-Sánchez, Laura de la Cruz Rosa Elena Hernández Fillor and Dany Naranjo Feliciano. **Methodology:** Laura de la Cruz and Adrián A. Díaz-Sánchez. **Supervision:** Evelyn Lobo Rivero. **Visualization**, **Writing-Original Draft:** Ivette Espinosa and Adrián A. Díaz-Sánchez.