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The analysis of antimicrobials epidemiological cut-off values of mycoplasma gallisepticum isolated from goose

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ABSTRACT

Mycoplasma gallisepticum (MG) poses a significant threat to the goose breeding industry, with antimicrobial agents commonly employed for its treatment. However, the absence of standardized criteria for antimicrobial selection complicates clinical decision-making regarding the choice and dosage of these agents, contributing to the emergence of clinical resistance. In this study, we isolated 102 clinical strains of MG from geese in Guangdong. We determined the minimum inhibitory concentration (MIC) values for various antimicrobials frequently used in MG treatment, including danofloxacin, enrofloxacin, tilmicosin, tylosin, tylvalosin, valnemulin, tiamulin, and spectinomycin. After conducting a statistical analysis of the susceptibility test results, we employed ECOFFinder to establish the wild-type cutoff values (ECOFFs) for these antimicrobials against the MG isolates. The findings revealed a significant reduction in sensitivity among the clinical isolates to the eight tested antimicrobial agents. While valnemulin and tiamulin maintained relatively good sensitivity, enrofloxacin, danofloxacin, and spectinomycin exhibited notably diminished effectiveness. The determined ECOFFs for MG isolated from geese against the aforementioned antimicrobials were 3.2, 3.2, 0.8, 0.8, 0.08, 0.05, 0.05, and 3.2 μg/mL, respectively. This study is crucial for identifying resistant strains and investigating resistance mechanisms, in addition to providing a precise assessment of the resistance levels of MG isolated from geese in Guangdong. The insights gained from this research will serve as a valuable reference for the prevention and treatment of MG infections and will promote more effective clinical strategies regarding the use of antimicrobials.

Introduction

In the global poultry industry, two significant issues are infectious sinusitis in turkeys and chronic respiratory disease (CRD) in broiler, layer, and breeder flocks. Both conditions are caused by infections with *Mycoplasma gallisepticum* (MG) (Marouf et al., 2022). Furthermore, *Mycoplasma gallisepticum* (MG) infections are known to increase viral load and reduce the efficacy of avian virus vaccinations. This interplay consequently elevates the risk of viral infections and subsequent bacterial complications (Lu et al., 2023). In addition to respiratory symptoms such as sneezing, rales, coughing, conjunctivitis, weeping, and mucus accumulation around the nostrils, birds affected by chronic respiratory disease (CRD) also exhibit reduced weight gain, decreased egg production, lower hatchability, and increased rates of embryonic mortality (Marouf et al., 2020).Guangdong Province is one of the regions with the highest populations of geese in China. In recent years, there have been reports of *Mycoplasma gallisepticum* (MG) infections in geese (Zhou et al., 2024). However, there are currently no studies on the isolation of mycoplasma from geese in China. *Mycoplasma gallisepticum* (MG) infection manifests with symptoms during the acute phase, with respiratory symptoms being the predominant clinical signs observed (Liu, Wang, et al., 2024). The treatment of mycoplasma infections is currently based on the use of antimicrobial agents (Tang et al., 2016), there is currently a lack of clear criteria for the selection of antimicrobials, which has emerged as a response to resistance. Furthermore, the symptoms of Mycoplasma gallisepticum (MG) infection are typically

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observed following large epidemics of this disease, leading to the misuse of antimicrobial agents (Hashem et al., 2022). Consequently, there is an urgent need for guidelines on the appropriate use of antimicrobials in the treatment of *Mycoplasma gallisepticum* (MG) infections.

Susceptibility discount points have been employed to evaluate the susceptibility of pathogens to antimicrobials and to identify acquired resistant strains of bacteria (Mouton et al., 2012). Resistance criteria are utilized to interpret and analyze the results of susceptibility testing, thereby determining the susceptibility of pathogens to antimicrobials (Turnidge et al., 2006). Knowledge of antibiotic susceptibility can be utilized to more quickly identify the appropriate therapeutic agent, develop rational dosing regimens, and minimize the emergence of clinical resistance (De Jong et al., 2021). CO_{WT} is derived from the results of in vitro antimicrobial susceptibility testing and has been used to differentiate between susceptible and resistant organisms, as well as to study levels of antimicrobial resistance. Additionally, studies have been conducted to analyze the antimicrobial susceptibility profiles of various mollicutes, such as *Mycoplasma pneumoniae* (Rui and Qiu, 2023), *Mycoplasma synoviae* (Catania et al., 2019) *Mycoplasma hominis* and *Ureaplasma urealyticum* (Lee et al., 2016), however, no antimicrobial susceptibility folds associated with *Mycoplasma genitalium* (MG) have been reported. Currently, the most clinically used fold points are those established by the authoritative international standard-setting organization, the Clinical and Laboratory Standards Institute (CLSI) in the United States (Toutain et al., 2017), but there is currently no reference standard for *Mycoplasma genitalium* (MG) in the standards developed by the Clinical and Laboratory Standards Institute (CLSI). The nutritional requirements and growth conditions necessary for the proliferation of each mycoplasma species are highly specific and distinct. Consequently, we conducted a determination of the minimum inhibitory concentration (MIC) values for MG to facilitate the establishment of antibacterial susceptibility breakpoints.

From 2021 to 2022, a total of 758 samples were collected from geese exhibiting respiratory symptoms in the Guangdong Province, specifically from Zhaoqing, Jiangmen, Foshan, Guangzhou, Qingyuan, Yangjiang, Jieyang, and Yunfu. Following isolation and purification, 102 clinical isolates of *Mycoplasma genitalium* (MG) were obtained. We selected several antibiotics commonly used to treat MG infections, including danofloxacin, enrofloxacin, tilmicosin, tylosin, tylvalosin, valnemulin, tiamulin, and spectinomycin, to determine the minimum inhibitory concentration (MIC) values. Subsequently, ECOFFinder was developed with the objective of establishing the wild-type fold points of these antimicrobials against MG isolated from geese. The present study offers a more comprehensive insight into the clinical resistance levels of MG isolates in Guangdong Province, provides a foundation for the prevention and control of MG infectious diseases, and serves as a valuable reference for the utilization of antimicrobials.

Materials and methods

Test strains

A total of 758 samples were collected from geese exhibiting respiratory symptoms in Guangdong Province between 2021 and 2022. The samples were obtained from the following locations: Zhaoqing, Jiangmen, Foshan, Guangzhou, Qingyuan, Yangjiang, Jieyang, and Yunfu. Following isolation and purification, 102 clinical isolates of *Mycoplasma gallisepticum* (MG) were obtained.

MG Standard S6 was obtained from the China Institute of Veterinary Antimicrobial Control.

Reagents

The antibacterial agents used in this study were of the highest quality. All the antibiotics are from the Guangdong Wenshi Dahuanong Biotechnology Co. A description of the anti-inflammatory antimicrobials is provided in Table 1.

Methods

Preparation of mycoplasma culture media

(1) Preparation of Liquid Culture Medium: Prepare the liquid medium according to the method outlined in the 2015 edition of the Veterinary Pharmacopoeia of the People's Republic of China, Part III. After preparation, store the medium at 4°C. (2) Preparation of Solid Medium: Add 1-1.5 % agar powder to the prepared liquid medium. Sterilize the mixture and pour it into sterile Petri dishes when it has cooled to 60°C in a laminar flow hood. After solidification, seal the dishes in bags and store them at 4°C.

Preparation of antimicrobial master batches

The antimicrobial solution was prepared at a concentration of 1280 μg/mL. An appropriate amount of the antibacterial agent was accurately weighed in a beaker using an analytical balance, dissolved in ultrapure water, and subsequently transferred to a 50 mL volumetric flask to adjust the final volume. The solution was then stored at −20°C.

G culture

(1) Resuscitation of *Mycoplasma gallisepticum* (MG): Add the liquid medium to a vial, followed by the appropriate quantity of lyophilized mycoplasma powder. The resulting solution should be incubated at 37°C. A change in color of the medium indicates the growth of MG and the initiation of the passaging process. (2) Passage Culture and Preservation of MG: Add liquid medium to a vial, then introduce 1/10th of the well-grown MG culture. Place the vial in a 37°C incubator. A subsequent change in color of the medium indicates that MG has reached full growth and can be preserved. To preserve the culture, add lyophilized liquid to the well-grown MG culture, freeze it, and then freeze-dry it using a vacuum freeze dryer. After drying, seal the vials for storage.

MIC determination

The minimum inhibitory concentration (MIC) was determined using the agar dilution method (Waites et al., 2011). The antimicrobial solution was prepared at a concentration of 1280 μg/mL.

(1) Preparation of Antimicrobial-Containing Plates: The diluted antimicrobial agent was combined with agar medium at a ratio of 1:19 to create antimicrobial-containing plates. The resulting mixture was allowed to solidify. Once solidified, the plates were inverted and labeled with the following information: type of medium, name and concentration of the antimicrobial agent, and date of preparation. Agar plates should be stored in a sealed plastic bag at 2 to 8°C for no more than three days. Prior to use, the plates should be removed from refrigeration and allowed to equilibrate to room temperature. Ensure that the surface of the agar is dry before inoculating with the bacterial suspension. If necessary, leave the Petri dish inverted for approximately 30 min to facilitate drying and temperature equilibration. (2) Preparation and Inoculation of Inocula: Clinical isolates were resuscitated and passaged five times. The culture was then diluted to approximately 10^4∼10^5 CFU/

Table 1
Antibacterial agent used in this trial.

Name	Purity/content
danofloxain mesylate	92 %
enrofloxacin sodium	92.1 %
tilmicosin phosphate	78 %
tylosin tartrate	80.45 %
tylvalosin tartrate	822 IU/mg
tiamulin fumarate	80.48 %
valnemulin hydrochloride	98.5 %
spectinomycin hydrochloride	750 IU/mg

This table shows the information of all antibacterial agents used in this test.

mL in an appropriate medium.The dilutions were incubated in ambient air at 37°C for two hours. Following incubation, aspirate and inoculate the surface of the antimicrobial-containing plates using a multipoint inoculator. After inoculation, the plates should be placed in a 37°C incubator with 5 % CO_2 for 7 to 10 days. (3) Judgment of Results: The minimum inhibitory concentration (MIC) was recorded as the lowest concentration of the antimicrobial agent that prevented colony formation at the time of reading,while no growth was observed on the antimicrobial control plate. Readings were taken with the aid of a microscope, and all dilutions were examined. For the negative control, the sterile culture substrate should show no signs of growth. The test should be repeated three times to ensure accuracy.

Establishment of antimicrobials for MG CO_{WT}

(1) Guerra (Guerra, 2003) et al. demonstrated that the distribution of minimum inhibitory concentrations (MICs) of wild-type strains conformed to a normal distribution when the sample size was sufficient. The determination of the clinical objective wild-type (CO_{WT}) requires the exclusion of interference from non-wild-type strains. To achieve this, a normality test was first performed to select strains that presumably conformed to a normal distribution: the MIC results of the strains were entered into SPSS (Statistical Product and Service Solutions) software, and a P-P plot test was conducted to assess the conformity of the MIC distribution to a normal distribution. (2) The range of MICs was then analyzed using nonlinear regression. Nonlinear regression was employed to fit the Log2 MIC data, starting from the smallest MIC and progressing to the largest MIC, incrementing the MIC gradient by one unit at each step. This process continued until the number of strains fitted deviated minimally from the actual number of strains, after which the log2 MIC was converted back to the original MIC scale. (3) Subsequently, the NORMINV function was utilized to ascertain the upper limit of the maximum value of wild-type strains. Following this, the NORMDIST function was employed to determine the probability associated with validating the aforementioned upper limit. Ultimately, the final CO_{WT} was established to encompass a minimum of 95 % of the wild-type strains. The Clinical and Laboratory Standards Institute (CLSI) integrates both the analysis and validation steps, and it is recommended to use the Ecoffinder macroform to directly fit the MIC distribution of wild-type strains in order to obtain the CO_{WT} (https://clsi.org/meetings/susceptibility-testing-subcommittees/ecoffinder/).

Results

MIC measurement results

The minimum inhibitory concentration (MIC) results for 102 Mycoplasma gallisepticum (MG) isolates from geese, evaluated against danofloxacin, enrofloxacin, tilmicosin, tylosin, tylvalosin, valnemulin,

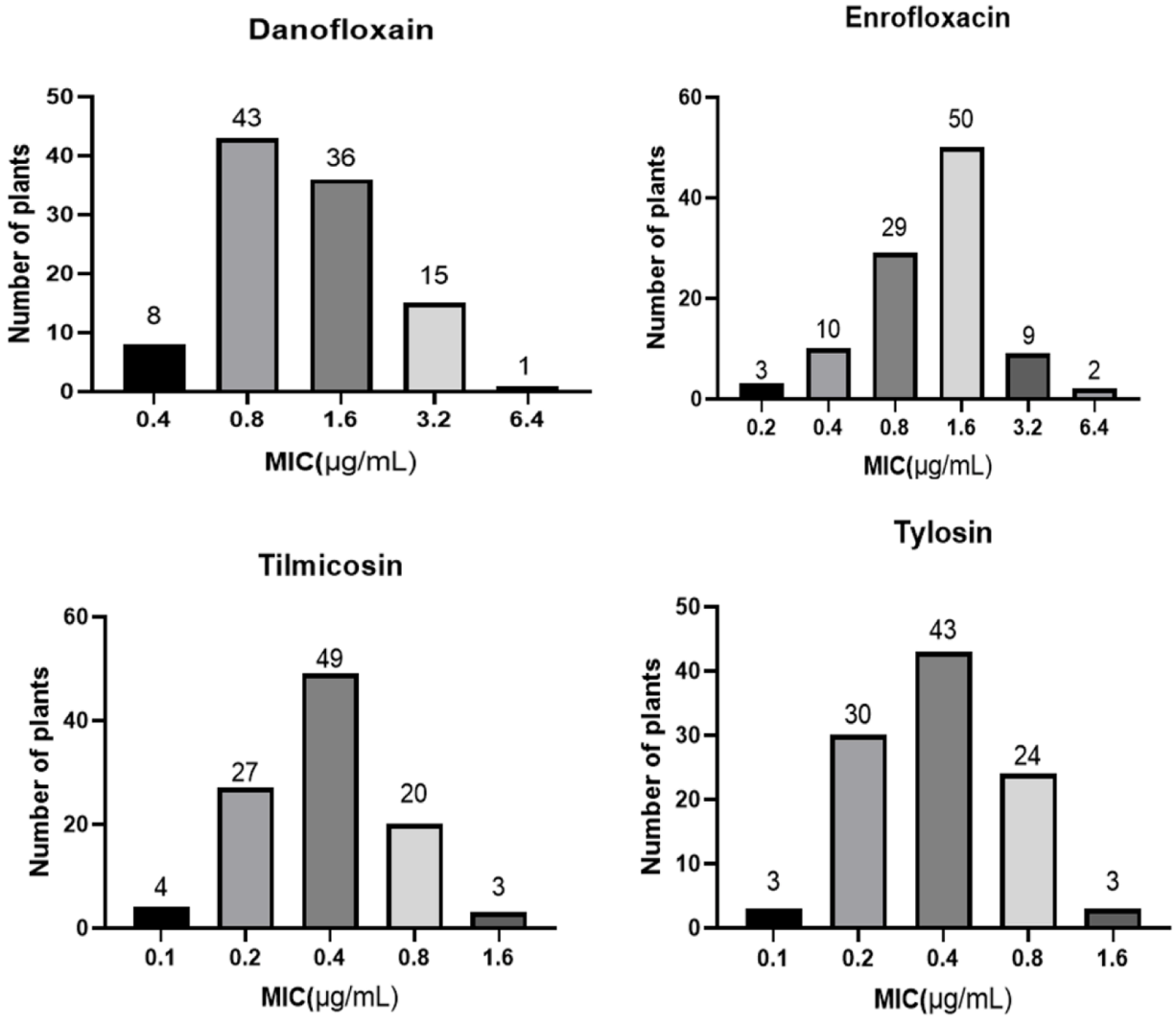

Fig. 1. Results of MIC measurement of clinical isolates
The figure shows the statistical results of MIC of 102 clinical isolates.

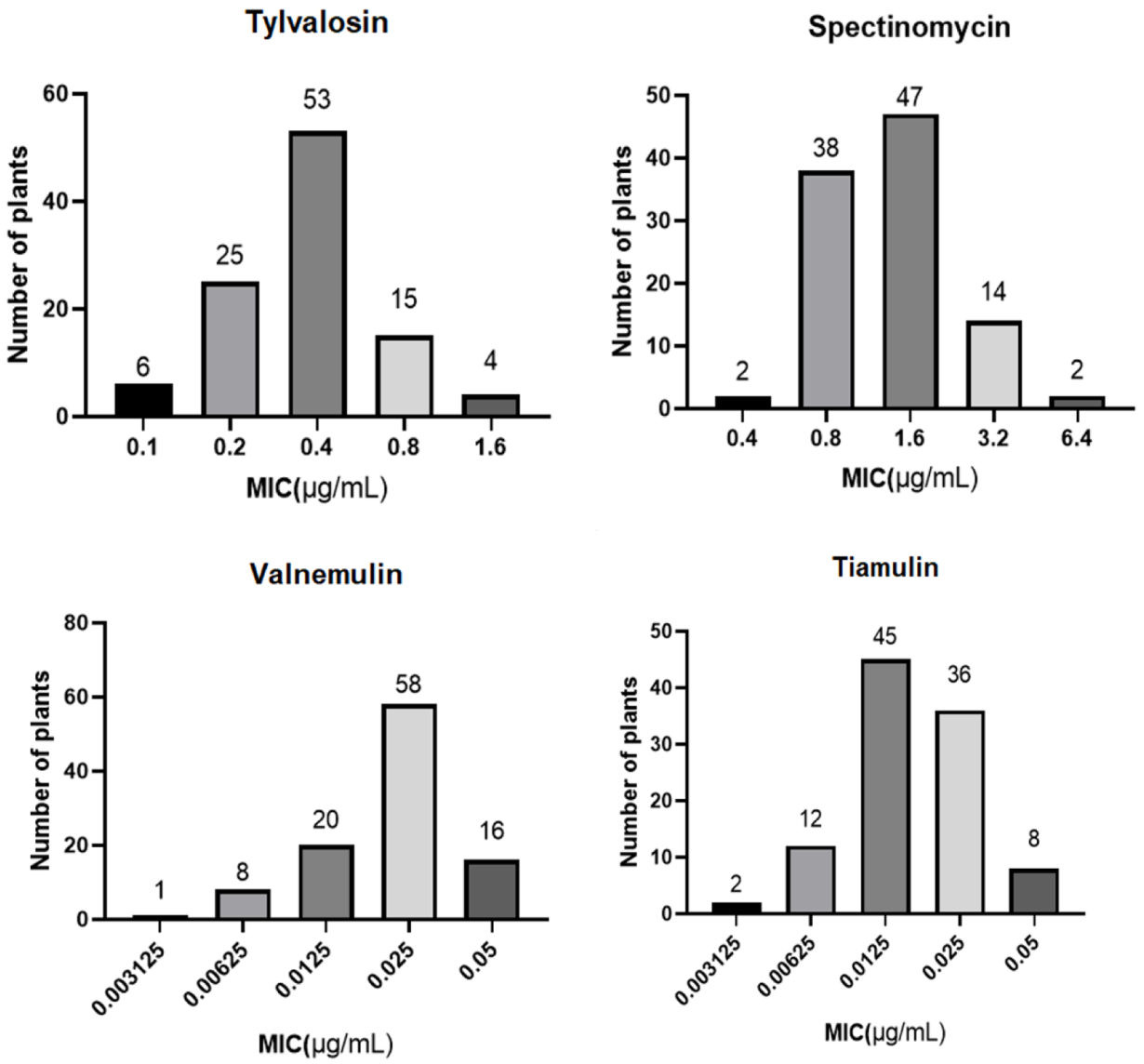

Fig. 1. (*continued*).

tiamulin, and spectinomycin, are presented in Fig. 1 and Table 2.

Following MIC testing, the highest MIC value for danofloxacin was found to have increased 16-fold compared to the MIC of the standard strain (from 0.4 μg/mL to 6.4 μg/mL), with the proportion of MG increasing to 50.98 % at this MIC. Similarly, the highest MIC for enrofloxacin increased 32-fold compared to the standard strain MIC (from 0.2 μg/mL to 6.4 μg/mL), and the percentage of MG reached 59.8 % at the MIC.For tilmicosin, tylosin, and tylvalosin, the highest MIC values also increased 16-fold compared to the standard strain MIC (from 0.1 μg/mL to 1.6 μg/mL), with the respective percentages of MG rising to 70.59 %, 68.63 %, and 70.59 % at the MIC.In the case of tiamulin and valnemulin, the highest MIC values increased 16-fold relative to the standard strain MIC (from 0.003125 μg/mL to 0.05 μg/mL), resulting in MG percentages of 87.25 % and 92.16 %, respectively, at the MIC.

Finally, the highest MIC for spectinomycin also increased 16-fold compared to the standard strain MIC (from 0.4 μg/mL to 6.4 μg/mL), with the proportion of MG reaching 60.78 % at this MIC. The complete

Table 2
MIC results of standard strain of MG S6 (μg/mL).

Name of antimicrobial	MIC
danofloxacin	0.8
enrofloxacin	0.4
tilmicosin	0.2
tylosin	0.2
tylvalosin	0.1
Tiamulin	0.00625
Valnemulin	0.00625
Spectinomycin	0.4

This table shows the MIC results of MG standard strain S6.

Table 3
Summary of MIC results for clinical strains of Mycoplasma fowlis (μg/mL).

Name	Maximum elevation of MIC value	Percentage of clinical strains with more than 4-fold elevated MIC values
Danofloxacin	16	50.98 %
Enrofloxacin	32	59.80 %
Tilmicosin	16	70.59 %
Tylosin	16	68.63 %
tylvalosin	16	70.59 %
tiamulin	16	87.25 %
valnemulin	16	92.16 %
spectinomycin	16	60.78 %

This table shows the statistical results of the multiple increase of MIC value of MG clinical strains.

results are detailed in Table 3.

Developing wild-type fold value

The normal P-P plot illustrating the MIC distribution of danofloxacin, enrofloxacin, tilmicosin, tylosin, tylvalosin, valnemulin, tiamulin, and spectinomycin against *Mycoplasma gallisepticum* (MG) isolates is presented in Fig. 2.

The impact of danofloxacin, enrofloxacin, tilmicosin, tylosin, tylvalosin, valnemulin, tiamulin, and spectinomycin on the MIC distribution of MG is depicted in Fig. 3.

The classification results of MG isolates based on exposure to danofloxacin, enrofloxacin, tilmicosin, tylosin, tylvalosin, valnemulin, tiamulin, and spectinomycin are shown in Table 4.

Table 5 presents the MIC_{50}, MIC_{90}, and ECOFF values of MG against the aforementioned antibiotics. The values for valnemulin and spectinomycin were relatively low, indicating that MG is highly sensitive to these agents, suggesting that pleuromutilin antibiotics should be preferred for the treatment of MG infections in clinical practice. Conversely, higher MIC_{50}, MIC_{90}, and ECOFF values were observed for danofloxacin, enrofloxacin, and spectinomycin. Given that MG shows reduced sensitivity to danofloxacin, enrofloxacin, and spectinomycin, these antibiotics may not provide the desired clinical outcome when utilized for treatment.

Discussion

Respiratory diseases in poultry significantly hinder the development of the poultry industry and pose a major threat to production. Mycoplasma infections have been reported with high incidence rates in geese, resulting in substantial economic losses for the goose-rearing sector. Among the various species in the genus Mycoplasma, *Mycoplasma gallisepticum* (MG) is the most pathogenic and represents a serious threat to poultry health. Benöina (Benöina et al., 1988) reported the isolation of

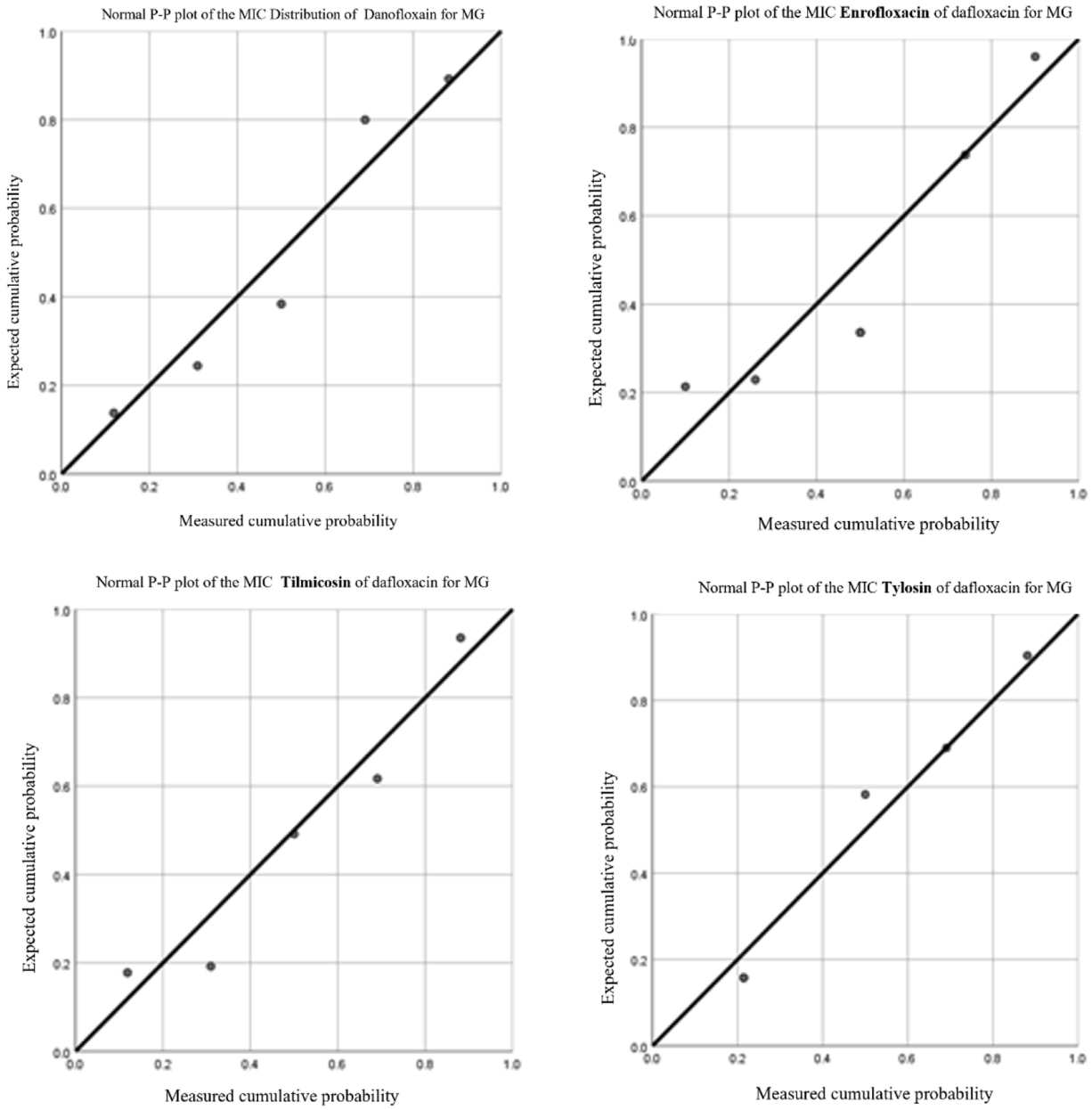

Fig. 2. Normal P-P plot of MIC distribution of antimicrobial against MG
This figure shows the normal distribution test of MIC value of 8 kinds of antibacterial drugs on MG clinical isolates.

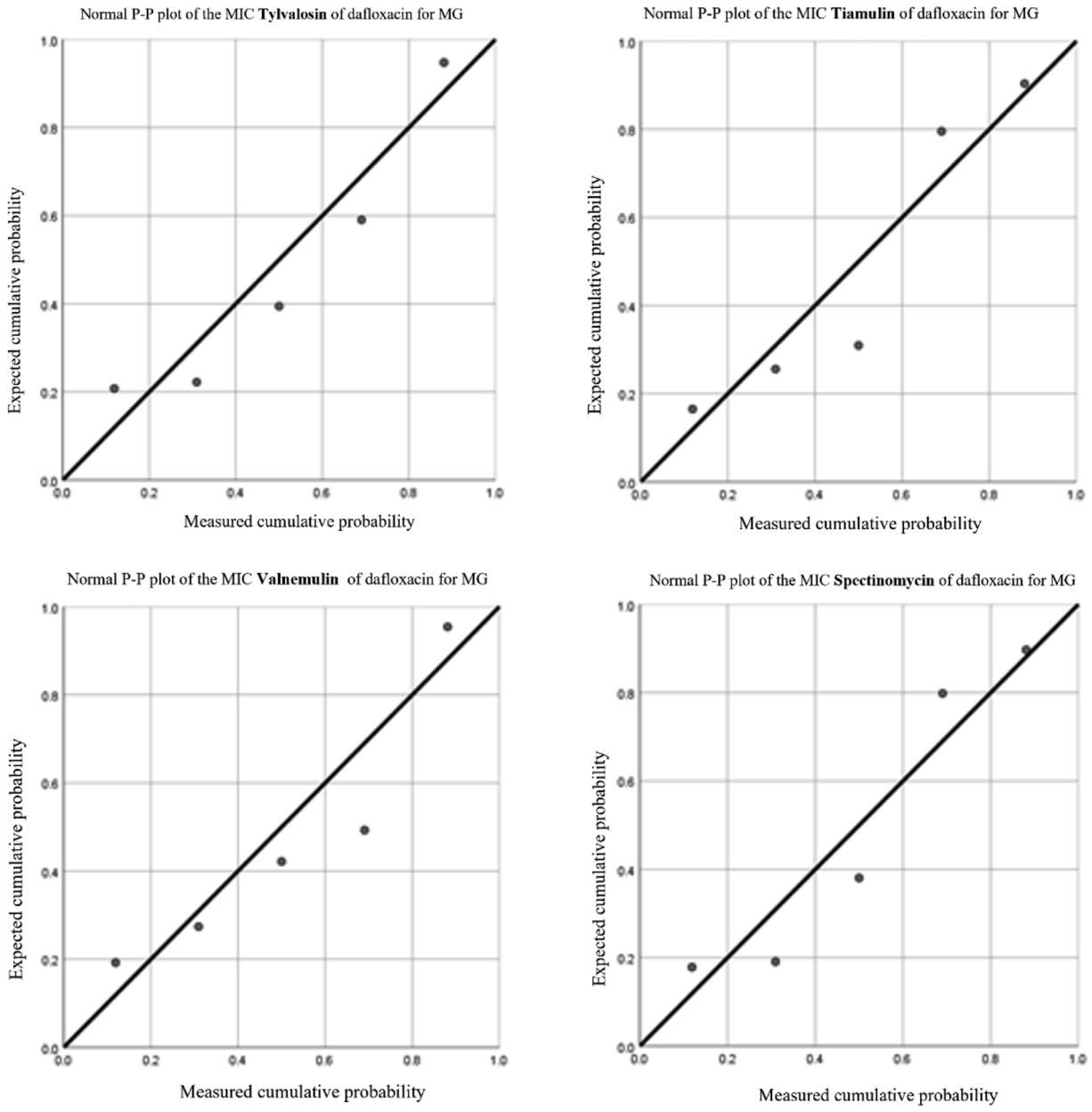

Fig. 2. (*continued*).

Mycoplasma gallisepticum (MG) from farmed geese, as well as the detection of MG antibodies in the serum of some individuals. Buntz (Buntz et al., 1986) conducted a study on Mycoplasma isolated from geese reared in the Landes region of southwestern France. In this study, three clinical strains of *Mycoplasma gallisepticum* (MG) were isolated from diseased materials (esophagus, trachea, cloaca) collected from 134 animals in a herd. Additionally, one clinical strain of MG was isolated from the feces of another flock of 70 geese. In a separate experiment, a total of 102 clinical strains of MG were isolated from diseased geese suspected of Mycoplasma infection in Guangdong Province.

The four main classes of antimicrobials commonly used to treat *Mycoplasma gallisepticum* (MG) infections in the poultry industry are tetracyclines, fluoroquinolones, macrolides, and pleuromutilins. The culture period for Mycoplasma is lengthy, and previous studies have demonstrated that tetracycline antibiotics are prone to degradation during culture. These antibiotics may discolor when exposed to sunlight and are not stable under acidic or alkaline conditions, resulting in poor reproducibility of test results (Zhang et al., 2018). In this experiment, we selected antimicrobials from three remaining classes to determine their minimum inhibitory concentrations (MICs) against 102 isolated clinical strains of *Mycoplasma gallisepticum* (MG). Although MG is generally not susceptible to aminoglycosides, some farmers continue to use these antimicrobials for treating MG infections. Therefore, we included spectinomycin in the evaluation of in vitro susceptibility against the clinical isolates of MG. The standard MG strain S6 was employed as a reference strain to assess the resistance levels of MG isolates in various regions of Guangdong, thereby providing a basis for the clinical use of antimicrobials. The final antimicrobials selected after screening included danofloxacin, enrofloxacin, tilmicosin, tylosin, tylvalosin, valnemulin, tiamulin, and spectinomycin.

The minimum inhibitory concentration (MIC) results indicated significantly reduced clinical sensitivity for all eight antibacterials. Among these, valnemulin and tiamulin exhibited the highest sensitivity, whereas enrofloxacin demonstrated the lowest sensitivity. Welchman (Welchman et al., 2022) investigated the resistance of 56 clinical isolates of *Mycoplasma gallisepticum* (MG) from the United Kingdom between

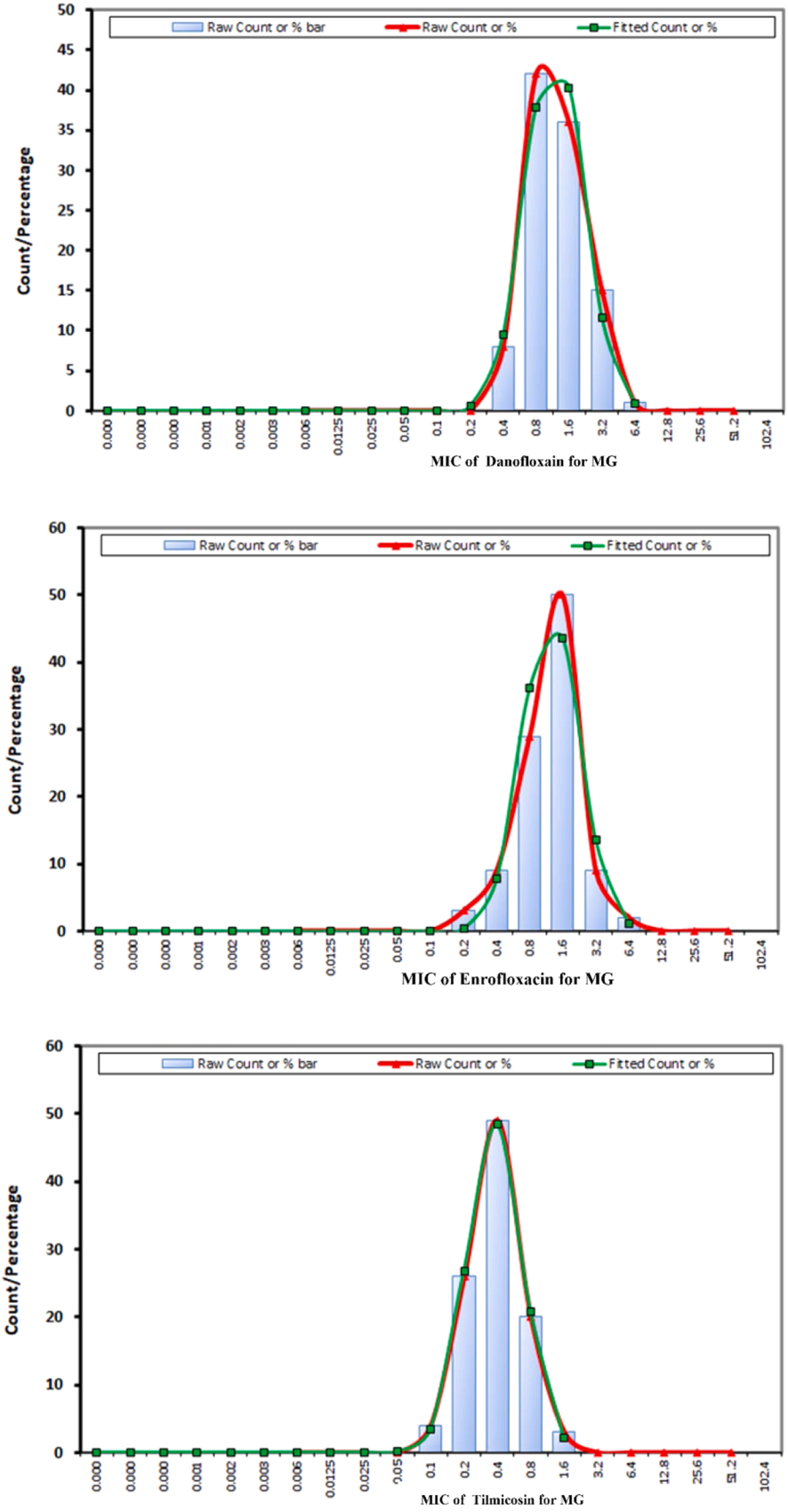

Fig. 3. Results of normal fitting of MIC distribution of antimicrobial to MG
The figure shows the influence of 8 antimicrobial agents on the distribution of MIC value of MG clinical isolates.

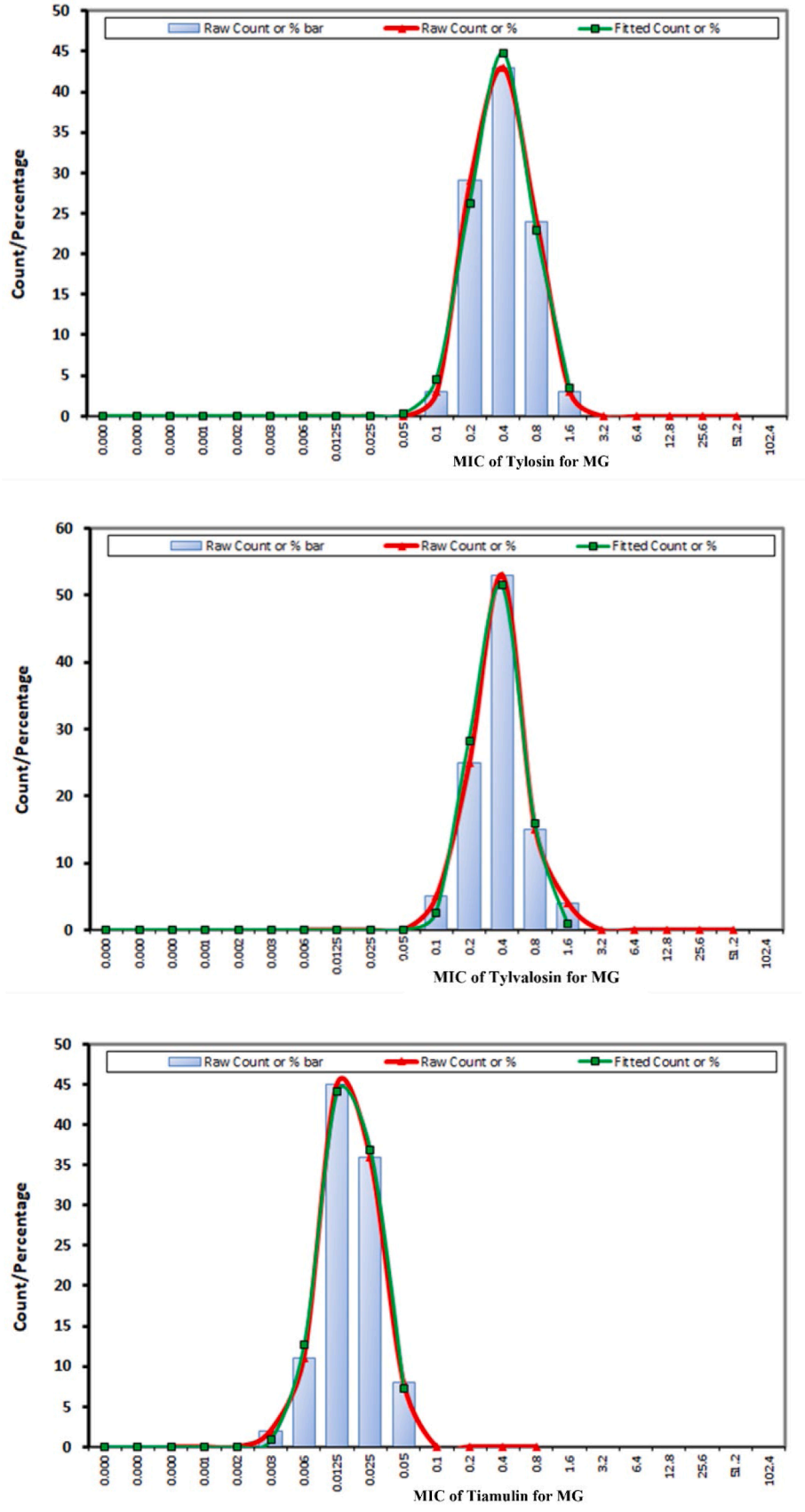

Fig. 3. (*continued*).

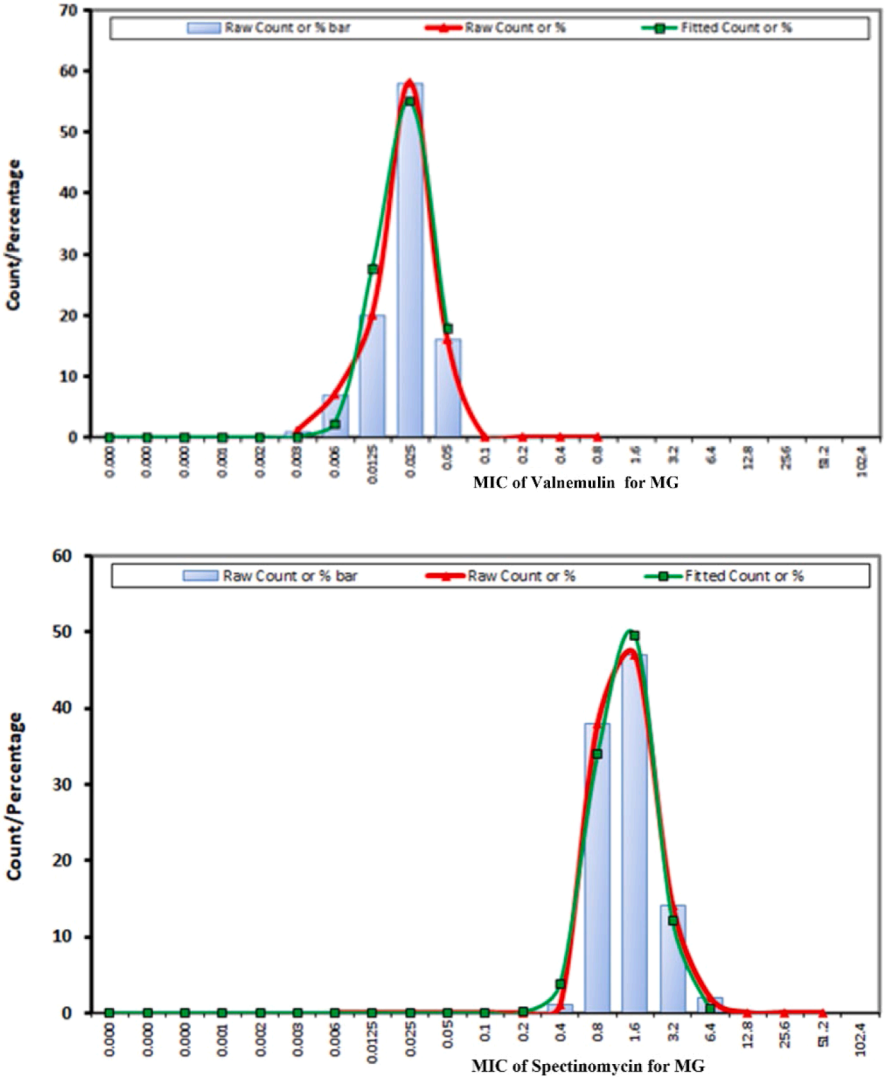

Fig. 3. (*continued*).

Table 4
CO_{WT} of antimicrobials to MG.

Name of antimicrobial	Number of best-fit strains	95 % upper limit of distribution	CO_{WT} (μg/mL)	≤ wild-type fold probability %
danofloxacin	100.7	2.1099	3.2	99.02
enrofloxacin	102.4	2.2055	3.2	98.03
tilmicosin	101.7	0.6478	0.8	97.06
Tylosin	102.2	0.7259	0.8	97.06
tylvalosin	99.1	0.5555	0.8	96.08
tiamulin	102.1	0.0279	0.05	100.00
valnemulin	103.5	0.0353	0.05	100.00
spectinomycin	99.8	2.0249	3.2	98.04

This table shows the statistical results of CO_{WT} value of MG clinical strains.

Table 5
MIC_{50}, MIC_{90} and ECOFF values of 8 antimicrobials against MG.

Veterinary antimicrobial	MIC_{50} μg/mL	MIC_{90} μg/mL	ECOFF μg/mL
danofloxacin	1.6	3.2	3.2
enrofloxacin	1.6	3.2	3.2
tilmicosin	1.6	3.2	3.2
tylosin	0.4	0.8	0.8
tylvalosin	0.4	0.8	0.8
tiamulin	0.4	0.8	0.8
valnemulin	0.025	0.05	0.05
spectinomycin	0.0125	0.025	0.05

This table shows the statistical results of MIC_{50},MIC_{90} and ECOFF values of 8 antimicrobial agents.

2016 and 2019. The results indicated that these 56 MG strains were susceptible to five antibacterials, which included tiamulin, tylosin, tylvalosin, doxycycline, and tetracycline. Gerchman (Gerchman et al., 2008) compared clinical isolates of *Mycoplasma gallisepticum* (MG) against enrofloxacin and difloxacin from 2005 to 2006 with previous clinical isolates obtained from 1997 to 2003. The study showed that the susceptibility of the clinical isolates to enrofloxacin and difloxacin was reduced over this time period. Bottinelli (Bottinelli et al., 2022) examined the minimum inhibitory concentrations (MICs) of 67 *Mycoplasma gallisepticum* (MG) isolates collected from Italy between 2010 and 2020, and found that 79.1 % of the isolates exhibited MIC values ≥ 8 μg/mL against enrofloxacin. The observed resistance may be attributed to several factors. Firstly, enrofloxacin is a broad-spectrum, slow-acting, and cost-effective antibacterial agent widely used for treating bacterial infections in livestock and poultry. Its misuse or overuse can lead to the emergence of antimicrobial resistance. Secondly, the resistance mechanisms of quinolones primarily involve mutations in the target genes, including *gyrA, gyrB, parC*, and *parE*, which can be stably inherited. Therefore, these results indicate a significant decrease in the

susceptibility of MG to fluoroquinolones, suggesting that continued use of fluoroquinolones in the clinical management of MG infections is not recommended.

The cumulative optimal working titer (CO_{WT}) of *Mycoplasma gallisepticum* (MG) against tiamulin and valnemulin is 0.05 μg/mL, which is the lowest value observed among the eight antibacterial agents tested. The mechanism of action of these antibiotics involves the inhibition of bacterial protein synthesis through binding to the 50S subunit of the bacterial ribosome. Furthermore, pleuromutilin possesses a specific target site that differs from those of other antibacterial agents (Liu, Zhou, et al., 2024). Furthermore, a published report indicated that microorganisms such as Mycoplasma and Staphylococcus aureus exhibit a low resistance mutation frequency against tiamulin, ranging from $10^{-9} \sim 10^{-11}$ () (Kosowska-Shick et al., 2006). Previous studies have demonstrated that the tiamulin resistance phenotype is primarily attributed to a mutation at position 149 (A445 G) in the ribosomal protein L3, which results in a conversion to asparagine (Asn) from aspartic acid (Asp). Moreover, resistance develops in a gradual manner (Bøsling et al., 2003). Thus, the specific mechanism of action and the identified resistance mutation site contribute to a mechanism that makes it difficult for bacteria to acquire resistance to pleuromutilin during the treatment of infectious diseases with truncated siderophore antimicrobials. Therefore, the use of tiamulin should be prioritized in the clinical management of *Mycoplasma gallisepticum* (MG) infections in geese.

Antimicrobial susceptibility breakpoints are utilized to determine whether a pathogen is resistant to a specific antimicrobial agent. In this context, CO_{WT} (clearance of wild-type) is employed to define wild-type colonies that do not contain resistance mechanisms, thereby allowing for the assessment of bacterial susceptibility to antimicrobials. These breakpoints are primarily used for epidemiological surveillance of antimicrobial-resistant phenotypes and to guide clinical antimicrobial usage (Meletiadis et al., 2017). The primary purpose is to conduct epidemiological monitoring of antimicrobial-resistant phenotypes and to guide clinical antimicrobial use. Huang (Huang et al., 2021) established the clinical fold points of tylosin based on the CO_{WT}, CO_{PD}, and CO_{CL} of tylosin against *Mycoplasma gallisepticum* (MG). Their results indicated that the CO_{WT} of tylosin against MG was 2 μg/mL. In the current study, the CO_{WT} of tylosin against MG of goose origin was found to be 0.8 μg/mL. Wang (Wang et al., 2022) demonstrated that the CO_{WT} of danofloxacin against *Mycoplasma gallisepticum* (MG) was 1 μg/mL. In the current experiment, the CO_{WT} of danofloxacin against goose-derived MG was found to be 3.2 μg/mL. The discrepancies observed between these results may be attributed to the different methodologies employed for minimum inhibitory concentration (MIC) determination. While the aforementioned studies utilized the micro broth dilution method, this study employed the agar dilution method. It has been established that the choice of determination method can significantly influence the resulting MIC values (Zhang et al., 2017). There are notable differences in the use of antimicrobials across different regions, as well as variations in the pathogen's resistance levels and mechanisms of resistance among infected animals. A combination of these factors may ultimately result in discrepancies in the fold points (Espinel-Ingroff and Turnidge, 2016).

In summary, this experiment evaluated the susceptibility of clinical isolates of goose-origin respiratory Mycoplasma gallisepticum (MG) to danofloxacin, enrofloxacin, tilmicosin, tylosin, tylvalosin, valnemulin, tiamulin, and spectinomycin. The results of the antimicrobial susceptibility tests revealed a significant decrease in susceptibility to all eight antimicrobials, with relatively good susceptibility observed for valnemulin and tiamulin. However, there was a more pronounced reduction in susceptibility to enrofloxacin. The wild-type fold points for danofloxacin, enrofloxacin, tilmicosin, tylosin, tylvalosin, valnemulin, tiamulin, and spectinomycin against clinical strains of goose-origin MG were calculated using the method recommended by the Clinical and Laboratory Standards Institute (CLSI) via ECOFFinder, yielding values of 3.2, 3.2, 0.8, 0.8, 0.8, 0.05, 0.05, and 3.2 μg/mL, respectively. The establishment of mycoplasma resistance criteria is advantageous for controlling mycoplasma resistance as it can be utilized not only to monitor and predict resistance patterns but also to guide clinical antimicrobial use and enhance the administration of antimicrobial regimens.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nan Zhang reports financial support was provided by National Natural ScienceFoundation of China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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