Susceptibility of caprine mastitis pathogens to tildipirosin, gamithromycin, oxytetracycline, and danofloxacin: effect of serum on the in vitro potency of current macrolides

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Received: 8 April 2022 / Accepted: 29 August 2022 © The Author(s) 2022

Abstract

Mastitis is a significant disease in dairy ruminants, causing economic losses to the livestock industry and severe risks to public health. Antibiotic therapy is one of the most crucial practices to treat mastitis, although the susceptibility of caprine mastitis pathogens to current antibiotics has not been tested under standard or modified incubation conditions. This work evaluated the in vitro activity of tildipirosin, gamithromycin, oxytetracycline, and danofloxacin against caprine mastitis pathogens incubated following standard conditions of Clinical and Laboratory Standards Institute (CLSI) and deviation method by 25% supplementation with goat serum. Mycoplasma agalactiae, Escherichia coli, Staphylococcus aureus, Streptococcus spp., and coagulase-negative Staphylococci (CNS) were isolated from dairy goats with mastitis in Spain. Minimum inhibitory concentrations (MICs) were determined using the broth microdilution technique. The lowest MIC90 under standard conditions was obtained with danofloxacin for mastitis-causing pathogens. An exception was M. agalactiae, where danofloxacin and oxytetracycline obtained low values. However, after adding serum, gamithromycin showed the lowest MIC50 for S. aureus, Streptococcus spp., and CNS. The lowest MIC50 was obtained with all the antibiotics tested (< 0.125 µg/ml) against M. agalactiae. Supplementing with serum resulted in a significant variation in tildipirosin and gamithromycin MIC values for CNS, S. aureus, M. agalactiae, and E. coli. In brief, the MIC for antibiotics used against mastitis should be determined under conditions closely resembling intramammary infections to obtain representative susceptibility patterns against mastitis pathogens. Caprine mastitis pathogens were broadly susceptible to danofloxacin under standard conditions. The potency of macrolides against caprine mastitis pathogens increases when serum is present in culture media.

Keywords Tildipirosin · Gamithromycin · Oxytetracycline · Danofloxacin · Mastitis · MIC · Mycoplasma agalactiae · Serum · Goats

Introduction

Mastitis is considered one of the most important diseases in dairy animals and frequently occurs due to the colonization of fungi, viruses, or bacteria, especially if poor milking management and unhygienic conditions are present in dairy farms (Ruegg 2017). Mammary gland inflammation is classified into clinical and subclinical infections. Subclinical mastitis is the most common presentation in goats (Persson and Olofsson 2011), where milk appears normal with no apparent abnormalities in mammary tissues. However, the subclinical form could lead to clinical form or establish a reservoir of pathogens that acts as a source of infection to healthy animals. In terms of livestock production, mastitis can result in relevant economic losses owing to reduced milk
yields, poor quality of the milk, early elimination of animals, and increased treatment costs (Gelasakis et al. 2015).

Staphylococci are the most common pathogen group present in mastitis (Virdis et al. 2010), where Staphylococcus aureus has been associated with clinical mastitis. In contrast, coagulase-negative Staphylococci (CNS) are considered the most prevalent in subclinical mastitis in dairy goats (Mishra et al. 2014). Streptococcus spp., is reported to be the most common cause of clinical and subclinical mastitis in goats after Staphylococci (Bergonier et al. 2013). Other isolated pathogens in goat mastitis include Mannhemia haemolytica, Escherichia coli, Clostridium perfringens, Mycoplasma spp., Pseudomonas and Nocardia species (Olechnowicz and Jaśkowiak 2014).

Mycoplasma spp. is the causative intracellular agent of contagious agalactia, a multi-aetiological syndrome that generates significant economic losses, principally in Mediterranean countries with small ruminant dairy industries (Gómez-Martín et al. 2013). Four different species have been associated with this disease: Mycoplasma agalactiae, Mycoplasma mycoides subsp. capri, Mycoplasma capricolum subsp. capricolum and Mycoplasma putrefaciens. Nevertheless, M. agalactiae is the most frequent agent isolated in sheep and goats. In endemic areas, the typical presentation of the disease is subclinical mastitis, with evolution to clinical mastitis in some animals. Clinical disease not only affects the mammary gland but can also be associated with arthritis, keratoconjunctivitis, septicaemia, pneumonia, and abortions (Bergonier et al. 2013; Gómez-Martín et al. 2013). Currently, tetracyclines, macrolides, and fluoroquinolones are widely recommended drugs against contagious agalactia (Gómez-Martín et al. 2013). Unfortunately, their current use and bacteriological/clinical outcomes are rarely reported. A few antibiotics are marketed explicitly for use in small ruminants; however, due to such circumstances, products authorized in cattle are used to treat contagious agalactia, rationalized on the cascade principle. Antibiotics provide clinical recovery in this disease but infrequently complete bacteriological cure (Bergonier et al. 1997), as was shown recently in two studies where long-acting oxytetracycline and macrolides were used (Agnello, et al. 2012; Giadinis et al. 2008). Mycoplasma bovis, another mastitis pathogen closely related to M. agalactiae, has been found to increase its antibiotic resistance level for almost all antimicrobials, except for fluoroquinolones, in contemporary bacterial isolates (Gautier-Bouchardon et al. 2014). In these circumstances, updated antibiotic susceptibility of M. agalactiae is essential. It has only been addressed within scarce studies (Poumarat et al. 2016).

Moreover, some experiments suggest that the antimicrobial activity of some macrolides may be undervalued if we only consider the MIC obtained by CLSI methods. For example, a study showed that tildipirosin MIC against Actinobacillus pleuropneumoniae incubated on standard methods was higher than those obtained by adding serum in different proportions to the culture medium (5%, 10%, 25%, and 50%) (Rose et al. 2013). For tulathromycin, calf serum has been reported to improve the control of pH media, consequently enhancing MIC determinations. Moreover, a buffer capacity has been attributed to plasma proteins, which are essential serum components (Lees et al. 2017), and low molecular weight proteinaceous components of the serum interact with azithromycin and other macrolides, such as roxithromycin and erythromycin, enhancing its antibacterial activities (Pruul and McDonald 1992). Therefore, the determination of macrolide dosages for therapeutic use should be derived from pharmacodynamic data obtained from biological fluids because in vitro measurement of MIC in broth, performed following international recommended methods, may be misleading for estimating the in vivo potency of these antibiotics. Thus, the objective of the present study was to evaluate the in vitro activity of tildipirosin, gamithromycin, oxytetracycline, and danofloxacin against M. agalactiae, E. coli, and S. aureus, Streptococcus spp., and CNS incubated on CLSI conditions and deviation from CLSI methods by 25% supplementation with goat serum.

**Materials and methods**

**Isolation and identification of pathogens**

The isolates included in the current study were obtained from the strain collection of the University of Murcia—Spain. The examined mastitis pathogens were isolated from individual mastitis samples in goat flocks during 2018 and 2020 in the southeastern region of Spain. Altogether, 107 isolates were included in the study, subdivided into 39 CNS, 37 S. aureus, 11 Streptococcus spp., 10 E. coli, and 10 M. agalactiae.

Ten microliters of each sample was spread onto the surface of blood agar plates, incubated aerobically at 37 °C and examined after 24, 48 and 72 h. A subclinical intramammary infection isolation threshold was established in five identical colonies (500 cfu/ml).

The identification of Gram-positive, catalase-positive cocci was performed according to the presence or absence of target hemolysis. For isolates with target hemolysis, a commercial latex agglutination kit for the identification and differentiation of S. aureus (Staphytec Plus, Oxoid, Basingstoke, UK) were used. Isolates without target hemolysis were inoculated into API Staph strips (bioMérieux) for identification. Gram-positive, catalase-negative cocci were identified as Streptococcus spp. To
investigate the presence of *Streptococcus agalactiae*, the CAMP test, esculin hydrolysis and hemolysis were considered.

Oxidase tests were performed for Gram-negative bacilli. If the oxidase test was negative, isolates were inoculated on MacConkey agar and investigated for indole production, utilization of citrate as the sole source of carbon, methyl red test and Voges-Proskauer test. Isolates lactose positive on MacConkey agar, indole positive, citrate negative, methyl red positive and Voges-Proskauer negative were identified as *E. coli*.

For the isolation of *M. agalactiae*, solid and liquid pH media were used (Kirchhoff and Rosengarten, 1984). Isolates from previously cloned single colonies of *M. agalactiae* from previously cloned single colonies of media were used (Kirchhoff and Rosengarten, 1984). Isolates were identified by PCR (Marenda et al. 2005).

**Antimicrobial susceptibility testing**

Tildipirosin, gamithromycin, oxytetracycline, and danofloxacin (Cymit Química, Barcelona, Spain) were selected for the study. Antibiotics were dissolved in suitable solvents to make stock solutions and then diluted in sterile distilled water following the guidelines of the Clinical and Laboratory Standards Institute (2009). Blood samples were obtained from ten healthy female Murciano-Granadina goats aged 3–5 years. The samples obtained were centrifuged at 1500xg for 10 min, and the freshly collected serum was pooled and divided into 1-ml portions, stored at −80 °C, and thawed immediately before the experiment.

**Antimicrobial susceptibility testing for CNS, *S. aureus*, *Streptococcus* spp., and *E. coli***

Standard conditions or modifications from CLSI methods by 25% goat serum supplementation were performed to determine the MIC of tildipirosin, gamithromycin, oxytetracycline, and danofloxacin. Minimum inhibitory concentration tests were performed by the microdilution broth technique (Clinical and Laboratory Standards Institute 2009) using U-bottom 96-well microtiter plates. Serial two-fold dilutions of the antimicrobial agents were prepared starting from the stock solution. Broth dilutions were made using Mueller–Hinton broth (MHB) (Merck, Madrid, Spain) for CNS, *S. aureus*, and *E. coli*. To investigate *Streptococcus* spp., cation-adjusted Mueller–Hinton broth (Merck, Madrid, Spain) with 5% defibrinated horse blood (Thermo Fisher Scientific, Massachusetts, USA) was used. Concentrations of all antibiotics ranging from 0.03 to 128 mg/l were used. Inocula were prepared by diluting an overnight MHB culture in buffered saline solution to a density of 0.5 on the McFarland Turbidity Scale and finally diluting again 40-fold before testing. The U-bottomed microtiter plates were incubated at 37 °C and observed 24 h later. The MIC was defined as the lowest concentration of antibiotic at which bacterial growth was completely inhibited. The reference strains *S. aureus* (ATCC 29213) and *E. coli* (ATCC 25922) were used as controls.

**Antimicrobial susceptibility testing for *M. agalactiae***

The minimal inhibitory concentration was determined according to the recommendations of Hannan (2000). Briefly, a stationary-phase culture of each isolate was carried out in mycoplasma medium without antimicrobials supplemented with phenol red (0.005%) in 96-well round-bottomed plates. Each antibiotic was added to achieve each of the pre-established final concentrations (from 32 to 0.006 μg/ml) and a final concentration of the mycoplasma cultures of 10^7–10^5 colour-changing units/ml. Positive (lacking antibiotic) and negative (lacking mycoplasmas) controls were also added. The plates were then sealed and incubated at 37 °C. After 48 h, the plates were examined for colour change. The MIC was defined as the lowest concentration of each antibiotic at which no *M. agalactiae* growth (no colour change) was observed.

**Statistical analysis**

Normality of the data was investigated using the Shapiro–Wilk test. The antibacterial susceptibility of isolates incubated in standard conditions was contrasted with 25% goat serum addition to culture media. Susceptibility differences between both methods (CLSI conditions and deviation from CLSI methods by 25% supplementation with goat serum) were determined by the Wilcoxon rank-sum test, and a significant difference was considered when p < 0.05. Statistical analysis was performed with IBM SPSS Statistics 24 (New York, NY, USA).

Susceptibility of CNS, *S. aureus*, *Streptococcus* spp., *E. coli*, and *M. agalactiae* are presented as minimal concentrations of tildipirosin, gamithromycin, oxytetracycline, and danofloxacin that inhibited 50% and 90% of these isolates (MIC\(_{50}\) and MIC\(_{90}\)).

The percentage of CNS, *S. aureus*, *Streptococcus* spp., *E. coli*, and *M. agalactiae* were determined by increasing concentrations of tildipirosin, gamithromycin, and oxytetracycline have a bimodal distribution, confirming that the data were plotted using ggplot2 [R version 4.0.4 (2021-02-15)].

**Results**

Modifying the culture media by adding goat serum resulted in appreciable variation of the MIC of tildipirosin, gamithromycin, and oxytetracycline for *S. aureus*, CNS, and *M.
agalactiae, but MIC values were unchanged or slightly increased for danofloxacin (Fig. 1).

The MIC50 and MIC90 of tildipirosin, gamithromycin, oxytetracycline, and danofloxacin are presented in Table 1.

Gamithromycin and tildipirosin showed a reduction of the MIC50 when the culture media was supplemented with goat serum for CNS and S. aureus. On the other hand, the MIC50 of oxytetracycline increased by 3 log2 dilution when goat serum was added for CNS and S. aureus. The lowest MIC50 and MIC90 values were obtained with danofloxacin under standard conditions and deviation from the CLSI method (MIC50 = 0.25 µg/ml for CNS and S. aureus; MIC90 = 8–32 µg/ml for CNS and 2–4 µg/ml for S. aureus).

Supplementing with serum the culture media caused a reduction of the MIC90 of both gamithromyc in and tildipirosin by approximately 5 log2 dilution, resulting in a significant decrease of the MIC90 (> 16 µg/ml) under standard conditions when compared to supplementation with goat serum (0.5 µg/ml) for M. agalactiae. In addition, the MIC50 and MIC90 of tildipirosin and gamithromycin were higher than those obtained with danofloxacin and oxytetracycline under standard conditions for M. agalactiae. Nevertheless, after modifying the culture medium conditions with serum, similar MIC50 and MIC90 values were obtained for gamithromycin, tildipirosin, danofloxacin, and oxytetracycline.

Danofloxacin showed the lowest MIC50 and MIC90 values against E. coli both under standard conditions and after adding goat serum. The supplementation with serum resulted in a decrease in MIC90 by 3 log2 dilution for tildipirosin and by 2 log2 dilution for gamithromycin (p < 0.05).

Supplementing the culture media with serum did not cause a variation in the MIC50 and MIC90 of gamithromycin, tildipirosin, danofloxacin, and oxytetracycline for Streptococcus spp. (p > 0.05). The lowest MIC50 and MIC90 for Streptococcus spp. were obtained with gamithromycin and danofloxacin, respectively.

Discussion

Contagious agalactia results in significant economic losses for farmers due to decreased milk yields, abortions, reduced growth rates, early culling in affected animals, and the expenses needed for control or treatment measures. Moreover, the impact of Mycoplasmas on milk quality is probably underestimated (Al-Farha et al. 2017; Contreras et al. 2008). Antimicrobial therapy is one of the most critical practices to control this syndrome but requires a withdrawal period resulting in significant milk production losses. Fluoroquinolones, macrolides, and tetracyclines are used as standard treatments for controlling contagious agalactia (Bergonier et al. 1997;
Gómez-Martín et al. 2013). In this study, danofloxacin and oxytetracycline were the most effective antibiotics, with an MIC$_{90}$ of 0.5 µg/ml. These values are in agreement with previously reported data (Antunes et al. 2008; Garnica et al. 2013). After adding 25% goat serum, similar values were obtained with the four antibiotics for M. agalactiae (MIC$_{90}$ of tildipirosin and gamithromycin = 0.5 µg/ml; MIC$_{90}$ of danofloxacin and oxytetracycline = 0.25 µg/ml). These results suggest that current macrolides may be helpful to treat contagious agalactia since they are potent weak bases that are ion-trapped within acidic intracellular compartments, such as lysosomes and phagosomes. A beneficial consequence of macrolide intracellular accumulation is its increased activity against intracellular pathogens (Ahmad et al. 2010; Fietta et al. 1997). In goats, tildipirosin concentrations in somatic cells following subcutaneous and intramuscular administrations were 22–27 times higher than simultaneous plasma concentrations (Galecio et al. 2022). It is crucial to know which antibiotics of veterinary use are effective against M. agalactiae isolated from goats to successfully treat contagious agalactia, especially in endemic areas such as Spain and neighboring Mediterranean countries. It is well known that the in vitro sensitivity of antibiotics is not always consistent with treatment effectiveness in field conditions, but antibiotics with high MIC values against these microorganisms are likely to be ineffective in successfully treating sick animals (Barlow 2011; Constable and Morin 2003). Staphylococci are the most common pathogen species isolated in dairy goats affected with subclinical mastitis, caus- ing subclinical to clinical mastitis, with various presentations from mild to acute toxic forms or even gangrenous mastitis, and are one of the most important causes of discarding dairy animals. Staphylococci grow on udder skin, inside the teat canal, and in mammary tissues and are disseminated through improper and unhygienic milking routines (Ruegg 2017). In the present study, the lowest MIC$_{90}$ values for CNS were obtained with danofloxacin (MIC$_{90}$ = 8 µg/ml), obtaining MIC$_{90}$ values higher than 128 µg/ml for the rest of the antibiotics tested. After adding 25% goat serum, gamithromycin showed a lower MIC50 value than danofloxacin (MIC$_{50}$ of gamithromycin = 0.125 µg/ml). Only scant data are found for MIC against CNS isolated from dairy goats, but the reported MIC$_{90}$ was obtained for oxytetracycline (MIC$_{90}$ = 1 µg/ml), and other fluoroquinolones (MIC$_{90}$ of ofloxacin = 1 µg/ml) were lower than those of our study (Virdis et al. 2010).

Table 1 Minimum inhibitory concentration values (µg/ml) of tildipirosin, gamithromycin, danofloxacin and oxytetracycline that inhibited 50% and 90% of the isolates, (MIC$_{50}$ and MIC$_{90}$, respectively) on CNS, S. aureus, Streptococcus spp., E. coli and M. agalactiae strains isolated from mastitic goat milk incubated in Mueller-Hinton Broth and Mueller-Hinton Broth supplemented with 25% goat serum.

| Strains            | Tildipirosin  | Gamithromycin | Danofloxacin | Oxytetracycline |
|--------------------|---------------|----------------|--------------|-----------------|
| CNS (n=30)         | 4             | >128           | 0.25         | >128            |
| Staphylococcus aureus (n=11) | >128          | >128           | >128         | >128            |
| Streptococcus spp. (n=10) | >128          | >128           | >128         | >128            |
| Escherichia coli (n=10) | >128          | >128           | >128         | >128            |
| Mycoplasma agalactiae (n=10) | >128          | >128           | >128         | >128            |
| MBH/MHB + 25% Goat Serum | <0.25         | <0.125         | <0.25        | <0.125          |

Staphylococcus aureus is one of the most important etiologic agents in ruminant mastitis. Intramammary infections are complicated to treat and eradicate because resistant strains of S. aureus frequently emerge after negligible antibiotic pressure. Strains tested in this study showed high MIC$_{90}$ values against tildipirosin, gamithromycin, and oxytetracycline (MIC$_{90}$ ≥ 128 µg/ml). The most effective antimicrobial tested against S. aureus strains isolated from milk was danofloxacin (MIC$_{90}$ = 2 µg/ml). Lower values for danofloxacin have been
reported previously [MIC90 = 0.25 µg/ml (Serrano-Rodríguez et al. 2017); MIC90 = 0.5 µg/ml (Marín et al. 2010)]. After adding 25% goat serum, the susceptibility of tildipirosin and gamithromycin against \textit{S. aureus} was not modified. The reason may be that the MIC90 values of the two macrolides under standard conditions were greater than 128 µg/ml, but the exact value was not quantified. Therefore, when adding serum, it is possible that MICs decreased but not enough to be detected; these values were still higher than 128 µg/ml.

The other commonly tested bacteria in the present study were \textit{E. coli}, which is responsible for environmental mastitis in dairy ruminants. Coliforms develop when farmers follow unsanitary housing and impair pre-milking teat disinfection (Ruegg 2017). Danofloxacin showed the lowest MIC90 and MIC90, for \textit{E. coli} under standard conditions and after adding goat serum. Strains tested in the current study showed high MIC90 values for oxytetracycline. Minimum inhibitory concentrations that inhibited 90% of caprine mastitis pathogens obtained with tildipirosin (4 µg/ml) and gamithromycin (8 µg/ml) were high, but after modifying the conditions with serum, MIC values decreased dramatically (MIC90 = 0.5 and 2 µg/ml for tildipirosin and gamithromycin, respectively).

References are available in the literature about the susceptibility of \textit{E. coli} strains isolated from milk to antimicrobial agents in dairy cows (Shinozuka et al. 2019; Thomas et al. 2015); however, data are unavailable for dairy goats. Similar high MIC90 values were found for different tetracyclines in strains isolated from dairy cows (MIC90 ≥ 64 µg/ml; Thomas et al., 2015) and lower values for other fluoroquinolones, such as enrofloxacin or marbofloxacin (MIC90 = 0.03 and 0.06 µg/ml, respectively; Thomas et al. 2015).

\textit{Streptococcus} spp. is also considered a common etiological cause of clinical and subclinical mastitis in goats after Staphylococci, with \textit{S. agalactiae}, \textit{Streptococcus uberis}, and \textit{Streptococcus dysgalactiae} frequently found in mammary gland infections (Bergonier et al. 2013). As with \textit{E. coli}, there are no published data about the susceptibility of \textit{Streptococcus} spp., isolated from goat mastitis with the antibiotics tested in this study. There are some studies on isolated \textit{Streptococcus} spp. strains from cow mastitis (McDougalla et al. 2013; Thomas et al. 2015) and MIC90 values reported to be lower than those in the present study for oxytetracycline (MIC90 = 1 and 4 µg/ml for \textit{S. uberis} and \textit{S. dysgalactiae}, respectively; McDougalla et al. 2013), another macrolide (MIC90 of tylosin = 2 and 1 µg/ml for \textit{S. uberis} and \textit{S. dysgalactiae}, respectively; Thomas et al. 2015) and different fluoroquinolones, such as enrofloxacin (MIC90 = 1 µg/ml for both species; McDougalla et al. 2013) and marbofloxacin (MIC90 = 2 µg/ml for \textit{S. uberis}; Thomas et al. 2015). Then, comparisons are difficult to establish.

The present study demonstrates that macrolides, but not danofloxacin and oxytetracycline, have markedly lower MICs against different pathogens when assayed in culture media broth supplemented with serum compared with MHB (CLSI recommendation for in vitro susceptibility testing studies). As antibiotic susceptibility is an essential property to contemplate when assessing its clinical value, these observations open a potentially important expectation concerning the clinical benefits of macrolides in goats affected with mastitis. Artificial growth matrices, such as MHB, are not undoubtedly predictive of bacterial growth in physiological fluids, and as a consequence, they may be poor predictive tools in some cases of antimicrobial drug activity in vivo. Considering these variations between biological fluids and artificial growth media, some authors advocate for the use of physiological fluids in studies of antimicrobial activity testing when the objective is to establish optimal dosing regimens for bacterial killing in vivo (Lees et al. 2017; Nightingale and Murakawa 2012).

Moreover, some buffering capacity has been attributed to proteins, which are major components of serum (Pruul and McDonald 1992; Lees et al. 2017). Antimicrobial activity-enhancing effects of proteinaceous serum components have also been assumed for azithromycin, erythromycin, roxithromycin (Pruul and McDonald 1992) and gamithromycin (Zhou et al 2020). Recent studies have shown that the high susceptibility of \textit{Pasteurella aeruginosa} to macrolides in RPMI 1640 medium (medium used for growing eukaryotic cells) compared to broths may be related to an increase in their accumulation within the bacteria, owing to an alteration of the outer-membrane integrity (caused by the nature of the medium in contact with bacteria) combined with an impairment of their active efflux (decreased expression of oprM) (Buyck et al. 2012). Moreover, tildipirosin and gamithromycin tested in this study, like other macrolides, may have various immunomodulatory benefits that probably contribute to successful clinical outcomes in different infections. Other reported benefits for macrolides may be influenced by enhanced degranulation and apoptosis of neutrophils, enhanced macrophage functions, and its ability to inhibit inflammatory cytokine production (Zarogoulidis et al. 2012).

The effect of milk as a matrix in the culture medium has also been investigated. Some studies have displayed a significant influence on MIC values and have revealed that much of the reduction in antibiotic activity is related to high binding to casein or lipids in milk (Kuang et al. 2009; Owens and Watts 1987). Although macrolides have low protein binding (18–30%) (Papich 2018; Stepanić et al., 2012), incorporating milk from healthy animals into the culture media determines a reduction in the antibacterial activity of erythromycin (Owens and Watts 1987); other milk components probably play an important role. Nevertheless, the milk from infected quarters has broad changes in its composition, with lower casein and lactose content but higher total protein and whey protein concentrations (Forsbäck et al. 2010), which could determine an increased potency of macrolides, as was shown...
in our study, but further experiments are needed to confirm this assumption.

**Conclusion**

Minimal inhibitory concentrations for antibiotics used on mastitis should be determined under conditions closely resembling intramammary infections to obtain representative susceptibility patterns against goat mastitis pathogens. Caprine mastitis pathogens were broadly susceptible to danofloxacin under standard incubation conditions. The potency of macrolides against susceptible caprine mastitis pathogens increases when serum is present in culture media.

**Acknowledgements** The authors would like to thank Fundación Carolina – España and Universidad San Francisco de Quito—Ecuador for their Ph.D. scholarship for Juan Sebastian Galecio.

**Author contributions** EE and PM: conceptualization, EE, PM and JSG: methodology, PM, JSG: software, EE, PM, JCC, VH and CF: validation, PM and JSG: formal analysis, JSG, VH, JCC, EG-R, PM: investigation, EE, JCC, CF and PM: resources, EE, JCC, CF, JSG and PM: data curation, JSG, VH, EE, JCC, CF, EG, and PM: writing—original draft preparation, EE, JSG and PM: writing—review and editing, JSG and PM: visualization, EE and PM: supervision, EE and PM: project administration, EE and PM: funding acquisition. All authors have read and agreed to the published version of the manuscript.

**Funding** Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors. This research was supported by the Faculty of Veterinary Medicine of the University of Murcia, Spain.

**Data availability** The data that support the findings of this study are openly available in Science Data Bank at https://doi.org/10.11922/sciencedb.01662 published on 2022–04-02.

**Declarations**

**Conflict of interest** The authors declare no conflict of interest.

**Ethical approval** The Bioethical Committee of the University of Murcia (Spain) approved the experimental protocol (CEEA 558/2019).

**Consent to participate** The authors have permission to participate.

**Consent for publication** The authors have permission for publication.

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