Short communication: Fluoroquinolone susceptibility of *Staphylococcus aureus* strains isolated from caprine clinical mastitis in southeast Spain

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ABSTRACT

The antibiotic susceptibility of 32 strains of *Staphylococcus aureus* isolated from the mastitic milk of dairy goats was evaluated. The antibiotics tested were 3 fluoroquinolones that have been developed especially for use in veterinary medicine: danofloxacin, marbofloxacin, and orbifloxacin. Minimum inhibitory concentration (MIC) tests were performed according to the microdilution broth method. The MIC₉₀ (concentration at which 90% inhibition is achieved) values obtained were 0.5, 1, and 1 mg/L for danofloxacin, marbofloxacin, and orbifloxacin, respectively. Danofloxacin was the most active fluoroquinolone tested against *Staph. aureus* strains isolated from milk; however, specific testing is required before using these drugs as therapy for the control of clinical mammary infections in goats.

Key words: *Staphylococcus aureus*, fluoroquinolone, mastitis, goat milk

Mastitis, which may be clinical or subclinical, is an important mammary gland disease that is usually caused by bacterial infection. If untreated, it constitutes a serious problem in dairy herds, with considerable economic consequences, mainly because of reduced milk production and discarded milk (Seegers et al., 2003). Although several bacterial pathogens can cause mastitis, *Staphylococcus aureus* is one of the most important etiologic agents in mastitis of cows, goats, and sheep (Mørk et al., 2005; Aires-de-Sousa et al., 2007). Moreover, *Staph. aureus* is probably the most important infectious agent because it causes a chronic and deep infection in the mammary glands that is extremely difficult to cure (Miles et al., 1992). The prevalence of *Staph. aureus* IMI in goats ranged from 5.6 to 17% in different countries (Silva et al., 2006).

Fluoroquinolones are antimicrobial drugs that generally have very good activity against a broad spectrum

of aerobic bacteria, including *Pasteurella* spp. and mycoplasma (Giles et al., 1991; Gutiérrez and Rodríguez, 1993; Hannan et al., 1997). The main target site for its bactericidal action is the DNA gyrase. This enzyme is required for supercoiling of DNA to facilitate the spatial arrangement of DNA in the bacterial cell. Furthermore, fluoroquinolones have good pharmacological characteristics for using in animal therapy such as large volumes of distribution, low plasma protein binding, and relatively low MIC against susceptible target microorganisms (Brown, 1996). Danofloxacin (**DAN**), marbofloxacin (**MAR**), and orbifloxacin (**ORB**), are relatively new synthetic fluoroquinolone antimicrobial drugs that have been developed especially for use in veterinary medicine, and they could be useful in lactating goats and other lactating species.

Therefore, this study was designed to evaluate the degree of in vitro activity of DAN, MAR, and ORB against 32 *Staph. aureus* strains isolated from the milk of goats with clinical mastitis in Spain.

Thirty-two *Staphylococcus aureus* strains were isolated from milk of goats with clinical mastitis in the southeastern region of Spain. Twenty microliters of each sample was plated on blood agar plates (5% washed sheep erythrocytes). The plates were incubated aerobically at 37°C and examined at 24, 48, and 72 h. Bacteria with target hemolysis were identified according to the recommendations of the National Mastitis Council (Harmon et al., 1990). Specific identification of *Staph. aureus* was made using commercial latex agglutination kit (Staphytec Plus, Oxoid, Basingstoke, UK). The strains were stored at –80°C in a nutrient broth enriched with 15% glycerol.

The antibiotics selected for the study were DAN, MAR, and ORB; DAN and MAR were provided by the manufacturer (Riedel-de Haën, Seelze, Germany), and ORB was obtained in pure form from Schering Plough Corp. (Kenilworth, NJ). Antibiotics were dissolved in suitable solvents to make stock solutions and then diluted in sterile distilled water according to the methods recommended by the Clinical and Laboratory Standards Institute (CLSI, 2009).

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Table 1. Minimal inhibitory concentrations of danofloxacin (DAN), marbofloxacin (MAR), and orbifloxacin (ORB) on *Staphylococcus aureus* strains isolated from mastitic goat milk (n = 32), as well as MIC₅₀ and MIC₉₀ values and minimum and maximum MIC values (range)

Species	Antibiotic	Number of strains with MIC (mg/L)									MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)	Range (mg/L)
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8			
<i>Staph. aureus</i>	DAN			8	16	4	4				0.25	0.5	0.12–1
<i>Staph. aureus</i>	MAR				16	8	5	3			0.5	1	0.25–2
<i>Staph. aureus</i>	ORB					13	15	3	1		1	1	0.5–4
Reference strains													
<i>E. coli</i> ATCC 25922	DAN												
	MAR												
	ORB												
<i>Staph. aureus</i> ATCC 29213						DAN	ORB						
						MAR							

Minimum inhibitory concentration tests were performed according to the microdilution broth method as recommended by CLSI (2009) using U-bottomed, 96-well microtiter plates. Serial 2-fold dilutions of the antimicrobial agents were prepared starting from the stock solution of each drug. Concentrations of DAN, MAR, and ORB ranging from 0.03 to 128 mg/L were used.

Inocula were prepared by diluting an overnight Mueller-Hinton broth 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 MIC was defined as the lowest concentration of antibiotic at which the bacterial growth was completely inhibited. The reference strains *Staph. aureus* (ATCC 29213) and *Escherichia coli* (ATCC 25922) were from Colección Española de cultivos tipo (CECT; Valencia, Spain) and used as controls for each plate.

Numerous data are available in the literature on the susceptibility to antimicrobial agents of *Staph. aureus* strains isolated from milk of dairy cows (Pengov and Ceru, 2003; Tikofsky et al., 2003); however, only scant data exist for dairy goats. The MIC values of the selected antibiotics are shown in Table 1, as well as MIC values of these drugs that inhibited 50 and 90% of the isolates (MIC₅₀ and MIC₉₀, respectively), and the minimum and maximum MIC values (range).

According to the susceptibility and resistance breakpoints, a strain is considered resistant when the MIC is ≥4 mg/L for MAR, and MIC is ≥8 mg/L for DAN and ORB (CLSI, 2008). None of the strains tested in the current study were resistant to these antibiotics. The most active fluoroquinolone tested against *Staph. aureus* strains isolated from milk was DAN (MIC₉₀ = 0.5 mg/L). A correlation between the MIC of DAN, MAR, and ORB was observed for each isolate tested.

The MIC₉₀ values reported in previous studies for ORB against *Staphylococcus intermedius* isolated from canine infections (MIC₉₀ = 1 mg/L; Ganiere et al., 2004) and *Staph. aureus* isolated from rabbits (MIC₉₀ = 2 mg/L;

Marín et al., 2008) were similar to those obtained in the present study. Grobbel et al. (2007) reported a MIC₅₀ = 0.25 mg/L for MAR against *Staph. aureus* strains isolated from bovine mastitis. This value is lower than the MIC obtained in this work (MIC₅₀ = 0.5 mg/L). Moreover, lower values of MIC₉₀ for MAR than in the present study were obtained by Schneider et al. (2004) against *Staph. aureus* isolated from bovine mastitic milk. Danofloxacin showed good activity against *Staph. aureus* in this work, and similar values were obtained by Grobbel et al. (2007). However, Fernández-Varón et al. (2007) obtained higher MIC₉₀ values (MIC₉₀ = 4 mg/L) against *Staph. aureus* isolated from rabbits.

Broad-spectrum antimicrobial drugs such as fluoroquinolones have been recommended and used to treat mastitis (Dosogne et al., 2002). Because the fluoroquinolones are relatively new antimicrobial agents, extensive research is needed to develop and implement appropriate specific dosing regimens that can maximize their clinical efficacy for use in production animals and reduce the risk of selection of resistant pathogens, particularly because these drugs are used for the treatment of multidrug-resistant infection in humans (Walker, 2000).

The ratios C_{max}/MIC₉₀ and AUC₂₄/MIC₉₀ are the best parameters for predicting the antimicrobial effect of fluoroquinolones, where C_{max} = peak or maximum plasma or milk concentration following extravascular administration, and AUC₂₄ = area under the plasma or milk concentration-time curve from 0 to 24 h. Previous investigations have shown that for fluoroquinolones, C_{max}/MIC₉₀ >3 produced 99% reduction in bacterial counts and C_{max}/MIC₉₀ of ≥8 prevented the emergence of resistant organisms (Craig, 1998). Furthermore, AUC₂₄/MIC₉₀ >100 h should be achieved to give maximum clinical and bacteriological efficacy (Turnidge, 1999). Therefore, if we take into account milk AUC and C_{max} parameters from a pharmacokinetic study with ORB in dairy goats (Marín et al., 2007), the optimal values of C_{max}/MIC₉₀ >8 and AUC₂₄/MIC₉₀ >100 h are not

reached. However, it must be noted that the numerical values of $C_{\max}/MIC_{90}$ and AUC_{24}/MIC_{90} , used as surrogate markers to predict optimal therapeutic outcomes, have been generated from experimental infections in laboratory animals or in human clinical trials (Toutain and Lees, 2004), and may not be applicable to goat infections or to animal infections in general. In fact, Lees and Shojaee Aliabadi (2002) studied, in ex vivo experiments, the ratio of AUC/MIC producing bacteriostasis, bactericidal activity, and elimination of bacteria with different fluoroquinolones and concluded, in all cases, that the surrogate markers for cattle, sheep, goat, and camel were lower than 100 to 125 h. For example, DAN was effective in terms of bacteriological results, milk production, and both systemic and local signs when it was used in the treatment of induced acute *E. coli* bovine mastitis (Poutrel et al., 2008). Therefore, although their pharmacokinetic/pharmacodynamic indices are not the optimum described in the literature, these antibiotics could be useful for treating mammary infections in goats. However, it is necessary to first carry out clinical efficacy studies in order to determine the proper dosage regimens. We conclude that DAN, MAR, and ORB, fluoroquinolone antimicrobial drugs developed especially for use in veterinary medicine, showed good in vitro activity against *Staph. aureus* strains isolated from mastitic goat milk.

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