STAPHYLOCOCCUS AUREUS METICILLIN RESITANT STRAIN (MRSA) MINIMUM INHIBITORY ENROFLOXACIN CONCENTRATION IN *STAPHYLOCOCCUS AUREUS* ISOLATIONS OBTAINED FROM COWS WITH SUBCLINICAL MASTITIS IN FAMILY DAIRY FARMS

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Introduction

Bovine mastitis is a disease that affects dairy herd production, characterized by considerable economical loss due to the diminished milk secretion, potential productive cow damage, increase in production costs and milk contamination. (Morin *et al.,* 1993; Tollersrud *et al.,* 2000). *Staphylococcus aureus* is considered one of the main bacteria causing bovine mastitis, which is widely distributed in different countries, with herd prevalences in between 41 and 19% of infected animals in the milking line (Nickerson, 1993). In Mexico, similar prevalences have been found (43.43 and 25%), with high frequencies in manual milking production farms in comparison to mechanical milking (Barba, 1992; Velázquez *et al* 1983). *S. aureus* and *Streptococcus agalactiae* intraglandular infection show an easy dissemination and transmisibility in dairy herds during milking, favouring infection levels in the herd (Bartlett *et al*., 1993). When treating *S. aureus* subclinical mastitis, resistent and multiresistent *in vitro* strains have been found (Sischo *et al*., 1993; Sol *et al*., 2000). β-lactamic an meticillin resistant (MRSA) strains are important and could contribute to increase human population infection pressure produced by animal origin strains (Lagunas *et al*., 2002). In *S. aureus* infection therapy caused by conventionally resistant strains, fluoroquinolone (Riddle *et al*., 2000) is a control alternative to penicillin resistance shown by β–lactamic and MRSA strains. In veterinary practice, enrofloxacin is a broad spectrum antibiotic used for treatment of localizad and systemic infections (Owens *et al*., 1997; Sol *et al*., 2000). These marks the necessity of maintaining rational use and fluoroquinolone resistance surveillance to prevent cross resistance strain appearance as an epidemiological strategy in dairy herds (Piddock *et al*., 2002). The objective of this research work was to determine enrofloxacin's minimum inhibitory concentration in *S.aureus* isolations obtained from cows with subclinical mastitis.

Material and methods

48 field *Staphylococcus aureus* isolations obtained from cows with subclinical and clinical mastitis in dairy herds from Toluca Valley, were preserved by freezing at –85°C in brain and heart broth with glycerol 20% v/v. Isolations were defrozen at 4°C for 60 minutes before strain subculturing. MIC was done according to Riddle *et al*. (2000) procedure and the European Diagnosis Laboratory Association. After defreezing the *S.aureus* strain, it was subcultured in brain and heart broth for 18 to 24 h at 37°C. 0.010mL were transferred to Müller–Hinton broth, incubated at 37°C for 4 hours. A similar volume by triplicate was placed on Müller–Hinton agar containing different concentrations of enrofloxacin (µg/mL): 0.5, 1.0, 3.0, 6.0, 10.0 and 60.0. The dishes were incubated at 37°C for 24 hours. Control strains were *S. aureus ATCC25293* and *S. epidermidis* ATCC1222. Growth inhibition was observed in the dishes, establishing MIC and MIC_{50} in the *S. aureus* strains. Results were evaluated using χ^2 test with 95% confidence level using HandyStat software version 2.0

Results

MIC characterization for control strains was: *S. aureus* was sensible to 1.0 µg /mL concentration and *S. epidermidis* at 3.0 µg/mL. From all the *S. aureus* evaluated field strains 8 (14.8%) inhibited its growth at lower concentrations than 3 µg/mL. 20 strains (41.7%) inhibited its growth in between 3 and 30.0 µg/mL. MIC_{50} was established at 30µg/mL, MIC distribution for growth inhibition in *S. aureus* strains (67%) was in between 3 y 120 µg/mL ($p<0.01$). 8 strins were not inhibited in the highest enrofloxacin concentration (Table 1).

Table 1. *Staphylococcus aureus* enrofloxacin minimum inhibitory concentration (MIC) obtained from cows with subclinical mastitis

Control strains	Enrofloxacin minimum inhibitory concentration	
Staphylococcus aureus ATCC 25293	1.00	
Staphylococcus epidermidis ATCC 12228	3.00	
Antibiotic concentration µg /mL	Number of inhibited strains	Percentage
0.5	6	12.6
1.0	1	2.1
3.0	1	2. 1
6.0	5	10.5
10.0	6	12.6
30.0	8	16.5
60.0	13	27.0
≥120	Number of unhinibited strains 8	Percentage 16.6
Strain total	48	100.0

Discussion and conclusions

Growth inhibition variation observed in *S. aureus* strains in the MIC test, indicates a low proportion of sensible strains to low enfrofloxacin concentrations (< 3.0 µg /mL), which is higher than the established concentration for enrofloxacin in other studies, which was in between 0.25 to 0.5 µg /mL. MIC_{50} was also superior to the established in other studies (De Oliveira *et al.,* 2000). The presence of tolerant strains to the maximum enrofloxacin concentration suggests the existence of *S. aureus* enrofloxacin resistance genetically mediated through topoisomerase IV (Piddock *et al.,* 2002). It has recently been suggested other mechanisms regulated by *gnr* gene that could take part in the control of the bomb flux from the bacterian wall, responsible for *in vitro* tolerance at high flouroquinolone concentrations (Riddle *et* al., 2000; Piddock *et* al., 1999) Evidence of possible enrofloxacin genetic resistance establishes an epidemiological alert over a potential veterinary public health risk due to the possibility of carrier strains resistance cross transmission for fluoroquinolones, widely used in the treatment of MRSA strains and other susceptible agents (De Oliveira *et* al., 2000; Lagunas *et* al.,2002). In veterinary practice of animals for food, regulations over ethics and rational use of fluoroquinoloens is necessary (Bartlett *et al.,* 1993; Sischo *et al.,* 1993; Sol *et al.,* 2000), due to the growing antibiotic resistance referred in intrahospital infections in man (Booth. *et al.,* 2001; Van Wamel. *et al.,*1995). It is concluded that the *S. aureus* studied strains from cows presenting subclinical mastitis showed a growth inhibition pattern at higher concentrations than the ones established by the test, in which tolerant *S. aureus* strain presence was seen at high enrofloxacin concentrations.

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