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Antimicrobial susceptibility of *Staphylococcus aureus* causing bovine mastitis in Argentine dairy herds

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ABSTRACT

We assessed the *in vitro* activity of selected antimicrobial agents against 95 *Staphylococcus aureus* strains causing both clinical and subclinical bovine mastitis belonging to 61 dairy farms from the Central dairy area of Argentina. Minimal inhibitory concentrations (MICs) of penicillin, oxacillin, gentamicin, erythromycin, enrofloxacin and florfenicol were estimated. In addition, the agar diffusion test was performed. MIC_{50} and MIC_{90} were as follows: penicillin, 0.05 and 4 µg/ml; oxacillin, 0.25 and 0.25 µg/ml; gentamicin, 0.25 and 0.5 µg/ml; erythromycin 0.125 and 0.25 µg/ml; enrofloxacin 0.25 and 0.5 µg/ml, and florfenicol 4 and 8 µg/ml. β-lactamase activity was detected in 89% of 46 penicillin-resistant strains. Apart from penicillin, antimicrobial resistance in *S. aureus* causing bovine mastitis remains rare in Argentine dairy farms.

Key words: antimicrobials, bovine mastitis, *Staphylococcus aureus*

RESUMEN

Sensibilidad a los antimicrobianos de *Staphylococcus aureus* causantes de mastitis bovina en rodeos lecheros de Argentina. Se evaluó la actividad *in vitro* de un grupo seleccionado de antimicrobianos contra 95 aislamientos de *Staphylococcus aureus* obtenidos de casos de mastitis bovina clínica y subclínica, en 61 rodeos lecheros de la cuenca central de Argentina. Fueron estimadas las concentraciones inhibitorias mínimas (CIM) de penicilina, oxacilina, gentamicina, eritromicina, enrofloxacina y florfenicol. Además se realizó la prueba de difusión en agar. Las CIM_{50} y CIM_{90} obtenidas fueron: penicilina 0,05 y 4 µg/ml; oxacilina 0,25 y 0,25 µg/ml; gentamicina 0,25 y 0,5 µg/ml; eritromicina 0,125 y 0,25 µg/ml; enrofloxacina 0,25 y 0,5 µg/ml y florfenicol 4 y 8 µg/ml. Se detectó actividad de β-lactamasa en el 89% de las cepas resistentes a la penicilina. A excepción de lo observado para penicilina, la resistencia a los antimicrobianos en *S. aureus* causantes de mastitis bovina en Argentina parece ser un fenómeno poco frecuente.

Palabras clave: antimicrobianos, mastitis bovina, *Staphylococcus aureus*

Staphylococcus aureus is a common cause of bovine intramammary infection (IMI) around the world which causes important economic losses for dairy farmers and industry (15). Antimicrobial therapy is one of the basis of control programs for mastitis caused by this organism; however, several factors at cow, pathogen, and antibiotic treatment levels affect the probability of cure of *S. aureus* IMI (15). Therefore, the appropriate selection of an antimicrobial agent for treatment of bovine mastitis should not only include knowledge about pharmacokinetics, but also about local susceptibility patterns of the main pathogens (3, 11). Furthermore, surveys on *in vitro* susceptibility patterns of *S. aureus*, performed by quantitative methods, are required for the detection of emerging resistance worldwide (3, 11). The objective of this study was to determine the *in vitro* susceptibility of *S. aureus* isolated from bovine IMI in Argentina to selected antimicrobial agents.

Ninety five *S. aureus* isolates recovered from clinical (n=25) and subclinical (n=70) IMI were evaluated. These isolates belonged to 61 dairy herds located in different dairy areas of Argentina. A minimum of one and a maximum of three isolates from each herd, were included as follows: 65 isolates from 42 herds located in Santa Fe Province, 17 from 12 herds in Córdoba Province, 10 from 6 herds located in Buenos Aires Province and 2 from a herd located in Entre Ríos Province. Isolates were obtained between 1998 and 2002, identified by biochemical standard procedures and stored at -80 °C in 10% glycerol brain heart infusion (Laboratorios Britania, Buenos Aires, Argentina) until use. Prior to susceptibility testing, bacteria were activated from frozen stocks by overnight culture at 35 °C on Columbia agar base (Laboratorios Britania) supplemented with 5% bovine defibrinated blood. Minimal inhibitory concentrations (MICs) of penicillin, oxacillin, erythromycin, enrofloxacin and gentamicin were

estimated by the E-test method following the manufacturer's instructions (AB BIODISK, Dalvägen, Solna, Sweden). MIC of florfenicol was estimated by a dilution method with Mueller-Hinton agar (Laboratorios Britania) according to the National Committee for Clinical Laboratory Standards guidelines (CLSI, former NCCLS) (9). The agar disk diffusion method was performed according to NCCLS guidelines (9) using the following disks: penicillin (10 U); oxacillin (1 μg); erythromycin (15 μg) and gentamicin (10 μg) (Laboratorios Britania); enrofloxacin (5 μg) (Neo-Sensitabs™, Rosco, Denmark) and florfenicol (30 μg) (Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA). Interpretive criteria for penicillin, oxacillin, gentamicin, erythromycin and florfenicol were those adopted by the NCCLS (9). Breakpoints for enrofloxacin were those suggested by Rosco (Neo Sensitabs™, Denmark). To further investigate oxacillin resistance, the disk diffusion test was performed with a 30-μg cefoxitin disk, as described elsewhere (1), against strains displaying a borderline inhibition zone around the 1-μg oxacillin disk (i.e. 11 to 16 mm) (13). Isolates showing an inhibition zone <20 mm around the cefoxitin disk were considered to be resistant. Control strains for MIC and agar diffusion method were *S. aureus* ATCC 29213 and *S. aureus* ATCC 25923, respectively. Penicillin resistant isolates were tested for β-lactamase production using a chromogenic cephalosporin disk method (DrySlide Nitrocefin, Difco Laboratories, Detroit, USA) following induction of β-lactamase production (13). The nitrocefin method was carried out and interpreted according to the manufacturer's instructions. *S. aureus* ATCC 29213 was used as a positive control.

Control strain values were within recommended ranges for all methods tested. MIC_{50}, MIC_{90} and MIC ranges against the evaluated isolates recovered from clinical specimens are given in Table 1. Only penicillin-resistant strains were detected by this method. No resistant isolates to oxacillin, enrofloxacillin and florfenicol were detected by the agar diffusion test, whereas 2% of the strains proved resistant to erythromycin and gentamicin. Fifty one percent of isolates were resistant to only one antimicrobial agent, 7.4% were simultaneously resistant to two antimicrobial agents (penicillin and erythromycin) and no multiresistant isolates were detected.

Penicillin MIC_{90} was higher than that observed in a collaborative study that included isolates from 11 countries (3) (0.5 μg/ml), and than that obtained for isolates from Chilean (1 μg/ml) (12) and Japanese (0.78 μg/ml) (14) dairy herds. Conversely, it was lower than that reported for isolates belonging to Uruguayan dairy herds (>8 μg/ml) (6). Although penicillin MIC_{50} was lower than that observed for isolates obtained from different dairy areas of Argentina (4), MIC_{90} was higher. Discrepancies between studies are difficult to analyze since, although isolates from both surveys belonged to the same provinces, except in the case of included isolates from Mendoza (4), no data about number of strains per dairy herd or province were provided in the former study. Nevertheless, penicillin resistance rates obtained in both studies and observed in a previous survey performed in the Central dairy area of Argentina (2) were similar. Selective pressure posed on bacterial populations through antibiotic use for bovine mastitis therapy does not seem to have changed susceptibility patterns over the last years. Among 46 penicillin-resistant isolates, 89.1% produced β-lactamase. Most β-lactamase-producing isolates in this study showed penicillin MIC ranging from 0.25 to 8 μg/ml, whereas two isolates showed a MIC of 0.25 μg/ml, and should be considered to be susceptible according to the interpretative criterion used (9). Although the MIC of these two isolates was close to the breakpoint of ≥0.25 μg/ml suggested by NCCLS, a recent study showed that β-lactamase producing *S. aureus* isolated from bovine mastitis had a MIC of 0.03 to 0.06 μg/ml; which suggests that this breakpoint could be too high to detect penicillin resistance, mainly in strains close to the detection limit (7). No oxacillin resistance was detected in our study, which is in accordance with results obtained in previous studies carried out in Argentina (2, 4), as well as in other

Table 1. *In vitro* susceptibility of 95 *Staphylococcus aureus* isolates obtained from bovine mastitis to six selected antimicrobial agents.

Antibiotic	Result (μg/ml) for:			Breakpoint	Resistance
	MIC_{50}	MIC_{90}	Range	μg/ml	%
Penicillin	0.06	4	0.03-8	≥0.25	48.4
Oxacillin	0.25	0.25	0.06-1	≥4	0
Gentamicin	0.25	0.5	0.03-2	≥16	2.1
Erythromicin	0.125	0.25	0.125-0.5	≥8	2.1
Enrofloxacin	0.25	0.5	0.125-1	≥2	0
Florfenicol[(1)]	4	8	4-8	≥8	0

[(1)]MIC was estimated by the agar dilution method, as E-test strips were not commercially available for this antibiotic.

countries (6, 7, 11, 12, 14). Since the disk diffusion test may lack accuracy to discriminate between susceptible and resistant strains (9), the cefoxitin disk included herein, enhances detection of methicillin-resistant strains (1). Seven isolates considered to be borderline on the disk diffusion test for oxacillin were detected (15 to 18 mm), oxacillin MIC range, 0.25 to 0.75 μg/ml, but all of them proved susceptible with the cefoxitin disk; therefore, ruling out resistance to methicillin. However, because methicillin resistance has been already described among coagulase-negative staphylococci isolated from bovine mastitis in our country (5), we consider that continuous surveillance is needed for early detection of this kind of resistance in *S. aureus.* In fact, during the last four decades, methicillin resistance by this pathogen has become a worrisome problem in both hospital-and community-acquired human infections in Argentina (13).

Macrolides are frequently used in Argentina for bovine mastitis treatment, since high concentrations in milk are obtained following parenteral administration. Erythromycin was evaluated as representative of this group and high activity was shown. MIC_{90} was slightly lower than that reported in studies performed in South American countries (4, 6), as well as that from the Northern Hemisphere (3, 7, 14).

Enrofloxacin is a quinolone approved in Argentina for treatment of various infectious diseases in cattle caused both by gram-positive and gram-negative bacteria, but it is not specifically recommended for bovine mastitis treatment; although high concentrations are reached and maintained in milk following parenteral administration. The MIC_{90} value found (0.5 μg/ml) was higher than that reported for isolates from several European countries and the USA (0.125 μg/ml) (3) and similar to that reported in studies from Chile, Uruguay and Japan (6, 12, 14). The isolates with the highest MIC against this antimicrobial agent were found in Norway (64 μg/ml) (3) and Chile (8 μg/ml) (12); which could be related to frequent use of this drug for bovine mastitis treatment.

Gentamicin was used as representative of the aminoglycoside group. Although these antimicrobials are used with care in dairy cattle because of the risk of extended residue presence in milk, members of this group are frequently included in combined preparations with β-lactams or macrolides to achieve synergic effects (3). The MIC_{90} found in this study was lower than that reported for *S. aureus* isolated from bovine mastitis in Argentina (4), Chile (12) and Uruguay (6), whereas it was similar to that reported for isolates from Japan (14).

Florfenicol is a synthetic fluorinated chloramphenicol and tiamphenicol analogue that is exclusively used in food producing animals for treatment of infectious diseases. Pharmacokinetic studies showed that high concentrations, both in serum and milk, are obtained following intramammary administration in cows, which led to propose its use for treatment of bovine mastitis. The MIC_{90} observed in this study was similar to MIC reported in a previous study carried out on 25 *S. aureus* isolated from bovine mastitis in Argentina (10). However, it was higher than the MIC_{90} reported for *S. aureus* isolated from bovine mastitis in Chile (12) (2 μg/ml) and Japan (14) (6.5 μg/ml), respectively. According to the interpretive criterion of the agar diffusion test used in the present study, no resistant isolates to florfenicol were detected. However, the MIC_{90} value found coincided with the tentative breakpoint recommended by NCCLS (9) for gram-negative organisms causing respiratory disease. This breakpoint has been considered doubtful since *S. aureus* from animal origin carrying *cfr* or *fexA* genes that codify for florfenicol resistance showed a MIC of at least 16 μg/ml, which suggests that 8 μg/ml should not be regarded as an appropriate breakpoint for *S. aureus* (8).

In conclusion, among the antimicrobial agents tested, the higher percentage of resistant isolates was observed for penicillin. Most penicillin resistant isolates produced β-lactamase and no oxacillin resistant isolates were detected. Tentative MIC breakpoints for penicillin and florfenicol for *S. aureus* isolated from bovine mastitis should be revised.

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