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Article

Frequency of genes encoding antibiotic resistance in strains of *Staphylococcus aureus* from udder skin of cattle from southwestern Mexico

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Abstract:

The presence of *Staphylococcus aureus* in the skin of bovine udder has been proposed as a source of upward contamination to the mammary gland, which causes the development of mastitis and the presence of this bacterium in milk. The entry of *S. aureus* into the food chain becomes a public health problem due to the dissemination of antibiotic-resistant strains from animals to food and therefore to humans. Therefore, this study aimed to determine resistance to antibiotics and associated genes in strains of *S. aureus* isolated from the skin of bovine udder. Antibiotic resistance was determined by minimum inhibitory concentration and the presence of antibiotic resistance genes by endpoint PCR. The strains of *S. aureus* showed a high frequency of resistance to beta-lactams, macrolides, lincosamides, and tetracyclines. The strains were resistant to cefoxitin but the *mecA* gene was not found; of the *ermA-C* genes, *ermB* was the most frequently found gene (16.39 %); the *msrA* gene was found in most strains with constitutive resistance to macrolides and lincosamides (88.46 %); the *tetM* gene was found in 64.28 % of the tetracycline-resistant strains. In conclusion, the strains of *S. aureus* isolated from bovine udder skin presented different degrees of resistance to different groups of antibiotics, which can be explained by the presence of associated genes.

Keywords: *Staphylococcus aureus*, Cow udder, Resistance genes, Antibiotics.

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Introduction

Bovine mastitis is the most prevalent infectious disease worldwide, which is associated with severe economic losses due to the reduction in milk production and quality, as well as the cost of treatments and animal health, increasing the risk of slaughter and replacement costs[1-3]. *Staphylococcus aureus* is recognized as the main pathogen worldwide that causes bovine mastitis, and this microorganism is considered contagious and difficult to treat due to its high rate of dissemination among infected animals and the ability to cause chronic or recurrent infections[1,4,5].

Antibiotic therapy is an important measure to control bovine mastitis. Nevertheless, there has been an increasing number of studies reporting that *S. aureus* is resistant to multiple classes

of antibiotics in response to selective pressure from their continued use, due to the overuse of these active substances in veterinary medicine and agriculture[6,7].

In addition to the use of antibiotics for mastitis control, it is important to identify epidemiological patterns and identify sources of contamination. Several potential sources of contamination have been identified, from barn air, milking equipment to udder skin[8]. However, results about the role of bovine udder skin as a source of *S. aureus* in intramammary infections are not yet conclusive. On the one hand, it has been shown that strains of *S. aureus* from the skin and canal of the udder of the cow may be a potential source for the development of bovine mastitis because, genetically, they are the same as the strains obtained in milk and because these can promote the chronicity of the infection[4,9]. On the other hand, another study showed that most of the *S. aureus* associated with mastitis cases belong to strains that are highly adapted to the mammary gland but different from the strains that come from the skin[8]. Although the role of strains isolated from cow udder skin is unclear, these strains can easily be transferred to raw milk during milking. Raw milk contaminated with *S. aureus* can become a public health problem due to the risk of food poisoning by this microorganism, in addition to the dissemination of antibiotic-resistant strains[4,10].

In a previous study, strains of *S. aureus* were isolated from the udder skin of cows from three dairy farms in southwestern Mexico, these strains presented the genes for enteroxins A, D and E. In addition, within these strains, clonal groups were identified that spread among the farms and remained in the rainy and dry seasons[11]. Due to the toxigenic potential of the strains, it is important to continue with the determination of other characteristics of epidemiological importance, such as antibiotic resistance, considering that their persistence in the farms may be related to high antibiotic resistance. Therefore, this study aimed to determine resistance to antibiotics and associated genes in strains of *S. aureus* isolated from the udder skin of cattle on farms in southwestern Mexico.

Material and methods

Bacterial strains

The strains of *S. aureus* from the teat of the cow udder were isolated in 2019 in two seasons of the year (rainy and dry) in three dairy farms located in three areas with different altitudes[11]. The strains are preserved in cryovials of glycerol at 15 % (v/v) at -20 °C in the strain collection of the Microbial Pathometabolism Research Laboratory (LIPM, for its initials in Spanish) of the Faculty of Chemical-Biological Sciences of the Universidad

Autonoma de Guerrero. These strains were characterized under the following phenotypic profile: Gram-positive coccus, mannitol, catalase and coagulase positive and molecularly identified by the *fem* gene. The reactivation of the strains was carried out by resuspending 20 µl of each cryovial in 1 ml of Mueller Hinton (MH) broth, which was incubated at 37 °C for 24 h. Once the growth was obtained, they were inoculated in BHI agar plates for conservation as working strains.

Minimum inhibitory concentration

Antimicrobial resistance was assessed using the Minimum Inhibitory Concentration (MIC) assay; bacterial inocula were prepared from 24-h cultures of each of the strains in MH broth and adjusted to 0.5 McFarland. In a 96-well microplate, each antibiotic was placed in different ranges of concentrations; penicillin (PEN 0.25-2 µg/ml), ceftriaxone (CRO 0.5-32 µg/ml), ciprofloxacin (CIP 4-32 µg/ml), clindamycin (DA 4-256 µg/ml), erythromycin (E 8-256 µg/ml), kanamycin (KAN 16-256 µg/ml), gentamicin (GEN 16-256 µg/ml), tetracycline (TET 16-256 µg/ml), vancomycin (NPV 16-256 µg/ml), chloramphenicol (CLF 16-256 µg/ml), and trimethoprim (TMP 16-256 µg/ml), indicated by the CLSI (Clinical and Laboratory Standards Institute)[12]. The bacterial inocula were then added and incubated at 37°C for 24 ± 1 h. As a positive control, the same strains were inoculated without antibiotics and as a negative control, a well with Mueller Hinton broth and antibiotics was used. Subsequently, the growth was read by absorbance at a wavelength of 492/630 nm in a spectrophotometer (Thermo Scientific®, GENESYS 200, USA). The MIC_{50} was then calculated and the strains were catalogued parametrically and according to the MIC values reported by the CLSI manual as Sensitive (S) or Resistant (R).

Resistance to beta-lactams

Each strain was incubated for 18 h in MH broth at 37 °C under static conditions; the culture was adjusted to 0.5 McFarland and inoculated in MH agar using the disc diffusion method with cefoxitin (FOX 30 µg) and penicillin (PEN 10 µg) discs and incubated at 35 ± 2 °C for 24 h[12]; after that time had elapsed, inhibition halos were measured and categorized as sensitive (S) (PEN ≥ 29 mm or FOX ≥25 mm) or resistant (R) (PEN ≤28 mm or FOX ≤24 mm)[12]. Then, 10 µl of nitrocefin (Oxoid Thermo Scientific™) was placed to determine the production of beta-lactamases by observing the change in the coloration of the halo: a reddish halo allowed them to be defined as producing and when no change in the coloration of the halo was observed as non-producing.

Determination of MLS_B resistance phenotypes

The phenotypes of resistance to MLS_B (Macrolides-Lincosamides-Streptogramin B) were determined by the results obtained from resistance to clindamycin (C-R) or erythromycin (E-R), classifying the resistances obtained as constitutive (C-R, E-R or both) or inducible (E-R, but sensitive to clindamycin)[12].

Antibiotic resistance genes

DNA extraction

The genomic DNA of all *S. aureus* strains was obtained from 1 ml of an 18-h static culture in brain heart infusion (BHI) incubated at 37 °C. The cells were centrifuged at 10,000 rpm for 10 min, resuspending the pellets in 300 µl of lysis buffer (10 mM Tris HCl, 1 mM EDTA pH 8.0, lysozyme 0.02 mg/ml) and incubating at 37 °C for 10 min. Subsequently, 200 µl of phenol/chloroform/isoamyl alcohol solution (25:24:1 v/v/v) was added, inverted 10 to 15 times, centrifuged again at 10,000 rpm for 10 min, 200 µl of the aqueous phase was recovered and transferred to 1 ml of cold ethanol (96 %). It was incubated at -20 °C for 24 h, then centrifuged at 10,000 rpm for 10 min, the supernatant was discarded, the cell pellets were left to dry at room temperature and resuspended in 20 µl of sterile water[13].

Genes that confer antibiotic resistance

The identification of genes conferring antibiotic resistance was determined by endpoint polymerase chain reaction (PCR). For the *mecA*, *blaZ*, *ermA-C*, *msrA*, and *tetM* genes, the following reaction mixture was used: 1X Taq 2x Master Mix RED, 1.5 $MgCl_2$ (Ampliqon, Denmark), 40 nM of each oligonucleotide, and 100 ng of DNA. In the case of *ermB*, the following reaction mixture was used: 1X Taq 2x Master Mix RED, 1.5 $MgCl_2$ (Ampliqon, Denmark), 160 nM of each oligonucleotide, and 100 ng of DNA. The ATCC 43300 strain of *S. aureus* subsp. *aureus* Rosenbach for *ermA*, *mecA*, *blaZ*; for the *ermB* gene, a clinical isolate of *S. aureus* characterized in the LIPM was used; the ATCC 25923 strain of *S. aureus* subsp. *aureus* Rosenbach and the strain 1084 of *S. hominis* characterized by the Microbiology Research Laboratory of the Faculty of Chemical-Biological Sciences of the Autonomous

University of Guerrero were used as controls for the *tetM* and *msrA* genes, respectively. Oligonucleotides and conditions are described in Table 1.

Table 1: Oligonucleotides used in this study

Gene	Sequence	Cycling	Expected size (bp)	Reference
blaZ	F-ATTTTGAAAAAGTTAATATTTTTAATTG R-CATTACACTCTTGGCGGTTTC	1 cycle 95°C for 5 min; 35 cycles at 95°C for 30 s, 52°C for 50 s, and 72°C for 50 s; and 1 cycle at 72°C for 10 min.	833	(14)
mecA	F- AAAATCGATGGTAAAGGTTGGC R- AGTTCTGCAGTACCGGATTTGC	1 cycle 94°C for 5 min; 32 cycles 94°C for 30 s, 57°C for 45 s, and 72°C for 1 min; and 1 cycle 72°C for 5 min.	583	(15)
ermA	F-GTTCAAGAACAATCAATACAGAG R-GGATCAGGAAAAGGACATTTTAC	1 cycle 94°C for 2 min; 32 cycles 94°C for 30 s, 52°C for 1 min, and 72°C for 30 s; and 1 cycle 72°C for 10 min.	421	(16)
ermB	F- CCGTTTACGAAATTGGAACAGGTAAAGGGC R- GAATCGAGACTTGAGTGTGC	1 cycle 95°C for 5 min; 32 cycles 95°C for 20 s, 52°C for 45 s, and 72°C for 40 s; and 1 cycle 72°C for 7 min.	359	
ermC	F- RGCTAATATTGTTTAAATCGTCAATTCC R-GGATCAGGAAAAGGACATTTTAC	1 cycle 94°C for 2 min; 32 cycles 94°C for 30 s, 52°C for 1 min, and 72°C for 30s; and 1 cycle 72°C for 10 min.	572	
msrA	F-GGCACAATAAGAGTGTTTAAAGG R-AAGTTATATCATGAATAGATTGTCCTGTT	1 cycle 95°C for 15 min; 32 cycles 95°C for 30 s, 52°C for 45 s, and 72°C for 1 min; and 1 cycle 72°C for 7 min.	940	(17)
tetM	F- GTGGACAAAGGTACAACGAG R-CGGTAAAGTTCGTCACACAC	1 cycle 95°C for 5 min; 32 cycles 95°C for 20 s, 52°C for 45 s, and 72°C for 40 s; and 1 cycle 72°C for 7 min	406	(16)

Results

Antibiotic resistance

It was determined that 95.1 % (58/61) of the strains are resistant to penicillin and 100 % (61/61) are resistant to ceftriaxone. It was found that only 1.6 % of the strains (1/61) were resistant to ciprofloxacin, gentamicin, and chloramphenicol, 98.4 % (60/61) to clindamycin, 42.6 % (26/61) to erythromycin, 62.3 % (38/61) to kanamycin, 22.9 % (14/61) to tetracycline, 13.1 % (8/61) to trimethoprim. Finally, no vancomycin-resistant strains were found.

Regarding the results of the antibiotic concentrations to which each of the strains analyzed in this work are inhibited. The 58 strains with penicillin resistance were distributed in MICs from 0.25 to 2.0 µg/ml; in the case of ceftriaxone, in the 61 resistant strains, MICs from 0.25 µg/ml to greater than 32 µg/ml were determined, of which 40 strains had the highest MIC (32 µg/ml).

In the MLS_B antibiotic group, clindamycin-resistant strains (60/61) had MICs from 8 to 256 µg/ml, with 44 strains having a MIC of 256 µg/ml; for erythromycin, the resistant strains had MICs from 16 to 256 µg/ml, of which four strains had a MIC of 256 µg/ml; of the fluoroquinolone group, only one strain with a MIC of 4 µg/ml of ciprofloxacin; in aminoglycosides, strains were determined with MICs from 32 to 256 µg/ml concentration of kanamycin and a strain with a MIC of 64 µg/ml of gentamicin.

Regarding tetracycline, there were strains with MICs from 32 to 256 µg/ml, of which 11 strains had a MIC of 256 µg/ml; in amphenicols, there was only one strain with a MIC of 128 µg/ml of chloramphenicol; finally, in the diaminopyrimidine group, values of 32 to 256 µg/ml were determined in strains with resistance to trimethoprim, observing two strains with a MIC of 256 µg/ml (Table 2).

Table 2: Minimum inhibitory concentration of *S. aureus* strains resistant to the different groups of antibiotics

Antibiotics	Concentrations used (µg/ml)											Resistant strains
	0.25	0.5	1	2	4	8	16	32	64	128	256	% (n), N= 61
Beta-lactams												
Penicillin	9	5	17	27								95.1 (58)
Cephalosporins												
Ceftriaxone		1	5	1	10	4	0	40				100 (61)
Fluoroquinolones												
Ciprofloxacin					1							1.6 (1)
Lincosamides												
Clindamycin						10	0	0	4	2	44	98.4 (60)
Macrolides												
Erythromycin							11	9	0	2	4	42.6 (26)
Aminoglycosides												
Kanamycin								10	9	7	12	62.3 (38)
Gentamicin									1			1.6 (1)
Tetracyclines												
Tetracycline								1	0	2	11	22.9 (14)
Phenicols												
Chloramphenicol										1		1.6 (1)
Diaminopyrimidines												
Trimethoprim								4	0	2	2	13.1 (8)

The parameters of the MIC (µg/ml) were determined according to the values established in the 31ed M100 Performance Standards for Antimicrobial Susceptibility Testing[12].

Antimicrobial resistance phenotypes

One hundred percent of the strains were resistant to cefoxitin and only one was beta-lactamase-producing (1.6 %). For erythromycin and clindamycin, resistance frequencies of 42.6 and 98.7 % were obtained, respectively, which were categorized within the group of phenotypes of constitutive resistance to MLS_B, highlighting that the same 42.6 % of strains were resistant to both antibiotics (erythromycin and clindamycin). No strains with resistance to erythromycin but sensitivity to clindamycin were determined, so there is no inducible resistance of an antibiotic; on the other hand, an atypical result of 55.8 % of strains with resistance to clindamycin (C-R), without being resistant to erythromycin (S), was found.

Eighty point two percent of the strains are resistant to at least three groups of antibiotics (Table 3)

Table 3: Antibiotic resistance phenotypes in *S. aureus* strains

Beta-lactam resistance phenotype	**Number of strains (%) N=61**
MRSA	61 (100.0)
MSSA	0 (0.0)
Production of beta-lactamases	1 (1.6)
Phenotypes MLS_B	**(%)**
$cMLS_B$	
E-R	26 (42.6)
C-R	60 (98.7)
E-R, C-R	26 (42.6)
$iMLS_B$	
Antibiotic resistance phenotypes	**(%)**
PEN, CRO	1 (1.6)
PEN, CRO, DAN	11 (18.2)
PEN, CRO, DAN, E	3 (4.9)
PEN, CRO, DAN, E, KAN	13 (21.3)
PEN, CRO, DAN, E, KAN, TET	4 (6.7)
PEN, CRO, DAN, E, KAN, CIP	1 (1.6)
PEN, CRO, DAN, E, KAN, TMP	1 (1.6)
PEN, CRO, DAN, E, TET	1 (1.6)
PEN, CRO, DAN, E, TMP	1 (1.6)
PEN, CRO, DAN, E, TMP, CLF, TET	1 (1.6)
PEN, CRO, DAN, KAN	14 (23.1)
PEN, CRO, DAN, KAN, GEN, TET	1 (1.6)
PEN, CRO, DAN, KAN, TET	3 (4.9)
PEN, CRO, DAN, TMP	3 (4.9)
PEN, CRO, DAN, TMP, TET	1 (1.6)
CRO, DAN, E	1 (1.6)
CRO, DAN, E, KAN, TET	1 (1.6)

PEN (penicillin), CRO (ceftriaxone), DA (clindamycin), E (erythromycin), KAN (kanamycin), GEN (gentamicin), TMP (trimethoprim), TET (tetracycline), CLF (chloramphenicol), CIP (ciprofloxacin), MRSA (methicillin-resistant *Staphylococcus aureus*), MSSA (methicillin-sensitive *Staphylococcus aureus*), MLS_B (Macrolides- Lincosamides- Streptogramin B), $cMLS_B$ (constitutive resistance to Macrolides- Lincosamides-Streptogramin B), E-R (resistance to erythromycin), C-R (resistance to clindamycin), $iMLS_B$ (inducible resistance to Macrolides-Lincosamides-Streptogramin B).

Antibiotic resistance genes

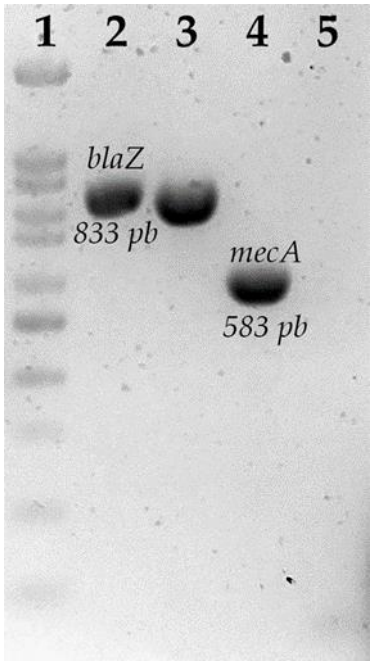
Absolute frequencies of the genes that confer resistance to each of the groups of antibiotics were determined. Of the beta-lactam-resistant strains, only three strains were found positive for the presence of the *blaZ* gene (4.91 %), one strain was positive for the nitrocefin test, highlighting that it was not positive for the presence of the *blaZ* gene. For *mecA*, no positive strains were found for the presence of this gene (Table 4) (Figure 1).

Table 4: Distribution of antibiotic resistance genes in *S. aureus* strains

Resistant strains		**Beta-lactams**		**MLS**				**TETRA**
Group of antibiotics	**Antibiotic**	***blaZ***	***mecA***	***ermA***	***ermB***	***ermC***	***msrA***	***tetM***
Beta-lactams								
	Penicillin (N=58)	3						
Cephalosporins								
	Ceftriaxone (N=61)	3						
	Cefoxitin (N=61)	3						
ML								
	Clindamycin/ Erythromycin (N=26)				9	1	23	
	Clindamycin (N=60)				1	1	29	
TETRA								
	Tetracycline (N=14)							9

TETRA= tetracycline.

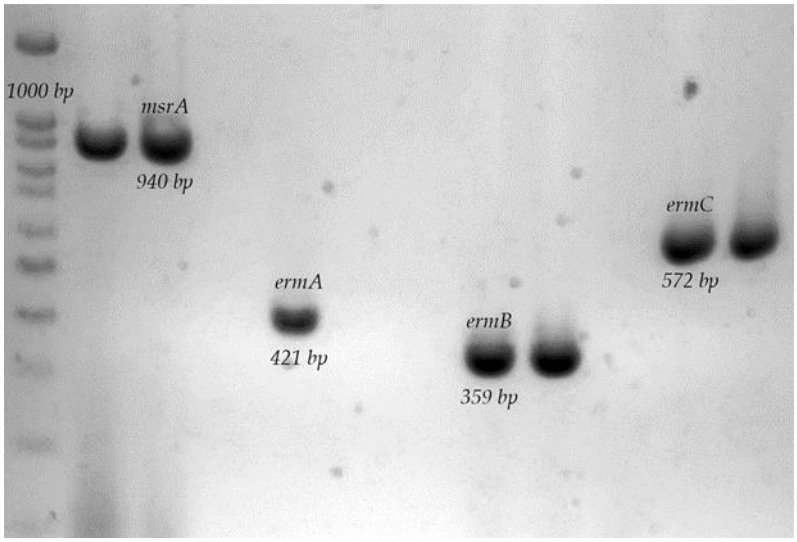
Figure 1: Beta-lactam resistance-associated genes in *S. aureus* strains isolated from bovine udder skin



Agarose gel electrophoresis of *blaZ* and *mecA* amplification. 1) 100 bp molecular weight marker; 2) *blaZ*, *S. aureus* ATCC 43300; 3) *blaZ*, *S. aureus* S680; 4) *mecA*, *S. aureus* ATCC 43300; 5) *mecA*, *S. aureus*.

From the MLS_B antibiotic group: 52 strains of the total MLS_B-resistant strains were determined with the presence of the *msrA* gene (86.6 %); of these, 29 strains belong to the C-R profile (55.7 %) and 23 corresponded to the ER-CR profile (44.23 %). In addition, there were 10 strains positive for the *ermB* gene (16.6 %), of which only nine correspond to the ER-CR phenotype (90 %) and the remaining strain is of the C-R phenotype (10 %). Likewise, two strains were determined with the presence of the *ermC* gene (3.3 %), of which one strain corresponds to the ER-CR phenotype and one strain to the C-R phenotype, and there were no *ermA*-positive strains (Figure 2).

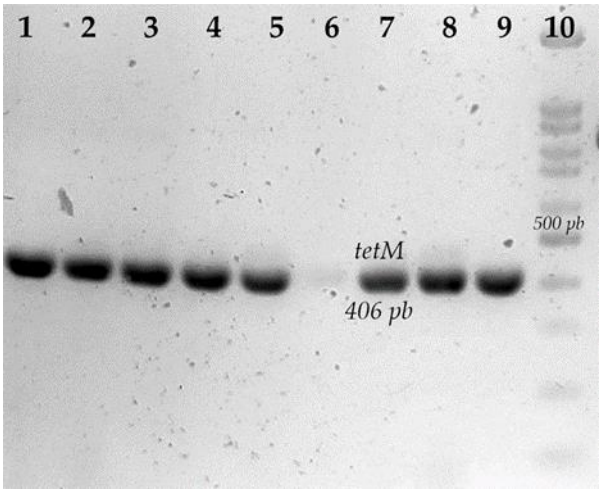
Figure 2: Macrolide resistance-associated genes in *S. aureus* strains isolated from bovine udder skin



Agarose gel electrophoresis of the amplification of *msrA*, *ermA-C* genes. 1) 100 bp molecular weight marker; 2) *S. haemolyticus* 1035, *msrA*+; 3) *S. aureus* S697; 4) Negative control; 5) *S. aureus* ATCC 43300, *ermA*+; 6) *S. aureus* S661; 7) Negative control; 8) *S. aureus* S697, *ermB*+; 9) *S. aureus* S694, *ermB*+; 10) Negative control; 11) *S. aureus* S662, *ermC*+; 12) *S. aureus* S661, *ermC*+.

In the determination of the *tetM* gene of 14 tetracycline-resistant strains, nine were determined with the presence of the *tetM* gene (64.28 %) (Figure 3); of these, six strains had a MIC of 256 µg/ml, one strain had a MIC of 128 µg/ml, and two strains had a MIC of 32 µg/ml.

Figure 3: Tetracycline resistance-associated genes in *S. aureus* strains isolated from bovine udder skin



Agarose gel electrophoresis of *tetM* amplification. 1) *S. aureus* S68; 2) *S. aureus* S689; 3) *S. aureus* S692; 4) *S. aureus* S693; 5) *S. aureus* S696; 6) *S. aureus* S697; 7) *S. aureus* S654; 8) *S. aureus* S662; 9) *S. aureus* D1; 10) 100 bp molecular weight marker.

Discussion

S. aureus is the main pathogen worldwide that causes bovine mastitis, characterized by causing chronic infections due to the low response to antibiotics, facilitating spreading among cattle. The antibacterial resistance of this microorganism originates from the extensive use of antibiotics to control mastitis, becoming a public health problem due to the emergence and dissemination of antibiotic-resistant strains to humans from food[18].

This study found a high resistance to beta-lactam antibiotics compared to other groups of antibiotics in *S. aureus* strains isolated from the skin of the bovine udder; resistance to these antibiotics is mediated by the synthesis of alternative penicillin-binding proteins (PBP2a, PBP2´), synthesis of beta-lactamases, and mutations in penicillin-binding protein (PBP) genes. Resistance to cefoxitin infers that the mechanism could be mediated by the synthesis of alternate proteins encoded in the SCCmec chromosomal cassette; nevertheless, in this study, the strains were negative for the amplification of the *mecA* gene. Strains negative for *mecA* but with a high oxacillin MIC have been reported as *mecA*-BORSA (borderline oxacillin-resistant *S. aureus*, *mecA* negative) in different foods, such as chicken, pork, milk, and even in the community[19], so its presence in bovine udder skin is not surprising. A key characteristic of the BORSA strains is the overproduction of beta-lactamases[20]; in this sense, to evaluate the presence of genes related to beta-lactamases, the *blaZ* gene was amplified, obtaining a low frequency for this gene (3/61, 4.91 %), the low frequency of beta-lactamase-producing strains encoded by *blaZ* and negative for *mecA* could be related to the presence of new beta-lactamases not encoded by *blaZ* but by plasmids[21]. On the other hand, to evaluate the production of beta-lactamases, nitrocefin hydrolysis was assessed, obtaining a low frequency (1/61, 1.64 %), emphasizing that the nitrocefin-test positive strain is negative for the *blaZ* gene. Regarding this point, up to four different beta-lactamases have been described in *S. aureus*, called A to D; however, not all of them can be determined by the nitrocefin, cefazolin or cephaloridine suspension test[22]. Although the main mechanism of resistance in BORSA strains is the overproduction of beta-lactamases, this is not the only mechanism; the presence of new beta-lactamases, mutations in PBP genes or the presence of efflux pumps could explain the behavior of these strains and could be studied in the future[20,21,23].

Macrolides, lincosamides, and streptogramins of group B, called MLS_B, have become a strategy for the control of methicillin-resistant *S. aureus*, as well as in patients with allergy to the beta-lactam group. Of the MLS_B group, clindamycin is important due to its application for the treatment of skin, soft tissue and bone infections due to its high permeability[24-26]. Regarding the above, the farmers who participated in this study report the use of this antibiotic as a preventive treatment for bovine udder infections, which is reflected in the high resistance to clindamycin (98.3 %, 60/61) in a constitutive phenotype with macrolides

(cMLS$_B$) in 42.6 % of the strains. MLS$_B$ resistance may be conferred by different mechanisms, such as target site modification, efflux pumps, and enzymatic inactivation of the antibiotic[27]. The modification of the target site is mediated by the presence of erythromycin resistance methylase (*erm*) genes. The main commonly found *erm* genes include *ermA* and *ermC*[17]. The *erm* genes encode methylases that cause conformational modifications of the 23S rRNA subunit, resulting in a decrease in the binding of MLS$_B$ antibiotics to their target site in the ribosomal 50S subunit[24]. One of the relevant data of this study is the high prevalence of the *ermB* gene in *S. aureus* strains isolated from bovine udder, which has also been found in coagulase-positive and negative *Staphylococcus* strains isolated from buffalo milk[28]; unlike strains of clinical origin where it is common to find the *ermA* and *ermC* genes[29-31]. As described, target site modification is not the only mechanism that explains constitutive resistance to MLS$_B$; another mechanism is the presence of efflux pumps, such as MsrA, which explains the constitutive resistance to macrolides[32]. The presence of both mechanisms could explain to some extent the constitutive phenotype of MLS$_B$ (E-R, C-R) found in this study, even leaving the circulation of other *erm* genes into consideration. In those strains of *S. aureus* that are only resistant to clindamycin (55.8 %), although it is an uncommon result, this could be explained by the presence of *linA* and *linB* genes that inactivate only lincosamides[33] in the circulating strains and that could be one of the future perspectives of this study.

Tetracycline is an antibiotic that has been used in livestock, humans, small animals, agriculture, and aquaculture for the last 40 yr[34]. At the same time, tetracycline is not only used for the treatment of infections in animals but also as a growth promoter or to improve the efficiency of fattening products, a practice that is still carried out today both in the United States of America and in other countries[35]. Therefore, it is not surprising that tetracycline-resistant strains of *S. aureus* from cattle[36] circulate and even that livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) of the CC9 clonal complex has tetracycline resistance as a distinctive characteristic[37]. In this sense, 14 strains with resistance to tetracycline were identified, noting that they share other characteristics of bacterial resistance, including clindamycin, erythromycin and cefoxitin, so it is not ruled out that these strains also belong to this clonal group. Because of this, complete characterization of these strains is necessary in the future. Tetracycline resistance is mediated by genes encoding efflux pumps and ribosomal protection proteins[35]. In the latter group, one of the main genes sought is *tetM*. In this study, the frequency of the *tetM* gene was 14.75 %, being found in 9 of the 14 resistant strains. This gene has commonly been reported in isolated strains of subclinical mastitis in Brazil, China, and Canada[38-40], samples of cow's milk in Brazil[41,42], and buffalo milk samples in Egypt[28]. In strains that are resistant to tetracycline but negative for the *tetM* gene, the search could focus on more than 25 genes related to tetracycline resistance[34,35].

The determination of resistance genes in *S. aureus* as well as in other microorganisms is important because many of these genes are located in mobile genetic elements (MGEs),

which, by horizontal transfer mechanisms, can generate new antibiotic-resistant strains. These MGEs have even been sought from environmental samples due to their importance[43,44].

Conclusions and implications

The circulation of strains of *S. aureus* from the skin of bovine udders with resistance to beta-lactams, macrolides, lincosamides, and tetracyclines with the presence of the genes *blaZ*, *ermABC*, mrsA and *tetM* genes was confirmed in the milk production units analyzed. Although the present results do not allow generalization, it is suggested that adequate monitoring of the development of mastitis cases, the implementation of good milking practices on ranches in the region, and the appropriate use of antimicrobials would help to prevent the circulation of *S. aureus* strains from the skin of the bovine udder to milk and eventually to humans by the consumption of milk and their derivatives, favoring animal health as well as human and environmental health.

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