Detection of Genes Responsible for Antimicrobial Resistance in Bacteria Isolated from Bovine Mastitis Milk

Byron Flores[1], Marjorie Mendoza[2], Rodolfo Cárdenas[2], Amaru Cruz[2], Brenda Mora-Sánchez[2], Jessica Sheleby-Elías[3]

[1]Universidad Internacional Antonio de Valdivieso, 47000, Rivas, Nicaragua

[2]Centro Veterinario de Diagnóstico e Investigación (CEVEDI), Departamento de Veterinaria y Zootecnia, Escuela de Ciencias Agrarias y Veterinarias, Universidad Nacional Autónoma de Nicaragua-León (UNAN-León), Carretera a La Ceiba 1 Km al Este, León, Nicaragua.

[3]Department of Morphology, Faculty of Veterinary Medicine and Zootechnics, Universidad Nacional Agricultura, 08003, Catacamas, Honduras

Keywords: *bovine mastitis, bacterial resistance, extended-spectrum β-lactamases, Nicaragua.*

Abstract. *Bovine mastitis is considered a problem that is impossible to eradicate, due to the indiscriminate use of antibiotics, mainly β-lactams. The aim of this study was to detect antimicrobial resistance genes in bacteria isolated from bovine mastitis in Western Nicaragua. The antimicrobial resistance profile of 30 bacterial strains was evaluated using the phenotypic method for the antibiotics amoxicillin/clavulanic acid (AMC), ceftriaxone (CRO), gentamicin (CN), cephalexin (CL), vancomycin (VA) for gram-positive bacteria, while the antibiotics amoxicillin/clavulanic acid (AMC), trimethoprim sulfamethoxazole (SXT), ceftriaxone (CRO), gentamicin (CN), cephalexin (CL) in gram-negative bacteria. In addition, the evaluation of the genotypic method was carried out using the PCR (Polymerase Chain Reaction) technique for the detection of extended-spectrum β-lactamases (ESBL) in gram-negative bacteria and the mecA gene for bacteria of the Staphylococcus genus (MRS). It was possible to identify that the bacterial isolates presented a greater resistance to amoxicillin and oxacillin with 24/30 and 10/14, respectively. On the other hand, 100% of the bacterial strains showed sensitivity to the antibiotics gentamicin (CN) and sulfamethoxazole (SXT). In the present study, 2 antimicrobial resistance genes were detected in the gram-negative isolates related to ESBL, the blaSHV gene coding for the strains: Enterobacter, Serratia and E. coli, while the blaTEM gene was detected in the strains: Enterobacter and Serratia. The blaCTX gene in gram-negatives and the mecA gene in Staphylococcus were not detected.*

Introduction

The use of antibiotics in veterinary medicine constitutes one of the main therapeutic tools used for the prevention, treatment and control of infectious diseases of bacterial origin (Ibrahim et al., 2020). However, there are publications that indicate the existence of bacterial multiresistance, not only to antimicrobials of the same family, but also to drugs with different structures and mechanisms of action (Artemyeva et al., 2020; Ríos Padilla, 2021; Arbab et al., 2021; Saeed et al., 2022).

Mastitis is one of the most important diseases in dairy cattle. It is a multi-etiological pathology recognized worldwide for causing harmful effects, either for animal welfare or for dairy farming (Ruegg, 2017). It is considered a multifactorial problem that is impossible to eradicate. Its control depends on the application of comprehensive systems such as the reduction in the rate of new infections and the appropriate use of drugs commonly used in the livestock sector, generally β-lactam antibiotics (Jiménez Velásquez et al., 2020).

Bovine mastitis is caused by gram-negative bacteria, from the coliform group (*Escherichia coli*, *Enterobacter, Klebsiella*), *Pseudomonas* and *Serratia* (Das et al., 2017), gram-positive bacteria (*Staphylococcus aureus*, coagulase-negative *Staphylococcus*, *Streptococcus agalactiae* and *Mycoplasmas* spp.), each of them expressing one or more types of antimicrobial resistance genes (Santiago et al., 2012; Liapi et al., 2021).

It is important to highlight that bovine mastitis has considerable effects on public health due to the transmission of zoonotic bacteria together with antibiotic resistance genes. These bacteria are present in animals and are exposed to antimicrobial pressure, thus developing strategies of survival through evolutionary adaptations (Allen and Stanton, 2014).

Enterobacteria acquire genes for β-lactamase enzymes that generate resistance to β-lactam antibiotics such as penicillins, 2nd, 3rd and 4th generation cephalosporins and monobactams (Lima et al., 2020). The detection of these genes is carried out with molecular techniques that determine extended-spectrum β-lactamases (ESBL) in gram-negative bacteria. For their part, methicillin-resistant *S. aureus* (MRSA) strains are resistant to all β-lactam antibiotics and resistance is mediated by the acquisition of *mecA*, which encodes a penicillin-binding protein (PBP) (Allen and Stanton, 2014).

Correspondence to Byron Flores; Universidad Internacional Antonio de Valdivieso, 47000, Rivas, Nicaragua.
E-mail: byronfloressomarriba@gmail.com

In Nicaragua, like in many countries in the region, antimicrobial susceptibility tests are not routinely performed as a diagnostic tool against any agent that causes bacterial diseases. The personnel in charge of certain livestock areas, such as cattle, use antibiotics as control and treatment measures for any clinical symptomatology, without prescriptions or veterinary consultations, contributing to an increase in antimicrobial resistance to most available treatments (Giono-Cerezo et al., 2020). In the country, resistance studies have been carried out in isolated enterobacteria from clinical samples in humans, with gram-negative bacteria found to be multidrug-resistant (92%; 50/54 isolates) by PCR (Dretler et al., 2020). However, the data relating to bacterial resistance to antibiotics in animals are scarce, especially in the case of bovine mastitis.

Antimicrobial resistance is a multisectoral problem because resistance genes are transmitted between food-producing animals and humans by direct exposure or through the food chain and the environment. This phenomenon between human health, veterinary and food production systems supports the need for health approaches (Quiñones Pérez, 2017). For this reason, the aim of this study is focused on the detection of antimicrobial resistance genes in bacteria isolated from the milk of cows with mastitis in bovines from Western Nicaragua.

Material and methods

A descriptive cross-sectional study was designed, reactivating 107 bacterial strains that had been isolated from the milk of cows with mastitis from 14 farms in Western Nicaragua from January to March 2021 and maintained in the strain collection of the Centro Veterinario de Diagnóstico e Investigación (CEVEDI). Farms were exclusively owned by small producers (less than 50 cattle), with extensive exploitation, dual-purpose herds (meat and milk), Brahman breeds, manual milking, and little technology.

The bacteria were stored in Brain Heart Infusion broth (ICC, OXOID) with 10% glycerol at a temperature of –20°C. For their reactivation, 50 µL of each strain were inoculated in 1 mL of ICC, incubated at 37°C for 24 hours. Then they were inoculated on 5% Sheep Blood Agar (ASC, OXOID) and on MacConkey agar (MC, OXOID), incubating the agar plates at 37°C for 24 hours. Only 30 strains from 8 farms were successfully reactivated without contamination, while 16 of them were classified as gram-negative bacteria and 14 as gram-positive. The pure growth of the bacteria was verified, and their identification was carried out again using API 20E (Biomériux®, Marcy l'Etoile, France) for gram-negative bacteria, while for *Staphylococcus* gram staining was used, catalase, coagulase DNase, (OXOID).

Antimicrobial susceptibility phenotypic test

Resistance patterns were determined by the agar diffusion method, according to the protocol established by the Clinical Laboratory Standards Institute (CLSI), *(Uddin et al.*, 2018). In plates with Müller Hinton agar (OXOID), the bacterial inoculums from the previously isolated colonies were seeded on the surface. Bacterial suspensions were prepared with a turbidity of 0.5 Mc Farland, to obtain a concentration equivalent to 1.5 x 10^8 cfu/mL. They were inoculated with a swab soaked in the suspension, using a conventional striation. The plate with the already seeded agar was allowed to dry for 5 minutes, and later the antibiotic discs for gram-negative bacteria were placed. These were: amoxicillin plus clavulanic acid (AMC 30), ceftriaxone (CRO 30), gentamicin (120), cephalexin (CL 30) and trimethoprim sulfamethoxazole (SXT 25). For gram-positive bacteria (*Staphylococcus*), in addition to the above antibiotics, vancomycin (VA 30) and oxacillin (OX 1) were also added. The plates were incubated at 37°C for 24 h, the inhibition halos were measured, and the results were recorded as resistant (R), intermediate (I) and sensitive (S) referring to the parameters already established for each antibiotic.

Molecular detection of resistance genes

A colony of each previously purified strain was diluted in 200 µL of nuclease-free water in 1.5 mL vials, vortexed for 20 seconds. Then the protocol described by the manufacturer (QIAamp DNA Mini Kit QIAGEN, Germany) was used.

For the identification of ESBL genes from enterobacteria, the following primers were used: *blaSHV*: forward (TGGTTATGCGTTATATTCGCC) and reverse (GGTTAGCGTTGCCAGTGCT) with an amplicon size of 868 bp; *blaCTX:* forward (TCTTCCAGAATAAGGAATCCC) and reverse (CCGTTTCCGCTATTACAAAC) with an amplicon size of 909 bp; *blaTEM*: forward (TCCGCTCATGAGACAATAACC) and reverse (TTGGTCTGACAGTTACCAATGC) with an amplicon size of 931 bp (Asir et al., 2015). The reaction mixture for the detection of the corresponding genes was carried out in a final volume of 15 µL, with the following elements: 3 µL of genomic DNA; 7.5 µL of master mix; 1 µL of forward; 1 µL of reverse; 2.5 µL of nuclease-free water.

For the detection of the *mecA* gene in *Staphylococcus* spp. forward (TGGCTATACGTGTCACAATCG) and reverse (CTGGAACTTGTTGAGCAGAG) primers were used with an amplicon size of 310 bp (Vannuffel et al., 1998). The reaction mixture for the detection of the *mecA* gene was carried out in a final volume of 15 µL, with the following elements: 3 µL of genomic DNA, 7.5 µL of master mix; 1 µL of forward; 1 µL of reverse; 2.5 µL of nuclease-free water.

The amplifications were performed in the Applied Byosystem 2720 thermocycler following the program that consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 denaturation cycles at 95°C for 30 seconds, hybridization at 58°C for 30 seconds, and extension at 72°C for 1 minute. The final extension

was performed at 72°C for 7 minutes. PCR products were visualized by agarose gel electrophoresis (2% w/v) run using TBE buffer at constant power, 110 W, for 2 hours and stained with ethidium bromide.

Statistical analysis

The results were analyzed as relative frequencies with their respective 95% confidence intervals. The Fisher exact test was applied to determine the significant association between categorical variables.

Results

In the present study, gram-negative bacteria represented 16/30 (53.33%, 95% CI: 33.82–72.85) of the bacterial species analyzed, resulting in 6/16 belonging to the genus *Escherichia coli*, 6/16 corresponding to the genus *Enterobacte*r spp. and 4/30 for the genus *Serratia* spp. On the other hand, gram-positive bacteria represented 14/30 (46.66%, 95% CI: 27.15–66.18), resulting in the genus coagulase cegative *Staphylococcus* (SCN) with the highest frequency with 12/14, while *Staphylococcus aureus* strains were 2/14. In the antimicrobial resistance profile of gram-negative strains analyzed, it was possible to identify that the bacterial isolates presented greater resistance to amoxicillin/clavulanic acid with 16/16 strains analyzed, while resistance was also found for cephalexin with 6/16 (Figure 1). In addition, 6/16 strains with multiresistance (AMC and CL) were identified. The most effective antibiotics were gentamicin, ceftriaxone and trimethoprim sulfamethoxazole, to which no resistant gram-negative strain was found. In gram-negative bacteria, a significant difference was observed in the frequency of resistance between antibiotics ($P < 0.001$).

In the case of antibiotics for *Staphylococcus* spp., 10/14 strains resistant to oxacillin, 8/14 strains resistant to AMC and 2/14 to vancomycin were identified. The most effective antibiotics were gentamicin and trimethoprim sulfamethoxazole, to which no resistant gram-positive strain was found (Figure 2). In gram-positive bacteria, a no significant difference was observed in the frequency of resistance between antibiotics ($P = 0.981$).

Two antimicrobial resistance genes were detected in the gram-negative isolates related to ESBL. The *blaSHV* gene was found in 8/16 strains, of which, 4 were found in *Enterobacter* spp., 2 in *E. coli*, and 2 in *Serratia* spp. The *blaTEM* gene was detected in 4/16 strains, of which 2 were identified in *Enterobacter* spp. and 2 in *Serratia* spp. (Figure 3). The *blaCTX* gene was not detected in any of the 16 strains analyzed. Molecular analyses for the detection of the *mecA* gene in *Staphylococcus* spp. were negative in all the isolates (Table 1).

Discussion

Within the isolated and reactivated strains, the species of *Staphylococcus* spp. and *E. coli* were found, which most frequently cause mastitis in cattle, similar to a study conducted in 2012 in western Nicaragua found that the most frequent bacteria in bovine mastitis were CNS with 55% and *S aureus* with 25% (Rivera Varela & Tórrez Cálix, 2012). The high frequency of *Staphylococcus* spp. may be influenced by the fact that the samples were taken in the dry months (January-March) since, as described, cows are more prone to this bacterial genus in the dry season, as reflected in the study carried out in Mexico in which they found a higher frequency for *S. aureus* in the dry season with 23.6%, in relation to the rainy season with 3.2% (Adame-Gómez et al., 2021). Strains such as *E. coli* and gram-negative bacteria found in bovine mastitis may be associated with poor hygienic-

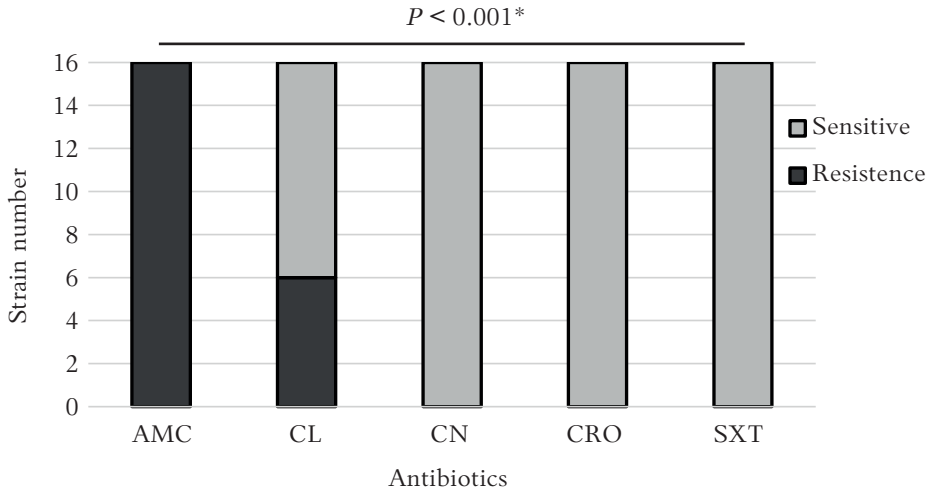

Fig. 1. Antimicrobial resistance profile of 16 gram-negative strains isolated from cows with mastitis in western Nicaragua

Amoxicillin/clavulanic acid (AMC), cephalexin (CON), gentamicin (CN), ceftriaxone (CRO), trimethoprim sulfamethoxazole (SXT).

* Significance according to the Fisher exact test

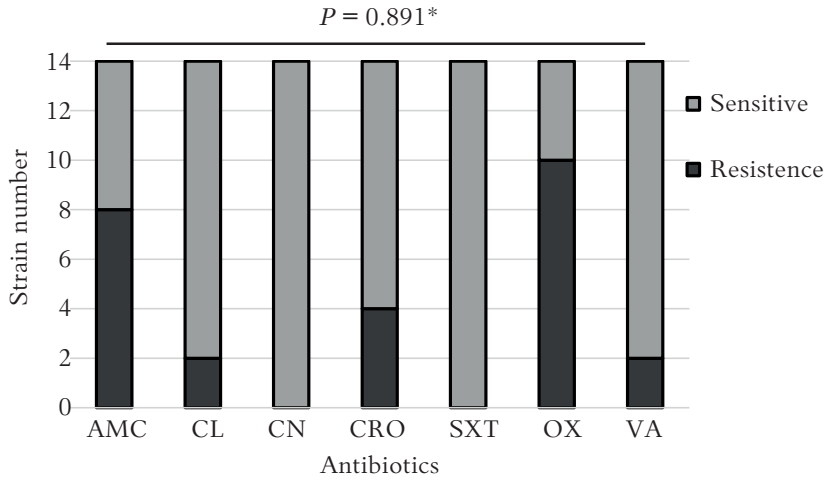

Fig. 2. Antimicrobial resistance profile of 14 *Staphylococcus* spp. strains isolated from cows with mastitis in western Nicaragua
Amoxicillin/clavulanic acid (AMC), cephalexin (CON), gentamicin (CN), ceftriaxone (CRO), trimethoprim sulfamethoxazole (SXT), oxaciline (OX), vancomicine (VA).
* Significance according to Fisher's exact test

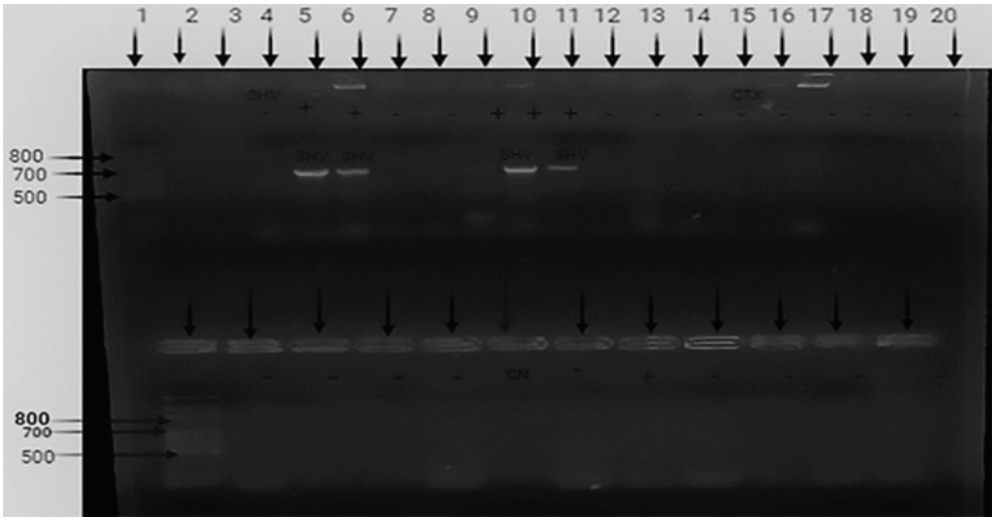

Fig. 3. Electrophoresis and identification of *blaSHV* genes in bacteria isolated from bovine mastitis in Nicaragua
Molecular weight marker (1), *Serratia* spp. positive to SHV (5), *Enterobacter* spp. positive to SHV (6), *Enterobacter* spp. positive to SHV (10). SHV-positive *Escherichia coli* (11).

sanitary practices at the time of handling the animals and their work environment. Being environmental pathogens, they have a greater possibility of causing clinical mastitis (Das et al., 2017).

In the analysis of the antimicrobial resistance profile, it was shown that AMC was the least effective (24/30). Furthermore, gram-negative bacteria showed a greater resistance to AMC with 16/16, while the gram-positive resistance frequency was lower with 8/14. This agrees with what was reported in 2012 in *La Paz Centro* in which sensitivity to AMC was only observed in 35.7% of the strains isolated from the milk of cows with mastitis (Chavarría Narváez and Meléndez Martínez, 2012). This can be attributed to the fact that this antibiotic is sold freely and is the most used in the country to empirically treat infections in humans and animals.

In this study, the bacterial strains did not show resistance to gentamicin (CN), similar to what was reported in a study carried out in the municipality of *La Paz Centro* in 2012, in which it was reported that the antibiotic gentamicin was the most effective against bacteria isolated from milk of cows with subclinical mastitis (Chavarría Narváez and Meléndez Martínez, 2012). This is because aminoglycosides are widely used in Nicaragua for the treatment of mastitis and there are no restrictions regarding their veterinary use.

The gram-positive bacteria were *S. aureus* (2/14) and SCN (12/14). In these bacteria, a high resistance to the antibiotic oxacillin was observed (10/14), which is a high result compared to a study carried out in the municipality of León in 2016, in which 18% of oxacillin-resistant *S. aureus* isolated from the

Table 1. Detection of genes for resistance to extended spectrum betalactamases (ESBL) and methacillin (MRSA) in bacteria isolated from cows with mastitis in Western Nicaragua

Strains	Bacterias	Resistance genes			
		blaSHV	*blaCTX*	*blaTEM*	*mecA*
35	*E.coli*	Negative	Negative	Negative	x
26	*E.coli*	Negative	Negative	Negative	x
38	*E.coli*	Negative	Negative	Negative	x
28	*E.coli*	Negative	Negative	Negative	x
34	*E.coli*	Positive	Negative	Negative	x
25	*E.coli*	Positive	Negative	Negative	x
31	*Enterobacter* spp.	Negative	Negative	Negative	x
22	*Enterobacter* spp.	Negative	Negative	Negative	x
11Mc	*Enterobacter* spp.	Positive	Negative	Negative	x
18Mc	*Enterobacter* spp.	Positive	Negative	Negative	x
3223	*Enterobacter* spp.	Positive	Negative	Positive	x
23	*Enterobacter* spp.	Positive	Negative	Positive	x
9	*Serratia* spp.	Negative	Negative	Negativo	x
4	*Serratia* spp.	Negative	Negative	Negativo	x
13	*Serratia* spp.	Positive	Negative	Positive	x
14	*Serratia* spp.	Positive	Negative	Positive	x
65	*Staphylococcus aureus*	x	x	x	Negative
68	*Staphylococcus aureus*	x	x	x	Negative
12Mb	Coagulase negative *Staphylococcus*	x	x	x	Negative
14Mb	Coagulase negative *Staphylococcus*	x	x	x	Negative
71	Coagulase negative *Staphylococcus*	x	x	x	Negative
79	Coagulase negative *Staphylococcus*	x	x	x	Negative
2	Coagulase negative *Staphylococcus*	x	x	x	Negative
17	Coagulase negative *Staphylococcus*	x	x	x	Negative
4mb	Coagulase negative *Staphylococcus*	x	x	x	Negative
6mb	Coagulase negative *Staphylococcus*	x	x	x	Negative
81	Coagulase negative *Staphylococcus*	x	x	x	Negative
84	Coagulase negative *Staphylococcus*	x	x	x	Negative
83	Coagulase negative *Staphylococcus*	x	x	x	Negative
86	Coagulase negative *Staphylococcus*	x	x	x	Negative

X: No test performed.

milk of cows with subclinical mastitis were reported (Thompson Bello and Ingram Oporta, 2017). This represents an increase in resistance; therefore, vigilance must be maintained in the use of veterinary antibiotics on livestock farms in the area.

Studies of resistance genes in bacteria in Nicaragua have been carried out on strains isolated from humans related to the hospital environment (Dretler et al., 2020; Sandoval-Rojas et al., 2022); there is no precedent for the detection of resistance genes in bacteria isolated from bovine mastitis.

In the analysis for the identification of genes associated with ESBL in gram-negative bacteria, it was shown that the *blaSHV* gene was the most frequent and the *blaTEM* gene was less frequent. The presence of ESBL in enterobacteria coincides with a study carried out by Timofte et al., where they show that *E. coli* obtained from milk samples from cows with mastitis could harbor *SHV-12* β-lactamases (Timofte et al., 2014). In another study, six ESBL-producing *E. coli* strains were also identified as carriers of the *blaTEM-1* gene; 3 also carried *blaCTX-M* genes and 3 carried *blaSHV* genes (Filioussis *et al.,* 2020). The finding of the study *blaSHV* gene was frequent in *E. coli*, *Enterobacter* and *Serratia* spp. different from what was described by Bradford, where

he points out that *SHV* β-lactamase is found more frequently in *K. pneumoniae*, because this bacterium has a greater ability to survive in the environment (Bradford, 2001). The ESBLs are enzymes that are phenotypically characterized by conferring resistance to penicillins and cephalosporins, including third and fourth generation ones. The frequency of these genes produced by enterobacteria is due to the disproportionate use of these families of antibiotics throughout the field of veterinary medicine, mainly in cattle (Álvarez Almanza, 2010).

Regarding the methacillin genotypic analysis, the *mecA* gene was not identified in any of the *Staphylococcus* spp. isolates. This coincides with the low methacillin frequency found by Monistero et al., who were able to detect the *mecA* gene in only 1.6% (2/120) *Staphylococcus aureus* isolates from different countries (Monistero et al., 2018). The low frequency of *Staphylococcus* spp. with *mecA* gene found in this study differs from those found by Velásquez et al. (2020), who detected the *mecA* gene in 26.7% of *S. aureus* strains, which in turn found resistant strains to cefoxitin that did not carry the *mecA* gene. Similarly, *mecA*-positive strains were sensitive to the same B-lactam antibiotic (Jiménez Velásquez et al., 2020).

In this study, high phenotypic resistance to OX was found in SCN and *S. aureus*; however, the *mecA* gene was not found in any of these strains, which has also been reported in SCN isolated from mastitis, in which 26.67% of the *mecA* negative isolates were found ti be resistant to oxacillin (Nayel et al., 2020). The discrepancy between phenotypic and genotypic tests could be linked to the presence of some other marker associated with methicillin resistance, such as the *mecC* homologous gene, which is located between the *mecA* gene and the AME genes, which could explain why many MRSA strains can be resistant to different antimicrobials (Aqib et al., 2018). It is *mecA* that causes resistance to methicillin in *S. aureus* isolates; however, it has been shown that some methicillin-resistant (MRSA) strains of *S. aureus* do not carry this gene but rather the new resistance gene called *mecC*, which is a homologue of *mecA* (Cikman et al., 2019).

Conclusion

Gram-negative bacteria showed greater resistance to amoxicillin, identifying the antimicrobial resistance genes encoding ESBL (*blaSHV* gene and *blaTEM* gene), with the exception of the *blaCTX* gene, while gram-positive bacteria showed significant resistance to oxacillin. However, the *mecA* gene encoding MRS was not identified.

Conflicts of interest

The authors declare they have no conflicts of interest.

Acknowledgments

We thank the small livestock producers in Western Nicaragua for accepting voluntary participation.

References

1. Adame-Gómez, R., Toribio-Jimenez, J., Castro-Alarcón, N., Talavera-Alarcón, K., Flores-Gavilan, J., Pineda-Rodríguez, S.-A., Ramírez-Peralta, A., 2021. Diversidad genética y factores de virulencia de cepas de *Staphylococcus aureus* aisladas de la piel de ubre bovina. *Revista mexicana de ciencias pecuarias*, 12, 3 : 655-680. DOI : 10.22319/rmcp.v12i3.5646.
2. Allen, H. K., Stanton, T. B., 2014. Altered Egos: Antibiotic Effects on Food Animal Microbiomes. *Annual Review of Microbiology*, 68, 1 : 297-315. DOI : 10.1146/annurev-micro-091213-113052.
3. Álvarez Almanza, D., 2010. Identificación de betalactamasas de espectro extendido en enterobacterias. *Revista Habanera de Ciencias Médicas*, 9, 4 : 516-524.
4. Aqib, A. I., Ijaz, M., Farooqi, S. H., Ahmed, R., Shoaib, M., Ali, M. M., Mehmood, K., Zhang, H., 2018. Emerging discrepancies in conventional and molecular epidemiology of methicillin resistant *Staphylococcus aureus* isolated from bovine milk. *Microbial Pathogenesis*, 116 : 38-43. DOI : 10.1016/j.micpath.2018.01.005.
5. Arbab, S., Ullah, H., Wang, W., Li, K., Akbar, A., Zhang, J., 2021. Isolation and Identification of Infection-Causing Bacteria in Dairy Animals and Determination of Their Antibiogram. *Journal of Food Quality*, 2021 : e2958304. DOI : 10.1155/2021/2958304.
6. Artemyeva, O., Nikanova, D. A., Kolodina, E., Brovko, F., Zinovieva, N. A., 2020. PSVIII-12 Estimations of treatment schemes efficiency of bacterial mastitis in dairy cow and antibiotic resistance of identified pathogens. *Journal of Animal Science*, 98, Suppl 4 : 256-257. DOI : 10.1093/jas/skaa278.463.
7. Asir, J., Nair, S., Devi, S., Prashanth, K., Saranathan, R., Kanungo, R., 2015. Simultaneous gut colonisation and infection by ESBL-producing *Escherichia coli* in hospitalised patients. *The Australasian Medical Journal*, 8, 6 : 200-207. DOI : 10.4066/AMJ.2015.2358.
8. Bradford, P. A., 2001. Extended-Spectrum β-Lactamases in the 21st Century: Characterization, Epidemiology, and Detection of This Important Resistance Threat. *Clinical Microbiology Reviews*, 14, 4 : 933-951. DOI : 10.1128/CMR.14.4.933-951.2001.
9. Chavarría Narváez, S. E., Meléndez Martínez, L. M., 2012. Identiicación de agentes bacterianos implicados en mastitis subclínica y perfil de resistencia en vacas que abastecen los Centros de Acopio Tecuaname y El Sauce en el Departamento de León, septiembre, noviembre 2011. Thesis.
10. Cikman, A., Aydin, M., Gulhan, B., Karakecili, F., Kurtoglu, M. G., Yuksekkaya, S., Parlak, M., Gultepe, B. S., Cicek, A. C., Bilman, F. B., Ciftci, I. H., Kara, M., Atmaca, S., Ozekinci, T., 2019. Absence of the mecC gene in methicillin-resistant *Staphylococcus aureus* isolated from various clinical samples: The first multi-centered study in Turkey. *Journal of Infection and Public Health*, 12, 4 : 528-533. DOI : 10.1016/j.jiph.2019.01.063.
11. Das, A., Guha, C., Biswas, U., Jana, P. S., Chatterjee, A., Samanta, I., 2017. Detection of emerging antibiotic resistance in bacteria isolated from subclinical mastitis in cattle in West Bengal. *Veterinary World*, 10, 5 : 517-520. DOI : 10.14202/vetworld.2017.517-520.
12. Dretler, A. W., Avila, J., Sandoval Lira, L., García Rener, M., Burger-Calderon, R., Hargita, M. N., Page, A. M., Waggoner, J. J., Satola, S. W., 2020. High Rates of New Delhi Metallo-β-Lactamase Carbapenemase Genes in Multi-Drug Resistant Gram-Negative Bacteria in Nicaragua. *The American Journal of Tropical Medicine and Hygiene*, 102, 2 : 384-387. DOI : 10.4269/ajtmh.19-0561.

13. Filioussis, G., Kachrimanidou, M., Christodoulopoulos, G., Kyritsi, M., Hadjichristodoulou, C., Adamopoulou, M., Tzivara, A., Kritas, S. K., Grinberg, A., 2020. Short communication: Bovine mastitis caused by a multidrug-resistant, mcr-1-positive (colistin-resistant), extended-spectrum β-lactamase–producing *Escherichia coli* clone on a Greek dairy farm. *Journal of Dairy Science*, 103, 1 : 852-857. DOI : 10.3168/jds.2019-17320.
14. Giono-Cerezo, S., Santos-Preciado, J. I., Rayo Morfín-Otero, M. del, Torres-López, F. J., Alcántar-Curiel, M. D., 2020. Resistencia antimicrobiana. Importancia y esfuerzos por contenerla. *Gaceta médica de México*, 156, 2 : 172-180. DOI : 10.24875/gmm.20005624.
15. Ibrahim, M., Ahmad, F., Yaqub, B., Ramzan, A., Imran, A., Afzaal, M., Mirza, S. A., Mazhar, I., Younus, M., Akram, Q., Ali Taseer, M. S., Ahmad, A., Ahmed, S., 2020. Chapter 4 - Current trends of antimicrobials used in food animals and aquaculture. *In* Hashmi, M. Z. (dir.). *Antibiotics and Antimicrobial Resistance Genes in the Environment*. Advances in Environmental Pollution Research series. Elsevier. DOI : 10.1016/B978-0-12-818882-8.00004-8.
16. Jiménez Velásquez, S. del C., Torres Higuera, L. D., Parra Arango, J. L., Rodríguez Bautista, J. L., García Castro, F. E., Patiño Burbano, R. E., 2020. Perfil de resistencia antimicrobiana en aislamientos de *Staphylococcus* spp. obtenidos de leche bovina en Colombia. *Revista Argentina de Microbiología*, 52, 2 : 121-130. DOI : 10.1016/j.ram.2019.05.004.
17. Liapi, M., Botsaris, G., Arsenoglou, C., Markantonis, N., Michael, C., Antoniou, A., Pipis, C., 2021. Rapid Detection of *Mycoplasma bovis, Staphylococcus aureus* and *Streptococcus agalactiae* in Cattle Bulk Tank Milk in Cyprus and Relations with Somatic Cell Counts. *Pathogens*, 10, 7 : 841. DOI : 10.3390/pathogens10070841.
18. Lima, L. M., Silva, B. N. M. da, Barbosa, G., Barreiro, E. J., 2020. β-lactam antibiotics: An overview from a medicinal chemistry perspective. *European Journal of Medicinal Chemistry*, 208 : 112829. DOI : 10.1016/j.ejmech.2020.112829.
19. Monistero, V., Graber, H. U., Pollera, C., Cremonesi, P., Castiglioni, B., Bottini, E., Ceballos-Marquez, A., Lasso-Rojas, L., Kroemker, V., Wente, N., Petzer, I.-M., Santisteban, C., Runyan, J., Veiga dos Santos, M., Alves, B. G., Piccinini, R., Bronzo, V., Abbassi, M. S., Said, M. B., Moroni, P., 2018. *Staphylococcus aureus* Isolates from Bovine Mastitis in Eight Countries: Genotypes, Detection of Genes Encoding Different Toxins and Other Virulence Genes. *Toxins*, 10, 6 : 247. DOI : 10.3390/toxins10060247.
20. Nayel, M., Abdeen, E., Basiony, E., Younis, G., Elnahriry, S., 2020. Phenotypic and Genotypic Characterization of Methicillin-Resistant Coagulase-Negative Staphylococci as an Etiological Agent of Bovine Mastitis in Egypt. *Journal of Current Veterinary Research*, 2, 2 : 31-40. DOI : 10.21608/jcvr.2020.121500.
21. Quiñones Pérez, D., 2017. Resistencia antimicrobiana: evolución y perspectivas actuales ante el enfoque « Una salud ». *Revista Cubana de Medicina Tropical*, 69, 3 : 1-17.
22. Ríos Padilla, C. A., 2021. Resistencia A Antibióticos En Bacterias Causantes De Mastitis Bovina.
23. Rivera Varela, M. I., Tórrez Cálix, M. A., 2012. Resistencia antimicrobiana de bacterias aisladas en leche de vaca con mastitis subclínica, en fincas que abastecen los Centros de Acopio de Achuapa y Larreynaga; septiembre - noviembre 2011. Thesis.
24. Ruegg, P. L., 2017. A 100-Year Review: Mastitis detection, management, and prevention. *Journal of Dairy Science*, 100, 12 : 10381-10397. DOI : 10.3168/jds.2017-13023.
25. Saeed, S. I., Mergani, A., Aklilu, E., Kamaruzzaman, N. F., 2022. Antimicrobial Peptides: Bringing Solution to the Rising Threats of Antimicrobial Resistance in Livestock. *Frontiers in Veterinary Science*, 9.
26. Sandoval-Rojas, K. A., Maliaños-Miranda, R., Marenco-Avilés, S., Castillo-Cano, M. A., Porras-Cortés, G. D., 2022. 1387. Prevalence and Risk Factors for Bacterial Resistance in Diabetic Foot Infections at a Hospital in Nicaragua. *Open Forum Infectious Diseases*, 9, Supplement_2 : ofac492.1216. DOI : 10.1093/ofid/ofac492.1216.
27. Santiago, J. M., Andrade, L. H., Ballesteros, E. A., Díaz, L. M. C., Valenzuela, D. H., 2012. Aislamiento bacteriano en bovinos de doble propósito con mastitis subclínica, en la costa de Guerrero, México. *REDVET. Revista Electrónica de Veterinaria*, 13, 7 : 1-11.
28. Thompson Bello, B. A., Ingram Oporta, A. D., 2017. Caracterización microbiológica de la leche proveniente de vacas con mastitis subclínica en fincas de doble propósito del departamento de León afiliadas a la asociación de ganaderos de león (ASOGAL) durante el período septiembre-octubre de 2016. Thesis.
29. Timofte, D., Maciuca, I. E., Evans, N. J., Williams, H., Wattret, A., Fick, J. C., Williams, N. J., 2014. Detection and Molecular Characterization of *Escherichia coli* CTX-M-15 and *Klebsiella pneumoniae* SHV-12 β-Lactamases from Bovine Mastitis Isolates in the United Kingdom. *Antimicrobial Agents and Chemotherapy*, 58, 2 : 789-794. DOI : 10.1128/AAC.00752-13.
30. Uddin, M. E., Akter, T., Sultana, P., Hasan, M. I., Lubna, M. A., Monem, H. A., Parvez, M. A. K., Nahar, S., Khan, M. S., 2018. Isolation, Identification and Antimicrobial Susceptibility Profile Analysis of *Vibrio cholerae* O1 from Stool Samples of Bangladesh. *Advances in Microbiology*, 8, 3 : 188-196. DOI : 10.4236/aim.2018.83013.
31. Vannuffel, P., Laterre, P.-F., Bouyer, M., Gigi, J., Vandercam, B., Reynaert, M., Gala, J.-L., 1998. Rapid and Specific Molecular Identification of Methicillin-Resistant *Staphylococcus aureus* in Endotracheal Aspirates from Mechanically Ventilated Patients. *Journal of Clinical Microbiology*, 36, 8 : 2366-2368.

Received 20 August 2023
Accepted 28 November 2023