RESUMO.- [Caracterização proteômica de Staphylococcus spp. oriundos de mastite caprina e avaliação fenogenotípica da resistência aos beta-lactâmicos.] A mastite ocupa lugar de destaque entre as doenças que acometem o rebanho leiteiro, em virtude de problemas econômicos e de saúde pública. Staphylococcus spp. são os agentes infecciosos mais envolvidos na etiologia das mastites caprinas, principalmente Staphylococcus coagulase negativo. Dezessete isolados de Staphylococcus spp. foram obtidos a partir de mastite subclínica. Todos os isolados foram caracterizados por MALDI-TOF MS, sendo 47,36% (9/19) identificados como S. epidermidis, 15,78% (3/19) como S. warneri, 10,52% (2/19) como S. aureus e S. caprae e 5,26% (1/19) tanto para S. lugdunensis, como para S. simulans e S. cohnii. Todos os isolados caracterizados pelo MALDI-TOF foram submetidos a polymerase chain reaction (PCR) para o gene 16S rRNA de Staphylococcus spp. para confirmar a espécie. Após determinar a espécie, foram realizados testes de detecção de resistência fenotípica aos beta-lactâmicos por disco de difusão, agar “screen” oxacilina e microdiluição (MIC) de cefoxitina. O teste de disco de difusão mostrou uma força de 58% (11/19) para penicilina G, 26,31% (5/19) para cefoxitina e 26,31% (5/19) para oxacilina. Todos os isolados foram suscetíveis à amoxicilina + clavulânico e agar “screen” oxacilina. Na microdiluição, 63,15% (12/19) dos microrganismos foram cefoxitina resistentes (MIC >4.0µg/ml). Em seguida, foram detectados os genes meca e seus reguladores mecl e mecR1, mecC e blaz. Dois dos isolados de S. epidermidis apresentaram a presença do gene meca. Todos os isolados foram negativos para os genes meca variante, mecl, mecR1, mecC e blaz. Estes dados reforçam a importância deste grupo de microrganismos na etiologia da mastite subclínica e abrem perspectivas para futuros estudos para investigar a epidemiologia da doença.

INDEX TERMS: Proteomics, Staphylococcus spp., goat, mastitis, resistance, beta-lactamics.
a reação em cadeia da polimerase (PCR) para o gene 16rRNA de *Staphylococcus* spp. para a confirmação do gênero. Após a determinação da espécie, foram realizadas as provas para a detecção fenotípica de resistência aos beta-lactâmicos: difusão em disco simples de oxacilina, cefoxitina, penicilina G e amoxicilina +ácido clavulânico, ágar “screen” de oxacilina e microdiluição em caldo (MIC) de cefoxitina. O teste de difusão em disco demonstrou resistência de 58% (11/19) para penicilina G, 26,31% (5/19) para cefoxitina e 26,31% (5/19) para oxacilina. Todas as amostras foram sensíveis a amoxicilina + ácido clavulânico e ao ágar “screen” de oxacilina. Pelo MIC, 63,15% (12/19) das amostras foram resistentes a cefoxitina (MIC > 4,0µg/ml). Em seguida os isolados foram submetidos a detecção dos genes de resistência mecA e seus reguladores (mec e mecRl), mecC e blaZ. Duas amostras de *S. epidermidis* apresentaram o gene mecA. Todos os isolados foram negativos para a variante do gene mecA, mecC, mecRl, mecC e blaZ. Tais achados reforçam a importância deste grupo de microrganismos na etiologia da mastite subclínica em caprinos e abrem perspectivas para futuras pesquisas para a investigação da epidemiologia da doença.

**TERMS DE INDEXAÇÃO:** Proteômica, *Staphylococcus* spp., mastite, caprinos, resistência a beta-lactâmicos.

**INTRODUCTION**

Mastitis, the inflammatory process of the mammary gland, is considered to be one of the major diseases affecting herds of dairy goats. This disease is associated with losses in production and economic losses to the farmer and industry (Almeida et al. 2013). Bacteria of the genus *Staphylococcus* spp. are among the main etiological agents of caprine mastitis and are frequently resistant to antimicrobials, especially beta-lactams, thus limiting the choice of antibiotic for the treatment of infections caused by this agent (Garino Junior et al. 2011, Almeida et al. 2013, Gomes & Henriques 2016).

Recent changes in the taxonomy of some species of the genus *Staphylococcus* have made species differentiation more difficult, especially in Veterinary Medicine. Thus, the use of molecular markers associated with the phenotypic diagnosis allows a more reliable and precise characterization (Motta et al. 2014).

The proteomic analysis by MALDI-TOF mass spectrometry has gained prominence due to advantages such as cost-benefit, fast and accurate results (Bannoehr & Guardabassi 2012), allowing the identification of different microorganisms at the species level, with a minimum sample amount, low cost, insignificant levels of chemical and biological waste generated and time to obtain extraordinary short results (Cherkouei et al. 2010, Alatoom et al. 2011, Carbonnelle et al. 2011).

*Staphylococcal* resistance to beta-lactam antibiotics is primarily due to two mechanisms: the production of extracellular beta-lactamase enzyme encoded by the *blaZ* gene and the production of PBP2a or PBP2, a low-affinity penicillin-binding protein encoded by *mecA* gene. The *blaZ* gene is usually located in plasmids and may also be chromosomal (Kuroda et al. 2001, Lowy 2003). The *mecA* gene is inserted into a mobile genetic element called Staphylococcal cassette chromosome mec (SCCmec), composed of several essential genetic elements: the mec complex, composed of the pathogenic island IS431, the mecA genes and their mecI and mecR1 regulators, and the complex ccr (Chromosome Recombinases Cassette), characterized by the presence of genes encoding recombinase (Weller 2000, Ma et al. 2002).

There are few studies conducted to investigate the profile of sensitivity and verification of resistance genes to beta-lactam antibiotics in *Staphylococcus* spp. isolated from caprine mastitis. Thus, this work aimed to characterize isolates using the MALDI-TOF technique and to identify the phenotypic profile of beta-lactam resistance in *Staphylococcus* spp.

**MATERIALS AND METHODS**

A total of 140 goat milk samples were collected from different cities of Rio de Janeiro, among them Seropédica, Niterói, Feitibusgo, Teresópolis, Paraíba do Sul, São Gonçalo, Engenheiro Paulo de Frontin, Tanguá and Valença. From these 140 samples, 19 isolates of *Staphylococcus* spp. were obtained from subclinical caprine mastites. These materials were stored in Brain Heart Infusion (BHI) broth with 10% glycerol at -80°C. The respective article obtained the number 593 from the Ethics Commission on Animal Use of the “Universidade Federal Fluminense” (UFF).

**Identification by MALDI-TOF MS** (Seng et al. 2010). The isolates were evaluated by the Matrix-Assisted Laser Ionization/ Desorption Flight Time (MALDI-TOF) technique. For the preparation of the samples, the isolates were cultured on BHI agar at 37°C for 24 hours. Each bacterial culture was transferred to the microplate (96 MSP, Bruker-Billerica, USA) and the bacterial pellet; sufficient lysis solution (70% formic acid, Sigma-Aldrich) was added to cover it. Then 1μl of matrix solution (alpha-cyano-4-hydroxy cinnamic acid diluted in 50% acetonitrile, and 2.5% trifluoroacetic acid, Sigma-Aldrich) was used to cover the bacterial extract, which was ready to be processed. The spectra of each sample were generated in a mass spectrometer (MALDI-TOF LT MicroflexBruker, Bruker®) equipped with a 337nm nitrogen laser in the linear mode controlled by the FlexControl 3.3 program (Bruker®). The spectra were collected in the mass range between 2,000-20,000m/s and later analyzed by the MALDI Biotype 2.0 program (Bruker®), with standardized configurations for bacterial identification. The results ranged from zero to three, and the higher the value, the more reliable the identification. In this study, those that presented values equal to or greater than two were considered as an acceptable identification.

**Extraction of total bacterial DNA** (Gregory et al. 2009). The 19 isolates studied were replicated in BHI agar (HIMEDIA) and submitted to DNA extraction for genotypic analyses. To extract the total DNA, each isolate was grown in 5 ml of BHI broth at 37°C for 12-16 hours at 150rpm. Then 1.5ml of the culture was transferred to 1.5ml microtubes and centrifuged for 5 minutes at 1239g, and the supernatant was discarded, with three replicates. Cells were resuspended in 600μl of extraction solution (200mM Tris-HCl pH 8.0, 25mM EDTA pH 8.0, 1% SDS, 25mM NaCl) and vortexed, followed by incubation at 65°C for 30 minutes. After the stipulated time, the tubes were cooled to room temperature and 600μl of phenol: chloroform: isoamyl alcohol [1:1 (24: 1)] was added, followed by homogenization for 2 minutes and centrifugation at 145rpm for 10 minutes. The upper phase was transferred to a new microtube (approximately 400μl), and 2 volumes of 100% ice-cold ethanol (PROQUIMOS) was added, followed by incubation at 20°C for 2 or 12 hours for precipitation of the DNA. Subsequently, the microtubes were centrifuged at 14549g for 30 minutes, the supernatant was discarded, and the pellet washed with 70% ethanol (approximately 500µL). After drying at room temperature in an exhaust hood, the pellets were resuspended in 30µl of ultra-pure water (QUATRO G)
and stored at -20°C. The DNA concentration estimation was done by comparison with the band intensity standard of the Lambda marker (λ) (Promega) at concentrations of 25 and 50ng. Quality was determined by the absence of traces along the gel.

**Polymerase Chain Reaction (PCR).** The concentrations used in all PCR reactions were 1X Buffer (10mM Tris-HCl, 50mM KCl, and 0.1% Triton X-100, 2.0mM MgCl2, pH 9.0), 0.3μM of each primer; 0.2mM dNTP (FERMENTAS), 1μL of Dream Taq™ Green DNA Polymerase (FERMENTAS) and milli-Q water to complete a total reaction volume of 20μL and about 20ng of total DNA. The fragments were evaluated by 1.5% agarose gel electrophoresis, containing SYBR Green dye (INVITROGEN) and visualized by the L-PIX EX (LoccusBiotechnology) image capture system. The genotypic characterization was performed by amplification using specific primers for the genus *Staphylococcus* (Zhang et al. 2004, Jaffe et al. 2000) (Table 1). The samples were applied with 1μL of SYBR Green (INVITROGEN) and diluted in the 0.8% agarose gel and submitted to electrophoresis. The gel was then developed with SYBR Green (INVITROGEN) by the L-PIX EX (LoccusBiotechnology) image capture system.

**Phenotypic detection of beta-lactam antimicrobial resistance.** Resistance phenotype detection tests were performed according to the standards established by the Clinical Laboratory Standard Institute (CLSI 2018). Disc diffusion technique was performed with Cefoxitin (30μg), Oxacillin (1μg), Penicillin G (10UI) and Amoxicillin + Clavulanic Acid (10μg). For the “Screen” Agar, the isolates of *Staphylococcus* spp. were seeded in culture medium with a final concentration of 6μg/mL oxacillin, and after 24 hours incubation at 37°C, any grown colony was considered resistant (CLSI 2018). Broth microdilution method (MIC) with antimicrobial cefoxitin was used to determine oxacillin resistance mediated by the *mecA* gene (CLSI 2018).

**Detection of oxacillin resistance genes from *Staphylococcus* spp.** PCR was performed to amplify the genes: *mecA* (Murakami et al. 1991), *mecA* variant (Melo et al. 2014), *mecI* (Oliveira & Lencastre 2002), *mecRI* and *blaZ* (Rosato et al. 2003) (Table 2).

### RESULTS AND DISCUSSION

The MALDI-TOF technique identified 47.36% (9/19) as *Staphylococcus epidermidis*, 15.78% (3/19) as *Staphylococcus warneri*, 10.52% (2/19) as *Staphylococcus caprae* and *Staphylococcus aureus* and 5.26% (1/19) as *Staphylococcus lugdunensis*, *Staphylococcus simulans* and *Staphylococcus schoenii*. This result corroborates with several studies on the etiology of caprine mastitis in Brazil that point to coagulase-negative *Staphylococcus* as the most frequent microorganisms (Langoni et al. 2006, Peixoto et al. 2010, Almeida et al. 2013, Cavalcante et al. 2013, Martins et al. 2017, Lima et al. 2018). Besides, all 19 samples were submitted to 16s rRNA gene PCR for confirmation of the species *Staphylococcus* spp.

The phenotypic resistance to oxacillin is extremely variable and dependent on *mecA* gene expression. This variability is recognized as phenotypic heteroresistance and is characterized by the fact that all heterogeneously resistant bacterial populations carry the *mecA* gene, the genotype marker of resistance, but not all phenotypically express their resistance in the same way (Cauwelier et al. 2004).

Two isolates of *S. epidermidis* were positive for the *mecA* gene as proposed by Murakami et al. (1991) (Table 1) similar to a study by Martins et al. (2017) and Coimbra-e-Souza et al. (2019). All the isolates studied were negative for the *mecA* gene as proposed by Melo et al. (2014), who characterized a variant of the *mecA* gene in bovine mastitis isolates. No positive isolates were found for the *mecI* and *mecRI* regulators, homologous to the *mecA*, *mecC* gene and the *blaZ* gene.

During some studies with isolates of *Staphylococcus* spp. from subclinical caprine mastitis in Brazil by Franca et al. (2012) and Peixoto et al. (2013), oxacillin resistance of 15.8% and 54.5%, respectively, were observed in the disc diffusion test, but in none of them was it positive for the *mecA* gene. However, 40.2% and 33.3%, respectively, of the same isolates contained the gene *blaZ*. For MIC, 63.15% (12/19) of

### Table 1. Primers used for identification of *Staphylococcus* spp.

| Gene/Fragment size | Sequence of primers (5’-3’) | Reference | Cycle* |
|--------------------|-----------------------------|-----------|--------|
| 16S rRNA/(756pb)    | AAC TCT GTT ATT AGG GAA GAA CA CCA CCT TCC TCC GGT GTT TGA CC | Zhang et al. (2004) | (94°C 40 s, 64°C 1 min, 72°C 1 min 12 s) x 30 |
|                    |                             | Jaffe et al. (2000) |        |

### Table 2. Primers used for amplification of resistance genes

| Gene/Fragment size | Sequence of primers (5’-3’) | Cycle* |
|--------------------|-----------------------------|--------|
| *mecA* (Murakami)  | AAA ATC GAT GGT AAA GGT TGG C | (94°C 1 min, 55°C 1 min, 72°C 1 min) x 30 and 72°C 10 min |
| (533 pb)           | AGT TCT GCA GTA CGG GAT TGG C |        |
| *mecA* (Melo)      | CAG GCA TGC AGA AAA ATC AA  | 95°C 5 min (94°C 1 min, 50°C 1 min, 72°C 1 min) x 30 and 72°C 10 min |
| (809 pb)           | TTG ACT GCA ACC AGG TGA TG  |        |
| *mecI*             | ATC AAG ACT TGG ATT CAG GC  | 94°C 4 min (94°C 30 s, 53°C 30 s, 72°C 1 min) x 30 and 72°C -4min |
| (209 pb)           | GCG GTT TCA ATT CAC TTT GC  |        |
| *mecRI*            | CGA AAC CGG ACA ACT AC  | 95°C 2 min (95°C 1 min, 53°C 1 min, 72°C 1 min) x 30 and 72°C 7 min |
| (234 pb)           | CGT GTC AGA TAC ATT TG  |        |
| *mecC*             | GAA AAA AGG GGT TAG AAC GCC TC | 94°C 15 min (94°C 30 s, 59°C 1 min, 72°C 2 min) x 30 and 72°C 10 min |
| (138 pb)           | GAA GAT CTT TTT GGT TTT CAG C  |        |
| *mecC*             | GAA AAA AGG TAG AAC GCC TC  | 94°C 15 min (94°C 30 s, 50°C 1 min, 72°C 1 min) x 35 and 72°C 10 min |
| (718 pb)           | CCT GAA TCC [W] GGT AAT ATT ATC  |        |
| *blaZ*             | TAC AAC GGT ATT ATC GGA GG  | 94°C 5 min (94°C 30 s, 58°C 30 s, 72°C 30 s) x 35 and 72°C 5 min |
| (861 pb)           | CAT TAC ACT CTT GGC GGT TT  |        |
the samples were resistant to cefoxitin, with 52.63% (10/19) with MIC >8.0μg/ml and 10.52% (2/19) with MIC >16μg/ml (Table 3). For MIC, the same isolate of *S. epidermidis* showed resistance to cefoxitin, and also produced resistance to oxacillin and penicillin G in the disk diffusion test, in addition to the positivity for the *meca* gene. However, in the remaining isolates that showed resistance in this test, the *meca* gene was not detected. This fact can be justified by the loss of the gene and the use of another form of resistance. This study corroborates with the work of Martins et al. (2017) and Lima et al. (2018) who reported a resistance profile to penicillin and oxacillin, antimicrobials of the beta-lactam class.

Van Griethuysen et al. (2005) conducted a study that demonstrated the loss of the *meca* gene in isolates kept under freezing. In this study, the isolates were maintained in this condition until molecular analysis. This result indicates that, despite its high specificity, and the fact that the technique for molecular genotype characterization is considered definitive to prove the presence of the gene, the sensitivity of the gene may vary with the conservation of the samples.

The identification of the *meca* gene in two isolates of *Staphylococcus* spp reinforces its epidemiological importance and significance in caprine mastitis. These strains tend to impair treatment, even when they are secondary etiological agents, increasing the tendency to chronicity of the disease and possibilities of dissemination among animals. The carriage of antimicrobial resistance genes by these microorganisms in a dairy environment is already a potential risk to animal health and public health, due to interspecies genetic transfer, including *S. aureus*, and direct transmission of resistant pathogens to humans.

Other classes of PBPs (e.g., PBP3 and PBP4) may be related to beta-lactam resistance. Memmi et al. (2008) reported that PBP4 is the key element for beta-lactam resistance in community-acquired methicillin-resistant *S. aureus* strains (CA-MRSA), and that PBP2a, the *meca* gene product, is not a determinant of resistance to oxacillin.

The high rate of phenotypic resistance and the low genotypic detection in *Staphylococcus* spp. isolates may be related to changes in genes triggered by mutations, phages, plasmids and transposons. Thus, the multiplicity of factors associated with beta-lactam resistance requires careful investigation. The detection of different genetic markers for resistance, as well as the study of the regulation of the gene expression of the mec system, allows to deepen the understanding of the real value of its detection in the prediction of the resistance to antimicrobial beta-lactams, as recommended by CLSI.

**CONCLUSION**

The study detected *Staphylococcus* spp. as a causative agent of subclinical mastitis in goats. Among *Staphylococcus* coagulase negative, the highest frequency found was for *S. epidermidis* and among *Staphylococcus* coagulase positive, *S. aureus* was confirmed as a prevalent species. Isolates of *Staphylococcus* spp. showed high resistance to penicillin, being the beta-lactam and beta-lactamase inhibitor the most effective antibiotic.

### Table 3. Phenogenotypic profile of beta-lactam resistance in *Staphylococcus* spp. isolates identified by MALDI-TOF and confirmed by PCR in isolates of goat’s milk with mastitis

| Identification by MALDI-TOF | CFO | OXA | PEN | AMC | MIC CFO (µg/ml) | meca (Murakami) |
|-----------------------------|-----|-----|-----|-----|----------------|-----------------|
| *S. epidermidis*            | S   | S   | R   | S   | S              | -               |
| *S. warneri*               | S   | S   | S   | S   | R = 8.0        | -               |
| *S. lugdunensis*            | S   | S   | S   | S   | R = 8.0        | -               |
| *S. epidermidis*            | S   | S   | R   | S   | S              | -               |
| *S. epidermidis*            | S   | R   | R   | S   | S              | -               |
| *S. epidermidis*            | S   | S   | R   | S   | R = 8.0        | -               |
| *S. warneri*               | R   | R   | S   | S   | R = 8.0        | -               |
| *S. epidermidis*            | S   | S   | R   | S   | S              | -               |
| *S. warneri*               | S   | S   | S   | S   | R = 8.0        | -               |
| *S. simulans*              | S   | S   | S   | S   | R = 8.0        | -               |
| *S. epidermidis*            | S   | R   | R   | S   | R = 8.0        | +               |
| *S. caprae*                | S   | S   | S   | S   | S              | -               |
| *S. caprae*                | S   | S   | S   | S   | S              | -               |
| *S. cohnii*                | R   | S   | R   | S   | R = 16.0       | -               |
| *S. epidermidis*            | S   | S   | R   | S   | R = 16.0       | +               |
| *S. aureus*                | R   | S   | S   | S   | R = 8.0        | -               |
| *S. aureus*                | R   | R   | R   | S   | R = 8.0        | -               |

CFO = cefoxitin, OXA = oxacillin, PEN = penicillin G, AMC = amoxicillin with clavulanic acid, MIC CFO = microdilution in broth with cefoxitin, R = resistant, S = sensitive; Parameters according to CLSI 2018: CFO and OXA = S≥22/ R≥21, PEN = S≥29/R≥28, AMC = S≥28/R≥27, MIC cefoxitin = S≤4/R≥8.
The low prevalence of mecA in beta-lactam resistant isolates reassert further molecular studies to detect the mechanisms involved in resistance.

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REFERENCES

Alatoom A.A., Cunningham S. A., Ilde S.M., Mandrekar J. & Patel R. 2011. Comparison of direct colony method versus extraction method for identification of gram-positive cocci by use of Bruker Biotyper Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry. J. Clin. Microbiol. 49(8):2868-2873. <https://dx.doi.org/10.1128/JCM.00566-11> 

Almeida J.F, Aquino M.H.C., Magalhães H., Nascimento E.R., Pereira V.L.A., Silva N.S., Barros C.G.G. Bannoehr J. & Guardabassi L. 2012. Staphylococcus pseudintermedius. Arq. Inst. Biológico, São Paulo, 78(1):103-107. <https://dx.doi.org/10.1590/S0100-736X2012000100012> 

Carboneille B., Gordts B., Descheemaecker P. & Van Landuyt H. 2004. Evaluation of a disk diffusion method with cefoxitin (30μg) for detection of methicillin-resistant Staphylococcus aureus. Antimicrob. Agents Chemother. 48(4):1169-1175. <https://dx.doi.org/10.1128/JCM.01881-09> 

Cherkaoui A., Hibbs J. & Emonet S. 2010. Comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry methods with conventional phenotypic identification for routine bacterial speciation. J. Clin. Microbiol. 48(4):1169-1175. <https://dx.doi.org/10.1128/JCM.01881-09> 

CLSI 2018. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. Approved Standards. 11th ed. Clinical and Laboratory Standards Institute, Wayne, PA. 

Goimes F. & Henriques M. 2016. Control of bovine mastitis: old and recent therapeutic approaches. Curr. Microbiol. 72(4):377-382. <https://dx.doi.org/10.1007/s00284-015-0958-8> 

Jaffe R.I., Lane J.D., Albury S.V. & Niemeyer D.M. 2000. Rapid extraction and from direct identification in clinical samples of methicillin-resistant staphylococci using the PCR. J. Clin. Microbiol. 38(9):3407-3412. <https://dx.doi.org/10.1128/JCM.38.9.3407-3412.2000> 

Kuroda M., Ohba T., Uchiyama L., Baba T., Yuzawa H.L., Kobayashi L., Cui A., Oguchi K., Aoki Y. & Nagai J. 2001. Plasmid-mediated resistance to vancomycin and teicoplanin in Enterococcus faecium. New Engl. J. Med. 319:157-161. 

Langoni H., Domingues P.F. & Baldini S. 2006. Mastite caprina: seus agentes e sensibilidade frente a antimicrobianos. Revsa Bras. Ciênc. Vet. 15(1):51-54. 

Oliveira D.C. & Lencastre H. 2002. Multiple PCR strategy for rapid identification of structural types and variants of the mec element in Methicillin-Resistant Staphylococcus aureus. Antimicrob. Agents Chemother. 46(7):2155-2161. <https://dx.doi.org/10.1128/AAC.46.7.2155-2161.2002> 

Lowy Ed. 2003. Antimicrobial resistance: The example of Staphylococcus aureus. J. Clin. Invest. 111(9):1265-1273. <https://dx.doi.org/10.1172/JCI18535> 

Ma X.X., Ito T., Tiensasitorn C., Jamklang M., Chongtrakool P., Boyle-Vavra S., Daum R.S. & Hiramatsu K. 2002. Novel type of staphylococcal cassette chromosome mec identified in community-acquired methicillin resistant Staphylococcus aureus strains. Antimicrob. Agents Chemother. 46(4):1147-1152. <https://dx.doi.org/10.1128/AAC.46.4.1147-1152.2002> 

Mota R.A. & Costa M.M. 2012. Antimicrobial resistance of Staphylococcus spp. isolated from milk of healthy sheep and animals with subclinical mastitis. J. Dairy Sci. 100(3):2184-2195. <https://dx.doi.org/10.3168/jds.2011-4949> 

Melo D.A., Coelho I.S., Motta C.C., Botelho L.A.B., Moreira B.M., Oliveira D.C & Lencastre H. 2002. Multiplex PCR strategy for rapid identification of methicillin-resistant Staphylococcus aureus isolates in bovine milk. J. Dairy Sci. 85(10):2966-2971. <https://dx.doi.org/10.3168/jds.2002-15723> 

Peixoto R.M., Mota R.A. & Costa M.M. 2010. Mastite em pequenos ruminantes no Brasil. Pesq. Vet. Bras. 30(3):754-762. <https://dx.doi.org/10.1590/S0100-736X2010000900008> 

Proteomics characterization of Staphylococcus spp. from goat mastitis and phenogeno-typical assessment of resistance to beta-lactamics
Peixoto R.M., Peixoto R.M, Alves A.P.P., Peixoto L.J.S., Reges A.M. & Costa M.M. 2013. Genes de resistência a antimicrobianos e produção de biofilme em Staphylococcus spp. isolados de caprinos leiteiros. Vet. Zootec. 20(2 Supl.1):343-344.

Rosato A.E., Kreiswirth B.N., Graig W.A., Eisner W., Climo M.W. & Aecher G.L. 2003. mecA-blaZ corepressors in clinical Staphylococcus aureus isolates. Antimicrob. Agents Chemother. 47(4):1460-1463. <https://dx.doi.org/10.1128/aac.47.4.1460-1463.2003> <PMid:12654694>

Seng P., Rolain J.M., Fournier P.E., La Scola B., Drancourt M. & Raoult D. 2010. MALDI-TOF-mass spectrometry applications in clinical microbiology. Future Microbiol. 5(11):1733-1754. <https://dx.doi.org/10.2217/fmb.10.127> <PMid:21133692>

Van Griethuysen A., Van Loo I., Van Belkum A., Vandembroucke-Grauls C., Wannet W., Van Keulen P. & Kluytmans J. 2005. Loss of the mecA gene during storage of methicillin-resistant Staphylococcus aureus strains. J. Clin. Microbiol. 43(3):1361-1365. <https://dx.doi.org/10.1128/JCM.43.3.1361-1365.2005> <PMid:15750108>

Weller T.M.A. 2000. The distribution of mecA, mecR1 and mecI and sequence analysis of mecI and the mec promoter region in staphylococci expressing resistance to methicillin. J. Antimicrob. Chemother. 43(1):15-22. <https://dx.doi.org/10.1093/jac/43.1.15>

Zhang K., Sparling J., Chow B.L., Elsayed S., Hussain Z., Church D.L., Gregson D.B., Louie T. & Conly J.M. 2004. New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of Staphylococcus aureus from coagulase-negative staphylococci. J. Clin. Microbiol. 42(11):4947-4955. <https://dx.doi.org/10.1128/JCM.42.11.4947-4955.2004> <PMid:15528678>