


## IDENTIFICATION AND SUSCEPTIBILITY TO ANTIBIOTICS OF BACTERIA ISOLATED FROM MASTITIC MILK FROM THE NORTHERN MESOREGION OF THE STATE OF RIO DE JANEIRO

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### ABSTRACT

Milk production is one of the main activities of Brazilian livestock. Among the diseases that cause the most economic losses to the producer, mastitis is the most recurrent. 805 cows were tested by the "California Mastitis Test" (CMT) in 54 farms in the northern mesoregion of the State of Rio de Janeiro. 45.96% (370) tested positive for subclinical mastitis. Next, milk samples were collected and submitted to culture and identification of microorganisms according to routine laboratory tests and soon after, the antibiogram was performed. *Staphylococcus aureus* was identified in 28.4% (105) of the samples, followed by 25.1% (93) *Streptococcus* spp., 10.8% (40) *Streptococcus agalactiae*, *Staphylococcus* spp., 8.4% (31), 7.8% (29) *Corynebacterium bovis*, 0.8% (3) *Streptococcus sanguis*, 23.2% (86) of the plates with culture medium had no growth. The antibiogram of 105 strains of *Staphylococcus aureus*, 83.8% (88) were resistant to penicillin, and 24.7% (26) were resistant to tetracycline. Of the 40 strains of *Streptococcus agalactiae*, 57.5% (23) were resistant to tetracycline, 18 (45%) were resistant to enrofloxacin, and 11 (27.5%) were resistant to gentamicin. The bacteria *Staphylococcus aureus*, *Staphylococcus* spp. and *Streptococcus agalactiae* were the most isolated and demonstrated resistance to antibiotics. These results are of concern to both animal and human health, as *Staphylococcus aureus* can infect humans.

**Keywords:** Antibiogram, Bacteria, Bovine, Milk, Mamites.

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## INTRODUCTION

For the milk producer, the economic losses generated by mastitis are due to the increase in the costs of labor, veterinary services, medicines and the early disposal of animals. For the industry, the losses are related to lower yield, due to changes in the levels of lactose, casein, fat and calcium. In the microbiology of "mammalic" milk, more than 200 microorganisms that cause bovine mastitis can be identified. Many of which are dangerous to human health. The objective of this study was to identify the microorganisms that cause bovine subclinical mastitis and to verify the bacterial susceptibility profile to antibiotics in order to verify the best therapy.

## MATERIAL AND METHODS

A total of 805 cows were tested by the California Mastitis Test (CMT) in 54 farms in the northern mesoregion of the State of Rio de Janeiro. Then, positive samples were collected for the CMT test and taken to the laboratory for bacterial culture. After centrifugation at 5000 rpm for 5 min, the precipitate generated was sown in Petri dishes containing Blood Agar and MacConkey Agar. Then, the Petri dishes were incubated in a microbiological incubator, at a temperature of 37°C for 24 hours. After 24 hours, laboratory identification was initiated using routine tests. The bacteria that had dubious tests were subjected to tests with biochemical galleries of the VITEK device – Biomérieux – France. After bacterial identification, they were submitted to the antibiogram according to the Kirby-Bauer method to obtain their susceptibility profile. The antibiotics tested were: Penicillin, Cephalexin, Tetracycline, Enrofloxacin and Gentamicin, which are the most used in therapy in veterinary medicine.

## RESULTS AND DISCUSSION

Of the 805 cows tested, 56% (370) tested positive for subclinical mastitis. Of the samples, 28.38% (105) identified *Staphylococcus aureus*, followed by 25.14% (93) *Staphylococcus spp.*, 10.8% (40) *Streptococcus agalactiae*, 7.8% (29) *Corynebacterium bovis*, 4.32% (16) *Streptococcus dysgalactiae*, 1.08% (4) *Streptococcus uberis* and 0.8% (3) *Streptococcus sanguis*. In 23.24% (86) of the plates with culture medium, there was no growth (Table 1). The identification does not differ much from those observed by several authors and described in the literature. However, the identification of *Streptococcus sanguis* is not common because it is a bacterium isolated from the oral cavity in humans, thus

demonstrating possible contamination in the animal by humans. The plates that did not have growth, leaves the doubt about pre-treatment, not communicated by the rural producer of the animals or microorganisms that do not grow in media used in the laboratory routine, such as *Mycoplasmas*, among others.

In the antibiogram of 105 strains of *Staphylococcus aureus*, 83.8% (88) were resistant to penicillin, and 24.7% (26) were resistant to tetracycline. Of the 40 strains of *Streptococcus agalactiae*, 57.5% (23) were resistant to tetracycline, 18 (45%) were resistant to enrofloxacin, and 11 (27.5%) were resistant to gentamicin. Albuquerque et al. (2020), identified resistance to tetracycline in 50% of *Staphylococcus aureus* samples, presenting resistance indices higher than those presented in this work. According to Alves et al. (2019), the microorganism *Staphylococcus aureus* has become resistant to several antibiotics and causing economic and public health impacts, emphasizing the importance of continuous research in order to map the susceptibility profile of *Staphylococcus aureus* to different antimicrobials, and encourage extension projects to raise awareness among producers. Kabelitz et al. (2021), observed that *Streptococcus agalactiae* has greater resistance to antibiotics than *Streptococcus uberis* and *Streptococcus dysgalactiae*, presenting a resistance rate of 46.2% to tetracycline, which corresponds to what was observed in this study. Resistance to gentamicin presupposes misuse of medications, making it necessary to have a stricter control of antibiotics intended for animal treatment (CADES, 2017).

Table 1: Bacteria isolated from cattle with subclinical mastitis in the Northern mesoregion of the State of Rio de Janeiro from 2017 to 2024.

|                                   |              |
|-----------------------------------|--------------|
| <i>Staphylococcus aureus</i>      | 28,38% (105) |
| <i>Staphylococcus spp.</i>        | 25,14% (93)  |
| <i>Streptococcus agalactiae</i>   | 10,8% (40)   |
| <i>Streptococcus spp.</i>         | 8,4% (31)    |
| <i>Corynebacterium bovis</i>      | 7,8% (29)    |
| <i>Streptococcus dysgalactiae</i> | 4,32% (16)   |
| <i>Streptococcus uberis</i>       | 1,08% (4)    |
| <i>Streptococcus sanguis</i>      | 0,8% (3)     |
| Boards without growth             | 23,24% (86)  |
| Total                             | 100% (370)   |

## **FINAL CONSIDERATIONS**

The bacterium *Staphylococcus aureus* was the most isolated, followed by *Staphylococcus* spp., *Streptococcus agalactiae* and *Streptococcus* spp. The bacterium *Streptococcus sanguis* was isolated in bovine mammites for the first time. It has been observed that the bacteria *Staphylococcus aureus* and *Streptococcus agalactiae* have a high resistance to the antibiotics Penicillin, Tetracycline, Enrofloxacin and Gentamicin. The increase in bacterial resistance to antibiotics is worrisome, as it influences the effectiveness of the treatment of diseases in both dairy and beef cattle. The considerable increase in antimicrobial resistance of the bacteria analyzed in this study worries veterinarians and human physicians, as many of the bacteria that can be isolated from bovine mastitis can infect humans.

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