ASSESSMENT OF MICROBIOLOGICAL QUALITY AND SAFETY OF RAW MILK AND CHEESES FROM DAIRY FARMS IN RIO DE JANEIRO, BRAZIL

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Introduction

Milk is an ideal substrate for the development of microorganisms and influences the quality and safety of its derivatives, so ensuring the quality and safety of the milk used as raw material is the best strategy to obtain safe and quality cheese. In turn, the microbiological quality and safety of raw milk are influenced by milking hygiene and the health of the dairy herd [1]. The most common disease in the dairy herd is mastitis, an inflammation of the mammary gland, usually caused by a bacterial infection that can manifest itself in the clinical or subclinical form. The extensive use of antibiotics in livestock, mainly in the treatment and prevention of mastitis, includes Brazil as an important focus of bacterial resistance. Furthermore, pathogenic strains of *Escherichia coli* are present at significant levels on dairy farms. Among them, Shiga toxin-producing *E. coli* (STEC) are zoonotic strains, which have cattle as their main reservoir. This study aimed to evaluate freshly milked raw milk for signs of mastitis; to identify the possible etiologic agent of mastitis; to evaluate the microbiological quality and safety, including STEC research, of the bulk tank milk (BTM) and cheese samples; to identify the isolated bacteria; and to evaluate the phenotypic profile of antimicrobial resistance in *Enterobacteriaceae* and *Staphylococcus* spp.

Material and Methods

From january to august 2022, milk from the teats of 90 cows (A= 43; B= 24; C= 23), from three dairy farms in Rio de Janeiro, Brazil, were evaluated for signs of clinical and subclinical mastitis. A total of 66 samples of freshly milked milk (A=32, B= 27, C= 7) were collected. In addition, seven samples of BTM (A= 3, B= 3, C= 1) and seven samples of cheese (A= 2, B= 2, C= 3) were collected. Freshly milked raw milk was subjected to isolation of *Enterobacteriaceae* and *Staphylococcus* spp. BTM and CH were evaluated for Standard Plate Count (SPC) and count of lactic acid bacteria, coliforms, *E. coli* and *Staphylococcus aureus* and detection of *Salmonella* spp. and *Listeria monocytogenes*. Isolates were identified by *Matrix-Assisted Laser Ionization Desorption Mass Spectrometry* (MALDI TOF MS; Microflex LT - Bruker Daltonik GmbH) and those identified as *Enterobacteriaceae*, and *Staphylococcus* spp. were evaluated for antimicrobial resistance by the disk diffusion test [2]. BTM and CH samples were also evaluated for STEC genetic marker (*stx1*) by polymerase chain reaction (PCR) [3].

Results and Discussion

Subclinical mastitis was more prevalent than clinical mastitis in the three farms. In farms A and B there was no detection of clinical mastitis and the highest prevalence of subclinical mastitis was verified in farm B. The most isolated microorganisms in freshly milked milk were *S. aureus* (37) and *Staphylococcus chromogenes* (22), the latter being isolated in all farms. The most isolated microorganism in freshly milked milk on farms A and B, located in the same city, was *S. aureus* and on farm C, *S. chromogenes*, which are the coagulase-positive and coagulase-negative *Staphylococcus* species, respectively, predominant in bovine mastitis cases and the possible etiological agents of mastitis in these herds. In properties A and C, no sample of BTM met the limit of SPC [4]. The highest counts of coliforms were found in BTM from farm A, possibly due to deficient hygienic conditions in the milking environment. The highest counts of *S. aureus* were found in the BTM from farm B,

possibly due to the high prevalence of subclinical mastitis in the herd. All samples of cheese showed unacceptable quality. The highest counts of coliforms were found in cheeses produced with milk from farms A and C. The most isolated microorganisms in BTM were *S. aureus* (22) and *Enterobacter cloacae* (15). In cheeses, *Klebsiella pneumoniae* (11) and *Lactococcus lactis* (9) prevailed. In BTM of all farms *Raoultella ornithinolytica*, *S. aureus* and *Staphylococcus* spp. were isolated. In the cheeses of all farms *Hafnia alvei* and *Proteus mirabilis* were isolated. *K. pneumoniae* was isolated in BTM and cheeses of all farms. *Staphylococcus* spp. isolates from freshly milked raw milk were predominantly resistant to Penicillin (26/28; 92.86%), followed by Erythromycin and Linezolid (7/28; 25%, each). In addition, 8 (28.57%) Multridug resistant (MDR) and 2 (7.14%) Methicillin-resistant *S. aureus* (MRSA) strains were detected. Also, 68 strains (BTM=56, CH=12) resistant to at least one of the tested antibiotics (RAM) were detected in BTM and cheese; 43 strains (BTM=34, CH=9) MDR, 11 strains (BTM=6, CH=5) resistant to third generation cephalosporins and 7 strains (BTM=2, CH=5) resistant to Carbapenems. *Enterobacteriaceae* and *Staphylococcus* spp. isolated from the BTM and from cheese were mostly resistant to Ampicillin and Penicillin, respectively. MRSA was detected in both (BTM=7; CH=4). Also, there was amplification of the *stx*1 gene in a sample of cheese made with milk from farm C. This gene is a marker of STEC and encodes the production of the STX1 toxin. STEC are zoonotic strains, which have cattle as their main reservoir.

Conclusion

The prevalence of subclinical mastitis in the properties studied is high and bulk tank milk and cheeses produced with milk from the studied farms can act as vehicles for the dissemination of MDR and MRSA strains and a potential vehicle for the spread of STEC.

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