ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF ENTEROBACTERALES ISOLATED FROM BOVINE MASTITIS MILK

Silva, G.C.L.[1,2*]; Souza, I.B.[1,2]; Azeredo, L.T.S.A.[1,2]; Alves, J.S.[2]; Souza, R.M.[2]; Gonzalez, A.G.M.[1]

[1] *Federal Fluminense University/Faculty of Pharmacy, Santa Rosa, Niterói, Rio de Janeiro, Brazil.*
[2] *Agricultural Research Company of the State of Rio de Janeiro (PESAGRO), Fonseca, Niterói, Rio de Janeiro, Brazil.*
*guilherme_castro@id.uff.br

Introduction

As the third-largest milk producer globally, dairy farming in Brazil is notable for being predominantly carried out by small and medium-sized farms [1]. However, inadequate management practices can increase the incidence of bovine mastitis. Bovine mastitis, a disease that affects milk production and animal health, causes inflammation of the mammary glands and can present in either clinical (CM) or subclinical (SCM) forms. Various microorganisms act as etiological agents of mastitis, among which Enterobacterales stand out due to their prevalence and pathogenic potential [2]. The intensive use of antimicrobials in the treatment of mastitis can promote selective pressure, leading to the emergence of resistant strains, which poses a significant challenge to public health.

Antimicrobial resistance (AMR) is recognized by the World Health Organization (WHO) as one of the top 10 global health threats [3]. Therefore, the evaluation of antimicrobial susceptibility profiles is essential for monitoring and preventing the spread of resistant strains, which can colonize and infect humans through the consumption of contaminated milk, particularly SCM milk. The aim of this study was to assess the antimicrobial susceptibility profiles of Enterobacterales isolated from freshly milked raw milk from cows with bovine mastitis on three family-owned dairy farms located in the state of Rio de Janeiro.

Material and Methods

A total of 35 Enterobacterales strains from the culture collection of the Biotechnology Laboratory at the Agricultural Research Company of the State of Rio de Janeiro (PESAGRO) were evaluated. These strains were identified using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) at the Professor Paulo de Góes Microbiology Institute, UFRJ. They were previously isolated from 28 raw milk samples obtained from 27 subclinical mastitis (SCM) mammary quarters and one clinical mastitis (CM) mammary quarter of 100 cows from three family-owned dairy farms in the state of Rio de Janeiro. The diagnosis of bovine mastitis was performed by a veterinarian from PESAGRO. Clinical mastitis (CM) was identified by visible pathological changes in the udder and/or using the strip cup technique, observing the presence of clots in the milk, while subclinical mastitis (SCM) was detected using the California Mastitis Test (CMT), which assesses the somatic cell count (SCC) in the milk at the time of milking [4]. The project was approved by the Animal Ethics Committee of PESAGRO-RIO, registered under nº. 001/21.

The antimicrobial susceptibility profile was assessed using the disk diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI) [5]. The sensitive (S) isolates were classified as susceptible, while intermediate (I) and/or resistant (R) isolates were categorized as non-susceptible, according to the interpretation provided in the CLSI manual (2021) [5]. Strains non-susceptible to at least three antimicrobials from different classes were classified as multidrug-resistant (MDR) [5,6]. *Escherichia coli* ATCC 25922 was used as a quality control. The strains non-susceptible to cefotaxime, ceftazidime, and aztreonam were classified as suspected of producing extended-spectrum beta-lactamase (ESBL) and were tested in the presence of the inhibitor, clavulanic acid [5]. Strains non-susceptible to imipenem, meropenem, and/or ertapenem were identified as carbapenem-resistant Enterobacterales [6].

Results and Discussion

Of the 35 strains, ten different species of Enterobacterales were identified, primarily from the family *Enterobacteriaceae*, including the genera *Enterobacter* (n=21), *Citrobacter* (n=5), *Escherichia* (n=3), *Klebsiella* (n=2), *Lelliottia* (n=1), and one strain belonging to the family *Yersiniaceae*, *Serratia* (n=1). The most prevalent species was *Enterobacter roggenkampii* (n=7, 20%). Although the *Enterobacteriaceae* family is commonly isolated from raw milk, the isolation of *E. roggenkampii* from raw milk originating from bovine mastitis is uncommon, though it has been reported in hospital sewage waste in China [7].

In the antimicrobial susceptibility profile, a total of 32 (91.4%) strains were non-susceptible to at least one antimicrobial, while three (8.6%) strains were susceptible to all tested antimicrobials. Additionally, 25 (71.4%) strains were identified as multidrug-resistant (MDR). Non-susceptibility to cefazolin (23/32; 71.9%) was the most frequently observed profile, followed by amoxicillin + clavulanic acid (19/32; 59.4%), ampicillin (17/32; 53.1%), cefotaxime (16/32; 50%), cefuroxime, and aztreonam (13/32; 40.6% each), trimethoprim, and trimethoprim-sulfamethoxazole (12/32; 37.5% each), fosfomycin, and nalidixic acid (11/32; 34.4% each), ceftazidime, and minocycline (8/32; 25% each), gentamicin, and piperacillin (5/32; 15.6%), tetracycline, and ciprofloxacin (4/32; 12.5%), imipenem (3/32; 9.4%), amikacin (2/32; 6.35%), meropenem, nitrofurantoin, ertapenem, and levofloxacin (1/32; 3.1% each). All strains were susceptible to chloramphenicol.

The ESBL phenotype was observed in two strains of *Klebsiella pneumoniae* (5.7%). Additionally, three strains (8.6%) (*E. roggenkampii* and two *Enterobacter* spp.) exhibited resistance to at least one carbapenem, classifying them as carbapenem-resistant Enterobacterales (CRE). The high prevalence of non-susceptible strains, along with the presence of MDR and ESBL-producing strains, is alarming, as antimicrobial non-susceptibility represents a serious public health issue with the potential to increase morbidity and mortality rates. The occurrence of CRE further exacerbates this situation [3].

Conclusion

Antimicrobial resistance poses a threat to both animal and public health, with the environment and food, such as milk, serving as vectors for dissemination. The detection of non-susceptible Enterobacterales and beta-lactamases in raw milk reflects the spread of resistance genes on farms. The presence of MDR strains in animal-derived products, such as raw milk, underscores the urgent need for control strategies. One Health approaches are essential for mitigating risks to human, animal, and environmental health.

Bibliographic References

[1] Ministry of Agriculture, Livestock, and Supply (MAPA). **Milk Map**, 2023.
[2] Locatelli, C. et al. CTX-M1 ESBL producing Klebsiella pneumoniae subsp. pneumoniae isolated from cases of bovine mastitis. **J. Clin. Microbiol.**, 2010, (48), p. 3822–3823.
[3] Centers for Disease Control and Prevention, (CDC). **Antibiotic resistance threats in the United States**, (U.S.), 2019.
[4] Souza, R. M. et al. Rapid diagnosis in the control of bovine mastitis in family production systems, 2019.
[5] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; **Twenty-Fifth Informational Supplement**. EUA, 2021.
[6] Magiorakos, A. P. et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. **Clin. Microb. and Infect.**, 2012, 18, (3), p. 268–81.
[7] Xu, T. et al. Identification of mcr-10 carried by self-transmissible plasmids and chromosome in Enterobacter roggenkampii strains isolated from hospital sewage water. **Environmental Pollution**, 2021, v. 268, p. 115706.