



PREVALENCE OF MULTIDRUG-RESISTANT STAPHYLOCOCCI IN OVINE MASTITIS MILK SAMPLES AS A POTENTIAL BIOLOGICAL RISK IN THE DAIRY SUPPLY CHAIN

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Objective. This study stems from the observation that there is a significant scientific gap and a general lack of interest in the epidemiology of ovine mastitis. To help address this gap, this preliminary research examined the bacterial population involved in subclinical mastitis in sheep. The objective was to map the antimicrobial resistance profiles of the isolated strains, assessing the issue from a food safety perspective, given that ovine milk is widely used to produce high-volume raw milk cheeses.

Methods. The research examined 17 initial samples, consisting of milk and mammary duct swabs collected from sheep affected by subclinical mastitis. Following initial bacterial isolation using culture-dependent methods, the strains were identified using MALDI-TOF technology. The relative abundance of the identified species was then evaluated, which allowed for the careful selection of 28 representative *Staphylococcus* spp. strains (14 *Staphylococcus aureus* and 14 coagulase-negative staphylococci). Antibiotic susceptibility testing was subsequently performed on these selected strains using the Kirby-Bauer disk diffusion method, following the criteria and interpretation guidelines of the European EUCAST committee.

Results. The data indicate a worrying presence of multidrug-resistant (MDR) strains, defined as those resistant to at least three different antibiotic classes. In the *S. aureus* group, tested against 15 antibiotics, 64.3% of the strains (9 out of 14) were MDR. In total, 67 resistances were recorded out of 210 tests performed (31.9%), with a mean Multiple Antibiotic Resistance (MAR) index of 0.30. This value is

above the critical threshold of 0.20, indicating strong selective pressure. This trend is confirmed by the Antibiotic Resistance Prevalence Assessment (ARPA) index, which reached 4.79, highlighting a significant prevalence of resistance across the entire group. Among the coagulase-negative staphylococci, species rarely investigated in ovine mastitis were identified, namely *S. equorum*, *S. xylosus*, *S. chromogenes*, and *S. simulans*. Of these 14 strains, tested against 14 antibiotics, MDR affected 71.4% of the isolates (10 out of 14), with 75 total resistances out of 196 tests (38.3%). In this second group, the mean MAR index rose to 0.38 and the ARPA value to 5.36, demonstrating an even higher overall burden and intensity of resistance compared to *S. aureus*.

Conclusions. The findings highlight substantial public health and food safety concerns. From an epidemiological perspective, the high incidence of staphylococci, particularly *S. aureus*, combined with the widespread practice of hand milking, suggests an operator-dependent transmission dynamics linked to hygiene gaps during mammary gland manipulation. Despite the lack of official data on the commercial destination of the examined milk batches, the introduction of genetic resistance determinants into the dairy chain poses a concrete risk to consumers. This threat manifests both in the potential survival of pathogenic strains in the finished product and in horizontal gene transfer mechanisms to the human gut microbiota. This underscores the urgent need to implement integrated epidemiological surveillance plans along the production chain, consistent with the One Health approach.