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AETIOLOGY OF GOAT INTRAMAMMARY INFECTIONS AND SUSCEPTIBILITY OF ISOLATED PATHOGENS TOWARD ANTIBIOTICS

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ABSTRACT

The aim of the study was to identify intramammary infection (IMI) in goats, focusing on *Staphylococcus* species, and to assess susceptibility pattern of isolated bacteria. A total of 78 half udder milk samples from 39 goats originated from an intensive dairy farm situated in northern Slovakia were submitted for microbiological analysis. The half udder milk samples were from White Shorthaired goats and Brown Shorthaired goats. Milk samples were cultured, and bacterial isolates were identified using MALDI-TOF MS. The results revealed a high prevalence of IMI, with 66.7% of milk samples testing positive for bacterial growth. Non-aureus staphylococci (NAS) were the most prevalent pathogens, accounting for 94.6% of the isolates, with *Staphylococcus epidermidis* and *Staphylococcus caprae* being the most common species. Antibiotic susceptibility testing showed that 17.0% of the isolates were resistant to at least one or more of the eight antibiotics tested, particularly penicillin, cefoxitin, and neomycin. The study emphasizes the importance of regular udder health monitoring and highlights the need for prudent antibiotic use. The findings underscore the necessity for targeted management strategies to control IMI and improve overall herd health.

Keywords: udder health, goats, *Staphylococcus* spp., non-aureus staphylococci, antibiotics

INTRODUCTION

Goat husbandry has a long-standing tradition in Slovakia, particularly within small-scale farming systems. The White Shorthair goat and the Brown Shorthair goat represent local dairy goat breeds originating from former Czechoslovakia, and they currently form the core of domestic goat production in the region **(Vostrý *et al.*, 2024; Gašper *et al.*, 2023)**. In 2024, a total of 17 922 goats were kept in Slovakia (**Repka, 2025**).

Milk quality and yield is significantly influenced by udder health – mastitis caused by penetrating microorganisms through the teat canal into udder (**Čobirka *et al.*, 2020**). Members of the genus *Staphylococcus* (*S.*) spp. are the most commonly reported bacterial species isolated from udder secretion of goats with intramammary infection (IMI) - mastitis. The genus is comprising a heterogeneous group of more than 50 species and subspecies (**Coimbra-e-Souza *et al.*, 2019; Ruiz-Romero and Vargas-Bello-Pérez, 2023; Smistad *et al.*, 2024**). The genus is divided into coagulase-positive staphylococci (*Staphylococcus aureus)* and coagulase-negative staphylococci (referred to as non-*aureus* staphylococci - NAS) based on their ability to coagulase plasma (**National Mastitis Council, 2017**). Contagious mastitis in goats is most frequently caused by a few contagious bacterial species such as *S. aureus* or *Streptococcus* (*Str.*) *agalactiae* manifested with elevated somatic cell count (SCC) in milk and possible clinical mastitis onset. However, NAS are considered the most significant causative agent of mastitis in goats, typically associated with mild intramammary immune response (mild increase of SCC) and subclinical mastitis occurrence (**Contreras *et al.*, 2007**). Despite this, *S. aureus* is recognized as the most important udder pathogen due to its ability to produce toxins that affect udder tissue and its capacity to persist and form abscesses (**Benić *et al.*, 2018)** and biofilm, which is one reason for the poor effect of antibiotics treatment (**Zigo *et al.*, 2022).** Similarly, some studies have shown the ability of NAS species to cause persistent infections with the potential for contagious spread (**Gosselin *et al.*, 2019),** and they are capable of forming a biofilm, facilitating genetic exchange and the transfer of antimicrobial resistance genes (**Coimbra-e-Souza *et al.*, 2019).** Since the start of the 21st century, with the application of MALDI-TOF MS in microbial identification, the most common *Staphylococcus* species reported from goat IMI have been NAS species, primarily *S. caprae, S. epidermidis*, *S. simulans*, *S. xylosus, S. warneri* and *S. chromogenes* (**Gosselin *et al*., 2019; Tvarožková *et al*., 2023; Smistad *et al*., 2024**). Despite the high frequency of NAS isolation in IMI cases, the epidemiology and the role of these pathogens as a causative agent of mastitis compared to *S. aureus* are not yet fully understood (**Smistad *et al*., 2024**). Thus, the aim of the study was to diagnose intramammary infections (IMI) in goats on selected farm, focusing on *Staphylococcus* species as the primary cause of IMI, and to evaluate their antibiotic susceptibility.

MATERIAL AND METHODS

Study population

Dairy goat farm is located in the north of Slovakia. At the time of the study, the goat herd was composed of approximately 80 lactating goats, with a predominance of White Shorthaired goats, and some Brown Shorthaired goats. All goats were kept in an intensive milk production system, with no outdoor access. The goats were housed in four separate pens containing around 20 goats per pen. Goats were fed in pen with hay (ad libitum), granulated complete feed mixture (0,6 kg/goat/day), oat (0.2 kg/goat/day), sugar beet pulp (0.5 kg/goat/day) and alfalfa (1.5 kg/goat/day). During the study period, the goats were milked once a day in a milking parlour, with no teat washing and brushing by a towel before machine milking. The average milk production was 2.0 to 2.5 litres per goat in this period (mid-lactation). The farmer also reported a low prevalence of clinical mastitis in the goats. In these cases, antimicrobials were used as prescribed by the veterinarian, but the treatment was unsuccessful, leading to irreversible loss of gland function or death of the goats.

Sampling

The total of 39 goats, without clinical signs of mastitis, were randomly sampled for bacteriological examination before evening milking, out of a total of 80 goats. The first few streams of milk were discarded, and the teat ends were disinfected with wipes containing 70% alcohol. Approximately 2 ml of milk from each udder half was collected into sterile tubes. The collected samples were frozen at -20°C, transported to the laboratory, and stored in a freezer until bacteriological culture. In total 78 half udder milk samples were available for examination.

Bacteriological culture

Aerobic bacterial culture was performed after thawing the tubes at room temperature (approximately 22°C). The 0.01 ml of the sample was plated on Colombia agar with 5% of ovine blood (MkB Test a.s., Rosina, SR) and incubated at 37°C for 24 to 48h. At 48 h. of incubation inoculate plates were evaluated for the presence of grown colonies. The grown colonies were primarily identified by colony morphology, Gram staining, type of hemolysis, and checked for catalase and oxidase production according to NMC guidelines **(National Mastitis Council, 2017)**. Gram and catalase-positive cocci were initially identified as *Staphylococcus* spp. and presumptive *Staphylococcus aureus* was identified using the clumping factor test (DiaMondiaL Staph Plus Kit, Germany). Small colonies that were gram-positive and catalase-positive were identified as coryneform bacteria. The bacteria were identified as *Bacillus* spp. based on their large colony size, Gram-positive staining, catalase-positive reaction, and ability to form endospores. Samples were classified as positive if ≥1 Colony Forming Units (CFU) of contagious pathogens (*S. aureus* or *Str. agalactiae*) was identified from 0.01 ml of milk, or ≥ 5 CFU of other pathogens were found. Samples were classified as negative if no growth was observed, or as contaminated if > 2 CFU of different colony types were isolated on blood agar. All presumptive staphylococcal isolates were subcultured onto blood agar plates to obtain pure colonies for species-level identification using MALDI-TOF MS **(Ozbey *et al.*, 2022).**

MALDI-TOF MS

Isolates were prepared using the ethanol/formic acid extraction method and analyzed in triplicate by MALDI-TOF MS. Briefly, 3 to 4 colonies were mixed in 300 µL of distilled water to which 900 µL of pure ethanol was added for protein extraction, mixed and centrifuged (12 000 rpm for 2 min). The supernatant was removed, and the pellet was allowed to dry. For cell disruption, the pellet was then mixed with 30 µL of 70% formic acid and 30 µL of acetonitrile and centrifuged again. One microliter of the supernatant was spotted on the MALDI-TOF target plate and allowed to dry, then overlaid with one microliter of matrix solution and allowed to dry. Generated spectra were assigned a score according to MALDI Biotyper 3.0 software (Bruker Daltonics). Instead of the standard score value of ≥ 2.000 typically used for reliable species identification, we employed a score value of ≥ 1.700, which also has been shown to improve the performance of MALDI-TOF MS in classifying NAS isolates in cows (**Taponen *et al.*, 2022; Freu *et al.*, 2024**). A using score of ≥1.700 for species identification also resulted in improved typeability, while minimally decreasing accuracy (**Gosselin *et al.*, 2018**). A score of ≥2.000 was considered only in 9 of a total of 29 isolates of *S. epidermidis* and 2 of a total of 12 isolates of *S. caprae*. On the other hand, 63.0% of the isolates had a score value ≥1.700, and at least two of the three repeats gave the same identification. A score below 1.700 were considered for unidentified bacterial species.

Testing of antibiotic susceptibility

On the base of farmer requirement, only 41 isolates were tested for antibiotic susceptibility. Isolates were tested *in vitro* using the disc diffusion method by evaluating the zone of inhibition to grow on Mueller-Hinton agar after 24 hours of incubation at 37°C, according to the CLSI manual **(CLSI, 2020)**. The following antimicrobial discs (OXOID Ltd., Basingstoke, Hants, UK) were used: tetracycline (TE 30 µg.disk^{-1}) amoxicillin-clavulanic acid 2:1 (AMC 30 µg.disk^{-1}), sulfamethoxazole-trimethoprim (SXT 25 µg.disk^{-1}), cefoxitin (FOX 30 µg.disk^{-1}), erythromycin (ERY 15 µg.disk^{-1}), lincomycin (MY 15 µg.disk^{-1}), penicillin (PEN 10 IU), neomycin (N 30 µg.disk^{-1}). The diameters of the inhibition zones were evaluated as susceptible, intermediate, or resistant according to CLSI breakpoints **(CLSI, 2020; Holko *et al.*, 2019a)**.

RESULTS AND DISCUSSION

Diagnosing an intramammary infection (IMI) using cultivation can help to determine treatment and prevention strategies on the farm, which can ultimately reduce both the incidence and prevalence of diseases of the udder (**Zigo *et al.*, 2019**). However, the animal environment is rich in microbiota, and milk provides an ideal medium for the growth of numerous bacterial species, which can cause IMI and may lead to mastitis when the conditions of infection are favourable. The development of mastitis is influenced by multiple factors, typically resulting from the interaction between the infectious agent, the animals, and the environment (**Benić *et al.*, 2018**).
From our previous study, we found a high percentage of goats with SCC above 1.10^6 cells in ml of milk (between 30-90%) in dairy farms (**Tančin *et al.*, 2024**). Also, at the study farm, the farmer did not perform any udder preparation before milking (no cleaning of the teats), nor was any post-milking teat disinfection applied. This corresponds to the high prevalence of intramammary infection (IMI) in our study (Fig 1). We found the presence of pathogens in 52 (66.7%) milk samples from 39 (82.1%) goats. Of these, 20 goats had an IMI in both udder halves, while 12 goats had IMI in only one udder half, with either a single type of colony or two different colony types. No growth was found in 26 (33.3%) samples, but only seven goats (17.9%) were found without IMI. The lower prevalence of bacteriologically positive samples from separate udder halves of goats was reported by **Tvarožková *et al.* (2023)** in June (13.1%) and July (12.7%) on another dairy goat farm in northern Slovakia. The prevalence of IMI in goats in Scandinavian countries is generally low, and the good udder health of goats reflects a strong focus on researching somatic cell count as an indicator of udder health in Norway (**Smistad *et al.*, 2021, 2024**) and Sweden (**Persson and Olofsson, 2011**). Compared to dairy cows or sheep, there is no comprehensive research on the udder health status of dairy goats in Slovakia. The markedly higher proportion of positive samples in our study may also be partially explained by the absence of udder preparation before milking and the lack of post-milking teat disinfection on the farm, practices which are known to influence the risk of IMI **(Zigo *et al.*,2019*).***

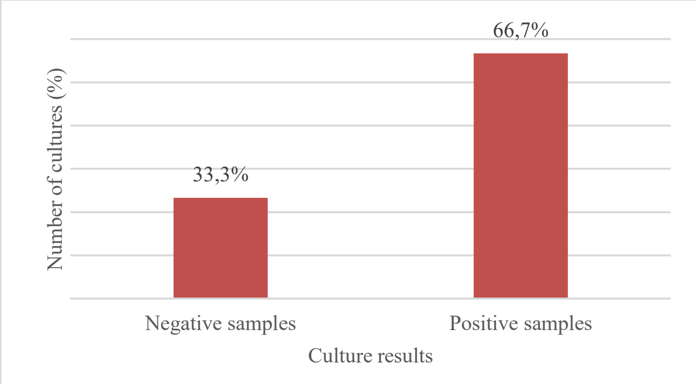

Figure 2 Culture results of 78 half-udder milk samples from goats from 39 goats

Several NAS species in goats are difficult to identify using phenotypic methods alone, which results in low typeability. Therefore, genotypic methods are regarded as more dependable for accurate identification (**Gosselin *et al.*, 2018**). It is important to note that phenotypic methods such as STAPHYtest 24 are useful for the routine identification of different NAS species in mastitic cows (**Zigo *et al.*, 2019; Farkašová *et al.*, 2024).** At present, the adoption of MALDI-TOF as the new identification tool for routine diagnosis of IMI in goats increases knowledge of NAS IMI in goats. In our study, NAS were the most common isolates (94.6%). Gram-positive bacteria such as *Corynebacterium* spp. and *Bacillus* spp. were minor pathogens in goat milk in our study. In other studies, the percentage of NAS causing IMI on the udder half level was 73.0% (**Tvarožková *et al.*, 2023)** and 59.0% (**Smistad *et al.*, 2021)**, which is notably lower than in your study. In general, to better understand NAS as the main microorganisms causing IMI in goats, it is necessary to identify the individual species, rather than just report an isolate as NAS. In our study, the most common NAS were *S. epidermidis* and *S. caprae* (Tab 1). Among these NAS species, **Smistad *et al.* (2024)** identified *S. caprae* and *S. epidermidis* as the first and second most frequent pathogens. The highest prevalence of *S. caprae* and *S. epidermidis* could be explained to their ability to persist in the mammary gland (**Gosselin *et al***., **2019**).

Table 1 Species identification of NAS isolations using MALDI-TOF MS

Species	A score ≥ 2.000	A score ≥ 1.700	A score <1.700
Staphylococcus epidermidis	9	20	
Staphylococcus caprae	2	10	
Staphylococcus simulans	3		
Staphylococcus cohnii	2		
Staphylococcus lugdunensis	1		
Staphylococcus microti		1	
Staphylococcus petrasii	1		
Unidentified			4

Instead of the standard score value of ≥ 2.000 typically used for reliable species identification, we employed a score value of ≥ 1.700, which also has been shown to improve the performance of MALDI-TOF MS in classifying NAS isolates in cows (**Taponen *et al.*, 2022; Freu *et al.*, 2024**). A score values below 1.7 were considered for unidentified bacterial species.
The antimicrobial resistance is one of the most important challenges in mastitis treatment in dairy animals. The increase of bacterial resistance toward antimicrobials is well documented, particularly in bacterial strains of bovine (**Holko *et al.*, 2019a**) and sheep origin (**Holko *et al.*, 2019b, Vasiľ *et al.*, 2018**). However, there is very limited information available on antimicrobial susceptibility of bacteria isolated from goats in Slovakia. In our study, less than fifth (7/41, 17%) of the NAS isolates were resistant to at least 1 or more of the 8 antimicrobials evaluated. The highest resistance rate of isolates was observed to penicillin (17%), cefoxitin (7%), and neomycin (5%) (Tab 2). Specifically, one *S. simulans* isolate and one *S. microti* isolate were resistant to all three mentioned

antibiotics. **Lauková *et al.* (2022)** reported, that more than 20 strains of staphylococci out of 35 tested isolates from goat milk collected in Slovakia were resistant to lincomycin and novobiocin, although most isolates were susceptible to commercial antibiotics. **Coimbra-e-Souza *et al.* (2019)** evaluated the antimicrobial susceptibility of 51 staphylococcal isolates of goats. They found resistance rates to ampicillin 39%, penicillin 26%, and erythromycin 12%. According to the **Norwegian Medicines Agency (2022)**, penicillin should be the first choice of treatment for intramammary infections (IMI) in goats caused by Gram-positive bacteria in Norway. In Slovakia, aminoglycosides, such as streptomycin and neomycin, are the most commonly used antibiotics for treating mastitic cows (**Holko *et al.*, 2019a).** In contrast, penicillin and ampicillin have been widely used in veterinary practice in Brazil for treating mastitis caused by Gram-positive bacteria (**Coimbra-e-Souza *et al.*, 2019**). The widespread use of antimicrobials without prior antibiogram testing may potentially contribute to the increasing resistance and the emergence of multidrug-resistant staphylococci (**Holko *et al*, 2019a,b**).

Table 2 Antimicrobial susceptibility of 41 NAS isolates from goats.

	TET	AMC	SXT	FOX	ERY	MY	PEN	NEO
Susceptible	40 (98%)	41 (100%)	41 (100%)	38 (93%)	39 (95%)	41 (100%)	34 (83%)	39 (95%)

TE- tetracycline, AMC- amoxicillin-clavulanic acid, SXT- sulfamethoxazole-trimethoprim, FOX- cefoxitin, ERY- erythromycin, MY- lincomycin, PEN- penicillin, N- neomycin

CONCLUSION

In conclusion, a high prevalence of intramammary infections (IMI) has been diagnosed in goats with a visually healthy udder, highlighting the importance of regular monitoring of udder health. An important finding in the present study was that no contagious pathogens, such as *Staphylococcus aureus*, were detected. Nevertheless, we also confirmed that non-*aureus* staphylococci were the most common pathogens responsible for causing intramammary infections in this study. Resistance to penicillin, cefoxitin, and neomycin was observed in 17.0% (7/41) of isolates, of which two isolates were resistant to all three mentioned antibiotics. This highlights the need for greater attention to non-*aureus* staphylococci in udder health management and control strategies should therefore account for these pathogens to ensure better management of intramammary infections and maintain overall herd health.

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