Research Article

Subclinical Mastitis in Albanian Dairy Cattle: Prevalence, Risk Factors, and Antimicrobial Resistance

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ABSTRACT

Subclinical mastitis is the most common form of mastitis in dairy cattle, often going undetected due to its lack of visible clinical signs. This condition leads to significant economic losses through reduced milk yield and increased culling. This study aimed to assess the national prevalence of subclinical mastitis in Albanian dairy herds, identify associated risk factors, and determine the antibiotic resistance patterns of major bacterial pathogens. Between December 2022 and April 2023, a total of 500 lactating cows from 62 farms across 12 Albanian counties were screened using the California Mastitis Test (CMT). Data on animal, management, and environmental variables were collected through structured interviews. Milk samples from CMT-positive quarters were cultured for bacterial isolation and tested for antibiotic susceptibility using the Kirby-Bauer method. The overall prevalence of subclinical mastitis was 59.6%, with the highest rates observed in Tiranë (76%) and Shkodër (75%). Multivariable logistic regression revealed three key risk factors: post-milking udder washing (OR=4.71), low farm altitude, and extensive management systems. The most frequently isolated pathogens were *Staphylococcus aureus* (33.7%), *Escherichia coli* (16.6%), and *Pseudomonas aeruginosa* (12%). Resistance was highest to penicillin and amikacin, while ciprofloxacin and gentamicin remained largely effective. Multidrug resistance was observed in over 50% of isolates. These findings highlight the need for better milking hygiene, targeted antibiotic use, and structured mastitis control. Routine CMT screening and farmer education are key to reducing disease and antimicrobial resistance in the Albanian dairy sector.

Keywords: Subclinical mastitis, Intramammary infection, Dairy cattle, CMT test, Bacterial isolation, Antibiotic resistance

INTRODUCTION

Mastitis is an inflammation of the udder that leads to abnormal milk and is one of the most common and economically damaging diseases in dairy cattle (Sinha et al. 2014). Although mastitis is considered a multifactorial disease, bacteria represent the main cause of the disease (Tezera and Ali 2021). Mastitis can be clinical, with visible udder inflammation and abnormal milk, or subclinical, with no apparent udder or milk changes (Quinn et al. 2002). While clinical mastitis also imposes significant economic losses ranging from approximately €61 to €97 per cow through treatment costs and milk discard, its visible nature facilitates immediate intervention. On the other hand, subclinical mastitis often goes undetected without routine screening, leading to cumulative losses estimated at €100–€300 per cow annually due to decreased milk production and increased culling rates (Hogeveen et al. 2011). Subclinical mastitis can also pose a public-health risk if zoonotic pathogens are shed in milk (Radostits et al. 2007; Goulart and Mellata 2022). There are several risk factors identified by many studies that increase the incidence of subclinical mastitis in dairy cattle. These factors are related to animal management and environment, such as age, milk yield, breed, floor type or bedding, milking hygiene, and teat disinfection (Hogan and Smith 2012; Meçaj et al. 2023).

Mastitis pathogens are classified by transmission mode as contagious or environmental. Contagious pathogens (e.g., *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma* spp.) spread from cow-to-cow

during milking (when hygiene is poor), whereas environmental pathogens (e.g., *Streptococcus uberis*, *Escherichia* coli, *Klebsiella* spp.) originate from the surroundings (manure, bedding etc) (Hogan and Smith 2012; Klaas and Zadoks 2018; Meçaj et al. 2023).

Indiscriminate antibiotic use in mastitis has raised serious concerns about antimicrobial resistance, and indeed rising drug resistance among mastitis pathogens has been documented (Kurjogi et al. 2019; Morales-Ubaldo et al. 2023). However, targeting therapy based on culture and sensitivity can significantly decrease antibiotic usage (by ~25–50%) without compromising effectiveness, while also reducing milk discard and drug residues (McDougall 2001; Neeser et al. 2006; Lago and Godden 2018). These findings underscore the importance of routine antibiotic susceptibility testing to ensure effective and prudent treatment.

Recent studies have reinforced the persistent global challenge of subclinical mastitis in dairy cattle, with prevalence rates varying by region and management practices, often aggravated by risk factors such as poor hygiene, breed susceptibility, and inadequate biosecurity measures (Demil et al. 2022; Ghebrehawariat et al. 2025). For instance, in Eritrea, SCM prevalence at the cow level was reported at 77.23%, influenced by factors like breed, body condition, and management practices, while antimicrobial susceptibility testing revealed high resistance to tetracycline, sulfonamides, and penicillin among isolated bacteria (Ghebrehawariat et al. 2025). Similarly, in Ethiopia, risk factors including udder hygiene and tick infestation have been linked to elevated subclinical mastitis prevalence, with *Staphylococcus* and *Streptococcus* species showing multidrug resistance patterns that endanger dairy production and public health (Demil et al. 2022; Farabi et al. 2024). Recent research also highlights the role of gram-negative pathogens in environmental subclinical mastitis, with extended-spectrum β-lactamase production contributing to antimicrobial resistance in regions with intensive farming (Abdel-Hameed and Abd-Elhafeez, 2025). Literature reviews further emphasize the need for advanced detection strategies to combat *Staphylococcus aureus*-driven subclinical mastitis, as resistance to methicillin and other antimicrobials continues to rise globally (Son and Duc 2024; Touaitia et al. 2025). In Eastern Europe, studies from Poland and Romania report similar trends, with molecular identification revealing virulence factors and biofilm formation in mastitis cultures, underscoring the priority for targeted interventions (Dobrut et al. 2024; Hutu et al. 2024). In Asia and South America, phage-based biocontrol and genomic analyses are being explored to address antimicrobial resistance in subclinical mastitis, providing potential models for countries like Albania where dairy farming faces comparable challenges (Aguirre-Sánchez et al. 2024; Son and Duc 2024; Moreno et al. 2025). The results highlight the complexity of subclinical mastitis and the importance of region-specific epidemiological data for designing sustainable control strategies.

To our knowledge, this is the first nationwide study in Albania investigating the prevalence, risk factors, bacterial pathogens, and antimicrobial resistance of subclinical mastitis in dairy cattle. This information would help to screen the situation and design proper control and preventive strategies.

MATERIALS AND METHODS

Ethical approval
Ethical exemption was granted by the Faculty of Veterinary Medicine's Ethics Committee, under national guidelines. However, verbal and written informed consent was obtained from all farm owners or managers before participation. All animal handling was carried out under the supervision of trained veterinary professionals to ensure animal welfare was maintained throughout the study.

Study animals
Farms were selected using a stratified random sampling approach, ensuring proportional representation across farm sizes (small, medium, large) and geographic distribution within each of the 12 Albanian counties. The tested breeds were Holstein Friesian, Jersey, Brown Swiss, Montbéliard, and crossbreeds. Farm management systems varied from extensive to intensive. Most farms used mechanical milking, though some practiced hand milking, and cattle housing was either tethered or free stall. On the basis of observation, most of the animals were apparently healthy and with normal udder and teat conformation.

Study area and sampling size
An epidemiological cross-sectional study was designed and carried out between December 2022 and April 2023 across all 12 counties of Albania. The sample size was estimated using the following formula for prevalence studies:

$$n = \frac{Z * p * q}{d^2}$$

Where: n = required sample size, Z=1.96 (standard normal value for 95% confidence), P = expected prevalence = 25% (based on previous regional studies), q=1−P =75%, d = desired absolute precision = 10%

$$n = \frac{(196)^2 * 0.25 * 0.75}{(0.10)^2} = \frac{3.8416 * 0.1875}{0.01} = 72.03 \approx 72$$

Since Albania has approximately 400 active dairy farms, we applied the finite population correction (FPC) to adjust the sample size:

$$n_{adj} = \frac{n}{1 + \frac{(n-1)}{N}}$$

Where N =400 (estimated population of dairy farms).

$$n_{adj} = \frac{72}{1 + \frac{71}{400}} = \frac{72}{1 + 0.1775} = \frac{72}{1.1775} \approx 61.1$$

To ensure greater statistical power and geographical representation, the study ultimately included 500 lactating cows from 62 farms across 12 counties.

Survey data recording
Before animals were tested, each farm owner was interviewed with a structured questionnaire with closed-ended questions. Livestock and housing were observed simultaneously to validate farmers' responses. This questionnaire included independent variables related to the animal (milking yield and breed), management (extensive, semi-intensive, and intensive), housing (tethered or free stall), management practices (milking frequency, milking technique, breeding system), milking hygiene (pre- and post-milking teat disinfection /washing, individual paper towel for cleaning), and altitude. The results obtained from

questionnaires were utilized to determine if there was a correlation between subclinical mastitis and any of the independent variables.

California Mastitis Test (CMT)

The CMT is a widely used screening tool for subclinical mastitis, but it has limitations, including variable sensitivity and specificity depending on the stage of lactation and the operator's experience, which may affect the accuracy of the results. These limitations are discussed further in the Discussion section. Milk from each animal was first checked for visible abnormalities, then screened using CMT. From each quarter, 2-3 streams of milk were placed into one of the four cups of the CMT paddle, which was inclined at 36–60° to equalize milk volume. Two milliliters of CMT reagent (Kerbl, Ref. No. 154) were added to each cup, and the mixtures were gently stirred horizontally for 15 seconds. CMT results were scored as 0 (negative: no precipitate), +1 (trace: slight gel), +2 (positive: mild gel) and +3 (strong positive: distinct gel) (Quinn et al. 2002; Birhanu et al. 2017). In each farm, a template for recording CMT results was filled and attached to the questionnaire. Positive quarters that scored +2 and +3 were taken for bacteriological analysis.

Milk sample collection and bacteriological analysis

Milk samples were collected from cows in the milking period that met three criteria: (1) positive CMT scores of +2 or +3, (2) absence of clinical signs of mastitis (e.g., normal udder and milk appearance, no fever or lethargy), and (3) no recent antibiotic use. At least one cow per herd was sampled, with up to four randomly selected cows sampled in larger herds. Approximately 10mL of milk was collected per animal immediately after CMT screening during farm visits.

Proper hygiene was ensured during sampling: dirty teats were cleaned and dried with disposable towels, then disinfected with 70% alcohol for one minute. The first milk streams were discarded, and samples were collected in sterile containers held at a 90° angle to minimize contamination. Containers were labeled with the animal ID, sample number and date, then transported in coolers to the laboratory.

To optimize bacterial isolation, samples were stored at −20°C and tested in batches. A 10μL aliquot was plated on blood agar (5% sheep blood), nutrient agar, MacConkey, and incubated at 37°C for 16–24 hours. Bacterial identification followed standard protocols: *Staphylococcus aureus* by morphology, hemolysis, and coagulase test; *Streptococci* spp. via CAMP and biochemical tests; *Escherichia coli* by PGUA and indole positivity; other Gram-negatives via oxidase reaction and API systems. Samples with ≥1 Colony-Forming Unit (CFU) of *Staphylococcus aureus* or *Streptococcus agalactiae*, or ≥3 CFUs of other pathogens, were considered positive. To ensure accurate diagnosis, we considered milk samples with three or more different bacterial species as contaminated. This classification was based on standard diagnostic protocols (Quinn et al. 2002; NMC 2017), which recommend this threshold to help distinguish true intramammary infections from external contamination, often a result of improper sampling or environmental exposure. By using this cutoff, we aimed to minimize the inclusion of misleading results in our bacterial analysis.

Antibiotic susceptibility

The antibiotic panel was selected to include antibiotics commonly used in veterinary practice in Albania and to represent a range of antibiotic classes, including aminoglycosides (amikacin, gentamicin, neomycin), beta-lactams (penicillin, ampicillin, cefotaxime), fluoroquinolones (ciprofloxacin), tetracyclines (tetracycline), and sulfonamides (sulfamethoxazole-trimethoprim). For testing the presence of microbial resistance, three of the most encountered bacteria (*Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*) were tested for their sensitivity. Antibiotic susceptibility of the isolates was determined using the Kirby-Bauer disk diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2022). A 0.5 McFarland standard suspension of each isolate was prepared and inoculated uniformly onto Mueller-Hinton agar plates. Antibiotic disks (Oxoid, UK) test included: A total of 10 antibiotics, including Amikacin (30μg), Ampicillin (10μg), Amoxicillin-clavulanic acid (30μg), Cefoxitin (30μg), Cefotaxime sodium (30μg), Ciprofloxacin (5μg), Gentamicin (10μg), Penicillin (10μg), Sulfamethoxazole-trimethoprim (25μg), Tetracycline (30μg). After incubation at 37°C for 18–24 hours, the diameter of inhibition zones was measured, and results were based on CLSI standards. In this study, we considered a bacterial strain to be multidrug-resistant (MDR) if it showed resistance to at least three different classes of antibiotics, in line with widely accepted epidemiological guidelines (Magiorakos et al. 2012).

Data Analysis

All data collected through questionnaires and laboratory results were entered and organized using Microsoft Excel 2016, and then imported into IBM SPSS Statistics Version 29.0.2.0 for statistical analysis. The overall prevalence of subclinical mastitis was calculated as the number of cows testing positive (at least one quarter with a CMT score of +1, +2 or +3) divided by the total number of cows tested. Descriptive statistics (frequencies and percentages) were used to summarize the distribution of subclinical mastitis by quarter and severity. To evaluate risk factors, the presence or absence of subclinical mastitis at the cow level (binary outcome: 1 = positive, 0 = negative) was used as the dependent variable.

Univariate analysis was performed:Chi-square tests (χ^2) were applied to assess the association between categorical independent variables (e.g., breed, management system, housing type, milking technique, teat disinfection, use of paper towels, washing before/after milking, breeding system) and subclinical mastitis status. Independent samples T-tests were used to compare continuous variables (milk production in kg/day and farm altitude in meters) between mastitis-positive and mastitis-negative groups. Variables with p-values <0.25 in univariate analysis were included in a multivariable logistic regression model to control for confounding and identify independent predictors of subclinical mastitis. The final model was built using stepwise forward selection. Associations were considered statistically significant at P≤0.05. For each

variable included in the logistic regression model, the odds ratio (OR), 95% confidence interval (CI), coefficient (β), and P-value were reported to assess the strength and precision of the association.

RESULTS

Overall prevalence of subclinical mastitis in Albania

Out of a total of 500 dairy cows examined using CMT, we found that 298 cows tested positive for subclinical mastitis, resulting in a prevalence rate of 59.6%. Our results revealed variation in subclinical mastitis prevalence across counties. We recorded the highest prevalence in Tiranë (76.0%) and Shkodër (75.0%), while we noted the lowest prevalence in Korçë (36.4%) and Vlorë (44.7%) (Fig. 1). These variations may be linked to differences in farm hygiene practices, management systems and regional veterinary services.
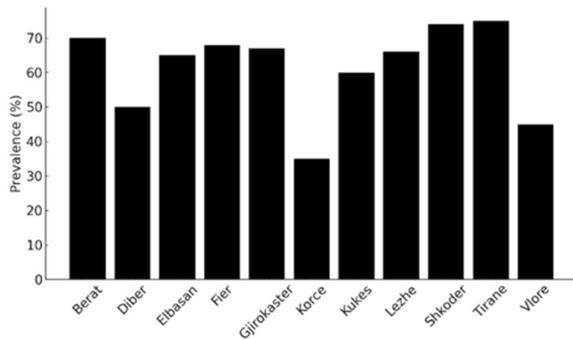

Fig. 1: Subclinical Mastitis Prevalence by County in Albania. Bar chart illustrating subclinical mastitis prevalence based on CMT screening of 500 dairy cows across 12 counties from December 2022 to April 2023. The highest prevalence was recorded in Tiranë (76%) and Shkodër (75%), with the lowest in Korçë (36.4%) and Vlorë (44.7%).

We did not test 30 of the 2000 quarters due to teat obstruction or malfunction. Regarding frequency at the quarter level, we found that the rear quarters had a higher frequency of subclinical mastitis (27%), while the anterior quarters were less affected (21%–24%). While we identified 127 and 113 positive cases of subclinical mastitis, respectively, for the right and left anterior quarters, and we confirmed 143 and 144 cases for the right and left posterior quarters (Fig. 2). This difference could be due to the rear quarters being more anatomically exposed to contaminants. Based on CMT scoring, we classified subclinical mastitis by severity. We found most cases as mild (+1) with 40% (208 out of 527) or moderate (+2) with 39% (205 out of 527), and fewer cases as severe (+3) with 21% (114 out of 527) (Table 1). This distribution suggests that most subclinical mastitis cases in the studied population were of lower severity, potentially indicating early-stage infections.

Relationship between risk factors and subclinical mastitis prevalence

We used univariate Chi-square analysis to examine the relationship between categorical risk factors (breed, management system, housing, milking technique, udder washing before and after milking, pre- and post-dipping teat

disinfection, use of individual paper towels, and breeding system) and the prevalence of subclinical mastitis. The Chi-square tests revealed significant associations with subclinical mastitis for two variables: management system (P=0.001) and post-milking udder washing (P<0.001) (Table 2). These results emphasize the critical role of both hygiene and production practices in the occurrence of mastitis. To assess the relationship between continuous variables and subclinical mastitis, we applied independent samples T-tests for daily milk production and altitude. While daily milk yield did not show a statistically significant association (P=0.749), altitude was significantly associated with mastitis occurrence (P=0.020), indicating that cows located at lower altitudes are at greater risk (Table 3).

Table 1: Severity classification of subclinical mastitis based on CMT score

Severity Grade	Proportion of Cases (%)
+1 (Mild)	40
+2 (Moderate)	39
+3 (Severe)	21

Analysis of CMT-positive quarters by severity: mild (+1), moderate (+2), and severe (+3), expressed as percentage of total positive quarters (n = 527).

Table 2: Chi-square test results for association between categorical risk factors and subclinical mastitis

Variable	Chi^2	P-value	Degrees of Freedom
Post-milking udder washing		<0.001	1
Management System		0.001	2
Housing (tethered vs free stall)		0.253	1
Single paper towel		0.828	1

A P<0.05 indicates statistical significance. Degrees of freedom (df) indicate the number of categories minus one for each variable

Table 3: T-test results for continuous variables associated with subclinical mastitis. Comparison of daily milk production (kg/day) and farm altitude (m) between mastitis-positive and mastitis-negative cows

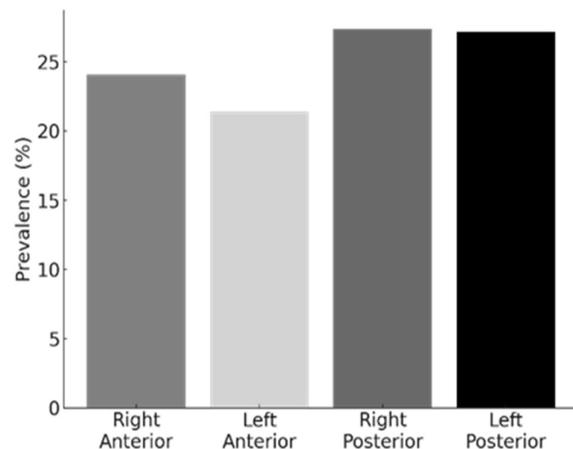| Variable	T-statistic	P-value
Milk Production (kg/d)	-0.32	0.749
Altitude (m)	-2.33	0.020

Statistical significance was defined as P<0.05

Fig. 2: Subclinical Mastitis Prevalence by Udder Quarter. Distribution of subclinical mastitis cases in 527 affected quarters of 500 dairy cows. Rear quarters showed higher prevalence (27%) compared to anterior quarters (21–24%). The right posterior and left posterior quarters had 143 and 144 positive cases, respectively.

A multivariable logistic regression model was constructed including all variables with P<0.25 from the univariate tests. The final model identified three significant predictors: Post-milking udder washing significantly increased the odds of subclinical mastitis (OR=4.71, P=0.001), likely due to improper drying or lack of individual towels that promote teat-end contamination. Altitude, treated as a continuous variable, was negatively associated with mastitis risk (β=−0.0011, P=0.020), meaning that each 1-meter increase in altitude slightly reduced the odds of infection. Management system also emerged as a significant factor: cows raised in intensive systems had 69% lower odds of subclinical mastitis compared to those in extensive systems (OR=0.31, P=0.001). Semi-intensive systems were borderline protective (OR=0.58, P=0.050) (Table 4).

These findings reinforce the relevance of post-milking hygiene, geographical positioning and structured management protocols in reducing the risk of subclinical mastitis.

Bacteria isolated

From 175 cultured samples, we found that 55% yielded a single bacterial species, we considered 19% contaminated (three or more species), and we identified 2%

yielded miscellaneous other bacterial species. We isolated a total of 85 bacterial strains. We identified the most frequent bacterial pathogens as *Staphylococcus aureus* (33.7%, 95% CI: 24.7–44.0%), *Escherichia coli* (16.6%, 95% CI: 10.1–26.0%), *Pseudomonas aeruginosa* (12.0%, 95% CI: 6.6–20.6%), *Enterobacter* spp. (10.3%, 95% CI: 5.4–18.8%), and *Staphylococcus epidermidis* (9.1%, 95% CI: 4.6–17.2%). We noted less frequent pathogens, including *Bacillus* spp. and *Candida albicans*, each at 1.1% (95% CI: 0.2–6.0%) (Fig. 3). Gram-positive bacteria accounted for a slightly higher proportion of isolates compared to Gram-negative ones. The predominance of *Staphylococcus aureus* suggests the importance of contagious transmission, while the presence of environmental pathogens like *Escherichia coli* and *Pseudomonas aeruginosa* reflects environmental associated infection.

Antibiotic susceptibility

We conducted antibiotic susceptibility testing for *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, summarized in Fig. 4. Among *Staphylococcus aureus* isolates, resistance was highest to penicillin (73%) and tetracycline (58%), while susceptibility remained moderate to gentamicin (72%).

Table 4: Multivariable logistic regression analysis of risk factors associated with subclinical mastitis.

Variable	Coefficient (β)	Std. Error	P-value	Odds Ratio	95% CI (OR)
Post-milking udder washing	1.52	0.51	0.003	4.71	1.68–12.40
Intensive management	-1.18	0.37	0.001	0.31	0.15–0.63
Semi-intensive management	-0.55	0.28	0.050	0.58	0.33–1.00
Altitude (continuous)	-0.0011	0.0005	0.020	0.9989	0.9979–0.9998

Odds ratios (OR) and 95% confidence intervals (CI) are reported. A P-value <0.05 was considered statistically significant
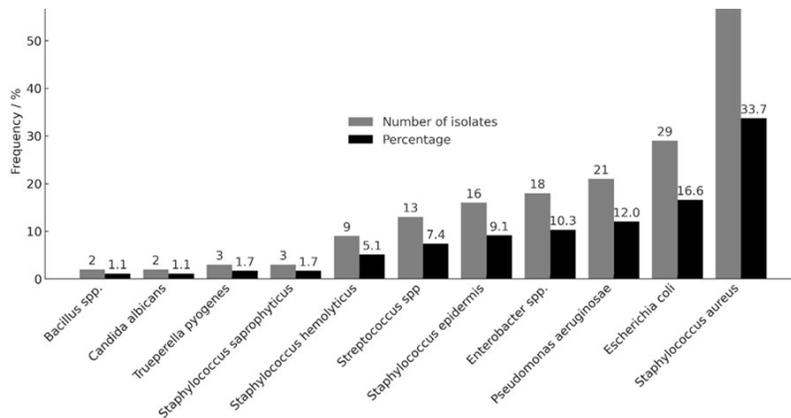

Fig. 3: Percentages of bacterial isolates from 175 positive milk samples. *Staphylococcus aureus* (33.7%) was the most frequent pathogen, followed by *Escherichia coli* (16.6%), *Pseudomonas aeruginosa* (12.0%), *Enterobacter* spp. (10.3%), and *Staphylococcus epidermidis* (9.1%). Less frequent isolates included *Bacillus* spp. and *Candida albicans* (1.1% each). Confidence intervals (95% CI) are shown.
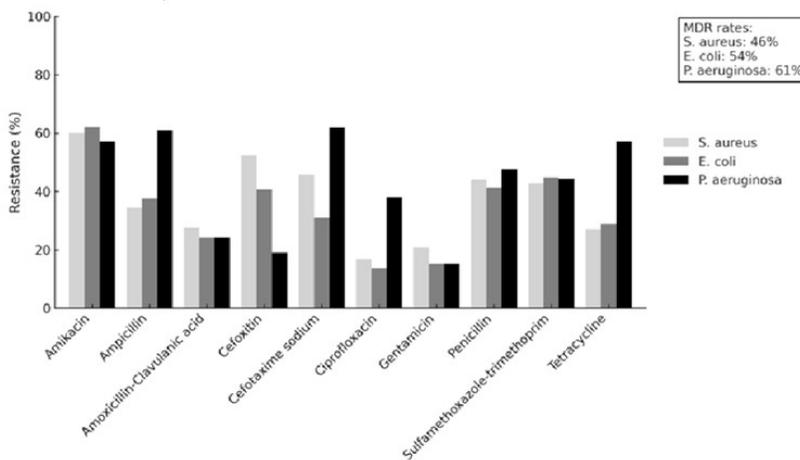

Fig. 4: Antibiotic resistance profiles of key mastitis pathogens. Resistance patterns of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* to 10 antibiotics using the Kirby-Bauer disk diffusion method. Ciprofloxacin and gentamicin were the most effective agents, while resistance to penicillin and amikacin was high across isolates.

Escherichia coli isolates showed marked resistance to ampicillin (82%) and cefotaxime (61%), with partial sensitivity to ciprofloxacin (56%) and trimethoprim-sulfamethoxazole (53%). Notably, *Pseudomonas aeruginosa* exhibited limited sensitivity to gentamicin (48%). The overall rate of multidrug resistance, defined as resistance to ≥3 antibiotic classes, was 61% in Pseudomonas aeruginosa, 54% in Escherichia coli, and 46% in *Staphylococcus aureus*. These findings underscore the growing antimicrobial resistance among mastitis-causing pathogens in Albanian dairy herds.

DISCUSSION

This study presents the first nationwide investigation into the prevalence and risk factors of subclinical mastitis in Albanian dairy cattle, alongside the identification of major bacterial agents and their antimicrobial resistance profiles. The CMT was used to screen for subclinical mastitis, with a reported sensitivity of approximately 82% and specificity of 81% for detecting intramammary infections in early lactation dairy cows (Sargeant et al. 2001; Dingwell et al. 2003). These limitations may result in some false positives or negatives, potentially affecting prevalence estimates. Seasonal factors (sampling from December 2022 to April 2023) and milking machine issues may introduce bias. While supplemented with bacteriological analysis, CMT's limitations may impact risk factor identification. Despite this, CMT remains practical for on-farm screening, though advanced diagnostics like automated SCC counting or PCR could improve accuracy in future studies. The overall prevalence of subclinical mastitis was high (59.6%), with notable regional variation across counties. Compared to similar studies in the Balkan region, such as Kosovo (22.3%) (Mazreku et al. 2024), North Macedonia (43.9%), (Janevski et al. 2019) and Greece (34.5%) (Themistokleous et al. 2020), the prevalence in Albania appears considerably higher. This may be due to insufficient hygiene protocols, poor udder health monitoring, and lack of mastitis prevention programs, particularly in extensive farms where resources and training are often lacking.

Subclinical mastitis was more frequent in rear quarters (27%), likely due to their lower anatomical position and greater exposure to environmental contaminants. This vulnerability has also been reported in similar studies (Singh et al. 2017; Ndahetuye et al. 2019). Most detected cases were mild to moderate in severity (+1 to +2), which are indicative of early-stage intramammary infections, before the manifestation of clinical signs. The predominance of mild infections may also reflect farmer unawareness or delayed recognition of udder health deterioration in the absence of obvious clinical symptoms, highlighting the importance of routine screening and preventive measures.

Among the most significant risk factors identified was post-milking udder washing significantly increased the risk of subclinical mastitis (OR=4.71, P<0.001) (Table 4), indicating that farms practicing post-milking udder washing had 4.71 times higher odds of subclinical mastitis compared to those that did not. This likely reflects inadequate post-milking hygiene, especially when teats are not properly dried or when reusable cloths are used across multiple cows. Previous studies also underline the importance of proper drying and disinfection (Galton et al. 1988; Fesseha et al. 2021). A key finding was the significantly lower prevalence of subclinical mastitis in intensive systems (OR=0.31, P=0.001). This aligns with earlier reports linking extensive management to increased mastitis risk (Kitila et al. 2021), likely due to less structured hygiene protocols and limited veterinary oversight. The association between semi-intensive management and subclinical mastitis was borderline significant (P=0.050), suggesting a potential trend but insufficient evidence to conclusively establish a significant relationship. Further research with larger sample sizes is needed to confirm this finding. Altitude, modeled as a continuous variable, was also significantly associated with mastitis risk (P=0.020). For every 1-meter increase in elevation, the odds of subclinical mastitis decreased by approximately 0.11%. This suggests that environmental conditions associated with higher altitudes, such as lower humidity, better drainage, and reduced pathogen load, may contribute to lower infection rates. These findings align with studies linking elevation to mastitis epidemiology (Abebe et al. 2016; Simbaña-Cifuentes et al. 2022).

In our study, samples with three or more bacterial species were considered contaminated and excluded from analysis, following standard protocols (Quinn et al. 2002; NMC 2017). While this approach ensures accurate pathogen identification, it may exclude rare cases of true polymicrobial infections, which are less common in mastitis but could slightly underestimate the prevalence of certain pathogens. Applying this criterion allowed us to focus on the most clinically relevant and reliable bacterial isolates. It also helped reduce the risk of drawing conclusions from potentially compromised samples. The predominant bacterial pathogen isolated in our study was *Staphylococcus aureus*, which aligns with findings from nearby countries such as Greece and other parts of Europe (Hoekstra et al. 2020; Kotzamanidis et al. 2021). Since *Staphylococcus aureus* is widely recognized as a contagious mastitis agent (Meçaj et al. 2023), its high occurrence likely points to issues related to milking hygiene and cow-to-cow transmission during this process. The bacteria are known to colonize the skin of the udder and the teat canal (Birhanu et al. 2017), making it easier for infection to spread from one quarter to another, especially when proper hygiene protocols are not followed. *Escherichia coli* was the second most common isolated bacteria. As a typical environmental pathogen (Hogan and Smith 2012; Klaas and Zadoks 2018; Meçaj et al. 2023), its presence highlights the influence of surrounding conditions, such as bedding and sanitation, on subclinical mastitis development. *Pseudomonas aeruginosa* was also among the most frequently identified bacteria. Though primarily considered an environmental pathogen, it can also be introduced through improperly cleaned equipment and has been associated with occasional clinical mastitis outbreaks (Hogan and Smith 2012; Huang et al. 2024).

The antimicrobial susceptibility analysis revealed that all tested isolates of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were non-susceptible to at least one antibiotic. Notably, a high level of resistance was observed against penicillin (80–90%) and amikacin (around 60%). On the other hand, ciprofloxacin,

gentamicin, and sulfamethoxazole-trimethoprim showed relatively strong efficacy, with 70–85% of isolates remaining susceptible (Fig. 4). For *Enterobacter* spp. and *Staphylococcus epidermidis*, preliminary results indicated promising susceptibility to ciprofloxacin (66.7 and 75%, respectively) and gentamicin (55.6 and 62.5%). However, due to the limited number of isolates tested, these findings should be interpreted cautiously and validated through further sampling.

Overall, the results indicate that ciprofloxacin and gentamicin represent viable options for targeted therapy against subclinical mastitis in Albanian dairy farms, provided that culture and sensitivity testing confirm pathogen identity and drug response in each case. Given the alarming levels of resistance, particularly to broad-spectrum agents like penicillin and amikacin, it is essential to adopt more prudent use of antimicrobials. We recommend routine antimicrobial sensitivity testing before initiating treatment, combined with improved milking hygiene and mastitis control protocols. Such strategies can help reduce the emergence of multidrug-resistant strains, minimize unnecessary antibiotic use, and lower the financial burden associated with prolonged treatments and milk withdrawal.

Conclusion

This study highlights a high prevalence of subclinical mastitis (59.6%) among Albanian dairy cattle, with substantial regional differences. We identified key risk factors (or protective factors) influencing mastitis risk: notably, improper post-milking udder washing and low-altitude, extensive management conditions were associated with higher infection odds, whereas intensive management was associated with lower odds. The most frequently isolated pathogens—*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*—exhibited considerable resistance to penicillin and amikacin, while ciprofloxacin and gentamicin remained effective. These findings underscore the need for immediate intervention. We recommend routine CMT screening, targeted antimicrobial susceptibility testing, and specific hygiene measures such as pre- and post-milking iodine-based teat disinfection and drying with disposable paper towels. Establishing a national mastitis control program is vital for standardizing farm practices, guiding antibiotic use, and supporting farmer education. Such a program should also provide subsidies for diagnostic tools and training.

DECLARATIONS

Conflict of Interest: The authors declare that they have no conflicts of interest

Data Availability: The data that support the findings of this study are available in this article. There is no other supporting data.

Ethics Statement: The authors declare no conflicts of interest that could influence the research outcomes.

Author's Contribution: Gerald Muça, Majlind Sulçe and Xhelil Koleci developed the study concept, collected the data, and performed the experimental procedures. Luigj Turmalaj and Pëllumb Zalla participated in animal testing, sample collection and manuscript writing. Doriana Beqiraj participated in study concept and manuscript writing and revision. Myqerem Tafaj participated in the critical evaluation and revision of the manuscript. All authors approved the manuscript for submission.

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