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Article

Diagnosis and Prevalence of Mastitis in a Dairy Farm from Cluj County, Romania

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Abstract: Mastitis, a prevalent and economically burdensome disease in dairy farming, impacts milk yield and quality. This study assesses the prevalence and diagnosis of mastitis in a dairy farm in Cluj County, Romania, focusing on field diagnostics and pathogen resistance profiling. The California Mastitis Test (CMT) revealed a 60% mastitis prevalence in lactating cows, with 51.7% of cases identified as subclinical and 8% as clinical. Laboratory analysis identified Staphylococcus spp. as the primary pathogen (43%), with a significant proportion displaying antibiotic resistance, notably to penicillin (85%) and erythromycin (75%). The study highlights the need for regular CMT screenings, targeted antimicrobial protocols, and enhanced farm hygiene practices to manage mastitis effectively, prevent resistance escalation, and optimize dairy productivity.

Keywords: mastitis, dairy cattle, California mastitis test, mastitis prevalence, antibiotic resistance

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1. Introduction

Mastitis, an inflammation of the mammary gland, is a pervasive issue in dairy cattle worldwide, leading to significant economic losses in dairy production due to reduced milk yield, altered milk composition, increased veterinary costs, and early culling of affected animals [1]. This condition not only affects milk production volume but also diminishes milk quality, affecting protein, fat, and lactose levels while increasing somatic cell counts, which are often used as indicators of milk quality [2]. The primary causative agents of mastitis are bacterial pathogens, which enter the mammary gland via the teat canal, particularly after milking when the canal is relaxed and susceptible to contamination [3].

Mastitis can present clinically, with visible symptoms such as udder swelling, redness, and pain, or subclinically, where no outward signs are evident, though both types affect milk quality and yield [4]. Subclinical mastitis is particularly challenging as it often goes undetected without specific diagnostic tests, allowing for transmission within the herd and prolonged milk contamination. In particular, pathogens like *Staphylococcus aureus* and *Streptococcus uberis* are known for their role in both clinical and subclinical mastitis, with *Staphylococcus spp.* accounting for a large portion of cases due to its contagious nature and ability to evade treatment through biofilm formation [5].

Mastitis shows variable incidence rates across regions due to differences in farm management practices, veterinary access, and herd size. In Romania, smaller farms with limited resources report significant rates of both clinical and subclinical mastitis, with studies showing that up to 35% of cows in smaller herds may have subclinical mastitis [6]. Limited veterinary infrastructure and insufficient preventive practices contribute to these high rates, a trend mirrored in other developing regions. Internationally, countries with larger, well-managed dairy operations, like the U.S. and parts of Western Europe, generally report lower clinical mastitis incidence (around 20-25 cases per 100 cows per year) due to advanced management and monitoring practices [7,8]. However, subclinical mastitis remains a widespread issue globally, with prevalence often exceeding 30% even in well-managed herds.

Routine diagnostic tests, such as the California Mastitis Test (CMT), have proven essential for early detection of mastitis, especially subclinical cases, in field conditions. The CMT is widely used for its rapid and cost-effective approach to detecting somatic cell count increases, providing a reliable indication of infection levels and guiding further laboratory testing for specific pathogen identification [9,10]. Laboratory diagnostics, including bacterial culturing and antibiogram testing, play a critical role in identifying causative pathogens and determining appropriate antibiotic treatments, which is increasingly important as resistance patterns emerge [11]. Recent studies have reported rising antibiotic resistance in common mastitis pathogens, underscoring the need for targeted antimicrobial use to manage infections effectively [12, 13,14].

This study aims to assess the prevalence of mastitis within a dairy herd, utilize on-site diagnostic tools to identify subclinical and clinical cases, and examine the distribution of bacterial pathogens and their resistance profiles. By combining field diagnostics with laboratory confirmation, this research contributes to a deeper understanding of mastitis management practices in dairy herds and highlights the need for evidence-based approaches to control this common and costly condition.

2. Materials and Methods

2.1 Farm Setting and Cattle Management

The study was conducted at a dairy farm in Cluj County, Romania, housing 253 cows, primarily of the Romanian Spotted breed. The herd was divided into specific sections for lactating cows (n=87), young stock (n=120), and calves (n=36), which facilitated targeted sampling and tracking. Milking occurred twice daily in a 24-station milking parlor designed to standardize milking practices and closely monitor milk output for each cow. Milking hygiene protocols were strictly adhered to, including pre- and post-milking teat disinfection and regular sanitation of equipment and facilities, to minimize the risk of contamination and maintain milk quality. This herd structure and adherence to hygiene standards contribute to a reliable assessment of mastitis prevalence and resistance patterns within this population.

2.2 Mastitis Screening Using California Mastitis Test (CMT)

The California Mastitis Test (CMT) was employed to identify subclinical and clinical mastitis in the lactating cows. The CMT is a rapid, field-based diagnostic tool that detects increases in somatic cell count, which indicate inflammatory responses within the udder [15]. The test involves mixing equal parts of milk and CMT reagent in a four-compartment plastic paddle, with each compartment corresponding to one quarter of the udder. The mixture was gently swirled, and after a few seconds, results were interpreted based on gel formation:

- **Negative**: No visible gel formation.
- **Mildly Positive**: Slight thickening, indicating a low increase in somatic cells.
- **Strongly Positive**: Distinct gel formation, indicating high somatic cell count [9].

All lactating cows on the farm were tested with CMT at the morning milking after removing a few drops of milk, cows with mild to strongly positive results were identified as mastitis cases and marked for further testing (bacteriological examination and antibiogram). This test was chosen for its ease of use in field settings, affordability, and reliability in detecting subclinical infections, which often go unnoticed without specific testing [16].

The CMT is limited by its subjective nature, as results can vary between observers and may be influenced by environmental conditions like lighting and temperature. It is more sensitive for high somatic cell counts (SCC) but less reliable at lower SCC levels, potentially missing early or mild infections and occasionally yielding false positives, especially in cows at the beginning or end of lactation when SCC may naturally fluctuate. Additionally, physiological factors such as stress, heat, or recent calving can temporarily elevate SCC, leading to inaccuracies. While useful for detecting subclinical mastitis, the CMT does not always correlate with the severity of clinical infections and cannot identify specific pathogens, which limits its effectiveness as a standalone diagnostic tool [17].

Sample Collection

Milk samples were collected aseptically from cows with positive CMT results. Each teat was cleaned with an antiseptic solution to prevent contamination, and the first few streams of milk were discarded. Approximately 10 mL of milk was then collected in sterile tubes (BD Falco 50 mL Conical Tubes, Dickinson and Company (BD), labeled, and transported on ice to the laboratory within two hours to maintain sample integrity [18].

2.3 Bacterial Culture and Identification

In the laboratory, milk samples were cultured on blood agar plates to allow for isolation and identification of pathogens. Each sample was streaked using a sterile loop, employing a quadrant streak method to isolate individual colonies. Plates were incubated at 37°C for 24–48 hours. Colony morphology, color, hemolytic activity, and growth patterns were observed to identify distinct bacterial colonies [19]. Gram staining was subsequently performed to classify the isolates as Gram-positive or Gram-negative, which facilitated preliminary identification of species. This approach enables reliable differentiation of common mastitis pathogens, such as *Staphylococcus aureus* and *Streptococcus spp.*, based on morphological characteristics [20].

To determine antibiotic susceptibility, an antibiogram was conducted using the Kirby-Bauer disk (Elta 90 Romania) diffusion method. Antibiotic discs—neomycin, amoxicillin, streptomycin, penicillin, erythromycin, ampicillin, and oxacillin—were placed on Mueller-Hinton agar (Elta 90, Romania) plates inoculated with bacterial isolates from the milk samples. Plates were incubated at 37°C for 24 hours, and zones of inhibition around each antibiotic disc were measured. Results were interpreted based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, which specify breakpoints for bacterial sensitivity and resistance, allowing for effective therapeutic recommendations [21].

The antibiogram results were critical for understanding the resistance profiles of pathogens present in the herd. Given the increasing prevalence of antibiotic resistance in common mastitis pathogens, the study aimed to identify effective treatment options tailored to the specific resistance patterns observed.

2.4 Data Analysis

Prevalence data from the CMT results were summarized as percentages, distinguishing between clinical and subclinical cases. Pathogen identification and antibiotic resistance profiles were analyzed to determine the most common bacterial agents and their corresponding resistance patterns.

3. Results

3.1 Prevalence of Mastitis

Out of the 87 cows tested using the California Mastitis Test, 52 cows (60%) were positive for mastitis. Among these, 45 cases (51.7%) were classified as subclinical (no visible symptoms), while 7 cases (8.0%) exhibited clinical signs, including udder swelling, redness, or pain and modifications in milk aspect. This distribution emphasizes the high prevalence of subclinical mastitis, which often goes unnoticed without specific testing. The prevalence of mastitis among the sampled cows, distinguishing between clinical and subclinical cases is presented in table 1.

Table 1 Mastitis prevalence

Percentage (%)	Number of Cases	Category
100%	87	Total Cows Tested
59.77%	52	Mastitis Positive
51.7%	45	Subclinical Mastitis
8.0%	7	Clinical Mastitis

3.2 Pathogen Identification

Bacterial culture results revealed that **Staphylococcus spp.** was the most frequently isolated pathogen, a counting for 43% of the positive samples. In 4 of the 52 samples examined, 2 types of colonies were identified. Other common pathogens included **Streptococcus spp.** (25%) and various Gram-negative bacilli (13%). These findings are consistent with previous research (Pascu) identifying *Staphylococcus aureus* and *Streptococcus spp.* as leading causative agents of both clinical and subclinical mastitis (Table 2).

Table 2 Breakdown of the isolated pathogens from the milk samples.

Percentage (%)	Number of Samples	Pathogen
43%	26	Staphylococcus spp.
25%	15	Streptococcus spp.
13%	8	Gram-negative bacilli
11%	7	Other bacteria

3.3 Antibiotic Resistance Patterns

Antibiogram testing showed significant resistance among *Staphylococcus spp.* isolates, especially to penicillin (85%) and erythromycin (75%). In contrast, neomycin and streptomycin retained relatively high effectiveness. Resistance was also observed in *Streptococcus spp.* and Gram-negative isolates, with some resistance patterns reflecting limitations in current antibiotic options (Figure 1).

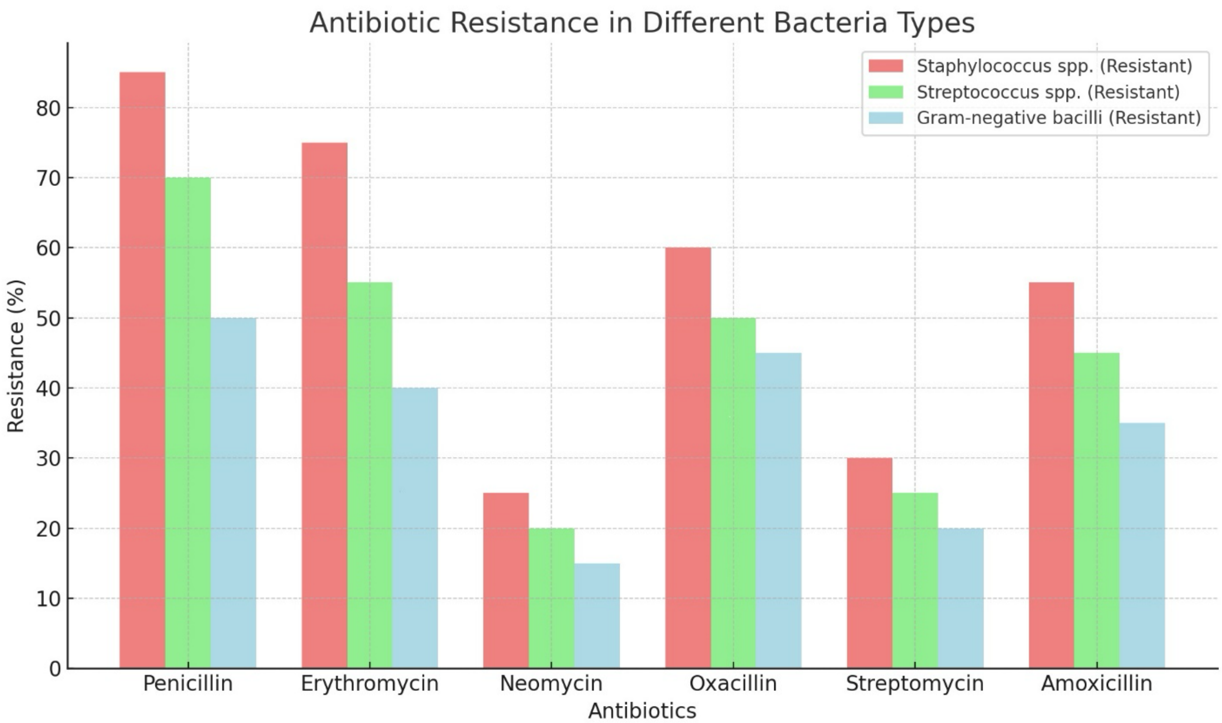

Figure 1. Antibiotic resistance for Staphylococcus spp., Streptococcus spp., and Gram-negative bacilli across different antibiotics.

4. Discussion

The study's finding of a 60% mastitis prevalence aligns with global reports indicating high rates of mastitis in dairy farms, particularly subclinical cases. Subclinical mastitis, which constituted 51.7% of the cases in this study, is often overlooked without routine testing due to the absence of visible symptoms, but it poses significant risks, including prolonged contamination of milk and increased pathogen transmission within the herd [22]. Studies have emphasized that subclinical mastitis contributes to higher somatic cell counts (SCC) and can reduce milk yield and quality [23]. Early detection methods, like the California Mastitis Test (CMT) used here, are essential for managing subclinical infections and reducing their economic impact on milk production [24].

The predominance of subclinical mastitis highlights the need for routine herd health monitoring, as subclinical infections are typically reservoirs for pathogen transmission within and between herds [25]. Regular CMT testing in field settings allows for rapid detection and facilitates timely intervention, mitigating the spread of infection and preserving milk quality.

The study identified Staphylococcus spp. as the predominant pathogen (43% of isolates), which is consistent with its known role as a common cause of both clinical and subclinical mastitis globally [23]. Staphylococcus aureus in particular is well-documented for its ability to form biofilms, enhancing its persistence within the mammary gland and complicating treatment [26]. A study by Rivas et al. (2020), [27] observed that *Staphylococcus aureus* was the most common pathogen associated with mastitis in dairy herds, accounting for 39% to 45% of cases. In Romania, a study by Popescu et al. (2016), [28] also identified *Staphylococcus aureus* as the leading cause of mastitis, although with varying prevalence rates between farms (35%-45%). The predominance of *Staphylococcus* in this study supports its role as a major pathogen in dairy mastitis worldwide. Furthermore, the study also highlighted the presence of *Streptococcus* species and Gram-negative bacilli, which are commonly associated with environmental sources of infection. This suggests that, like other studies, environmental factors—such as insufficient sanitation practices—play a crucial role in mastitis outbreaks [16]. This is consistent with findings from other studies, including a Romanian study by Matei et al. (2018), [29] which reported a similar resistance pattern, with *Staphylococcus aureus* showing 80% resistance to penicillin and 70% resistance to erythromycin. The emergence of beta-lactam resistance due to the production of beta-lactamase enzymes complicates treatment options, highlighting the urgent need for more targeted therapies. In contrast, the study found lower resistance rates for neomycin and streptomycin, which is in line with global findings that suggest these antibiotics may still be effective against certain strains of mastitis pathogens. However, antibiotic resistance remains a significant challenge, and ongoing monitoring of susceptibility patterns is essential to prevent further resistance development. A study from Italy by Gallo et al. (2019), [30] stressed the importance of routine antibiogram testing for optimizing antibiotic use and minimizing the overuse of broad-spectrum antibiotics.

Environmental control measures, including proper bedding management, post-milking teat disinfection, and regular sanitation of the milking area, have been shown to reduce the incidence of environmental pathogens [16]. This multifaceted approach to infection control could significantly reduce both the occurrence and spread of mastitis within the herd. The pathogen profile observed here supports previous research, which advocates for integrated management practices targeting both contagious and environmental sources of infection [31].

Antibiotic resistance, particularly among Staphylococcus spp., poses a critical challenge in treating mastitis. In this study, high resistance rates were observed against commonly used antibiotics, such as penicillin (85%) and erythromycin (75%), which echoes recent findings of increased resistance in mastitis pathogens [32]. The resistance of Staphylococcus spp. to beta-lactam antibiotics, like penicillin, is largely due to the production of beta-lactamase enzymes, which render these treatments ineffective. This resistance can limit treatment options and necessitates the use of more targeted therapies, potentially increasing treatment costs and duration [33].

The lower resistance rates observed with neomycin and streptomycin indicate that these antibiotics remain viable treatment options; however, continued monitoring of susceptibility is crucial to prevent further resistance development. The need for routine antibiogram testing as part of mastitis control programs is increasingly emphasized, as it allows for tailored therapy, reducing the reliance on broad-spectrum antibiotics and promoting more effective treatment outcomes [34]. Implementing evidence-based antibiotic selection could significantly enhance treatment efficacy and help mitigate the development of resistant bacterial strains.

These findings underscore the need for comprehensive mastitis management strategies in dairy farms. Regular CMT testing can facilitate early detection of subclinical cases, allowing for prompt treatment and reduced pathogen transmission. Additionally, integrating susceptibility testing into routine herd health protocols will enable more effective use of antibiotics, optimizing treatment while reducing the risk of resistance development. Antibiotic stewardship in veterinary medicine is increasingly important, with research suggesting that targeted therapy can significantly reduce the overuse of antibiotics in dairy herds [35].

Enhanced hygiene practices, such as improving bedding quality, maintaining milking equipment, and ensuring proper post-milking teat disinfection, are critical to reducing both contagious and environmental sources of infection. Evidence shows that improving udder hygiene can lower the incidence of mastitis by reducing pathogen load on teat surfaces [25]. Future research might explore alternative treatments, such as bacteriophages or probiotics, which have shown promise in reducing mastitis pathogens without contributing to antibiotic resistance [34].

While this study provides valuable insights into mastitis prevalence and pathogen profiles, it has several limitations. The reliance on CMT for detecting subclinical mastitis, as mentioned earlier, may lead to false negatives. Additionally, the study's scope is limited to one farm, which may not be representative of broader regional trends. A more comprehensive study involving multiple farms and additional diagnostic tools would provide a more accurate overview of mastitis prevalence and pathogen dynamics across different dairy systems in Romania. Moreover, the lack of data on the farm's management practices, such as milking techniques and sanitation protocols, limits the ability to draw conclusions about the exact factors contributing to the observed pathogen profiles and resistance patterns.

5. Conclusions

This study underscores the critical need for effective diagnostics and targeted treatment of mastitis in dairy herds. The high prevalence of mastitis and the significant presence of antibiotic-resistant Staphylococcus spp. highlight the importance of routine susceptibility testing. Field diagnostics, such as the CMT, allow for rapid, practical screening that can inform timely intervention and treatment decisions. Implementing evidence-based approaches, including rigorous hygiene practices and targeted antibiotic use, is essential to improve mastitis management and enhance the health and productivity of dairy herds. Penicillin and erythromycin were more potent against resistant bacterial strains and could be used as first-line treatments when an antibiogram is not performed.

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