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Article

Zoonotic Mastitis Pathogens in Dairy Cows: Prevalence and Risk Factors of *E. coli* and *S. aureus* in the West Arsi Zone, Ethiopia

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Abstract

Mastitis is a leading disease in dairy cows, significantly affecting milk quality and yield, and posing notable public health risks due to milk contamination with zoonotic pathogens. This study aimed to isolate and determine the prevalence of *Staphylococcus aureus* and *Escherichia coli*, and assess their associated risk factors in mastitic milk samples from selected dairy farms in Shashamane and Arsi Negelle districts, West Arsi Zone, Ethiopia. A total of 83 mastitis-positive milk samples were examined bacteriologically. The results revealed that 50.6% of the samples tested positive for *S. aureus* and 24.1% for *E. coli*, confirming their prominent role in bovine mastitis. Risk factor analysis showed that only the stage of lactation was significantly associated with *S. aureus* prevalence ($p < 0.05$), with early lactation cows being most affected. Additionally, significant associations ($p < 0.05$) were identified between both pathogens and hygiene-related variables, including the cleanliness of milking processes, milking equipment, handlers' hands, and cow housing conditions. In contrast, variables such as breed, age, parity, production system, and settlement type did not show statistically significant associations. These findings emphasize the need for enhanced hygiene management, regular mastitis screening, and farmer education to reduce the burden of mastitis and improve milk safety. The study provides critical evidence for designing context-specific intervention strategies and highlights the necessity for further research into antimicrobial resistance profiles of mastitis pathogens in the region.

Keywords: mastitis; dairy cows; *E. coli*; *S. aureus*

Introduction

Mastitis, the inflammation of the mammary gland, is one of the most economically devastating diseases affecting the dairy industry globally. It significantly compromises milk yield, quality, and animal welfare while simultaneously posing substantial public health threats through contamination of raw milk with zoonotic pathogens. Among the primary causative agents of mastitis are *Staphylococcus aureus* and *Escherichia coli*, both of which are frequently isolated from clinical and subclinical mastitic milk samples (Weldemelak Girmay et al., 2020; Zeryehun et al., 2013).

S. aureus is a gram-positive bacterium known for its ability to cause chronic infections due to its resistance to host immune defenses and common antimicrobial treatments. It spreads primarily through contagious routes, including milking equipment, handlers' hands, and direct udder contact. Its ability to persist within the mammary gland makes it particularly difficult to eliminate once established. Additionally, *S. aureus* has demonstrated considerable antimicrobial resistance exacerbating the difficulty of treatment and raising concerns about potential transmission to humans through raw milk consumption (Balcha et al., 2022).

In contrast, *E. coli* is a gram-negative, opportunistic environmental pathogen that frequently causes acute, sometimes severe, mastitis. It typically enters the udder through contaminated bedding, floors, and milking equipment. Unlike *S. aureus*, *E. coli* infections tend to be short-lived but can cause significant clinical symptoms, particularly in the early stages of lactation when cows are immunocompromised (Cheesbrough, 2006; Brenner et al., 2005).

In Ethiopia, mastitis remains widespread due to numerous factors such as limited veterinary infrastructure, lack of awareness among farmers, traditional milking practices, and inadequate hygiene measures (Shimels et al., 2014). The use of bare hands for milking, shared towels, absence of pre- and post-milking teat disinfection, and the general absence of routine health screening are common in smallholder dairy farms. These contribute not only to high mastitis prevalence but also increase the risk of milk-borne zoonoses.

Furthermore, the lack of effective control strategies and poor implementation of good milking practices exacerbate the problem. Studies have shown that risk factors such as breed, age, stage of lactation, parity, and type of production system may influence the prevalence of mastitis-causing organisms. However, there remains a critical gap in linking these factors to actual pathogen prevalence using statistically grounded evidence, particularly in specific local contexts like the West Arsi Zone.

Given these challenges, this study aims to fill the knowledge gap by examining the prevalence of *E. coli* and *S. aureus* in mastitic milk from dairy cows in the Shashamane and Arsi Negelle districts of Ethiopia and by evaluating key risk factors influencing their occurrence. A clear understanding of the relationship between pathogen prevalence and specific environmental and management-related risk factors is essential for designing targeted interventions that can enhance milk safety and productivity in the region.

By identifying the primary sources of contamination and quantifying the risk posed by various management practices, this research contributes critical data to both veterinary and public health domains. The findings are expected to support evidence-based recommendations for mastitis control programs tailored to the realities of small- and medium-scale dairy farming systems in Ethiopia.

Ethical Considerations

The study involved only non-invasive milk sample collection from lactating cows suspected of mastitis, following standard aseptic procedures and without causing harm or distress to the animals. However, informed consent was obtained from all participating farm owners prior to sample collection, and animal welfare considerations were strictly observed throughout the study. Confidentiality of farm-level information was also maintained throughout the study.

Methodology

Study Area and Design

This cross-sectional study was conducted between December 2023 and October 2024 in two selected districts—Shashamane and Arsi Negelle—located in the West Arsi Zone of Oromia Region, Ethiopia. These districts are recognized for their high concentration of dairy farms, both traditional and semi-commercial, making them suitable for assessing the prevalence of mastitis and its causative pathogens. The study areas exhibit typical highland agroecology with mixed crop-livestock farming systems and varying hygienic and management practices.

The study design was aimed at determining the prevalence of *E. coli* and *S. aureus* in mastitic dairy cows and evaluating associated risk factors through bacteriological isolation and structured questionnaires. The target population included lactating cows showing clinical signs of mastitis as well as subclinical cases identified using the California Mastitis Test (CMT).

Sampling Technique and Sample Size

A purposive sampling technique was employed to select 200 lactating cows suspected of having mastitis from smallholder and semi-intensive dairy farms across the study districts. Mastitis-positive cows were identified through clinical examination and/or CMT. From these, 83 milk samples were confirmed as mastitis-positive and subsequently used for bacteriological analysis.

Data Collection Instruments

Data were collected through three main methods:

1. **Milk Sample Collection:** Following strict aseptic protocols, milk was collected into sterile universal bottles. The udder and teats were thoroughly cleaned, and the first few streams of milk were discarded to avoid contamination. Each sample was labeled appropriately for traceability.
2. **Transport and Storage:** Collected samples were transported to the microbiology laboratory of Adami Tulu Agricultural Research Center (ATARC) in ice-cooled containers. If immediate processing was not feasible, samples were refrigerated at 4°C and processed within 24 hours.
3. **Questionnaire Survey:** Structured questionnaires were administered to 40 farm owners and attendants to gather information on risk factors such as breed, age, parity, lactation stage, hygiene practices, production system, and environmental conditions.

Bacteriological Examination

The identification of *S. aureus* and *E. coli* followed standard bacteriological techniques recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018) and described in Bergey's Manual of Determinative Bacteriology (Brenner et al., 2005).

- *S. aureus* was isolated by culturing milk on Mannitol Salt Agar (MSA) and 5% Sheep Blood Agar. Colonies showing golden yellow pigmentation and β -hemolysis were Gram-stained and subjected to catalase and slide coagulase tests. Catalase-positive, coagulase-positive, Gram-positive cocci arranged in clusters were identified as *S. aureus*.
- *E. coli* was isolated by streaking milk onto MacConkey Agar and Eosin Methylene Blue (EMB) Agar. Colonies with a metallic green sheen on EMB were Gram-stained and subjected to biochemical tests, including Indole, Methyl Red, Voges-Proskauer, and Citrate (IMViC). Indole- and Methyl Red-positive but VP- and Citrate-negative results confirmed the presence of *E. coli*.

Data Analysis

Descriptive statistics were used to calculate the overall prevalence of *S. aureus* and *E. coli*. Risk factor analysis was conducted using the chi-square (χ^2) test to examine associations between the

prevalence of pathogens and potential risk factors such as breed, age, parity, lactation stage, hygienic practices, and production systems. Statistical significance was accepted at $p \leq 0.05$.

All analyses were carried out using SPSS software version 27. The results were interpreted in the context of known epidemiological patterns and previous research findings, with a focus on implications for dairy herd health and zoonotic risk mitigation.

Results

Bacterial Isolation and Prevalence

Out of the 83 milk samples collected from clinically and subclinically mastitic cows, 62 (74.7%) tested positive for either *S. aureus* or *E. coli*. Among these, 42 samples (50.6%) yielded *S. aureus* and 20 samples (24.1%) were positive for *E. coli* (Table 1). These findings confirm the high occurrence of these pathogens in mastitic milk within the study area.

Table 1. Prevalence of *S. aureus* and *E. coli* in Mastitis-Positive Milk Samples (n = 83).

Pathogen	Number of Isolates	Percentage (%)
<i>S. aureus</i>	42	50.6
<i>E. coli</i>	20	24.1

Association of Risk Factors with Pathogen Prevalence

Breed: Though numerical variation was observed, with crossbred cows showing higher prevalence rates of both pathogens (*S. aureus* = 47.62%, *E. coli* = 50.00%) (Table 2), statistical analysis did not reveal a significant association ($p > 0.05$).

Table 2. Prevalence of *S. aureus* and *E. coli* by Breed.

Breed	<i>S. aureus</i> (%)	<i>E. coli</i> (%)
Cross	47.62	50.00
Exotic	35.71	30.00
Local	16.67	20.00

Age: The highest prevalence of *S. aureus* (42.86%) was found in cows aged 6–9 years, while *E. coli* was most prevalent (40.00%) among cows aged 3–6 years. However, the differences across age groups were not statistically significant.

Parity: Cows with 3–6 parities had the highest rates of both *S. aureus* (40.48%) and *E. coli* (40.00%). The association between parity and pathogen isolation was not statistically significant ($p > 0.05$).

Lactation Stage: A significant association ($p < 0.05$) was observed between lactation stage and *S. aureus* prevalence. Cows in early lactation (≤ 4 months) were more susceptible to infection (Table 3). No such significance was observed for *E. coli*.

Table 3. Prevalence by Lactation Stage.

Lactation Stage	<i>S. aureus</i> (%)	<i>E. coli</i> (%)
≤ 4 months	38.10	45.00
4–8 months	35.71	30.00
> 8 months	26.19	25.00

Hygienic Practices and Pathogen Association

Significant associations ($p < 0.05$) were observed between hygiene-related variables and the prevalence of both *S. aureus* and *E. coli*. These include:

- Milking process hygiene
- Milking equipment hygiene

- **Milker's hand hygiene**
- **Pen and floor cleanliness**

Farms with poor hygiene in these areas reported the highest rates of pathogen isolation. For instance, 60.00% of *E. coli* cases and 47.62% of *S. aureus* cases (Table 4) were linked to poor overall hygiene levels.

Table 4. Prevalence by Overall Hygienic Practices.

Hygiene Level	<i>S. aureus</i> (%)	<i>E. coli</i> (%)
Poor	47.62	60.00
Medium	35.71	30.00
Good	16.67	10.00

Other Variables

No significant associations were found with other variables such as production system (intensive, semi-intensive, or extensive), location (district/kebele), or settlement type (urban, periurban, rural). However, variations in prevalence across these categories suggest potential indirect contributions, possibly through their influence on hygiene and management practices.

These results reinforce the critical role of hygiene management and early lactation care in reducing the prevalence of mastitis-causing pathogens.

Discussion

The findings of this study demonstrate that mastitis in dairy cows remains a considerable concern in the West Arsi Zone of Ethiopia, particularly due to its association with zoonotic pathogens *Staphylococcus aureus* and *Escherichia coli*. The observed prevalence rates—50.6% for *S. aureus* and 24.1% for *E. coli*—are consistent with previous findings by Zeryehun et al. (2013), Amer et al. (2018), and Weldemelak Girmay et al. (2020), which identified these two bacteria as dominant mastitis-causing agents in Ethiopia and similar developing regions.

The relatively higher prevalence of *S. aureus* compared to *E. coli* may be attributed to its contagious nature and chronic persistence within the udder tissues, as also supported by Balcha et al. (2022). Conversely, *E. coli* typically represents an environmental contaminant and often causes acute infections, especially in suboptimal hygienic settings.

This study found a statistically significant association between the stage of lactation and the presence of *S. aureus* ($p < 0.05$). Cows in early lactation (≤ 4 months) showed increased susceptibility to infection, possibly due to physiological stress and hormonal imbalances that compromise immune responses. This finding agrees with reports by Shimels et al. (2014), who emphasized the vulnerability of the udder during early lactation.

More importantly, strong and statistically significant associations were observed between hygiene-related variables and the prevalence of both *S. aureus* and *E. coli*. Specifically, poor hygienic practices in the milking process, improper cleaning of milking equipment, unclean hands of milkers, and dirty pens and floors were all significantly correlated with higher pathogen isolation rates ($p < 0.05$). These results are in line with studies by Harrigan and McCance (2014) and Langer et al. (2020), which underlined that environmental hygiene plays a critical role in the prevention of mastitis in dairy herds.

Although trends in breed, age, and parity were observed—such as higher prevalence of both pathogens in crossbred cows and cows with 3–6 parities—these factors did not show statistically significant associations in this study. Nonetheless, the numerical differences suggest the need for further longitudinal studies with larger sample sizes to explore these trends more thoroughly.

Interestingly, production systems and scale of farming did not show significant associations with pathogen prevalence, indicating that even smallholder and extensive systems may be at risk if

basic hygienic practices are not maintained. This reinforces the concept that management practices, rather than the scale or type of production alone, are the key determinants in mastitis prevention and control.

Overall, this study underscores the importance of improving on-farm hygiene and early detection strategies. Regular training of dairy farm workers, implementation of standard milking procedures, and routine veterinary checks could significantly reduce the burden of mastitis and its associated public health risks. Moreover, further research should also investigate the antimicrobial resistance profiles of the isolated pathogens to guide effective treatment regimens.

The findings highlight a clear pathway toward intervention through hygiene management. The statistically validated associations, especially with environmental and procedural hygiene, provide actionable targets for both policy makers and farm-level stakeholders aiming to enhance dairy health and productivity in the region.

Conclusion

This study highlights the considerable prevalence of zoonotic pathogens *Staphylococcus aureus* and *Escherichia coli* in mastitis-positive milk samples from dairy cows in the Shashamane and Arsi Negelle districts of the West Arsi Zone, Ethiopia. The findings underscore that 50.6% and 24.1% of the examined samples were positive for *S. aureus* and *E. coli*, respectively, emphasizing their importance as major contributors to bovine mastitis and potential public health risks through milk-borne transmission.

Among the various risk factors assessed, only the stage of lactation was significantly associated with *S. aureus* infection, with early lactation (≤ 4 months) presenting the highest prevalence. Moreover, hygiene-related variables—including cleanliness of the milking process, equipment, handlers' hands, and cow housing environment—were strongly and significantly associated with the presence of both pathogens. These associations highlight the critical role of proper sanitary practices in reducing the risk of mastitis and controlling zoonotic bacterial spread in dairy farms.

Although other factors such as breed, age, and parity showed numerical differences in prevalence, their associations were not statistically significant, indicating that pathogen prevalence may be more directly influenced by management and environmental conditions rather than inherent animal characteristics.

Therefore, the study calls for urgent implementation of improved farm-level hygiene measures, targeted education programs for dairy farmers, and routine mastitis screening to mitigate the spread of these pathogens. Additionally, policies and training that support milking hygiene and cow housing sanitation should be prioritized to ensure both animal productivity and public health safety.

Further research is recommended to explore antimicrobial resistance profiles of the isolated organisms and to evaluate longitudinal outcomes of hygiene-focused interventions on mastitis incidence and milk safety in the region.

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