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Epidemiology of Subclinical Mastitis in Dairy Cows of Monaragala District, Sri Lanka: Prevalence, Risk Factors, Bacterial Etiology, and Antimicrobial Resistance

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ABSTRACT

A cross-sectional study was conducted in the Monaragala District of Sri Lanka to assess the prevalence, bacterial causes, antibiotic resistance patterns, and associated risk factors of subclinical mastitis (SCM) in dairy cows. Five veterinary surgeon (VS) divisions were selected using a simple random sampling method, and 607 lactating cows from 175 dairy farms were chosen through multi-stage stratified random sampling. SCM was diagnosed in each cow via the California Mastitis Test (CMT). Cows testing positive for CMT (≥2+ for any quarter) without clinical symptoms were considered SCM positive. Milk samples from positive cows were bacteriologically isolated and tested for antimicrobial susceptibility. Risk factors for SCM incidence were identified using multivariable generalized estimating equation (GEE) models and descriptive statistical analysis. The SCM prevalence was reported as 18.7% at the cow level, 26.8% at the farm level, and 10.75% at the quarter level. ***Staphylococcus aureus*** **(79.1%) and** ***Staphylococcus epidermidis*** **(16.4%) were the most commonly isolated pathogens. Antimicrobial susceptibility testing demonstrated significant resistance to ampicillin (67.6%), neomycin (89.8%), and sulfamethoxazole-trimethoprim (60.2%). In contrast, the majority of isolates remained susceptible to enrofloxacin (81.1%). Multidrug resistance was noted in 31.8% of isolates, with** ***S. aureus*** **reporting the highest prevalence. Multivariable analysis detected parity, lactation stage, mastitis history, breed, production system, floor hygiene, and cleaning frequency as risk factors. In conclusion, SCM in Monaragala District was predominantly associated with contagious pathogens and influenced by both animal- and farm-management factors. Antimicrobial resistance was common among mastitis-associated bacterial isolates.**

Keywords: antimicrobial resistance; bacterial etiology; dairy cattle; risk factors; subclinical mastitis

INTRODUCTION

Mastitis, an inflammation of the mammary gland, is characterized by changes in udder tissue, along with physical, chemical, bacterial, and sensory abnormalities in the milk (Kibebew, 2017). This is the most prevalent and substantial barrier to dairy productivity in Sri Lanka and an economically significant illness affecting dairy cows worldwide (Ranasinghe *et al.*, 2021).

Reduced milk quality and quantity, milk discarded during treatment, veterinary and pharmaceutical costs, increased labor requirements, early culling, and cow mortality in extreme circumstances are contributing factors to the financial losses connected with mastitis (Kibebew, 2017; Kerro Dego, 2021). Subclinical mastitis (SCM), which is much more prevalent than clinical mastitis in dairy cattle, is the most insidious type. However, it does not display obvious clinical symptoms in lactating cows. As a result, SCM often remains unnoticed for prolonged periods, causing significant hidden financial losses and acting as a recurring source of infection in dairy herds (Silva *et al.*, 2021). According to Puerto *et al.* (2021), SCM alone accounts for 20%-60% of the economic losses associated with clinical mastitis, mostly due to decreased milk production and rejection. It has a complex multifactorial etiology that includes environmental and infectious pathogens. Long-lasting subclinical mastitis infections are often caused by contagious microorganisms such as *Staphylococcus aureus* and *Streptococcus agalactiae*, which are well adapted to survive within the mammary gland and spread during milking practices.

On the other hand, housing and floor materials are the primary sources of environmental pathogens, including coagulase-negative staphylococci, streptococci, and coliforms (Anwar *et al.*, 2022; Shoaib *et al.*, 2023). Additionally, factors associated with the host and management, such as parity, lactation stage, milk yield, hygienic procedures, and prior mastitis history, have a critical impact on the prevalence of SCM.

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Numerous studies have shown significant occurrences of SCM in Sri Lanka, although estimates of its occurrence vary markedly by area. Notably, *S. aureus* is consistently detected as the predominant pathogen, with cow-level prevalence ranging from roughly 12% to over 55% in several districts (Ranasinghe *et al.,* 2021). Previous studies have reported the contribution of *Escherichia coli, Klebsiella* spp., and *Streptococcus* spp., suggesting the involvement of both environmental and contagious pathogens in the prevalence of SCM in Sri Lankan dairy systems (Sanotharan *et al.,* 2016; Rahularaj *et al.,* 2019). These results collectively demonstrate that SCM is a widespread problem across the country's many agroecological regions.

The extensive use of antimicrobials for mastitis treatment and prevention has heightened concerns about antimicrobial resistance (AMR) in dairy production systems. In Sri Lanka, increasing resistance levels among mastitis-related pathogens, especially *S. aureus*, to frequently utilized antimicrobials have been documented, potentially undermining treatment effectiveness and posing substantial risks to both animal and public health (Jayaweera *et al.,* 2018; Priyantha *et al.,* 2021). Nevertheless, region-specific data on antimicrobial susceptibility patterns and the frequency of multidrug resistance among SCM-associated pathogens are scarce, fragmented, and insufficient to inform evidence-based therapeutic decision-making (Bari *et al.,* 2022).

The Monaragala District in the Uva Province is distinguished by primarily small- to medium-sized dairy farms managed by widespread, semi-intensive, and intensive systems. Despite the ongoing growth of the dairy sector in this region, issues such as inadequate milking hygiene, insufficient veterinary assistance, and low farmer awareness persist, thus hindering proper mastitis management. Many studies have reported the prevalence of SCM and patterns of antimicrobial resistance in Sri Lanka; however, there are still few region-specific studies that use robust multivariable analytical techniques to integrate epidemiology, hierarchical risk factors, bacterial etiology, and antimicrobial resistance.

Using a population-averaged multivariable modeling methodology, the present study integrates SCM prevalence, risk-factor analysis, bacterial characterization, and antibiotic resistance profiling to provide the first comprehensive assessment in Monaragala District. Therefore, the objectives of the current study were to: (i) detect the prevalence of SCM; (ii) identify the risk factors of SCM; (iii) identify the bacterial pathogens causing SCM; and (iv) evaluate their antimicrobial resistance profile. The findings of this study will support evidence-based mastitis control strategies and promote prudent antimicrobial use in Sri Lanka's dairy sector.

MATERIALS AND METHODS

Ethical Approval

This observational study collected non-invasive milk samples from dairy cows, following a standard procedure integrated into farm management protocols. All animal interactions were executed in strict adherence to the animal welfare directives established by the Department of Animal Production and Health in Sri Lanka. Ethical clearance was unnecessary for this study as the experimental investigation involved no manipulations, invasive techniques, or actions that could harm the animals.

Study Area

A cross-sectional epidemiological study was conducted from January to December 2025 in five Veterinary Surgeon (VS) divisions of Monaragala District, Uva Province, Sri Lanka.

Study Design and Study Population

Lactating cows from intensive, semi-intensive, and extensive production systems were included. Each cow was examined for clinical mastitis before sampling, and those showing clinical signs such as udder inflammation, abnormal milk, udder pain, or systemic illness were excluded. Only the cows without visible symptoms were selected. Farms were eligible if located in the selected VS divisions, actively producing milk during the visit, had at least three lactating cows at the time of visit, and agreed to udder examination, milk sampling, and data collection from the questionnaire. Individual cows were qualified if they were actively lactating, had four working udder quarters, and showed no clinical mastitis signs. Cows that were dry, with clinical mastitis, severely damaged udders, or could not be safely examined were excluded.

Sample Size Determination and Sampling Procedure

Sample size determination: The sample size (number of farms) was calculated using the single-proportion sample size formula described by Cochran (1977).

$$N= \frac{Z^2\ P\,(1-p)}{E^2}$$

Where Z= 1.96 for a 95% confidence interval, P was the assumed farm-level prevalence of SCM (0.30), and E was the standard error (0.07).

Based on the formula, the minimum required sample size was 161 dairy farms. To improve the farms' representativeness across different VS divisions and management conditions, the 175 farms were selected.

Sampling procedure: A stratified two-stage cluster sampling approach was used to select dairy farms and lactating cows for the study.

In this study, VS divisions were defined as strata for the stratified sampling design. Monaragala District is administratively divided into 14 VS divisions by the Department of Animal Production and Health, Sri Lanka. Out of these, five divisions, including Badalkumbura, Madulla, Buttala, Monaragala, and Siyabalanduwa, were randomly selected using a simple random sampling method.

Within each selected VS division, dairy farms were identified using farm registers maintained by the VS offices. These divisions had 226 registered dairy farms in Badalkumbura, 209 in Madulla, 384 in Buttala, 502 in Monaragala, and 582 in Siyabalanduwa. A total of 175 farms were chosen, proportionally to the farm population in each division: 21 farms from Badalkumbura, 19 from Madulla, 35 from Buttala, 46 from Monaragala, and 54 from Siyabalanduwa.

Subsequently, cows were selected through a two-stage cluster sampling approach. In the first stage, the selected farms constituted the clusters, and in the second stage, eligible lactating cows within each farm were randomly sampled. Approximately 50% of eligible lactating cows per farm were randomly sampled. Although no fixed statistical rule exists for determining the optimal number of sampling units within clusters, increasing the number of animals sampled per cluster improves the precision of prevalence estimates. It enhances the robustness of statistical analyses in cluster-based epidemiological studies (Thrusfield & Christley, 2018).

The selected proportion, therefore, represents a pragmatic balance between statistical efficiency and logistical feasibility, while ensuring adequate representation of within-farm variability. Accordingly, the study included 607 lactating cows (Figure 1).

Screening and Case Definition of Subclinical Mastitis

All udder quarters of 607 cows were examined using the California Mastitis Test (CMT) as described by Quinn *et al.* (1999). Among these cows, 114 cows tested positive for SCM. Equal volumes of milk and CMT reagent (Chimertech, Chennai, India) were mixed in a four-compartment paddle and gently swirled for 10-15 seconds. Reactions were visually evaluated. CMT values of 0-1 were negative, while scores of 2 or higher were considered positive (Gunawardana *et al.*, 2014; Kayesh *et al.*, 2014). Cows were classified as having SCM if at least one udder quarter had a CMT score of 2 or higher. In total, 2,428 udder quarters were checked.

Collecting Milk Samples

During the study period, all milk samples were collected once per positive cow, a total of 114 samples, with no repeated sampling. If multiple udder quarters tested positive, milk was collected from the one with the highest CMT score. Teats were cleaned with water, dried with disposable paper towels, and disinfected with 70% ethanol. The first few streams of milk were discarded, and then 10 mL of milk was aseptically collected into sterile screw-capped tubes. Samples were transported to the Veterinary Investigation Center (VIC), Monaragala, in insulated ice boxes at 4-8 °C, and processed within 6 hours after collection.

Bacteriological Isolation and Identification

Milk samples were cultured on milk agar (M163, HiMedia Laboratories Pvt. Ltd., Mumbai, India) and blood agar (TM360, Titan Biotech Ltd, Delhi, India) plates. Plates were incubated aerobically at 37 **°C for 24**-48 h. They were examined for colony morphology, hemolytic patterns, and lactose fermentation following incubation. Next, representative colonies were sub-cultured to obtain pure isolates.

Bacteria were identified using Gram staining followed by standard biochemical tests: Catalase, coagulase, oxidase, indole, methyl red, Voges–Proskauer, citrate, urease, and triple sugar iron (TSI) tests according to established microbiological procedures (Hafezi & Khamar, 2024).

Samples reporting heavy mixed growth (>2 colony morphotypes) were excluded from further analysis

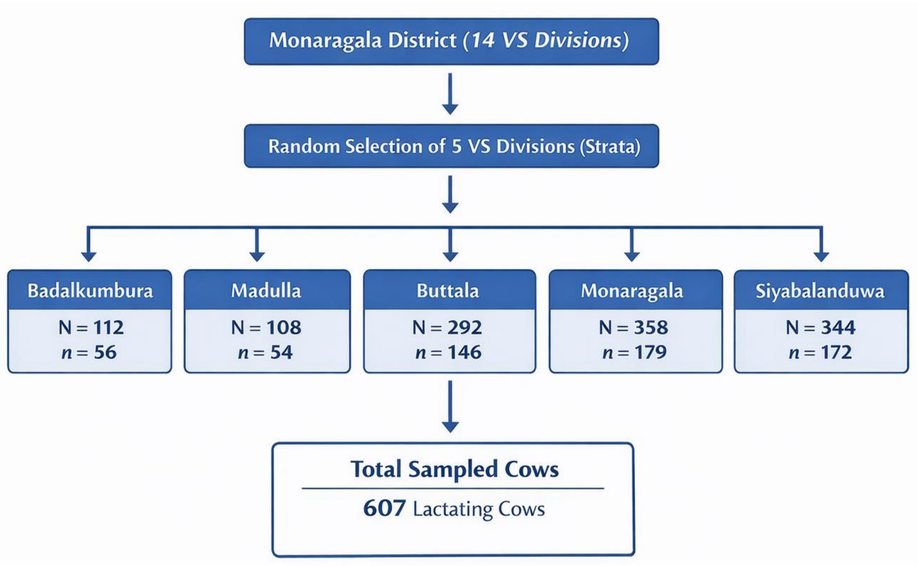

Figure 1: Stratified two-stage cluster sampling framework illustrating the distribution of total lactating cows (N) and sampled cows (n) across selected Veterinary Surgeon (VS) Divisions (strata) in Monaragala District, Sri Lanka. Note: The number of cows sampled per division varied depending on the number of farms selected and herd sizes within each farm.

to minimize contamination bias and ensure that the antimicrobial susceptibility testing was performed on pure bacterial isolates. This may further improve the accuracy of pathogen identification and reduce the risk of misinterpretation of the study's antimicrobial resistance results.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method, using Mueller–Hinton agar (Oxoid Ltd., Basingstoke, UK), according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2018).

A bacterial suspension equivalent to a 0.5 McFarland turbidity standard was prepared for each isolate. They were uniformly inoculated onto the agar surface with a sterile swab. Antibiotic disks were placed on the inoculated plates and incubated at 37 °C for 18-24 h. Inhibited zones were measured in millimeters after incubation. Results interpreted as susceptible, intermediate, or resistant, according to CLSI guidelines (CLSI, 2018).

The tested antimicrobials were enrofloxacin, cloxacillin, ampicillin, amoxicillin, tetracycline, neomycin, Sulfamethoxazole-trimethoprim, and cephalexin. Due to occasional variations in antibiotic disc availability, not all isolates were tested against the same number of antimicrobials. Therefore, pathogen resistance and multiple antibiotic resistance (MAR) were interpreted descriptively.

Definition of Multidrug Resistance (MDR)

Sweeney *et al.* (2018) defined multidrug resistance as resistance to at least one antimicrobial agent in three or more antimicrobial classes.

Risk-Factor Data Collection

A pretested structured questionnaire was used to collect data on cow-related factors (breed, parity, lactation stage, milk yield, body condition score, and previous mastitis history), farm-related factors (management system, herd size, feeding practices), and hygiene-related factors (floor type, floor cleaning frequency, floor hygiene status, udder hygiene, and teat dipping practices). Whenever feasible, questionnaire responses were verified through on-site observations.

Calculation of SCM Prevalence

The SCM prevalence was calculated at three levels as per Gómez-Quispe *et al.* (2015) and Thrusfield and Christley (2018).

Cow level prevalence= (No. of CMT positive cows / Total cows examined) x 100

Quarter level prevalance= (No.of positive quarters / Total quarters examined) x 100

Farm level prevalence= (No. of farms with at least one SCM positive cow / Total farms surveyed) x 100

Statistical Analysis

After entering data into Microsoft Excel (version 2016) for cleaning and validation, they were analyzed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). SCM results were defined as CMT-positive or CMT-negative status and treated as a binary outcome variable. The study variables were summarized using descriptive statistics. Variables with a $p\text{-value}\leq 0.20$ in bivariate analyses were considered qualified for addition in the multivariable analysis.

Multivariable analysis was performed using generalized estimating equation (GEE) models to identify independent risk factors for SCM, accounting for clustering of cows within farms. Multicollinearity among explanatory variables was assessed using variance inflation factors (VIF). Model adequacy was evaluated using the quasi-likelihood under the independence model criterion (QIC). Statistical significance was set at $p<0.05$.

Antimicrobial Resistance Analysis

Based on Clinical and Laboratory Standards Institute (CLSI) breakpoints (CLSI, 2018), antimicrobial resistance was summarized descriptively. It was calculated as the proportion of resistant isolates for each bacterial species and antimicrobial agent.

RESULTS

Prevalence of Subclinical Mastitis (SCM)

At the cow level, 114 of the 607 examined cows (18.7%) tested positive for SCM (CMT score ≥2). At the farm level, 46 of the 175 surveyed farms (26.8%) had at least one CMT-positive cow. At the quarter level, prevalence was relatively uniform across all udder quarters, with no marked variation between front and rear quarters (Table 1).

Risk Factors Associated with Subclinical Mastitis

The multivariable GEE model identified several significant animal- and management-level factors associated with SCM (Table 2). Pure exotic breeds and cows in higher-parity groups were more likely to develop SCM compared to their respective reference groups. In contrast, cows in early lactation and those with lower milk yield showed reduced odds of SCM.

A previous history of mastitis was also an important predictor, with cows lacking such a history showing a lower likelihood of infection. At the farm level, production system intensity was strongly associated with SCM occurrence, with intensive and semi-intensive systems showing a higher risk compared to extensive systems.

Hygiene-related factors demonstrated a strong protective effect, as frequent floor cleaning and good floor hygiene were associated with reduced SCM occurrence.

Table 1. Quarter-wise prevalence of subclinical mastitis determined using the California mastitis test

Quarter	Total quarters examined	SCM positive quarters	SCM-Positive quarters (%)	C.I. (95%)
RF	607	65	10.71	8.0-13.0
RB	607	62	10.21	8.0-13.0
LF	607	71	11.70	9.0-14.0
LB	607	63	10.38	8.0-13.0
Total	2428	261	10.75	-

Note: SCM = subclinical mastitis; RF = right front; RB = right back; LF = left front; LB = left back; C.I. = confidence interval.

Table 2. Combined multivariable generalized estimating equation model identifying risk factors for subclinical mastitis in dairy cows

Variables	Category	Adjusted OR	95% CI	p-value
Breed	Local	0.80	0.20-3.19	0.749
	Pure exotic	2.10	1.20-3.66	0.009
	Crossbred	Reference	-	-
Parity*	1-3	1.11	0.50-2.46	0.801
	4-6	2.30	1.07-4.95	0.033
	≥ 7	Reference	-	-
Lactation stage	Early	0.44	0.24-0.81	0.008
	Mid	0.69	0.37-1.30	0.249
	Late	Reference	-	-
Milk yield (L/day)	≤8	0.49	0.28-0.85	0.011
	>8	Reference	-	-
Previous mastitis history	Yes	Reference	-	-
	No	0.38	0.19-0.79	0.010
Farming system	Intensive	16.40	3.26-82.60	0.001
	Semi-intensive	4.23	1.27-14.10	0.019
	Extensive	Reference	-	-
Floor cleaning frequency	Daily	0.05	0.01-0.32	0.002
	Weekly	Reference	-	-
Floor hygiene status	Good	0.25	0.11- 0.55	0.001
	Poor	Reference	-	-

Note: OR = odds ratio; CI = confidence interval. *For parity, cows with ≥7 parities were specified as the reference category in the generalized estimating equation (GEE) model; therefore, all odds ratios for parity are interpreted relative to this group.

Table 3. Culture outcome of milk samples collected from cows positive for subclinical mastitis

Parameter	Number	Percentage (%)
CMT-positive samples cultured	114	100.00
Culture-positive samples	110	96.49
Contaminated (excluded)	4	3.51

Note: CMT = California mastitis test.

Bacterial Etiology of Subclinical Mastitis

Culture outcome: Of the 114 milk samples collected from 114 CMT-positive cows, bacterial growth was observed in 110 samples, which were included in the bacteriological analysis. Four samples (3.5%) were excluded due to contamination (Table 3).

Bacterial species distribution: *Staphylococcus aureus* was the predominant bacterial species isolated from milk samples, indicating the major role of contagious pathogens in the occurrence of SCM. Other bacterial species, including coagulase-negative staphylococci and environmental pathogens, were detected at considerably lower frequencies (Table 4).

Epidemiological classification of isolates: The bacterial isolates were classified into contagious and environmental pathogen groups, indicating that SCM in the study area is primarily driven by contagious transmission. In contrast, environmental pathogens contributed only minimally to the overall pathogen profile (Table 5).

Table 4. Distribution of bacterial species isolated from milk samples of cows with subclinical mastitis

Bacterial species	Number of isolates	Prevalence (%)
Staphylococcus aureus	87	79.09
Staphylococcus epidermidis	18	16.36
Escherichia coli	2	1.81
Bacillus spp.	2	1.81
Klebsiella spp.	1	0.93
Total	110	100.00

Antimicrobial Susceptibility Patterns of Mastitis Isolates

Antimicrobial susceptibility profile: Antimicrobial susceptibility testing revealed substantial variation in resistance patterns among the tested antibiotics (Table 6). High resistance levels were observed for commonly used antimicrobials, particularly within the penicillin, aminoglycoside, and sulphonamide classes. In contrast,

Table 5. Epidemiological classification of bacterial isolates recovered from subclinical mastitis cases

Pathogen group	Included species	Number of isolates	Percentage (%)
Contagious pathogens	*Staphylococcus aureus, Staphylococcus epidermidis*	105	95.45
Environmental pathogens	*Escherichia coli, Klebsiella spp., Bacillus spp.*	5	4.55

Table 6. Antimicrobial susceptibility profile of bacterial isolates recovered from subclinical mastitis cases

Tested antibiotic	Antibiotic class	Number of isolates tested	Resistant(%)	Intermediate(%)	Susceptible(%)
Enrofloxacin	Fluoroquinolones	110	10.0 (11)	9.1 (10)	80.9 (89)
Cloxacillin	Penicillins	29	31.0 (9)	3.4 (1)	65.5 (19)
Ampicillin	Penicillins	66	68.2 (45)	7.6 (5)	25.8 (17)
Tetracycline	Tetracyclines	106	11.3 (12)	33.0 (35)	55.7 (59)
Neomycin	Aminoglycosides	106	89.6 (95)	8.5 (9)	1.9 (2)
Amoxicillin	Penicillins	107	42.3 (46)	15.9 (17)	41.1 (44)
Sulfamethoxazole/Trimethoprim	Sulphonamides	107	58.9 (63)	23.4 (25)	17.7 (19)
Cephalexin	Cephalosporins	104	38.5 (40)	15.4 (16)	46.1 (48)

Note: The number of isolates tested varied by the antibiotic.

most isolates remained susceptible to fluoroquinolones, indicating the continued effectiveness of these agents. Moderate resistance patterns were observed for other antimicrobial classes.

Multiple Drug Resistance (MDR): A considerable proportion of bacterial isolates exhibited multidrug resistance, indicating a notable level of antimicrobial resistance within the study population (Table 7). Among the predominant pathogens, *Staphylococcus aureus* had the highest occurrence of multidrug resistance.

The extremely high levels of multidrug resistance observed in certain bacterial species should be interpreted with caution, as these findings were based on a limited number of isolates.

Multiple Antibiotic Resistance (MAR): The MAR index indicated a relatively high level of antimicrobial resistance among bacterial isolates (Table 8). A large proportion of isolates exhibited MAR values above the commonly accepted threshold, suggesting exposure to environments with frequent antimicrobial use. Staphylococcal species exhibited comparatively lower MAR values. Higher MAR indices observed in certain bacterial species should also be interpreted with caution, given the limited number of isolates.

DISCUSSION

The cow-level prevalence of SCM in Monaragala District was 18.7%, which was substantially lower than the prevalence reported in Kurunegala (57.5%) and Ampara (45.5%), but higher than that reported in selected areas of Nuwara Eliya (11.8%) (Sanotharan *et al*., 2016; Rahularaj *et al*., 2019; Ranasinghe *et al*., 2021). This moderate prevalence may be attributed to differences in agroecological conditions, herd structure, housing systems, hygiene management practices, and production intensity in Monaragala District, where small- to medium-scale herds are predominantly managed under semi-intensive and extensive systems (Sanotharan *et al.,* 2016).

Other South Asian smallholder dairy systems have reported comparable levels of SCM prevalence. For example, reports reveal that the cow-level SCM prevalence ranges from 20% to 75% in Bangladesh,

Table 7. Multidrug resistance (MDR) profile of bacterial isolates recovered from subclinical mastitis

Pathogen	Number of isolates tested	MDR (n)	MDR (%)
Staphylococcus aureus	87	29	33.3
Staphylococcus epidermidis	18	2	11.1
Escherichia coli	2	2	100.0
Bacillus spp.	2	2	100.0
Klebsiella spp.	1	0	0.0
Overall	110	35	31.81

Note: MDR = resistance to ≥1 antimicrobial agent in ≥3 antimicrobial classes.

Table 8. Mean multiple antibiotic resistance (MAR) index of mastitis pathogens

Pathogen	Number of isolates	Mean MAR±SD	MAR range	% MAR ≥ 0.2
Staphylococcus aureus	87	0.421±0.25	0.90	80.46
Staphylococcus epidermidis	18	0.422±0.21	0.90	94.44
Escherichia coli	2	0.600±0.28	0.60	100.00
Bacillus spp.	2	0.75±0.07	0.10	100.00
Klebsiella spp.	1	0.30	-	100.00

Note: MAR index calculation is based on the ratio of the number of antibiotics to which an isolate was resistant to the total number of antibiotics tested.

18% to 87% in India, and 17% to 81% in Pakistan (Senthilkumar *et al.*, 2020; Bari *et al.*, 2022).

The quarter-wise prevalence was relatively uniform in the present study, indicating limited evidence of quarter-specific predisposition under the prevailing management conditions. This, in turn, supports the view that SCM transmission is influenced more by management- and hygiene-related factors. The observed farm-level prevalence results further suggest that SCM is widely distributed across herds and does not concentrate in a few, highly affected farms. This finding emphasizes the importance of implementing herd-level rather than cow-level control strategies.

Dairy herds in Bagmati Province, Nepal, have reported a comparable prevalence pattern (Tiwari *et al.*, 2022). In a similar context, Hussein *et al.* (2022) observed a relatively higher quarter-level SCM prevalence (30.2%) among dairy farms in Qena Governorate, Egypt. In contrast, other studies have reported a substantially higher SCM prevalence in forequarters than in hindquarters. It suggests that potential anatomical or exposure-related differences exist across specific production systems (El-Kholy *et al.*, 2018; Mourya *et al.*, 2020), possibly contributing to the observed differences in SCM prevalence between forequarters and hindquarters in dairy farms.

Animal-level factors significantly influenced SCM occurrence in the study area. Pure exotic breeds were more susceptible than crossbred cows, probably due to their higher production potential, increased metabolic stress, and reduced adaptability to local environments. These factors have been consistently associated with immune suppression and an increased likelihood of SCM occurrence (Taponen *et al.*, 2017; Curone *et al.*, 2018). As parity increases, enlargement of the teat size, relaxation of the teat sphincter, and pathogen exposure may further facilitate bacterial entry into a cow's udder (Yimam *et al.*, 2020).

The lactation stage also influenced the risk of SCM, with lower odds in early lactation compared with late lactation. This pattern likely reflects prolonged exposure to mastitis pathogens and a gradual decrease in teat sphincter competence toward the end of lactation (Qayyum *et al.*, 2016; Bari *et al.*, 2022).

Similarly, lower-yielding cows exhibited reduced odds of SCM than high-yielding animals. High milk production is associated with prolonged opening of the teat canal before and after milking and increased physiological stress. Both these factors enhance susceptibility to intramammary infections (Bari *et al.*, 2022). These findings are consistent with those of Biswas and Sakar (2017), who reported a substantially lower prevalence of SCM in low-yielding cows (33.3%) than in high-yielding cows (87.5%).

In addition, a history of mastitis emerged as a strong predictor of current SCM, highlighting the persistence of intramammary infections and the importance of effective post-treatment monitoring. Cows previously affected by mastitis are at a higher risk of reinfection. It suggests that repeated microbial challenges to mammary tissues, coupled with other stressors, may increase susceptibility to infections (Bronzo *et al.*, 2020). This association may also result from incomplete elimination of infective agents during treatment. Clinical signs are suppressed, but infection persists in a subclinical form.

At the farm level, production system intensity was a significant determinant of SCM risk. Cows managed under intensive and semi-intensive systems exhibited higher odds of SCM, due to intensification without corresponding improvements in hygiene and management. Pathogen exposure is increased through higher stocking densities and greater environmental contamination. Moreover, in intensive systems, cows tend to remain in confined areas for prolonged periods, which may be sanitized inadequately. Overcrowding may also exacerbate physiological stress. Similar associations between intensive systems and increased SCM prevalence have been widely reported in South Asia and other regions (Tedla *et al.*, 2018; Bihon *et al.*, 2019; Pugliese *et al.*, 2021). Implementing appropriate housing interventions, such as the use of clean, comfortable bedding materials, has enhanced cow comfort and reduced SCM prevalence (Singh *et al.*, 2020).

Hygiene-related practices have been demonstrated to be strongly protective against SCM. For instance, frequent floor cleaning and good overall housing hygiene are associated with reduced SCM occurrence. Additionally, proper disposal of urine and manure, together with routine cleaning and disinfection of barns, lowers the risk of environmental mastitis caused by coliform bacteria. It limits the transmission of contagious mastitis pathogens such as *Staphylococcus aureus* (Klaas & Zadoks, 2018). Differences in how hygiene-related variables are defined and measured across the studies have led to variability in the identification of specific risk factors. Nevertheless, the lack of separation of infected cows, poor hygienic practices, and the absence of teat dipping have been consistently reported as significant risk factors for SCM (Sanotharan *et al.*, 2016).

S. aureus was the predominant pathogen isolated from CMT-positive milk samples, indicating that SCM in Monaragala District is primarily driven by contagious transmission. A similar predominance of *S. aureus* has been reported in smallholder dairy systems operating under comparable management conditions in Bangladesh (Abebe *et al.*, 2016).

The high prevalence observed in the present study likely reflects prevailing smallholder management practices, suboptimal milking hygiene, widespread reliance on hand-milking, and limited implementation of dry cow therapy. Consistent with this interpretation, previous studies from Sri Lanka have also documented poor adoption of post-milking hygienic practices, which may facilitate transmission and persistence of *S. aureus* infections (Ranasinghe *et al.*, 2021).

The persistence of *S. aureus* is further enhanced by its ability to invade deep mammary tissues and establish chronic intramammary infections that are difficult to eliminate during lactation using conventional antimicrobial therapy (Calvinho & Dallard, 2023). In comparison, the relatively low prevalence of Gram-negative and other environmental pathogens may suggest limited environmental mastitis pressure in the study area. The dominance of contagious pathogens stresses the need to prioritize improvements in milking hygiene and herd-level control measures rather than relying solely on environmental interventions.

The antimicrobial resistance patterns observed among mastitis pathogens raise important concerns for mastitis management and antimicrobial stewardship. Resistance to commonly used antimicrobials, along with the presence of multidrug-resistant isolates, underscores the potential consequences of prolonged, unregulated antimicrobial use in dairy herds. The resistance patterns should be interpreted with caution due to differences in antimicrobial testing across isolates; however, the findings are consistent with regional reports and indicate increasing resistance among mastitis pathogens (Jayaweera *et al.*, 2018; Priyantha *et al.*, 2021).

These results emphasize the need to prioritize preventive mastitis control strategies, reduce reliance on antimicrobial therapy, and promote rational drug use. Improving hygiene and management practices may substantially reduce the burden of SCM in Monaragala District. Interventions focused on milking hygiene, regular floor cleaning, and udder sanitation may lead to meaningful reductions in SCM prevalence and, consequently, lower dependence on antimicrobial treatments. The observed antimicrobial resistance patterns underscore the urgency of strengthening mastitis prevention programs and antimicrobial stewardship in the Sri Lankan dairy sector.

CONCLUSION

SCM prevalence among dairy cows in Monaragala District was 18.7%. This was primarily related to contagious pathogens, particularly *Staphylococcus aureus*. Furthermore, SCM occurrence was significantly associated with some animal-level factors, including breed, parity, lactation stage, milk yield, and previous mastitis history, as well as farm management intensity and hygiene practices. Importantly, the detection of antimicrobial resistance highlights concerns regarding treatment efficacy in smallholder dairy systems. Given these findings, strengthening farm hygiene, mastitis prevention strategies, and prudent antimicrobial use is therefore essential to improve udder health, milk quality, and the sustainability of dairy production in Sri Lanka.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT to improve readability and language. The authors reviewed and edited the content after using this tool as needed and take full responsibility for the publication's content.

REFERENCES

Abebe, R., Hatiya, H., Abera, M., Megersa, B., & Asmare, K. (2016). Bovine mastitis: Prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. BMC Veterinary Research, 12, 270. https://doi.org/10.1186/s12917-016-0905-3

Anwar M.A., Aziz S., Ashfaq K., Aqib A.I., Shoaib M., Naseer M.A., Alvi M.A., Muzammil I., Bhutta Z.A., Sattar H., Saleem A., Zaheer T., Khanum F., & Mahmood, A. (2022). Trends in frequency, potential risks, and antibiogram of *E. coli* isolated from semi-intensive dairy systems. Pakistan Veterinary Journal, 42(2): 167-172. https://pvj.com.pk/pdf-files/42_2/167-172.pdf

Bari, M. S., Rahman, M. M., Persson, Y., Derks, M., Sayeed, M. A., Hossain, D., Singha, S., Hoque, M. A., Sivaraman, S., Fernando, P., & Ahmad, I. (2022). Subclinical mastitis in South Asian dairy cows: A review of risk factors and etiology to prioritize control measures. Veterinary Research Communications, 46(3), 621–640. https://doi.org/10.1007/s11259-022-09948-x

Bihon, A., Syoum, A., & Assefa, A. (2019). Assessment of risk factors and isolation of *Staphylococcus aureus* and *Escherichia coli* from bovine subclinical mastitic milk in Northwest Ethiopia. Tropical Animal Health and Production, 51(4), 939–948. https://doi.org/10.1007/s11250-018-1777-2

Biswas, D., & Sarker, T. (2017). Prevalence of subclinical mastitis in Banaripara Upazila, Barisal. Bangladesh Journal of Veterinary Medicine, 15(1), 21–26. https://doi.org/10.3329/bjvm.v15i1.34050

Bronzo, V., Lopreiato, V., Riva, F., Amadori, M., Curone, G., Addis, M.F., Cremonesi, P., Moroni, P., Trevisi, E., & Castiglioni, B. (2020). The role of innate immune response and microbiome in resilience of dairy cattle to disease: the mastitis model. Animals, 10(8), p.1397. https://doi.org/10.3390/ani10081397

Calvinho, L.F., & Dallard, B.E. (2023). *Staphylococcus aureus* chronic intramammary infections in dairy cows: Pathogen-specific characteristics. CABI Reviews, (2023), 1-13. https://doi.org/10.1079/cabireviews.2023.0007

Clinical and Laboratory Standards Institute (CLSI). (2018). Performance standards for antimicrobial susceptibility testing (28th ed., CLSI supplement M100). Clinical and Laboratory Standards Institute. https://www.calameo.com/read/007001748ccef4b9fdfe5

Cochran, W. G. (1977). Sampling techniques (3rd ed.). John Wiley & Sons.

Curone, G., Filipe, J., Cremonesi, P., Trevisi, E., Amadori, M., Pollera, C., Castiglioni, B., Turin, L., Tedde, V., Vigo, D., & Moroni, P. (2018). Mastitis resistance in Holstein Friesians and a local cattle breed. Research in Veterinary Science, 116, 88–98. https://doi.org/10.1016/j.rvsc.2017.11.020

El-Kholy, A. M., Hassan, G. M., Zeinhom, M. M. A., & El-Klawy, M. A. A. M. (2018). Detection of subclinical mastitis in a dairy farm in Beni-Suef City, Egypt. Assiut Veterinary Medical Journal, 64(157), 18–24. https://doi.org/10.21608/avmj.2018.168897

Gómez-Quispe, O. E., Santivañez-Ballón, C. S., Arauco-Villar, F., Espezua-Flores, O. H., & Manrique-Meza, J. (2015). Criterios de interpretación para California Mastitis Test en el diagnóstico de mastitis subclínica en bovinos. Revista de Investigaciones Veterinarias del Perú, 26(1), 86–95. https://doi.org/10.15381/rivep.v26i1.10912

Gunawardana, S., Thilakarathne, D., Abeygunawardana, I. S., Abeynayake, P., Robertson, C., & Stephen, C. (2014). Risk factors for bovine mastitis in the Central Province of Sri Lanka. Tropical Animal Health and Production, 46, 1105–1112. https://doi.org/10.1007/s11250-014-0602-9

Hafezi, A., & Khamar, Z. (2024). The method and analysis of some biochemical tests commonly used for microbial identification: a review. Comprehensive Health and Biomedical Studies, 3(3). https://doi.org/10.5812/chbs-160199

Hussein, O. H., Abdel Hameed, K. G., & El-Malt, L. M. (2022). Prevalence and public health hazards of subclinical mastitis in dairy cows. SVU-International Journal of Veterinary Sciences, 5(3), 52–64. https://doi.org/10.21608/svu.2022.131652.1189

Jayaweera, T. S. P. J., Randika, J. L. P. C., Gamage, H. G. C. L., Udawatta, N. N., Ellepola, W. U. N. T. S., Ruwanjith, H. A. D., & Ruwandeepika, H. A. D. (2018). Isolation of *Escherichia coli*, *Klebsiella spp*., and *Staphylococcus* spp. from bovine mastitic milk in Nuwara Eliya District, Sri Lanka and their antibiotic sensitivity. South Asian Journal of Research in Microbiology, 1(2), 1–13. https://doi.org/10.9734/sajrm/2018/v1i2766

Kayesh, M. E. H., Talukder, M., & Anower, A. K. M. M. (2014). Prevalence of subclinical mastitis and its association with bacteria and risk factors in lactating cows of Barisal District, Bangladesh. International Journal of Biological Research, 2(2), 35–38. https://doi.org/10.14419/ijbr.v2i2.2835

Kerro Dego, O. (2021). Bovine Mastitis: Part I. In Animal Reproduction in Veterinary Medicine. IntechOpen. https://doi.org/10.5772/intechopen.93483

Kibebew, K. (2017). Bovine mastitis: A review of causes and epidemiological point of view. Journal of Biology, Agriculture and Healthcare, 7(2), 1–14. https://api.semanticscholar.org/CorpusID:55458788

Klaas, I.C. & Zadoks, R.N. (2018). An update on environmental mastitis: Challenging perceptions. Transboundary and Emerging Diseases, 65, 166-185. https://doi.org/10.1111/tbed.12704

Mourya, A., Shukla, P. C., Gupta, D. K., Sharma, R. K., Nayak, A., Singh, B., Jain, A., & Pradhan, S. (2020). Prevalence of subclinical mastitis in cows in and around Jabalpur, Madhya Pradesh. Journal of Entomology and Zoology Studies, 8(4), 40–44. https://www.researchgate.net/publication/343826574

Priyantha, M. A. R., Fernando, P. S., & De Alwis, P. S. (2021). Emerging antimicrobial resistance in coagulase-positive *Staphylococci* and *Escherichia coli* isolated from bovine clinical mastitis in Sri Lanka. Asian Journal of Research in Animal and Veterinary Sciences, 7(4), 29–35. https://doi.org/10.9734/ajravs/2021/v4i2153

Puerto, M. A., Shepley, E., Cue, R. I., Warner, D., Dubuc, J., & Vasseur, E. (2021). The hidden cost of disease: I. Impact of the first incidence of mastitis on production and economic indicators of primiparous dairy cows. Journal of Dairy Science, 104(7), 7932–7943. https://doi.org/10.3168/jds.2020-19584

Pugliese, M., Biondi, V., Passantino, A., Licitra, F., Alibrandi, A., Zanghì, A., Conte, F., & Marino, G. (2021). Welfare assessment in intensive and semi-intensive dairy cattle management systems in Sicily. Animal Science Journal, 92(1), e13546. https://doi.org/10.1111/asj.13546

Qayyum, A., Khan, J. A., Hussain, R., Avais, M., Nisar, A., Khan, A., & Khan, M. S. (2016). Prevalence and association of possible risk factors with subclinical mastitis in Cholistani cattle. Pakistan Journal of Zoology, 48(2), 519–525. https://api.semanticscholar.org/CorpusID:36385727

Quinn, P. J., Carter, M. E., Markey, B., & Carter, G. R. (1994). Mastitis. In Clinical Veterinary Microbiology (pp. 327–344). Wolfe Publishing.

Rahularaj, R., Deshapriya, R. M. C., & Ranasinghe, R. M. S. B. K. (2019). Influence of bovine subclinical mastitis and associated risk factors on calving interval in crossbred lactating cows in Sri Lanka. Tropical Animal Health and Production, 51(8), 2413–2419. https://doi.org/10.1007/s11250-019-01957-4

Ranasinghe, R. M. S. B. K., Deshapriya, R. M. C., Abeygunawardana, D. I., Rahularaj, R., & Dematawewa, C. M. B. (2021). Subclinical mastitis in dairy cows in major milk-producing areas of Sri Lanka: Prevalence, associated risk factors, and effects on reproduction. Journal of Dairy Science, 104(12), 12900–12911. https://doi.org/10.3168/jds.2021-20223

Sanotharan, N., Pagthinathan, M., & Nafees, M. S. M. (2016). Prevalence of bovine subclinical mastitis and its association with bacteria and risk factors in milking cows of Batticaloa District, Sri Lanka. International Journal of Scientific Research and Innovative Technology, 3(6), 1–9. https://www.researchgate.net/publication/331829654

Senthilkumar, A., Murugesan, S., & Balamurugan, P. (2020). Prevalence and detection of subclinical mastitis using California mastitis test in dairy farms of Theni District, Tamil Nadu, India. International Journal of Current Microbiology and Applied Sciences, 9(7), 988–993. https://doi.org/10.20546/ijcmas.2020.907.116

Shoaib, M., He, Z., Geng, X., Tang, M., Hao, R., Wang, S., Shang, R., Wang, X., Zhang, H., & Pu, W. (2023). Emergence of multidrug-resistant and virulence gene–carrying *Escherichia coli* in the dairy environment. Frontiers in Microbiology, 14, 1197579. https://doi.org/10.3389/fmicb.2023.1197579

Silva, A. C., Laven, R., & Benites, N. R. (2021). Risk factors associated with mastitis in smallholder dairy farms in Southeast Brazil. Animals, 11(7), 2089. https://doi.org/10.3390/ani11072089

Singh, A. K., Kumari, T., Rajput, M. S., Baishya, A., Bhatt, N., & Roy, S. (2020). A review: Effect of bedding material on production, reproduction and health and behavior of dairy animals. International Journal of Livestock Research, 10(7), 11-20.

Sweeney, M. T., Lubbers, B. V., Schwarz, S., & Watts, J. L. (2018). Applying definitions for multidrug resistance, extensive drug resistance and pandrug resistance to clinically significant livestock and companion animal bacterial pathogens. Journal of Antimicrobial Chemotherapy, 73(6), 1460-1463. https://doi.org/10.1093/jac/dky043

Taponen, S., Liski, E., Heikkilä, A. M., & Pyörälä, S. (2017). Factors associated with intramammary infection in dairy cows caused by major mastitis pathogens. Journal of Dairy Science, 100(1), 493–503. https://doi.org/10.3168/jds.2016-11465

Tedla, M., Mehari, F., & Kebede, H. (2018). Cross-sectional survey and follow-up study on major dairy health problems in urban farms of Mekelle, Ethiopia. BMC Research Notes, 11, 236. https://doi.org/10.1186/s13104-018-3347-0

Thrusfield, M., Christley, R., Brown, H., Diggle, P. J., French, N., Howe, K., Kelly, L., O'Connor, A., Sargeant, J., & Wood, H. (2018). Describing disease occurrence. In M. Thrusfield, R. Christley, H. Brown, P. J. Diggle, N. French, K. Howe, L. Kelly, A. O'Connor, J. Sargeant, & H. Wood (Eds.), Veterinary epidemiology (4th ed., pp. 58–85). Wiley-Blackwell. https://doi.org/10.1002/9781118280249.ch4

Tiwari, B. B., Subedi, D., Bhandari, S., Shrestha, P., Pathak, C. R., Chandran, D., Pandey, G., Mohankumar, P., & Dhama, K. (2022). Prevalence and risk factors of staphylococcal subclinical mastitis in dairy animals of Chitwan, Nepal. Journal of Pure and Applied Microbiology, 16(2), 1392–1403. https://doi.org/10.22207/JPAM.16.2.67

Yimam, T. M., Kasse, G. E., & Yitie, M. T. (2020). Prevalence of bovine subclinical mastitis and associated risk factors in smallholder dairy farms of Mecha District, Ethiopia. Epidemiology International Journal, 4(5), 000161. https://doi.org/10.23880/EIJ-16000161