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RESEARCH ARTICLE

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Prevalence of subclinical mastitis in a selected group of cows in Matara district of Sri Lanka: Risk factors, pathogens and antimicrobial resistance

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Abstract: This study aimed to determine the prevalence of subclinical mastitis (SCM), identify associated risk factors, characterize major bacterial pathogens, and evaluate their antimicrobial resistance in dairy cows in the Matara district, Sri Lanka. The study was conducted from October 2024 to March 2025, involving 220 lactating cows from 60 randomly selected groups. SCM was diagnosed using the California mastitis test (CMT), and positive milk samples were subjected to bacteriological identification. Antimicrobial susceptibility testing was performed following Clinical and Laboratory Standards Institute (CLSI) guidelines. Farm- and animal-level data were collected using a structured questionnaire and analyzed using descriptive statistics, chi-square tests, and binary logistic regression using IBM SPSS (version 25) Statistics. The overall cow-level prevalence of SCM was 30.0%, with a quarter-level prevalence of 11.5%. Logistic regression analysis identified older age (>5 years), higher parity, early lactation stage, and high milk yield as significant animal-level predictors of SCM ($p < 0.05$). Friesian crossbred cows showed comparatively higher susceptibility than other breeds. At the farm-level, larger herd size, once-daily milking, poor udder hygiene, poor housing cleanliness, and absence of supplementary feeding were significantly associated with increased SCM risk. *Staphylococcus aureus* (54.6%) was the predominant isolate, followed by *Escherichia coli* (19.7%) and *Streptococcus* spp. (13.6%). Antimicrobial susceptibility testing revealed high resistance of *S. aureus* to amoxicillin, while *E. coli* showed high resistance to enrofloxacin and amoxicillin. However, gentamicin and neomycin remained highly effective against *E. coli,* and enrofloxacin showed complete efficacy against *Streptococcus* spp. In conclusion, SCM remains a significant constraint to dairy productivity in southern Sri Lanka. The findings highlight the need for improved milking hygiene, routine bacteriological screening, and prudent antimicrobial use to mitigate antimicrobial resistance and improve udder health within a One Health framework.

Keywords: Antimicrobial resistance, dairy cattle, risk factors, subclinical mastitis, *Staphylococcus aureus.*

INTRODUCTION

Mastitis, an inflammation of the mammary gland, remains one of the most economically impactful diseases in dairy production worldwide. It significantly reduces milk yield and quality, increases veterinary expenses, leads to premature culling, and contributes to substantial economic losses for dairy farmers (Seegers et al., 2003; Ruegg, 2017). Mastitis manifests in two primary forms. Clinical mastitis is characterized by visible signs such as udder swelling, heat and abnormal milk secretion. The subclinical mastitis (SCM) lacks visible symptoms but silently affects milk production and facilitates the transmission of pathogens within the herd (Halasa et al., 2007; Sharma et al., 2011).

SCM is considerably more prevalent than its clinical counterpart. It represents a major challenge to udder

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health particularly in low- and middle-income countries (LMICs) where resources for routine surveillance and control are limited (Mekonnen et al., 2017). A global meta-analysis estimated the average prevalence of SCM at approximately 42.0%. However, some regions had reported even higher rates, such as 86.2% in Uganda and 60.0% in Egypt (Abrahmsén et al., 2014; Bonatto et al., 2021). The absence of visible signs often delays detection, allowing infections to persist and spread unnoticed thereby undermining herd health interventions. Early detection and effective management of SCM are essential to sustain dairy productivity and ensuring milk safety, particularly in LMICs (Molina-Flores et al., 2020).

The most common pathogens responsible for mastitis include *Staphylococcus aureus*, *Streptococcus* spp., and *Escherichia coli*. The distribution of these pathogens varies with geography, hygiene, and farm management practices (Bradley, 2002; Kalmus et al., 2011). Among them, *S. aureus* is often linked to persistent subclinical infections due to its ability to evade host immune defenses through intracellular survival and biofilm formation (Zaatout et al., 2020).

Antibiotic therapy remains the cornerstone of mastitis treatment. However, the excessive and often unregulated use of antimicrobials in dairy production has led to the emergence of antimicrobial resistance (AMR). It is posing risks to both animal and public health (WHO, 2016). Resistance among mastitis pathogens, including *S. aureus, E. coli*, and *Streptococcus* spp., to widely used antibiotics such as tetracyclines, β-lactams and fluoroquinolones has been increasingly reported (Zhang et al., 2021; Velasco et al., 2025). Resistance genes such as blaZ, blaSHV, and blaTEM contribute to this trend. AMR surveillance in dairy systems is now recognized as an integral part of global One Health strategies to mitigate risks of transmission via the food chain (Van et al., 2015; WHO, 2025).

In Sri Lanka, the dairy sector is a vital component of rural livelihoods and national food security. Yet, the country meets only about 40.0% of its domestic milk requirement. The remainder is met through imports (Department of Animal Production and Health, 2019). SCM remains a major but underdiagnosed issue among local herds particularly under small- and medium-scale farming systems. Studies have shown that *S. aureus* is the predominant SCM pathogen, with increasing resistance to commonly used antibiotics such as cephalexin and amoxicillin (Jayaweera et al., 2018; Priyantha et al., 2021). However, comprehensive regional data on SCM prevalence, bacterial aetiology, and AMR remain limited and fragmented. According to Bari et al. (2022), only two published studies in Sri Lanka have investigated SCM pathogens with considerable variation across regions, underscoring the need for localized research.

The Matara district, situated in the Southern province, features predominantly small to medium dairy herds managed under extensive, semi-intensive, or intensive systems. While dairy farming is expanding in this region, constraints such as poor milking hygiene, inadequate veterinary support and lack of awareness hinder effective mastitis control. Understanding the epidemiology and AMR patterns of SCM in this context is essential for developing targeted interventions and guiding rational antibiotic use. Therefore, this study was undertaken to: (i) determine the prevalence of SCM, (ii) identify the associated bacterial pathogens and their antimicrobial resistance profiles, and (iii) assess animal and farm-level risk factors (determinants) associated with SCM. The findings aim to inform evidence-based mastitis control strategies and promote sustainable dairy development at the regional level.

MATERIALS AND METHODS

Ethical clearance statement

This study involved no experimental intervention on animals. All procedures were conducted as part of routine veterinary investigation services. Informed verbal consent was obtained from all participating farmers. The study adhered to the guidelines set by the Department of Animal Production and Health, Sri Lanka, for animal welfare and field data collection.

Study area and design

This cross-sectional study was conducted from October 2024 to March 2025 in 12 veterinary surgeon (VS) divisions of the Matara district of Sri Lanka.

Sample size determination

A prevalence of 50.0% was assumed for the purpose of sample size calculation as this provides the maximum variability and yields the most conservative estimate. The desired margin of error was set at 7.0% (0.07), with a 95.0% confidence level.

Although dairy farmers are typically registered under VS divisions, updated and reliable records of lactating cow populations at the district level were not available at the time of the study. Therefore, the total cattle population (n = 7,580) reported by the Department of Census and Statistics (Department of Census and Statistics, 2024) was used to estimate the lactating population. Based on regional herd structure and management practices, it was assumed that approximately 40.0% of cattle were lactating, yielding an estimated population size of 3,032 cows.

The initial sample size for an infinite population was calculated using the standard formula for prevalence studies.

$$n = \frac{Z^2 \times P \times (1 - P)}{d^2}$$

Where:
Z = 1.96 (95% confidence)
p = 0.5 (assumed prevalence; 50.0%)
d = 0.07 (precision)
n = population size (3032)

This value was adjusted for the finite population of lactating cows using the finite population correction formula.

$$nadj = \frac{n \times N}{N + n - 1}$$

Where,
nadj = adjusted sample size for finite population
n = initial sample size calculated for an infinite population
N = total population size (estimated number of lactating cows in the study area)

Accordingly, the minimum required sample size was estimated at 184 cows. To enhance the statistical power and allow for potential exclusions, a total of 220 lactating cows with about 880 teat quarters were considered for the study. The 220 cows were distributed across 12 VS divisions in the Matara district, representing similar agro-climatic conditions. All study locations fall within the low-country wet zone, characterized by high rainfall and humidity, minimizing climatic variation across sampling sites. The number of cows sampled from each division varied depending on herd availability and farmer participation (Table 1).

Table 1: Distribution of sampled lactating dairy cows across Veterinary Surgeon (VS) Divisions in the Matara District, Sri Lanka (n = 220)

VS Division	Sampled cows (n)	Percentage (%)
Akuressa	09	4.1
Dikwella	04	1.8
Hakmana	10	4.5
Kamburupitiya	26	11.8
Kekanadura	26	11.8
Malimbada	20	9.1
Matara	28	12.7
Mulatiyana	11	5.0
Pasgoda	17	7.7
Pitabeddara	16	7.3
Weligama	26	11.8
Welipitiya	27	12.3
Total	220	100.0

Detection of subclinical mastitis (SCM)

All lactating cows were screened during the morning milking period. Cows exhibiting clinical signs of mastitis were excluded. SCM was diagnosed using California mastitis test (CMT), as described by Quinn et al. (1999). 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. Each quarter was scored from 0 to 4 based on gel formation. A score of ≥2 in any quarter in the absence of visible abnormalities in milk or udder was considered positive for SCM. All CMTs were conducted by the same trained veterinarian to ensure consistency. All four quarters of each selected cow were tested and evaluated separately.

Composite foremilk samples (15 mL) from CMT positive quarters were aseptically collected into sterile glass vials after cleaning the teats with 70.0% ethyl alcohol and discarding the initial streams. Samples were kept on ice and transported to the Matara Veterinary Investigation Centre (VIC) for microbiological analysis.

Questionnaire survey

A questionnaire was designed to gather farm and animal-level data through face-to-face interviews with the farmer during the farm visit. It was pretested with 10 randomly selected farmers. Animal-level data included age, breed, parity, lactation stage, body condition score (BCS), and daily milk yield. Farm-level information captured herd size, farming system, milking frequency, udder hygiene practices, supplementary feeding, and house cleanliness. Interviews were conducted in the local language by a trained investigator. Clarifications and follow-up visits were conducted to ensure data accuracy.

Defining categorical variables

Categorical variables used in the analysis were defined based on field observations and standard management practices. Age was categorized as ≤3 years, 3–5 years and >5 years. Parity was grouped as 1, 2 and ≥3. Lactation stage was classified as early (≤3 months), mid (3–6 months) and late (>6 months). Daily milk yield was categorized as low (1–4.9 L/day), average (5–6.9 L/day) and high (7–12 L/day). BCS was classified as poor (≤2.5), average (2.75–3.0) and good (≥3.5) following Edmonson et al. (1989). The breed of the animals was recorded as Jersey cross, Friesian cross, and Sahiwal cross.

Udder hygiene was categorized as 'good' when teats were washed and dried before milking, and 'poor' when no cleaning or inadequate cleaning was observed. House cleanliness was classified as good (clean, dry housing with regular manure removal), average (moderately clean with occasional manure accumulation) and poor (dirty, wet conditions with visible manure accumulation). Milking frequency was categorized as once or twice daily. Supplementary feeding was recorded as 'yes' when concentrate or additional feed was provided and 'no' otherwise. Herd size was categorized as small (≤10 cows) and medium (11–50 cows). Farming systems were classified based on animal housing and grazing practices. Management systems were defined as intensive where there is zero-grazing (animals housed and fed for 24 hours), semi-intensive where cows were tethered in the morning (around 08:00 h) and returned to sheds by 14:30 h (Bandara et al., 2011), and extensive where animals were predominantly managed under free grazing conditions with minimal housing.

Bacteriological examination

Milk samples were processed at VIC, Matara within six hours of collection. Samples were brought to room temperature and aseptically streaked onto blood agar and MacConkey agar plates. The plates were incubated aerobically at 37 °C for 24-48 hours. Colonies were characterized based on morphology and haemolysis.

Gram staining was used to classify isolates. Gram-positive cocci were subjected to catalase testing to differentiate *Staphylococcus* spp. (catalase-positive) from *Streptococcus* spp. (catalase-negative). Coagulase testing was used to identify *Staphylococcus aureus*. Gram-negative isolates were sub-cultured on MacConkey agar and presumptively identified as *E. coli* based on lactose fermentation. Final confirmation was done using standard biochemical tests including triple sugar iron (TSI), urease, citrate, and indole tests.

Antimicrobial sensitivity testing (ABST)

Antimicrobial susceptibility was determined using the Kirby-Bauer disk diffusion method (Bauer et al., 1966) following the Clinical and Laboratory Standards Institute (CLSI - 2023) guidelines. Five commonly used antibiotics were tested, including gentamicin (10 µg), oxytetracycline (30 µg), amoxicillin (10 µg), enrofloxacin (10 µg) and neomycin (30 µg). Antibiotic discs (Himedia, India) were placed on Mueller-Hinton agar inoculated with overnight cultures grown in Luria–Bertani broth. The plates were incubated at 37 °C for 18–24 hours. Zones of inhibition were measured by one technician to reduce observer bias. The zone of inhibition was measured in millimeters and interpreted based on CLSI breakpoint standards for each species (CLSI, 2023).

Statistical analysis

All data collected from field observations, laboratory analysis, and questionnaire surveys were coded and entered into IBM SPSS Statistics Version 25 for statistical analysis. Descriptive statistics were used to summarize the data. SCM prevalence was calculated at both the cow and quarter levels according to Gómez-Quispe et al. (2015).

$$Cow\ level\ prevalance = \frac{No.of\ CMT\ positive\ cows}{Total\ cows\ examined} x\ 100$$

$$Quarter\ level\ prevalance = \frac{No.of\ positive\ quarters}{Total\ quarters\ examined} x\ 100$$

The association between explanatory variables and SCM status (positive/negative) was initially assessed using the Chi-square (χ^2) test of independence. Variables with a significance level of $p < 0.05$ in the Chi-square analysis were considered for further modeling.

To identify independent risk factors associated with SCM, binary logistic regression analysis was performed using a backward stepwise approach. Separate models were developed for animal and farm-level variables to avoid multicollinearity and improve interpretability. The dependent variable was SCM status (1 = positive, 0 = negative). Independent variables were entered as categorical predictors based on predefined classifications. The general form of the binary logistic regression model is expressed as follows:

$$logit\ (p) = In\left(\frac{p}{(1-p)}\right) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \cdots$$

where,
p = Probability of SCM occurrence
β_0 = Intercept
β_i = Regression coefficient
X_i = Independent variables

The strength of association was expressed using Adjusted Odds Ratios (AORs) with corresponding 95% confidence intervals (CI). Statistical significance was declared at $p < 0.05$.

Model adequacy was evaluated using standard diagnostic measures including the Hosmer–Lemeshow goodness-of-fit test, and multicollinearity among predictors was assessed using variance inflation factors (VIF), ensuring that all included variables met acceptable thresholds.

All statistical tests were two-tailed and results were presented in tables with corresponding test statistics, p-values, and confidence intervals.

RESULTS AND DISCUSSION

Prevalence of subclinical mastitis (SCM)

The overall prevalence of SCM in the present study was 30.0% (66/220 cows) as determined by the CMT. Of the 880 quarters examined, 101 quarters (11.5%) were found positive for SCM. These results indicate a considerable burden of subclinical infections in lactating cows in the Matara district. Compared to previous studies in

Table 2: Association between animal-level factors and subclinical mastitis (SCM) prevalence in lactating dairy cows (n = 220).

Variable	Category	n	%	SCM positive (n)	Prevalence (%)	X^2	*P* value
	≤3 years	97	44.1	18	18.6	14.68	0.001
Age	3-5 years	93	42.3	32	34.4		
	> 5 years	30	13.6	16	53.3		
	Jersey cross	100	45.5	20	20.0	20.22	0.000
Breed	Friesian cross	36	16.4	6	16.7		
	Sahiwal cross	84	38.2	40	47.6		
	1 parity	71	32.3	17	23.9	8.90	0.012
Parity	2 parity	65	29.5	14	21.5		
	≥ 3 parity	84	38.2	35	41.7		
	Early	109	49.5	46	42.2	15.31	0.000
Lactation stage	Mid	55	25.0	10	18.2		
	Late	56	25.5	10	17.9		
	Poor (≤ 2.5)	89	40.5	28	31.5	2.67	0.263
BCS	Average (2.75-3.0)	125	56.8	38	30.4		
	Good (≥ 3.6)	6	2.7	0	0.0		
	Low (1-4.9 l/d)	84	38.2	15	17.9	13.89	0.001
Milk yield	Average (5-6.9 l/d)	101	45.9	33	32.7		
	High (7-12 l/d)	35	15.9	18	51.4		

BCS: body condition score

Sri Lanka, the observed prevalence is relatively lower. For instance, Gunawardana et al. (2014) reported prevalence rates ranging from 43.0% to 71.0% while Rahularaj et al. (2019) recorded a prevalence of 49.0% based on CMT testing. This variation may be attributed to differences in study settings, herd sizes and management practices including hygiene and milking systems.

Internationally, reported SCM prevalence varies widely. In Bangladesh, studies have shown prevalence rates ranging from 20.0% to 75.0% depending on herd characteristics and sampling methodology (Sarker et al., 2013; Bari et al., 2022). A meta-analysis by Krishnamoorthy et al. (2021) documented a global SCM prevalence of 42.0% with higher prevalence in India (45.0%) and African countries like Uganda (86.2%, Abrahmsén et al., 2014).

Association between animal-level factors and subclinical mastitis (SCM)

Results of the Chi-square test revealed significant associations between SCM prevalence and age, breed, parity, lactation stage and milk yield. No statistically significant association was found with BCS (Table 2). These significant variables were subsequently included in a binary logistic regression model to identify independent predictors.

Animal-level determinants of subclinical mastitis (SCM)

The binary logistic regression model identified age, breed, parity, milk yield, and lactation stage as statistically significant animal level determinants of SCM ($p < 0.05$) (Table 3).

Table 3: Binary logistic regression analysis of animal-level determinants of subclinical mastitis (SCM) in lactating dairy cows (n = 220)

Variable	B	S.E.	*P* value	AOR (Exp(B))	95% CI
Age					
≤3 years	-2.415	0.587	0.000	0.089	0.028-0.282
3-5 years	-0.590	0.540	0.275	0.554	0.192-1.598
Breed			0.002		
Sahiwal cross	-1.420	0.413	0.001	0.242	0.107-0.543
Jersey cross	-1.106	0.573	0.054	0.331	0.108-1.017
Parity			0.004		
Parity 1	-1.586	0.512	0.002	0.205	0.075-0.559
Parity 2	-1.426	0.516	0.006	0.240	0.087-0.660
Milk yield			0.002		
Low (1-4.9 l/d)	-2.050	0.570	0.000	0.129	0.042-0.394
Average (5-6.9 l/d)	-1.249	0.489	0.011	0.287	0.110-0.747
Lactation stage			0.020		
Early lactation	1.408	0.507	0.005	4.088	1.514-11.036
Mid lactation	0.934	0.623	0.134	2.545	0.751-8.626

AOR: Adjusted Odds Ratio; CI: Confidence interval; Reference groups: Age: > 5 years; Breed: Friesian cross; Parity: ≥ 3 parity; Milk yield: High (7-12 l/d); Lactation stage: late lactation. The Hosmer–Lemeshow test indicated good model fit ($p > 0.05$), and no significant multicollinearity was detected among predictors (VIF < 10).

Logistic regression analysis revealed that older cows (>5 years) were significantly more prone to SCM, likely due to age-related teat and immune changes (Rainard & Riollet, 2006; Radostits et al., 2007; Moges et al., 2011; Zeryehun et al., 2013). Younger cows had significantly lower odds of infection, supporting earlier findings.

Breed also significantly influenced SCM risk with Sahiwal and Jersey crosses being less susceptible than Friesian crosses. Breed-related differences reflect both genetic susceptibility and variations in management practices. Friesian crosses, being higher-yielding, showed greater risk of SCM, particularly under sub-optimal conditions like outdoor milking and temporary housing with poor hygiene (Curone et al., 2018; Ranasinghe et al., 2021). The present study observed that farmers used outdoor areas for milking and brought the cows outside the rearing pens and tethered them to a tree before milking. Those farmers were poor and had minimal facilities such as temporary housing. Further, it was observed that those farmers were not concerned about the hygienic aspects of milking. Although these farmers are encouraged to keep high-yielding exotic animals, this is not accompanied by the awareness of proper prevention and control of mastitis in the study area.

Cows with higher parity had greater SCM risk, which is consistent with prior findings (Zeryehun et al., 2013). Some studies reported that compared with primiparous cows, multiparous cows were 2.51 times more likely to have SCM (Ranasinghe et al., 2021). Age-related teat end changes, tissue degeneration and pendulous udders may predispose to infection (Neijenhuis et al., 2001; Bhutto et al., 2010; Zeryehun et al., 2013).

High yielding cows had increased SCM risk, likely due to elevated metabolic stress and udder pressure (Taponen et al., 2006; Biswas & Sarker, 2017). This association is likely due to the elevated nutrient demands in high producing cows which can compromise the immune system and make the mammary glands more susceptible to infection (Schalm et al., 1971; Sharma et al., 2011). However, some studies have reported that the incidence rate of mastitis was higher in low (55.2%) and moderate (58.0%) yielders than high (41.0%) yielders (Jingar et al., 2014; Elmaghraby et al., 2017).

Early lactation was significantly associated with SCM (AOR = 4.09). Zeryehun et al. (2013) also reported that the early lactation stage had higher relative prevalence (87.2%) than late (73.1%) and mid (65.9%) lactation stages. The absence of a dry cow therapy regime could possibly be the major factor contributing to high prevalence at early lactation and early infection associated with delayed diapedesis of neutrophils into the mammary glands (Schalm et al., 1971). This may also due to physiological stress and immunosuppression of cows in the study region (Schalm et al., 1971; Sharma et al., 2011).

Poor BCS has been suggested as a risk factor in other studies. For instance, Fesseha et al. (2021) have reported that poor BCS had higher (60.4%) relative prevalence than that of moderate (52.5 %) and good (30.0%) BCS. However, it was not significant in the present analysis possibly due to limited sample representation.

Table 4: Association between farm-level factors and subclinical mastitis (SCM) prevalence in lactating dairy cows (n = 220).

Variable	Category	n	%	SCM positive (n)	Prevalence (%)	X^2	*P* value
Herd size	Small (≤ 10)	70	31.8	8	11.4	16.86	0.000
	Medium (11-50)	150	68.2	58	38.7		
Farming system	Intensive	33	15.0	17	51.5	9.08	0.011
	Semi intensive	162	73.6	44	27.2		
	Extensive	25	11.4	5	20.0		
Milking frequency	Once daily	126	57.3	52	41.3	17.83	0.000
	Twice daily	94	42.7	14	14.9		
Udder hygiene	Good	97	44.1	10	10.3	32.03	0.000
	Poor	123	55.9	56	45.5		
Supplementary feeding	Yes	137	62.3	26	19.0	21.00	0.000
	No	83	37.7	40	48.2		
House cleanliness	Good	36	16.4	6	16.7	49.03	0.000
	Average	117	53.2	18	15.4		
	Poor	67	30.5	42	62.7		

SCM: Subclinical mastitis

Association between farm-level factors and subclinical mastitis (SCM)

Chi-square analysis revealed significant associations between SCM and several farm-level variables, including herd size, farming system, milking frequency, udder hygiene, supplementary feeding and house cleanliness (Table 4). These variables were subsequently included in the logistic regression model (Table 5).

Table 5: Binary logistic regression analysis of farm-level determinants of SCM in lactating dairy cows (n = 220)

Variable	B	S.E.	*P* value	AOR (Exp(B))	95% CI
Herd size (Small)	-1.892	0.516	0.000	0.151	0.055-0.415
Milking frequency (once daily)	1.375	0.469	0.003	3.954	1.576-9.920
Udder hygiene (Good)	-1.535	0.469	0.001	0.216	0.086-0.541
Supplementary feeding (No)	1.299	0.417	0.002	3.666	1.618-8.308
House cleanliness			0.001		
House cleanliness (Good)	-1.771	0.649	0.006	0.170	0.048-0.607
House cleanliness (Average)	-1.470	0.425	0.001	0.230	0.100-0.529
Farming system			0.033		
Farming system (Intensive)	1.444	0.872	0.098	4.239	0.767-23.433
Farming system (Semi Intensive)	0.084	0.772	0.913	1.088	0.240-4.938

AOR: Adjusted odds ratio; CI: Confidence interval; Reference groups: Herd size: Medium herd (11-50); Milking frequency: twice daily; Udder hygiene: poor; Supplementary feeding: Yes; House cleanliness: Poor; Farming system: Extensive. The Hosmer–Lemeshow test indicated good model fit ($p > 0.05$), and no significant multicollinearity was detected among predictors (VIF < 10).

Cows reared in small herds (≤10 cows) had significantly lower odds of SCM compared to those in medium-sized herds. This supports findings by Islam et al. (2012) and Ranasinghe et al. (2021), suggesting that larger herds may face increased pathogen exposure due to crowding, shared equipment, and less individualized attention to udder health and hygiene. Bari et al. (2022) also explained that the larger herds are usually managed under an intensive management system to be commercially viable. Therefore, they are likely to have increased pathogen exposure due to high stocking density and dirty condition of the sheds or equipment.

Poor udder hygiene, poor house cleanliness, and absence of supplementary feeding were all significantly associated with higher SCM risk. Cows milked once daily had a significantly higher likelihood of SCM compared to those milked twice daily. This may be due to increased intramammary pressure and bacterial proliferation caused by prolonged milk retention (Stelwagen & Lacy-Hulbert, 1996; Green et al., 2006). Schreiner & Ruegg (2003) demonstrated that dirty udders were associated with higher pathogen load in milk (1.5 times more likely to have major pathogens compared to clean udders). Post-milking teat disinfection, use of gloves and keeping cows standing after milking are effective preventive measures (Dufour et al., 2011). In the present study, cows that did not receive supplementary feeding exhibited a significantly higher prevalence of SCM compared to those that received supplementary feeding. This suggests that improved nutritional support may enhance immune function and udder health, thereby reducing susceptibility to infection (Green et al., 2007; Sordillo et al., 2009). Farms with good and average hygiene practices had substantially lower odds of SCM. Dirty housing environments contribute to increased bacterial load on the udder and teat surface facilitating intramammary infections (Abrahmsén *et al.*, 2014; Mureithi & Njuguna, 2016; Kagira et al., 2022).

Although there was a significant difference among farming systems, pairwise comparisons indicated no significant difference between intensive and extensive systems. An intensive system may contribute to more frequent infections as the cow stays longer within a small area that may be less clean and in addition, cows may be more stressed because of overcrowding (Barker et al., 2001).

Bacterial pathogens of milk

Bacteriological examination of milk samples from SCM positive cows revealed four major bacterial groups: *Staphylococcus aureus*, *Streptococcus* spp., *E. coli* and other coagulase negative *Staphylococcus species* (CNS) (Table 6). Based on Gram staining, colony morphology, and biochemical tests, *S. aureus* was confirmed by its Gram-positive cocci appearance in clusters, catalase and coagulase positivity, and golden-yellow colonies on nutrient agar. *Streptococcus* spp. appeared as Gram-positive cocci in chains, were catalase negative, and produced small translucent colonies. *E. coli* was identified as Gram-negative rods producing pink lactose fermenting colonies on MacConkey agar and confirmed by gas production on triple sugar iron (TSI) slants and negative urease and citrate tests.

Table 6: Distribution of bacterial pathogens isolated from SCM positive milk samples (n = 66)

Bacteria species	Number of isolates (n)	Prevalence (%)
S. aureus	36	54.6
Staphylococcus spp (CNS)	8	12.1
Streptococcus spp.	9	13.6
E. coli	13	19.7
Total	66	100.0

CNS: Coagulase-negative Staphylococcus

Several studies in Sri Lanka have reported the major causal agents for SCM to be *Staphylococcus spp*., *Klebsiella spp*., *E. coli*, *Streptococcus spp*., *Pasteurella spp*., *Corynebacterium spp*., and *Proteus spp.* (Samarakoon et al., 2014).

S. aureus was the most prevalent isolate among SCM cases followed by *E. coli*, *Streptococcus* spp. and other coagulase-negative *Staphylococcus* species in this study. This aligns with previous studies in Sri Lanka and South Asia, where *S. aureus* is consistently recognized as the primary pathogen associated with SCM (Bhattacharyya et al., 2016; Bari et al., 2022). The dominance of *S. aureus* is attributed to its strong udder colonization capacity, intracellular persistence and resistance to immune clearance enabling it to cause chronic subclinical infections and facilitate transmission during milking (Radostits et al., 2007).

E. coli and *Streptococcus* spp. were also important pathogens identified. These are often classified as environmental mastitis pathogens, commonly originating from contaminated housing, bedding, or milking equipment. The presence of *E. coli* highlights poor hygiene or water contamination in some farms. Similar bacterial profiles have been reported across tropical dairy systems in Bangladesh (Islam et al., 2014), India (Shome et al., 2012; Prabhu et al., 2013), and Pakistan (Khan et al., 2013), where *S. aureus*, NAS, and *Streptococcus* spp. predominate.

The identification of both contagious pathogens like *S. aureus* and environmental pathogens such as *E. coli* and *Streptococcus* spp. in the current study highlights the dual challenge of mastitis control. Effective prevention must therefore integrate milking hygiene, cow cleanliness, housing sanitation and targeted treatment strategies to mitigate both routes of infection.

Antimicrobial resistance patterns

Staphylococcus aureus

Among 36 *S. aureus* isolates, high susceptibility was observed to enrofloxacin, gentamicin and neomycin (Table 7), which is consistent with previous findings that aminoglycosides and fluoroquinolones retain significant efficacy against *S. aureus* infections (Pyorala & Taponen, 2009; Kalmus et al., 2011). However, marked resistance to amoxicillin was observed raising concern regarding the sustained effectiveness of β-lactam antibiotics in mastitis control. Moderate resistance levels to oxytetracycline reflect its widespread and often indiscriminate use raising concerns about its future efficacy in mastitis management (Barkema et al., 2006) (Table 7).

Table 7: Antimicrobial susceptibility profile of *S. aureus* isolates (n = 36)

Antimicrobial agent	Disc concentration (µg)	Susceptible (%)
Enrofloxacin	10	94.9
Amoxicillin	10	35.9
Gentamicin	10	84.6
Neomycin	30	82.1
Oxytetracycline	30	59.0

Interpretation of antimicrobial susceptibility was performed according to Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines. Intermediate results were not observed.

Escherichia coli

All 13 *E. coli* isolates demonstrated complete susceptibility to gentamicin and neomycin (Table 8) reinforcing the continued value of these antimicrobials for treating Gram-negative infections. Similar susceptibility patterns have been reported in calf isolates (Orden et al., 2000; Diwakar, 2014). However, resistance to enrofloxacin was notably high, a troubling observation given the drug's broad-spectrum profile. The emerging resistance to fluoroquinolones may be attributed to their frequent and indiscriminate use in veterinary practices within the study area. Furthermore, field observations revealed that antibiotics were often self-administered by farmers without adequate veterinary supervision. High resistance was also observed against amoxicillin, which is consistent with studies in France and South Asia (Islam et al., 2014; Boireau et al., 2018). Moderate resistance to oxytetracycline underscores the growing concern about tetracycline-class antibiotic efficacy, as reflected in resistance trends reported by Johns & Adams (2015) (Table 8).

Table 8: Antimicrobial susceptibility profile of *Escherichia coli* isolates (n = 13)

Antimicrobial agent	Disc concentration (µg)	Susceptible (%)
Enrofloxacin	10	9.1
Amoxicillin	10	27.3
Gentamicin	10	100.0
Neomycin	30	100.0
Oxytetracycline	30	72.7

Interpretation of antimicrobial susceptibility was performed according to Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines. Intermediate results were not observed.

Streptococcus spp.

All 9 *Streptococcus* spp. isolates were fully susceptible to enrofloxacin indicating robust activity of this fluoroquinolone against streptococcal pathogens (Table 9). This finding is in consistent with earlier findings of Kaczorek et al. (2017) and Yurin et al. (2020). However, amoxicillin and oxytetracycline exhibited equal rates of resistance and susceptibility (50%) indicating variable therapeutic outcomes depending on strain and context. Such inconsistencies have also been reported by Ceniti et al. (2017) and Diri et al. (2022). Gentamicin and neomycin were not tested due to the absence of CLSI-approved interpretive breakpoints for *Streptococcus* spp. (CLSI, 2023) to be in accordance with best practices to avoid misleading interpretations.

Table 9: Antimicrobial susceptibility profile of *Streptococcus* spp. isolates (n = 9)

Antimicrobial agent	Disc concentration (µg)	Susceptible (%)
Enrofloxacin	10	100
Amoxicillin	10	50.0
Oxytetracycline	30	50.0

Interpretation of antimicrobial susceptibility was performed according to Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines. Intermediate results were not observed.

Overall, the findings suggest that enrofloxacin demonstrated high *in vitro* efficacy against major mastitis pathogens of *S. aureus* and *Streptococcus spp.,* while gentamicin or neomycin could be considered for *E. coli* infections. In situations where mixed bacterial infections are suspected, a combination approach may offer broader efficacy.

Implications for mastitis management and public health

The observed antimicrobial resistance patterns in the present study may be attributed to multiple factors, including frequent empirical treatment without culture-based diagnosis, incomplete dosage regimens and the widespread availability of antibiotics without veterinary prescription. The high resistance observed against amoxicillin and enrofloxacin suggests prolonged and possibly indiscriminate use of β-lactams and fluoroquinolones in the study area, leading to increased selective pressure and the emergence of resistant strains. In smallholder dairy systems, such practices are often exacerbated by limited access to veterinary guidance.

Environmental factors may also contribute to resistance development, as improper disposal of drug residues, manure contamination, and horizontal gene transfer among bacterial populations facilitate the persistence and spread of resistant organisms. These findings highlight the urgent need for antimicrobial stewardship programs, farmer education and stricter regulatory control of veterinary drug use.

It is important to note that *in vitro* antimicrobial susceptibility does not necessarily translate into clinical efficacy due to host factors, drug pharmacokinetics, and infection dynamics. From a One Health perspective, the detection of antimicrobial resistance in milk pathogens poses potential risks to public health through the transmission of resistant bacteria or resistance genes via the food chain. This aligns with global concerns regarding the role of livestock in the dissemination of antimicrobial resistance (WHO, 2025). Therefore, integrating mastitis control strategies with prudent antibiotic use, farmer training, and routine surveillance is essential for safeguarding both animal and human health.

CONCLUSIONS

The present study revealed that the overall and quarter-level prevalences of SCM in the Matara district of Sri Lanka are 30.0% and 11.5% respectively. Key animal-level factors including age, breed, parity, lactation stage, and milk yield, as well as farm-level practices such as herd size, milking frequency, udder hygiene, supplementary feeding, and housing cleanliness were significantly associated with SCM prevalence. *S. aureus*, *E. coli*, and S*treptococcus* spp. were identified as the predominant pathogens of SCM. High resistance rates to commonly used β-lactam antibiotics and moderate resistance to oxytetracycline raise concerns about the continued efficacy of empirical treatment protocols and emphasize the need for antibiotic stewardship in dairy farming. These findings highlight the importance of targeted management interventions, routine bacteriological testing and integrated AMR surveillance as part of a One Health approach.

This study relied solely on the CMT for the detection of SCM. It is inherently subjective and may fail to detect infections with low somatic cell count elevations or pathogens. Furthermore, the cross-sectional nature of the study limits the ability to capture seasonal patterns in SCM prevalence and AMR over time. Additionally, the study did not include molecular characterization of the bacterial isolates. Furthermore, the study did not assess farmer knowledge, attitudes, or practices regarding mastitis control and antibiotic usage which could significantly influence treatment outcomes and resistance development.

Future research should employ longitudinal designs to monitor seasonal variations in SCM prevalence and AMR trends. Incorporating molecular tools for pathogen identification and resistance gene profiling is recommended. In addition, integrating socio-behavioral assessments such as farmer knowledge and antibiotic usage practices would provide a more holistic understanding. It would support the design of effective, locally adapted mastitis control interventions.

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