

Multiple *Staphylococcus* species and modifiable production-related risk factors associated with bovine subclinical mastitis in Kenyan cattle systems

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Abstract

Background Bovine subclinical mastitis (SCM) remains a major constraint to dairy productivity and udder health in Kenya. However, most studies have not resolved non-aureus *Staphylococcus* (NAS) species beyond the genus level, limiting epidemiological understanding. We assessed *Staphylococcus* species diversity and modifiable production-related risk factors associated with SCM across heterogeneous cattle production systems.

Methods A cross-sectional study was conducted involving 436 composite milk samples collected from 436 lactating cows in 152 herds. SCM was defined using somatic cell count thresholds and bacterial isolates were characterized using matrix-assisted laser desorption/ionization-time of flight mass spectrometry. Risk factors were assessed using mixed-effects logistic regression models to account for herd-level clustering and unmeasured heterogeneity.

Results SCM prevalence was 53.0% (229/436) at the cow-level and 73.0% (111/152) at the herd-level. Culture-positive samples were 58.0% (251/436), yielding 174 bacterial isolates. NAS species accounted for 44.3% of isolates, exceeding *S. aureus* (35.6%). *S. chromogenes* was the predominant NAS species (19.0%), followed by *S. succinus* (5.2%). Additional pathogens included *E. coli* (19.5%) and *P. aeruginosa* (0.6%). Mixed bacterial growth occurred in 8.8% of culture-positive samples, with NAS present in 5.0%. The odds of having SCM were significantly higher in indigenous cattle compared with exotic cattle (adjusted odds ratio [aOR]=2.51, 95% CI: 1.16–5.57, $p=0.020$), in cattle housed in temporary (aOR=6.27, 95% CI: 1.97–21.0, $p=0.002$) or semi-permanent structures (aOR=4.63, 95% CI: 1.91–11.8, $p<0.001$) compared with permanent housing, and in medium-sized herds compared with small-sized herds (aOR=2.43, 95% CI: 1.19–5.06, $p=0.016$).

Conclusion SCM remains prevalent across Kenyan cattle systems and is characterized by substantial *Staphylococcus* spp. diversity, including *S. aureus* and multiple NAS species, with occasional mixed bacterial growth. In resource-limited settings, sustainable mastitis control strategies should prioritize system-specific interventions integrating scalable diagnostic tools with species-level pathogen surveillance and targeted improvements in modifiable production-related risk factors, particularly housing quality and herd management.

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Keywords Bovine subclinical mastitis · Species-level characterization · Non-aureus *Staphylococcus* spp. · Cattle production systems · Production-related risk factors · Kenya

Introduction

Bovine mastitis, an inflammation of the cow's mammary gland commonly caused by an infection, remains one of the most consequential diseases affecting cattle production worldwide. Its economic burden is substantial, arising from reduced milk yield and quality, irreversible damage to udder tissue, veterinary costs (including antimicrobial use), discarded milk, and premature culling of chronically affected animals. Collectively, these losses amount to billions of dollars annually across the global dairy sector [1]. Beyond productivity constraints, mastitis also poses important public-health risks, as associated zoonotic pathogens and antimicrobial-resistant (AMR) bacteria may enter the milk value chain, particularly in settings where milk hygiene systems are weak and informal marketing channels predominate [2]. These concerns are particularly relevant in low-resource settings, where dairy products constitute a major dietary component for vulnerable populations, including children under five years of age, and where consumption of raw or inadequately processed milk remains common [3].

Mastitis is not a single disease entity but occurs in two principal forms: clinical mastitis (CM) and subclinical mastitis (SCM). CM is less prevalent and readily identifiable, presenting with visible signs of udder inflammation such as swelling, heat, pain and redness, often accompanied by abnormal milk appearance, including clots, discoloration, blood, or purulent secretions. In contrast, SCM is far more prevalent yet difficult to detect as it presents without overt clinical signs. It is primarily diagnosed through elevated somatic cell counts (SCC), typically exceeding 200,000 cells/mL, which reflect an intramammary inflammatory response dominated by leukocytes regardless of the underlying causative agent. Although pen-side diagnostic tools such as the California Mastitis Test (CMT), White-side, surf field and bromothymol-blue tests are widely used for SCM detection [4–8], their implementation remains inconsistent in many resource-limited settings due to cost, availability, and operational constraints. Consequently, SCM frequently escapes farmer detection, persists within herds and contributes disproportionately to production losses and pathogen transmission compared with CM [3–6, 8–10].

The aetiology of bovine mastitis is multifactorial, involving a diverse range of infectious agents, including bacteria, mycoplasmas, yeasts, algae and viruses [7, 11]. Among these, bacterial pathogens predominate and are broadly classified as either contagious or environmental pathogens based on their primary reservoirs and transmission pathways. Contagious pathogens, including *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma* spp., and *Corynebacterium bovis*, primarily reside within the udder and may spread between quarters and among cows, particularly during milking. In contrast, environmental pathogens such as *Escherichia coli*, *Klebsiella* spp., *Streptococcus dysgalactiae*, and *Streptococcus uberis* originate from bedding, soil, manure and housing environments. Consequently, mastitis infections may therefore occur as mono- or polymicrobial infections, reflecting simultaneous exposure to multiple ecological reservoirs and transmission routes [7, 12].

Within this broader bacterial landscape, non-aureus *Staphylococcus* (NAS) species, historically grouped as coagulase negative *Staphylococci* (CONS), have gained increasing attention in mastitis epidemiology. NAS are common commensals of the teat skin and bovine udder and were traditionally regarded as minor opportunist contaminants. However, accumulating evidence indicates that several NAS species can colonize the mammary gland, establish persistent intramammary infections and contribute to elevated SCC [13, 14]. Moreover, as control of major contagious pathogens such as *S. aureus* improves in some settings, NAS are increasingly emerging as mastitis-associated bacteria, including potential reservoirs of AMR genes [2, 13–15]. Studies from diverse regions, including South Africa and Bangladesh, have reported considerable heterogeneity in NAS species associated with mastitis in dairy cattle and other livestock systems [14, 16], reinforcing the importance of species-level characterization in epidemiological investigations.

Despite this growing recognition, most SCM studies in many African settings including Kenya, have generally reported NAS at the genus level, thereby limiting epidemiological understanding. Previous research from selected regions in Kenya have documented high SCM prevalence and identified *S. aureus*, NAS species (often reported collectively), *Strep. agalactiae* and *E. coli* as common mastitis-associated pathogens [3, 17, 18]. The lack of species-level characterization of NAS obscures understanding of their diversity and AMR profiles, constrains evaluation of their associations with intramammary inflammation across breed types and cattle production systems, and limits comparability across studies in Kenya and the wider East African region. Enhancing species-level characterization is therefore essential for strengthening mastitis surveillance and advancing understanding of the intramammary microbial diversity within a One Health framework.

Kenya provides a particularly informative setting in which to examine these dynamics, given the diversity of its cattle production systems. These include extensive uncontrolled production systems (pastoralism), semi-intensive production systems (agropastoralism), intensive controlled production systems (zero grazing), and extensive controlled production systems (ranching), as defined by FAO [19]. These systems differ markedly in herd size or structure, breed type or composition, housing quality, lactation and parity status, manure management practices, animal mobility, and access to veterinary services [3, 20–22]. Such differences are likely to influence both exposure to mastitis-associated pathogens and variability in susceptibility to intramammary infection across production contexts.

Against this backdrop, the present study aimed (i) to characterize *Staphylococcus* species diversity, with particular emphasis on NAS species, and (ii) to evaluate modifiable production-related risk factors associated with SCM across heterogeneous cattle production systems in Kenya.

Materials and methods

Study design and area

This cross-sectional observational study was conducted between October and December 2021 as part of a broader research program, for which detailed descriptions of the study sites, sampling frame, and sampling strategy have been described previously [23]. In brief, comprehensive sampling frames of all registered cattle farms with lactating cows in Machakos, Makueni and Narok counties were compiled with support from county veterinary officers. Farms or herds were subsequently stratified according to cattle production systems and projected herd sizes, after which 152 herds were randomly selected to ensure proportional and representative coverage across the production systems and herd densities.

Sample size estimation

The required sample size of lactating cows was estimated using Fisher's formula, assuming a SCM prevalence of a 50%, a 95% confidence level, and a 5% margin of error. Given a lactating cow population exceeding 10,000, the sample size was calculated as:

$$N = \frac{z^2 PQ}{e^2}$$

Where N represents the required sample size; P is the assumed SCM prevalence (0.5) across Kenyan cattle production systems; Q is variance/difference with the prevalence ($1 - P$) given as (0.5); Z is the Z-value corresponding to a 95% confidence level (1.96); and e is the allowable margin of error (0.05). This yielded a minimum required sample size of 385 lactating cows.

Study questionnaire and data capture

Structured interviews were conducted with farm owners, managers, or designated representatives following explanation of the study objectives and provision of free verbal informed consent. Interviews were administered in English or Kiswahili, with vernacular translation provided where necessary to ensure accurate communication.

Data were collected using the validated International Livestock Research Institute Antimicrobial Management Use in Livestock Production - ILRI's AMUSE questionnaire tool v2 [24], which was customized for this study. Modifications included the addition of a lactating cow-specific module to capture breed type, cow and calf ages, lactation and parity status, history of clinical mastitis, milking methods, housing characteristics and veterinary services including antimicrobial use and vaccine practices (see Supplementary file 1). Modules unrelated to cattle production were removed to enhance contextual relevance and reduce respondent burden.

The adapted tool was pilot-tested in a subset of cattle herds in Machakos County. Minor refinements were subsequently made to improve clarity and usability, without substantive structural changes. Final data collection was conducted electronically using Open Data Kit (ODK), enabling real-time data entry and quality control. Variables included in the analysis are summarized in Table 1.

Animal selection

Eligible study animals were lactating cows, with or without suckling calves, which showed no observable signs of CM at the time of sampling, had no history of systemic or intramammary antibiotic treatment within the preceding 14 days, and were physically present during the time of study visit. Importantly, no penside screening tests such as the California mastitis test (CMT) were used to guide cow selection, thereby minimizing outcome-dependent sampling bias. Cows were excluded if they exhibited signs of CM, had received recent antimicrobial treatment, were non-lactating, or were unavailable at the time of sampling.

Within-herd, cow selection was conducted using systematic (k th) random sampling. In herds without written animal lists, all eligible lactating cows present at the time of the visit were ordered as they were presented for milking or handling. A random starting point between 1 and k was selected, after which every (k th) eligible cow was selected until the predetermined target number of cows per herd was achieved. In herds with only one or two eligible lactating cows present, those cows were selected by default.

To account for clustering, potential non-response and logistical constraints, a total of 436 lactating cows were sampled from 152 herds distributed across Machakos (32 herds, 78 cows), Makueni (18 herds, 44 cows), and Narok (102 herds, 314 cows) counties. Stratified sampling by county and dominant production system ensured representation of heterogeneous cattle production systems and herd densities.

Sample collection and handling

Following herd-level interviews and animal selection, the pre-selected lactating cows were humanely restrained using minimal manual force in a crush or, where unavailable, in an open field conditions. Sampling procedures were conducted without disrupting routine suckling practices to minimize animal stress and maintain farmer compliance.

All selected cows subsequently underwent physical examination for evidence of CM. Udder preparation followed standard aseptic procedures: removal of gross debris (dirt, hair and bedding), washing with sterile distilled water, stripping and discarding of foremilk, and visual inspection and palpation of all quarters to exclude CM. Only cows without observable signs of CM signs proceeded to composite milk sampling, after which cow-specific epidemiological data were recorded.

Teat ends were disinfected using sterile cotton gauze soaked in 70% alcohol and allowed to air-dry for at least 30 s in accordance with National Mastitis Council guidelines [25]. Composite milk samples were collected aseptically by pooling equal volumes from all four quarters into 50 mL conical centrifuge tubes (Falcon[®], Corning,

Table 1 Stratified production systems and management variables of the sampled lactating cow population ($n=436$), presented at the cow-level

Variables	Extensive Controlled, $n=29$ ¹	Extensive Uncontrolled/ Pastoralism, $n=170$ ¹	Intensive-Zero grazing, $n=87$ ¹	Semi-Intensive/ Agropastoralism, $n=150$ ¹	p -value ²
Herd size/structure					<0.001
Small (1–20)	0 (0%)	34 (20%)	46 (53%)	97 (65%)	
Medium (21–90)	7 (24%)	68 (40%)	37 (43%)	49 (33%)	
Large (≥ 91)	22 (76%)	68 (40%)	4 (4.6%)	4 (2.7%)	
Housing structure					<0.001
Temporary	11 (46%)	91 (100%)	4 (4.6%)	35 (57%)	
Semi-permanent	3 (13%)	0 (0%)	40 (46%)	24 (39%)	
Permanent	10 (42%)	0 (0%)	43 (49%)	2 (3.3%)	
No data	5	79		89	
Manure management					-
Composting	0 (0%)	15 (8.8%)	33 (38%)	2 (1.3%)	
Dry stack	20 (69%)	93 (55%)	14 (16%)	70 (47%)	
Stockpiling	9 (31%)	62 (36%)	40 (46%)	78 (52%)	
Milkshed availability					<0.001
Present	16 (55%)	50 (31%)	60 (69%)	70 (47%)	
Absent	13 (45%)	110 (69%)	27 (31%)	80 (53%)	
No data		10			
Milking method					0.004
Hand	29 (100%)	155 (97%)	75 (86%)	139 (93%)	
Machine	0 (0%)	5 (3.1%)	8 (9.2%)	11 (7.3%)	
Do not milk	0 (0%)	0 (0%)	4 (4.6%)	0 (0%)	
No data		10			
Breed type/composition					<0.001
Exotic	20 (69%)	44 (26%)	64 (74%)	61 (41%)	
Indigenous	9 (31%)	126 (74%)	23 (26%)	89 (59%)	
Cows' Age					0.4
<5years	16 (55%)	84 (49%)	53 (61%)	77 (51%)	
≥ 5 years	13 (45%)	86 (51%)	34 (39%)	73 (49%)	
Lactation status					<0.001
Suckling calf	20 (69%)	141 (83%)	47 (54%)	116 (77%)	
No suckling	9 (31%)	29 (27%)	40 (46%)	34 (23%)	
Calves' age					>0.9
≤ 6 months	13 (65%)	91 (65%)	28 (60%)	73 (63%)	
≥ 7 months	7 (35%)	50 (35%)	19 (40%)	43 (37%)	
No data	9	29	40	34	
Parity status					>0.9
Primiparous	8 (28%)	45 (26%)	23 (26%)	115 (77%)	
Multiparous	21 (72%)	125 (74%)	64 (74%)	35 (23%)	
Clinical mastitis history					0.013
Has history	7 (24%)	22 (13%)	22 (27%)	17 (12%)	
No history	22 (76%)	146 (87%)	59 (73%)	121 (88%)	
No data		2	6	12	

Exotic and crossbreed cattle were classified together as exotic breeds, while indigenous cattle were categorized separately to represent locally adapted or pure indigenous breeds. Housing structures were categorized as permanent, semi-permanent or temporary based on their level of permanence and presumed hygiene standards.¹ Values are presented as number (n) and percentage (%)² Statistical comparisons were performed using Pearson's Chi-squared test or Fisher's exact test, where appropriate

USA), beginning with the nearest teat and proceeding to the farthest, while avoiding contact between the teat and container rim.

Samples were properly labelled, placed immediately on ice, transported at 4 °C in insulated cooler boxes, and processed within 8 h of collection. Milk volumes ranged from <2 mL to 4 mL; 122 samples yielded <2 mL, primarily due to low milk availability at the time of sampling. Each enrolled lactating cow ($n=436$) contributed one composite milk sample; thus, a total of 436 composite milk samples were collected and processed as described in the laboratory sample processing (Figs. 1 and 2) and data analysis sections.

Laboratory analysis

Somatic cell counts (SCC)

To determine SCM status, SCC were measured for all samples ($n=436$) using an automated fluorescence-based cell counter (Luna II, Logos Biosystems, Anyang, South Korea), operated in accordance with the manufacturer's instructions and routine calibration procedures. Fresh milk samples (1 μ L) were diluted 1:99 (100-fold) in methylene blue stain to a final volume of 100 μ L (0.1mL). A 10 μ L (0.01mL) aliquot of the stained suspension was loaded onto a disposable counting slide and analyzed immediately.

Samples with SCC values > 200,000 cells/mL were classified as SCM-positive, whereas those with SCC values $\leq$ 200,000 cells/mL were classified as SCM-negative [4–6, 8–10, 26]. In this study, SCM was operationally defined as an elevated SCCs (> 200,000 cells/mL) in a composite milk sample obtained from a lactating cow without CM at the time of sampling. This standardized case definition enabled consistent comparison of SCM occurrence across individual cows, herds and diverse cattle production systems.

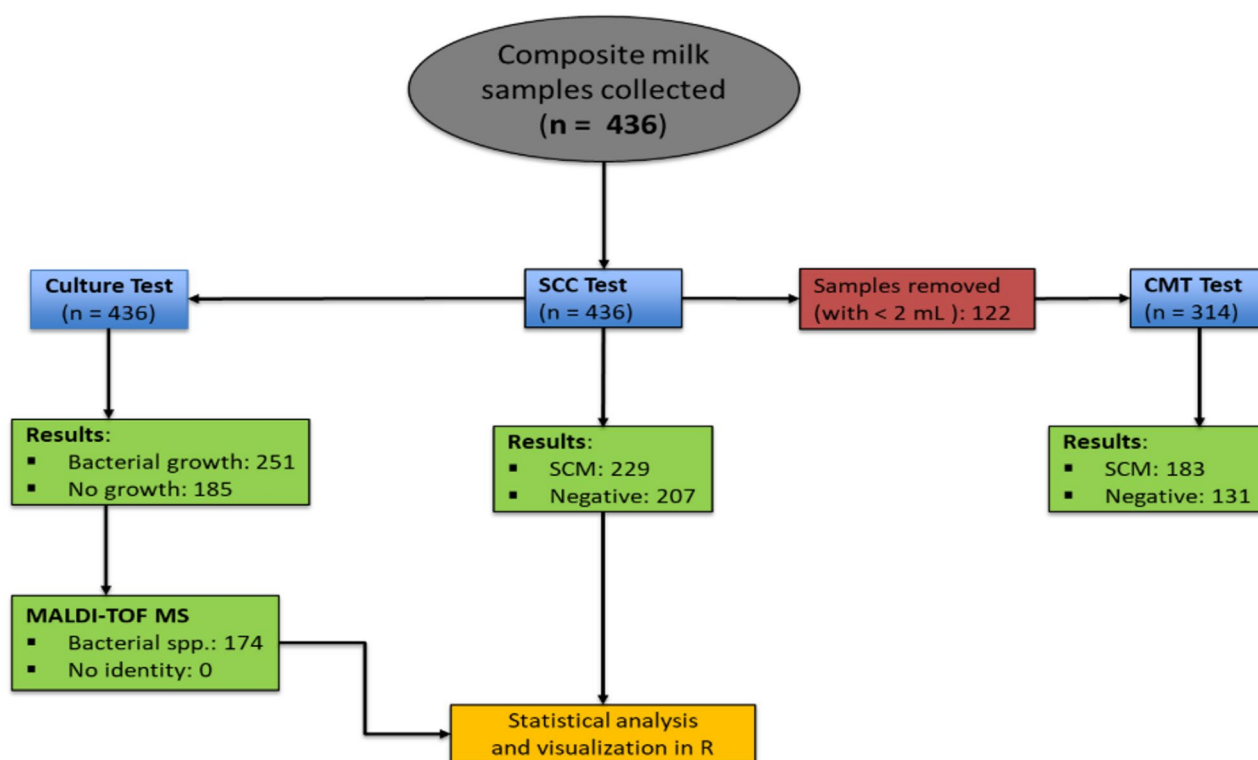


Fig. 1 Flowchart illustrates the number of composite milk samples collected and the subsequent analytical steps undertaken in this study. (Abbreviations: SCC–Somatic cell counts; CMT–California mastitis test; SCM–Subclinical mastitis (positive); MALDI-TOF MS–Matrix-assisted laser desorption/ionization-time of flight mass spectrometry; R v.4.5.2)

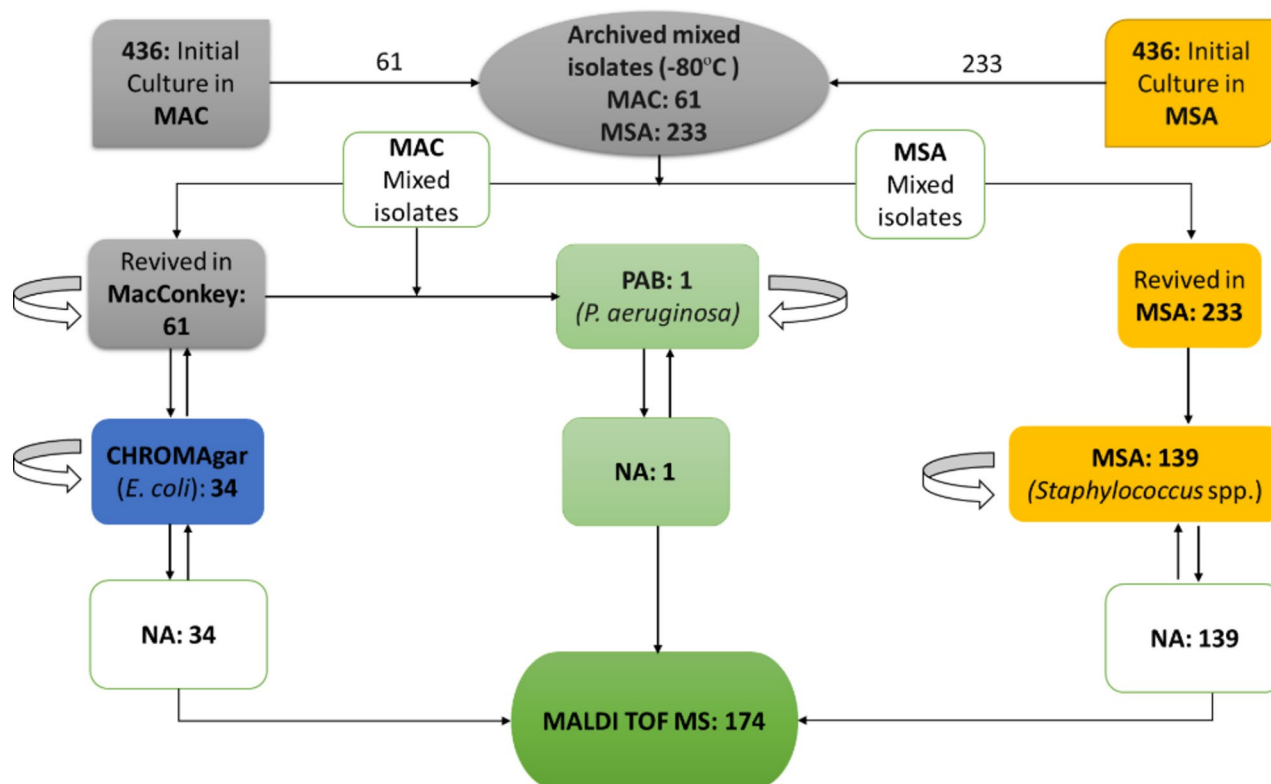


Fig. 2 Workflow illustrating the bacteriological culture, isolation and identification of bacterial species from primary cultures using selective media and MALDI-TOF MS. Arrows indicate successive subculturing steps performed to obtain pure presumptive bacterial isolates. Numbers denote the number of samples or isolates obtained at each stage of the workflow. (Abbreviations: **MAC**–MacConkey; **MSA**–Mannitol Salt Agar; **PAB**–Pseudomonas gar base; **NA**–Nutrient Agar; **MALDI-TOF MS**–Matrix-assisted laser desorption/ionization-time of flight mass spectrometry)

California mastitis test (CMT)

The CMT was performed on composite milk samples with sufficient residual milk volume (≥ 2 mL; $n=314$) using the ImmuCell California Mastitis Test Kit (ImmuCell Corporation, Portland-Oregon, USA) in accordance with manufacturer’s instructions. Samples with insufficient residual milk volumes (< 2 mL; $n=122$), which may yield unreliable results,

For each assay, equal volumes (2 mL) of milk and CMT reagent were dispensed into pre-sterilized paddle cups and mixed by gentle circular rotation on a horizontal plane for approximately 15 s. Samples showing visible gel formation or increased viscosity were classified as SCM-positive, whereas absence of gel formation was considered negative.

Bacteriological culture analysis

To characterize bacterial diversity, with particular emphasis on *Staphylococcus* species, bacteriological culture was performed on all composite milk samples ($n=436$). Importantly, culture results were not used for SCM classification but solely for pathogen identification.

All samples were cultured directly, without enrichment using standard bacteriological procedures. Mannitol Salt Agar (MSA) was used for presumptive isolation of Gram-positive *Staphylococcus* spp., while MacConkey agar (MAC) for Gram-negative lactose fermenting *E. coli*, and non-lactose fermenting *P. aeruginosa* (Figs. 1

and 2). All media were prepared according to the manufacturer's instructions (Oxoid Limited, Hampshire, UK). *Strep. agalactiae* and *Strep. uberis* were not investigated because they were outside the scope of the present study.

Briefly, 100 µL of each composite milk sample was aseptically inoculated and incubated aerobically at 37 °C for 18–24 h. Presumptive colonies were selected by colony morphology and medium reaction, then archived in Trypticase Soy Broth (TSB) supplemented with 20% glycerol at –80 °C (Fig. 2). For purification, presumptive *Staphylococcus* spp. were subcultured on MSA; lactose fermenting isolates on CHROMagar for *E. coli*, and non-lactose fermenting isolates onto *Pseudomonas* agar base (PAB) for *P. aeruginosa*. All isolates were subsequently subcultured onto Nutrient agar (NA) to obtain pure cultures prior to definitive species identification. Quality control reference strains (*S. aureus* ATCC 29213, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) were included throughout the procedure to ensure analytical validity.

Species-level characterization

All pure presumptive isolates ($n = 174$), comprising 139 *Staphylococcus* spp., 34 *E. coli*, and one *P. aeruginosa* (Fig. 2) were subjected to species-level identification using Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) following the manufacturer's instructions (MALDI Biotyper Invitro Diagnosis Bruker v.10, Karlsruhe, Germany). MALDI-TOF MS was selected due to its rapid turnaround time, high analytical accuracy and superior species-level resolution compared with conventional biochemical methods. This enhanced resolution is particularly critical for distinguishing closely related NAS species, which are often difficult to differentiate using phenotypic assays [27–30].

For protein profiling, two sample preparation strategies were applied depending on bacterial group. A direct spotting method was used for Gram-negative *E. coli* and *P. aeruginosa* isolates. Fresh overnight colonies were spotted on MSP 96 sterile target plates, air-dried for approximately 5 min, overlaid with 1 µL of α -Cyano-4-hydroxycinnamic acid (HCCA) matrix to facilitate protein crystallization and air-dried before analysis. An indirect (formic-acid extraction) method was applied for Gram-positive *Staphylococcus* spp., whereby 1 µL formic acid was applied to the dried colony spot on sterile plates, air-dried, then overlaid with 1 µL HCCA matrix and air-dried before analysis.

Protein spectra generated using both preparation methods were analyzed using the MALDI Biotyper MTB IVD reference library, which contains spectra from over 4,230 bacterial species across more than 680 genera. Identification scores ≥ 2.0 were considered high-confidence species, while scores between 1.7 and 1.99 were regarded as acceptable species-level identifications in line with published validation studies [27]. Scores < 1.7 were deemed unreliable and re-analyzed. For this study, scores ≥ 1.7 were accepted as valid species-level assignments.

Data analysis

Data curation and variable definition

All statistical analyses were performed using R (v4.5.2) under relevant packages. Two datasets were analyzed: (i) the primary dataset comprising 436 composite milk samples with SCC and bacteriological culture results, and (ii) a subset of 314 samples additionally tested using the CMT (Figs. 1 and 2). Predictor variables were defined and categorized a priori based on biological relevance and empirical data distribution (Table 1).

Herd size (or herd structure) was categorized as small (1–20 cattle), medium (21–90 cattle) or large (≥ 91 cattle) based on the interquartile range (IQR = 70) to account for skewness and ensure analytically meaningful groupings. Breed type was grouped as indigenous or exotic, with cross-bred cattle classified as exotic. Housing structures were categorized according to structural durability and presumed hygiene conditions into permanent (e.g., concrete), or semi-permanent (e.g., wood or bamboo), or temporary (e.g., stick-based structures) [19]. Cow and calf ages were categorized as < 5 years vs. ≥ 5 years, and ≤ 6 months vs. ≥ 7 months, respectively while parity status was grouped as primiparous (one calf) or multiparous (≥ 2 calves).

Descriptive and concordance analyses

Cow- and herd-level SCM prevalence estimates were derived using SCC-based classifications and presented as proportions with 95% confidence intervals (CI). Cow-level prevalence was defined as the proportion of SCM-positive cows, while herd-level prevalence was defined as the proportion of herds with at least one SCM-positive cow. Group differences were assessed using Pearson's Chi-squared tests or Fisher's exact tests, where appropriate.

Agreement between CMT- and SCC-based SCM classifications was assessed in the subset of samples ($n = 314$) using a 2×2 contingency table, and the agreement beyond chance quantified using Cohen's Kappa (κ) statistic and interpreted using standard benchmarks [31]. Additional diagnostic performance metrics (e.g., sensitivity, specificity or regression-based diagnostic comparisons) were not pursued. Instead, concordance findings were interpreted descriptively to illustrate the complementary performance of SCC and CMT in field-based SCM surveillance rather than diagnostic validation. Interpretation was further contextualized using composite milk samples, which limit quarter-level inference.

Multicollinearity assessment

Prior to multivariable modelling, collinearity among explanatory variables was assessed, with particular attention to production systems, housing types, manure management practices and herd size due to their conceptual and structural interrelatedness. Variance inflation factors (VIF) were calculated from a fixed-effects logistic regression model excluding herd random effects. A VIF threshold of < 5 was considered acceptable collinearity [32]. Where moderate pairwise correlations ($0.40 > r > 0.69$) were identified [33], variables were retained only if they were biologically distinct and contributed independently to model performance. Otherwise, the variable with the greatest epidemiological relevance and interpretability was prioritized for inclusion in the final model.

Mixed-effects logistic regression modelling

Risk factors associated with SCC-defined SCM were evaluated using mixed-effects logistic regression to account for clustering of cows (level 1) within herds (level 2). Herd was included as a random intercept in all models to account for within-herd clustering and unmeasured herd-level heterogeneity. SCM status (positive vs. negative) was modelled as a binary outcome assuming a binomial error distribution using the glmer function.

Variable selection and model building

Each hypothesized explanatory variable (Table 1) was initially screened using univariate mixed-effects logistic regression models. Variables with a likelihood ratio test p -value ≤ 0.20 were considered for inclusion in the multivariable analysis. In addition, biologically plausible covariates and potential confounders, including cow age, calf age, history of clinical mastitis, lactation and parity status and milking methods, were considered irrespective of their univariate significance to ensure adequate control of confounding.

Multivariable model refinement followed a manual backward stepwise elimination approach, guided by likelihood ratio tests (p -value ≤ 0.20 , Akaike Information Criterion (AIC), confounding assessment ($> 20\%$ change in effect estimates), acceptable multicollinearity ($VIF < 5$), and biological plausibility. Variables with substantial missingness or sparse observations (e.g., calf age, cow age, history of clinical mastitis and parity status) were excluded where they produced unstable parameter estimates. Milking methods (machine vs. hand vs. do not milk) were excluded because of quasi-complete separation caused by the small number of machine-milked cows ($n = 24$). Penalized likelihood methods (e.g., Firth correction) were not applied, as inclusion of this variable did not materially contribute to the primary epidemiological objectives and or yield stable, interpretable estimates.

The final multivariable model prioritized parsimony and interpretability, retaining herd-level variables (production system, herd size/structure, housing type, milkshed availability and manure management practices)

alongside key cow-level variables (breed type and lactation status). Model coefficients were exponentiated and reported as adjusted odds ratios (aOR) with 95% CI, describing the association between each predictor and the likelihood of having SCM. Regression outputs were summarized (Table 3) using the *tbl_regression* function from the *gtsummary* package. Statistical significance was defined at $\alpha=0.05$.

Random-effects variance and intra-class correlation (ICC)

Between-herd variability was quantified from the variance of the random intercept of the final multivariable model. The ICC was estimated using the *icc ()* from the *performance* package to assess the proportion of total variance attributable to herd-level clustering and justify the use of mixed-effects modelling.

Results

Characteristics of cattle production systems

The number of lactating cows sampled (Table 1) per herd ranged from 1 to 10 (mean=2.87; median=3). According to FAO's production-system classifications, cattle were managed predominantly under extensive uncontrolled/pastoralism (38.9%, 170/436), followed by semi-intensive/agropastoralism (34.4%, 150/436), intensive controlled/zero grazing (20.0%, 87/436) and extensive controlled/ranching systems (6.7%, 29/436). Herd level distribution was comparable, with the highest proportion of herds classified as agropastoralism (41.4%, 63/152), followed by pastoralism (35%, 53/152), intensive-zero grazing (17.1%, 26/152) and ranching (6.6%, 10/152).

Marked heterogeneity was observed in housing characteristics across production systems. In intensive-zero grazing systems, cattle were predominantly housed in permanent or semi-permanent housing (49% vs. 46%). In contrast, temporary housing predominated in pastoral (100%) and agropastoral (57%) systems.

Similarly, herd structure, used as a proxy for cattle contact intensity, varied across the production systems. Medium-sized herds were most common overall. Maximum herd cattle interactions represented by large-herd size was more common in extensive controlled/ranching (76%) and pastoral systems (40%). Small herds were more frequently kept in agropastoral (65%) and intensive zero-grazing systems (53%).

Breed type or composition also differed systematically by production systems with indigenous cattle predominated within pastoral systems (74%), whereas exotic cattle were more common in intensive zero-grazing (74%) and extensive controlled/ranching systems (69%). Agropastoral systems exhibited mixed breed composition (59% indigenous; 41% exotic). Additional summaries are presented in Table 1.

Prevalence of bovine SCM

Based on SCC assessments, the overall cow-level prevalence of SCM was 53.0% (229/436; 95% CI: 48–57) while 73.0% of herds (111/152; 95% CI: 66–80) had at least one SCC-defined SCM-positive lactating cow. Among the subset of cows additionally tested using CMT, CMT-defined SCM prevalence was 58.0% (183/314; 95% CI: 53–64).

When stratified by production systems, SCC-defined SCM prevalence was highest in extensive uncontrolled/pastoralism (57%; 95% CI: 50–65), followed by semi-intensive/agropastoralism (55%; 95% CI: 47–63). Prevalence was 52% (95% CI: 35–69) in extensive controlled/ranching and lowest in intensive-zero grazing (40%; 95% CI: 31–52). Notably, within intensive zero-grazing systems, SCM prevalence differed by diagnostic method, with CMT-based classification yielding higher estimates (62%; 95% CI: 51–73) compared with SCC-defined prevalence (40.0%; 95% CI: 31–52).

Staphylococcus species relative abundance

Bacteriological culture growth occurred in 58.0% (251/436; 95% CI: 53–63) of the composite milk samples, yielding 174 bacterial isolates identified by MALDI-TOF MS (Figs. 1, 2 and 3). *Staphylococcus* species predominated, accounting for 79.9% (139/174) of the isolates. From this group, NAS species represented 44.3% (77/174) exceeding *S. aureus* (35.6%; 62/174).

Among NAS, *S. chromogenes* were the most prevalent species (19%; 33/174), followed by *S. succinus* (5.2%; 9/174). *Mammaliicoccus sciuri* (formerly *Staphylococcus sciuri* [34] and *Staphylococcus saprophyticus* each accounted for 4% (7/174). Among non-staphylococcal pathogens, the environmental bacterium *E. coli* accounted for 19.5% (34/174) of the isolates, while *P. aeruginosa* was infrequently detected (0.6%; 1/174).

Comparison of SCC, CMT and bacteriological culture outcomes

Comparison of SCC-defined SCM status with bacteriological culture outcomes (growth vs. no growth) showed that 60% (138/229) of SCC-positive samples yielded bacterial growth, while 40% (91/229) showed no growth. Conversely, among SCC-negative samples, 55% (113/207) were culture-positive and 45% (94/207) had no detectable growth. Overall, the likelihood of bacterial isolation did not differ significantly between SCC-positive and SCC-negative samples (OR = 1.26, 95% CI: 0.86–1.85; $p = 0.23$).

Agreement between CMT- and SCC-defined SCM classifications was limited. The overall agreement was 60.2% (189/314), comprising 111 true positives and 78 true negatives. Cohen's kappa statistics indicated slight concordance between the two diagnostic methods ($\kappa = 0.20$; 95% CI: 0.09–0.31).

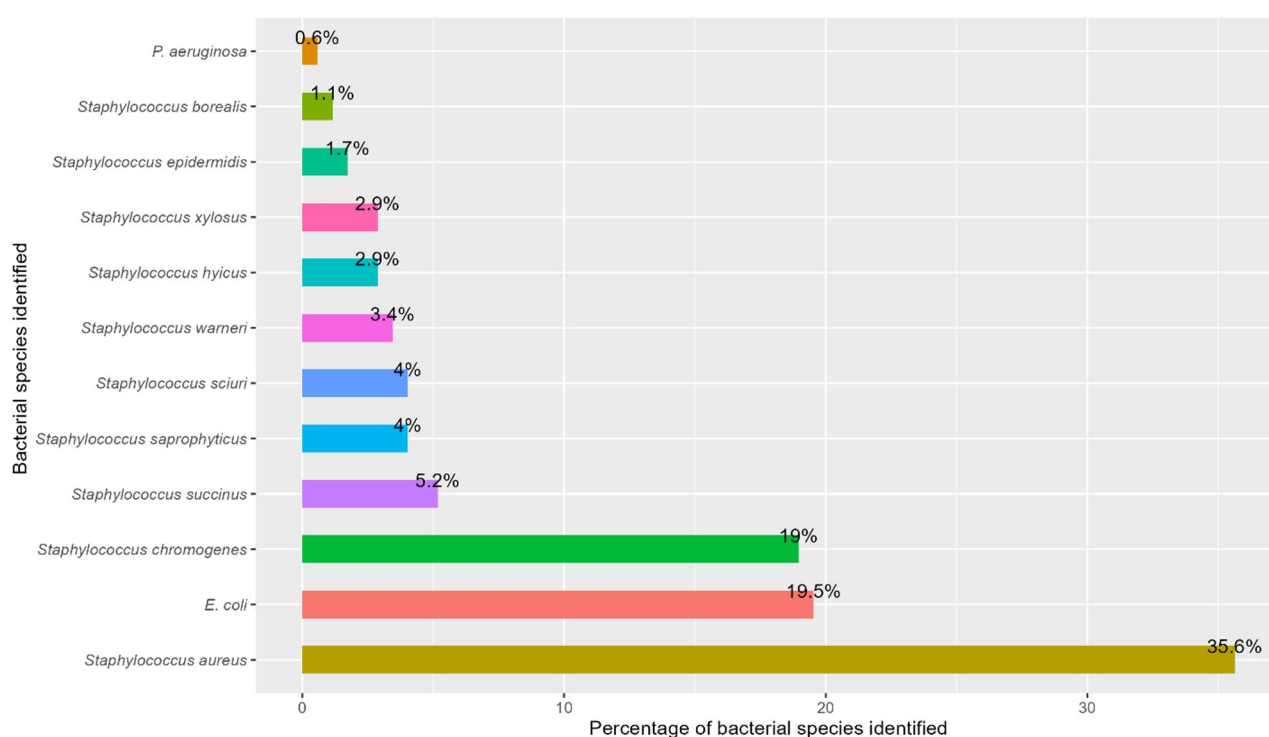


Fig. 3 Distribution of bacterial isolates showing relative abundance of *Staphylococcus* spp. diversity and Gram-negative lactose fermenting and non-lactose fermenting bacteria ($n = 174$). Bars represent the percentage contribution of each bacterial species to the total number of isolates. *Staphylococcus* species constituted 79.9% of all isolates, of which non-aureus *Staphylococcus* (NAS) species accounted for 44.3%

Pathogen distribution across the cattle production systems

Bacterial isolates were predominantly recovered from cattle reared under extensive uncontrolled/pastoral systems (51.1%; 89/174) and semi-intensive/agropastoral systems (32.7%; 57/174). Lower proportions of isolates were obtained from intensive zero-grazing (10.3%; 18/174) and extensive controlled/ranching systems (5.7%; 10/174).

NAS were detected in cattle across production systems. Within pastoral systems, *S. aureus*, *S. chromogenes*, *E. coli* and *S. succinus* were the most frequently isolated organisms. Overall, *S. aureus* was the predominant pathogen in both pastoral (16.1%, 28/174) and semi-intensive/agropastoral systems (14.9%, 26/174). A detailed breakdown of pathogen distribution across production systems is presented in Fig. 4.

Breed-wise distribution of *Staphylococcus* spp

NAS species were detected in both indigenous and exotic cattle, although their distribution differed between breed groups. *S. chromogenes* was more frequently isolated from indigenous cattle (13.2%; 23/174) than exotic cattle (5.7%; 10/174). *S. succinus* occurred in low proportions in both exotic cattle (2.9%; 5/174) and indigenous cattle (2.3%; 4/174). Similarly, *S. saprophyticus* was detected in low frequencies in exotic cattle (2.3%; 4/174) and indigenous cattle (1.7%; 3/174). *M. sciuri* showed a comparable pattern, accounting for 2.9% (5/174) of isolates from exotic cattle and 1.7% (3/174) from indigenous cattle.

S. aureus was more frequently isolated from indigenous cattle (23.6%; 41/174) than in exotic cattle (12.1%; 21/174). Likewise, *E. coli* was detected more in indigenous cattle (13.2%; 23/174) compared to exotic cattle (6.3%; 11/174). Overall bacterial isolation frequency was higher in indigenous cattle (61.0%; 106/174) than from exotic cattle (39.0%; 68/174).

Mixed bacterial growth

Mixed bacterial growth was inferred when two or more distinct colony morphotypes were recovered from a single composite milk sample and was interpreted conservatively (Table 2). Mixed growth was identified in 8.8% (14/160) of culture-positive samples (after species identification) and occurred exclusively as two-species combinations, whereby NAS species-*E. coli* combinations accounted for 5% (8/160), while *S. aureus*-*E. coli* combinations accounted for 3.8% (6/160). When stratified by breed type, mixed bacterial growths were more frequently observed in indigenous cattle (6.3%; 10/160) than in exotic cattle (2.5%; 4/160). Notably, no mixed bacterial growth was detected among cattle managed under intensive zero-grazing systems (Table 2).

Production-related risk factors associated with SCC

Prior to multivariable modelling, all explanatory variables demonstrated acceptable collinearity (VIF range = 1.41–5.21, Supplementary file 2), indicating no evidence of problematic multicollinearity that could bias parameter estimates. Substantial clustering at the herd-level was observed (adjusted ICC=0.29, 95% CI: 0.10–0.50), justifying the use of a mixed-effects logistic regression model with herd included as a random intercept.

After accounting for herd-level clustering, unmeasured heterogeneity and confounding, several herd- and cow-level factors were significantly associated with SCC-defined SCM (Table 3). Housing conditions demonstrated the strongest association with SCM occurrence. Compared with permanent structures, cattle reared under semi-permanent structures were associated with 4.63-fold higher odds of having SCM (adjusted odds ratio [aOR]=4.63, 95% CI: 1.91–11.8, $p<0.001$), while those in temporary structures had the highest odds of having SCM (aOR=6.27, 95% CI: 1.97–21.0, $p=0.002$).

Breed type and herd size were also independently associated with SCM. Indigenous cattle had more than twice the odds of having SCM compared with exotic breeds (aOR=2.51, 95% CI: 1.16–5.51, $p=0.020$). Medium-sized

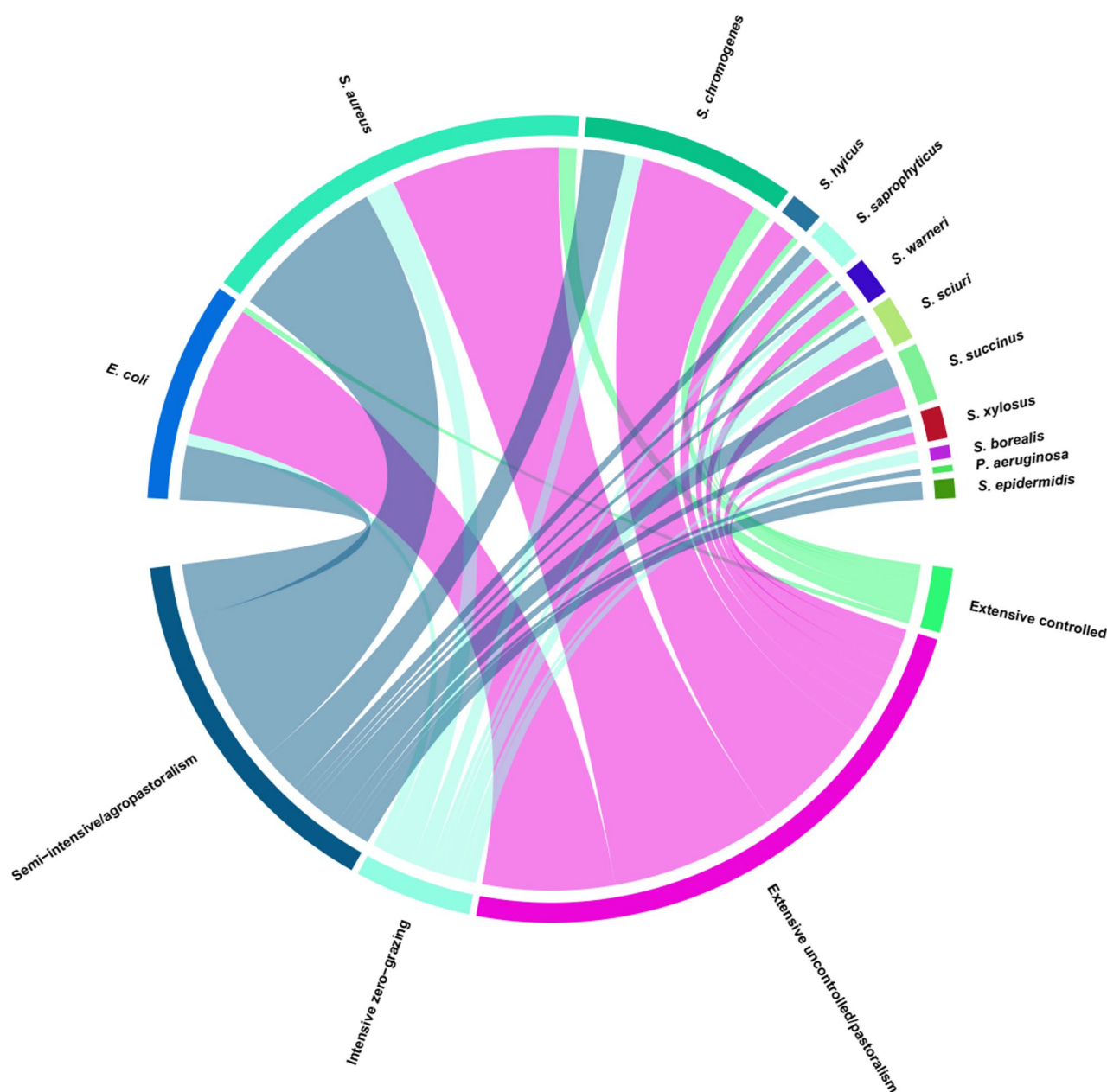


Fig. 4 Abundance and distribution of bacterial species across cattle production systems ($n=174$). The outer ring is subdivided into segments representing bacterial species (e.g., *E. coli*, *S. aureus*, *S. chromogenes*) and cattle production systems (e.g., pastoralism). Chord thickness reflects the number of isolates, with thicker indicating higher counts. Arc length is proportional to occurrence frequency, with longer arcs representing greater species abundance

herds were associated with increased odds of SCM relative to small herds (aOR=2.43, 95% CI: 1.19–5.06, $p=0.016$), whereas large herd size showed no significant association ($p=0.70$).

Several variables, including milkshed availability, lactation status, manure management, and production systems (semi-intensive/agropastoralism and extensive uncontrolled/pastoralism) were associated with SCM at the univariable analyses but were not retained as independent predictors in the final adjusted model. Additionally, biologically relevant covariates screened using the univariable model, including calf age (≤ 6 months vs. ≥ 7 months; OR=1.68, 95% CI: 1.05–2.69, $p=0.030$), parity status (primiparous vs. multiparous; OR=1.36, 95% CI: 0.88–2.09, $p=0.2$), cow age (< 5 years vs. ≥ 5 years; OR=1.39, 95% CI: 0.95–2.02, $p=0.091$), history of clinical

Table 2 Mixed bacterial growth involving *Staphylococcus spp.* and *E. coli* across the cattle breed types and production systems

Mixed bacterial growth was identified in 8.8% (14/160) of culture-positive samples and was conservatively interpreted as representing contamination rates. **Barcodes** represent individual composite milk samples; (**Staph spp.**—*Staphylococcus* species; *M. sciuri*/*S. sciuri*; **Pastoral**—Pastoralism)

Barcode	Staph spp. Id	<i>E. coli</i> Id	Breed type	Production system
FMK000092	<i>S. epidermidis</i>	<i>E. coli</i>	Exotic	Semi-intensive/Agropastoralism
FMK000159	<i>S. chromogenes</i>	<i>E. coli</i>	Exotic	Semi-intensive/Agropastoralism
FMK000062	<i>S. succinus</i>	<i>E. coli</i>	Indigenous	Semi-intensive/Agropastoralism
FMK000297	<i>S. aureus</i>	<i>E. coli</i>	Indigenous	Extensive uncontrolled/Pastoral
FMK000533	<i>S. xylosus</i>	<i>E. coli</i>	Exotic	Extensive uncontrolled /Pastoral
FMK000551	<i>S. aureus</i>	<i>E. coli</i>	Indigenous	Semi-intensive/Agropastoralism
FMK000458	<i>S. hyicus</i>	<i>E. coli</i>	Indigenous	Extensive controlled/Ranching
FMK000422	<i>S. xylosus</i>	<i>E. coli</i>	Indigenous	Extensive uncontrolled/Pastoral
FMK000552	<i>S. sciuri</i>	<i>E. coli</i>	Exotic	Extensive uncontrolled/ Pastoral
FMK000342	<i>S. chromogenes</i>	<i>E. coli</i>	Indigenous	Extensive uncontrolled/ Pastoral
FMK000516	<i>S. aureus</i>	<i>E. coli</i>	Indigenous	Extensive uncontrolled/Pastoral
FMK000450	<i>S. aureus</i>	<i>E. coli</i>	Indigenous	Extensive uncontrolled/Pastoral
FMK000515	<i>S. aureus</i>	<i>E. coli</i>	Indigenous	Extensive uncontrolled/Pastoral
FMK000530	<i>S. aureus</i>	<i>E. coli</i>	Indigenous	Extensive uncontrolled/Pastoral

Table 3 Mixed-effect logistic regression model estimating the odds of having SCC-defined bovine SCM at the cow- and herd-levels in Kenya, 2021

Levels	Variables	Univariable			Multivariable		
		OR	95% CI	p-value	aOR	95% CI	p-value
Herd	Production systems						
	Intensive controlled (Zero grazing)	1			1		
	Extensive controlled (Ranching)	1.59	0.68–3.74	0.3	2.42	0.68–8.77	0.2
	Semi-intensive (Agropastoralism)	1.79	1.05–3.08	0.033	1.34	0.51–3.50	0.6
	Extensive uncontrolled (Pastoralism)	1.97	1.17–3.36	0.011	1.61	0.49–5.25	0.4
	Herd size/structure						
	Small (1–20)	1			1		
	Medium (21–90)	1.88	1.22–2.91	0.004	2.43	1.19–5.06	0.016
	Large (>91)	1.46	0.89–2.40	0.13	0.85	0.32–2.22	0.7
	Housing structure						
	Permanent	1			1		
	Semipermanent	3.19	1.52–6.94	0.003	4.63	1.91–11.8	<0.001
	Temporary	4.05	2.10–8.12	<0.001	6.27	1.97–21.0	0.002
	Milkshed availability						
	Absent	1			1		
	Present	0.60	0.41–0.88	0.009	1.51	0.79–2.97	0.2
	Manure management						
	Composting	1			1		
	Dry stack	1.90	1.01–3.61	0.047	0.70	0.22–2.20	0.5
	Stockpiling	1.65	0.88–3.15	0.12	2.15	0.78–6.20	0.15
Cow or animal	Breed type/composition						
	Exotic	1			1		
	Indigenous	1.85	1.26–2.71	0.002	2.51	1.16–5.51	0.020
	Lactation status						
	Without suckling calf	1			1		
	With suckling calf	2.27	1.47–3.56	<0.001	0.92	0.45–1.89	0.8

OR = crude Odds Ratio, aOR = adjusted crude Odds Ratio, CI = Confidence Interval, **Bold** p-values were considered statistically significant ($p \leq 0.05$)

mastitis (no history vs. previous occurrence; OR = 0.73, 95% CI: 0.43–1.23, $p = 0.2$) and milking method (hands vs. machines), were excluded in the final adjusted model due to substantial sparse data or model instability, notably quasi-complete separation for milking method.

Discussion

Advancing the understanding of bovine SCM increasingly depends on species-level pathogen characterization, including that of NAS species, which enables a more precise characterization of bacterial diversity beyond established aetiological frameworks. Such enhanced characterization strengthens the evidence base for development of context-specific and sustainable mastitis control strategies, especially in resource-limited settings.

In the present study, SCM was prevalent at both the cow (53%) and herd (73%) levels, consistent with previous reports from Kenya [3, 17, 18], and neighbouring East African countries, including Rwanda [35], Uganda [36] and Ethiopia [21]. Stratification by production systems revealed a substantial SCM burden across all systems, with prevalence highest in pastoral (57%) followed by agropastoral (55%), extensive controlled/ranching (52%) and intensive zero-grazing (40%). Given that Kenya's cattle production systems broadly reflect those across sub-Saharan Africa, these observations may have wider regional relevance for bovine mastitis epidemiology. Collectively, the findings suggest that SCM is not merely an isolated cow- or herd-level condition, but rather a widespread and endemic constraint across diverse production systems, potentially reflecting persistent gaps in mastitis prevention, biosecurity implementation and overall herd health management practices [37, 38]. Nevertheless, variation in reported cow-level SCM prevalence across a few studies, including lower estimates (33% vs. 16.3%) from certain regions of Kenya [39] and Zimbabwe [40] respectively, may be attributable to differences in diagnostic methods, seasonal conditions and management intensity, rather than to substantial epidemiological divergence.

The multifactorial aetiology and largely asymptomatic nature of SCM further complicate its detection and interpretation. Even in relatively well-managed herds, low-level intramammary infections may persist undetected [1, 37, 38, 41]. This may influence herd-level SCM classification in the present study, such that herds classified as SCM-negative, particularly those with limited within-herd sampling (< 3 lactating cows), may still have harboured undetected intramammary infections. Thus, the observed 27% of herd-level SCM-negative status likely, at least in part, reflects constraints in sampling sensitivity rather than the true absence of intramammary infection. In contrast, the higher SCM burden observed in pastoral and agropastoral systems is biologically plausible and aligns with previous studies associating elevated mastitis risk with suboptimal hygiene practices, limited access to veterinary services, frequent inter-herd interactions and mobility-related stress linked to extensive livestock movement [3, 21, 37, 42, 43]. Together, these factors may increase exposure to mastitis-associated pathogens and facilitate their transmission and persistence within herds.

Within this epidemiological context, species-level characterization of *Staphylococcus* diversity offers critical insights into the aetiology of bovine staphylococcal mastitis in Kenya. *S. aureus* remained the predominant pathogen, particularly in pastoral and agropastoral systems, consistent with its well-established epidemiological role as one of the primary contagious mastitis agent. Its biological capacities for chronic intramammary colonization, intracellular persistence, and reduced responsiveness to commonly used antimicrobials, particularly β -lactams such as penicillins [7, 44], provide a mechanistic basis for its sustained circulation. These traits are likely amplified in settings characterized by close animal contact, suboptimal milking hygiene, and limited feasibility of test-and-cull strategies due to the absence of compensation mechanisms, as commonly occurs in resource-constrained pastoral and agropastoral production systems [3]. Inadequate antimicrobial stewardship and limited access to veterinary services in pastoral and smallholder systems [23], may further reinforce pathogen persistence and transmission.

Notably, substantial diversity was observed among NAS species, with *S. chromogenes* (19%) as the predominant NAS species, followed by *S. succinus* (5.2%), and lower frequencies in *S. saprophyticus* and *M. sciuri*.

Comparable NAS profiles have been documented across diverse geographical settings in South Africa, Canada, Belgium, USA, Cambodia, and among cattle in Bangladesh [2, 14–16, 45], supporting the broader external validity of these findings. Collectively, these observations reinforce growing evidence that NAS do not constitute a homogeneous group but rather comprise species with distinct ecological niches, host associations and pathogenic potential, including the capacity to act as reservoirs of AMR genes [2, 13–15]. The predominance of *S. chromogenes* is particularly noteworthy given its documented ability to establish persistent intramammary colonization and its association with elevated SCC [13, 14, 46, 47]. Moreover, the widespread detection of NAS species across production systems and cattle breeds suggests the presence of both environmental and host-associated reservoirs, underscoring their emerging epidemiological importance in bovine staphylococcal mastitis [2, 14, 45]. These findings further highlight the value of species-level resolution, particularly in contexts where improved control of major contagious pathogens may shift the relative importance of historically under-recognized pathogens such as NAS species.

With the emerging epidemiological importance of NAS species in bovine staphylococcal mastitis, inferred mixed bacterial growth involving *E. coli* in combination with NAS species or *S. aureus* was detected in a minority of samples (8.8%). Comparable mixed infections involving NAS and other mastitis-associated pathogens have been reported elsewhere [45, 48]. However, given the use of composite milk sampling and targeted culture-based approach, these observations should be interpreted cautiously and may reflect concurrent colonization, early or transient infections, or bacteria originating from different quarters rather than confirmed intramammary co-infections. Nonetheless, recovery of two or more bacterial species from the same sample is consistent with an increasingly recognized polymicrobial component of bovine mastitis [7, 11, 12]. This microbial complexity has practical implications for diagnostics and therapy, as reliance on a single-pathogen diagnostic tool may underestimate disease complexity and contribute to suboptimal treatment outcomes, particularly in production systems where polymicrobial infections and environmental pathogen exposure are more likely to occur.

Beyond microbial diversity, several production-related risk factors were independently associated with SCM, underscoring the central role of husbandry practices in disease occurrence. Housing quality emerged as one of the strongest and most consistent production-related risk factors for SCM. Cattle housed in temporary or semi-permanent structures had significantly higher odds of having SCM compared with those in permanent facilities. Such suboptimal housing conditions may increase exposure to moisture, manure and bedding contaminations, particularly during wet seasons, thereby creating conducive conditions to pathogen survival and transmission [20]. These findings identify housing improvement as a key, practical and modifiable intervention, especially within resource-limited pastoral and agropastoral systems where infrastructural constraints are common and structured mastitis control programs are often limited or absent.

In addition to housing, herd size or structure also showed a clear association with SCM occurrence, with medium-sized herds exhibiting the highest risk. Expanding herd size may increase animal density and frequency of inter-animal interactions while simultaneously placing greater demands on hygiene practices and veterinary support, thereby facilitating pathogen transmission. Similar associations between herd size and mastitis risk have been reported in Ethiopia and other settings [21, 22], supporting herd structure as a key epidemiological determinant. This underscores the importance of aligning herd expansion with proportional improvements in management capacity and biosecurity to mitigate increased disease risk.

Breed was also associated with SCM occurrence, with indigenous cattle showing higher odds of having SCM than exotic cattle. This observation contrasts with reports from high-input dairy systems, where increased mastitis susceptibility has been described among high-producing exotic cattle, often attributed to udder or teat conformation and production-related stress [39, 49]. In the present setting, however, the observed association is more plausibly explained by context-specific production conditions rather than inherent breed-level genetic resistance. Exotic cattle were more commonly managed under intensive production systems characterized by improved housing qualities and more standardized milking routines, conditions that likely reduced environmental exposure and pathogen transmission. In contrast, indigenous cattle were more frequently managed under pastoral or semi-intensive agropastoral systems, where prolonged lactation periods, greater exposure to contaminated

environments and inconsistent milking hygiene may increase exposure and susceptibility to both contagious and environmental mastitis pathogens [50]. Nonetheless, residual confounding related to seasonality, nutrition, access to veterinary services, composite sampling dilution effects and exclusion of streptococcal pathogens may also have influenced the observed breed-associated patterns, including differences in bacterial recovery and distribution.

Methodological considerations are also important in interpreting these findings. Differences between CMT- and SCC-based prevalence estimates, particularly in intensive zero-grazing systems, likely reflect inherent methodological differences between the two assays, including variation in sensitivity and the subjective nature of CMT scoring compared with the quantitative, threshold-based SCC measurements. Discordance between SCC-defined SCM status and bacteriological culture outcomes may additionally reflect intermittent bacterial shedding, prior antimicrobial exposure, low-level intramammary infections below culture detection thresholds, host-adapted infections, absence of pre-enrichment, exclusion of streptococcal pathogens, involvement of non-bacterial or mixed aetiologies. Similar sources of disagreement between CMT results and bacteriological culture have been reported previously [3]. In addition, the use of composite milk samples may dilute quarter-level infections, underestimate pathogen diversity and limit quarter-level inference thereby complicating interpretation of mixed bacterial growth. Collectively, these factors provide a plausible explanation for the observed low concordance between CMT- and SCC-based classifications (overall agreement = 60.2%; $\kappa = 0.20$). This limited agreement underscores the constraints of relying on a single field-based diagnostic method and supports the use of SCC and CMT as complementary rather than interchangeable tools for SCM surveillance. Furthermore, composite milk sampling remains a pragmatic approach for large-scale surveillance in resource-limited settings where quarter-level sampling is logistically and financially prohibitive.

The present study is not without limitations. The primary limitation was the targeted focus on *Staphylococcus* spp., which precluded comprehensive characterization of other important mastitis pathogens, particularly *Streptococcus* spp. Consequently, inference on the full aetiological spectrum of mastitis and overall intramammary microbial diversity is necessarily limited. This restriction may also have influenced the proportion of SCC-positive culture-negative samples and the interpretation of mixed bacterial growth patterns. However, the focused design enabled detailed species-level characterization of *Staphylococcus* spp., particularly NAS species, within available resources. The cross-sectional design precludes causal inference and limits assessment of temporal or seasonal dynamics; observed associations should therefore be interpreted conservatively as epidemiological correlates of management conditions rather than causal effects. Additionally, limited within-herd sampling may have resulted in misclassification of herds as SCM-negative, particularly where classification was based on a relatively small number of sampled lactating cows.

Despite these constraints, this study provides one of the most detailed species-level resolution of NAS in Kenyan cattle to date, integrating SCC-defined SCM classification, complementary CMT-based SCM screening, and MALDI-TOF MS-based bacterial identification across diverse production systems. The combined use of quantitative inflammatory markers and high-resolution microbial identification strengthens inference on SCM epidemiology across heterogeneous cattle production in Kenya, while also highlighting the value of aligning phenotypic and microbiological approaches in field-based surveillance. Future longitudinal studies should build on these findings by incorporating larger within herds sample sizes, quarter-level sampling, AMR profiling, and culture-independent and omics-based approaches (e.g., whole-genome sequencing, metagenomics, phylogenomics, functional profiling) to more comprehensively define intramammary microbial diversity. Emphasis should be placed on comprehensive characterization of *Streptococcus* spp. and other non-staphylococcal pathogens to better capture the full spectrum of mastitis aetiology in Kenya.

Conclusions

In summary, SCM in Kenyan cattle systems remains prevalent and continues to constrain dairy productivity. The condition is characterized by substantial *Staphylococcus* diversity, encompassing both *S. aureus* and multiple NAS species, particularly *S. chromogenes* and *S. succinus*, with occasional evidence of mixed bacterial presence. SCM occurrence was independently associated with modifiable production-related risk factors, particularly housing quality and herd structure, whereas observed breed-associated differences likely reflected variations in management practices rather than intrinsic biological susceptibility. Effective control in resource-limited settings should therefore prioritize system-specific interventions that integrate accessible diagnostic tools with targeted improvements in housing, hygiene and herd management. In addition, continued species-level surveillance will be critical for supporting sustainable, context-appropriate mastitis control strategies and addressing emerging challenges, including antimicrobial resistance.

Abbreviations

AIC	Akaike information criterion
AMR	Antimicrobial resistant or resistance
AMUSE	Antimicrobial management use
aOR	Adjusted crude odds ratio from the multivariable regression model
CI	Confidence interval
CM	Clinical mastitis
CMT	California mastitis test
CONS	Coagulase negative Staphylococci
ICC	Intra-class correlation
FAO	Food and Agriculture Organization
ILRI	International Livestock Research Institute
MAC	MacConkey agar
MALDI-TOF MS	Matrix-assisted laser desorption/ionization-time of flight mass spectrometry
MSA	Mannitol salt agar
NA	Nutrient agar
NAS	Non-aureus Staphylococcus species
OR	Crude odds ratio from the univariable regression model
PAB	Pseudomonas agar base
SCC	Somatic cell count(s)
SCM	Subclinical mastitis
TSB	Trypticase soy broth
VIF	Variance inflation factors

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1186/s12866-026-05745-7>.

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Author contributions LW and WOO conceived and designed the study. WOO, DR, LN, LW, HK, LO, and LJ conducted field and laboratory investigations. LW, HNV and EF provided managerial and logistical support. LW, MMM and MWM supervised the project. WOO, JA, DR and MMM developed and performed the statistical analyses. WOO drafted the initial

manuscript. LW and WOO reviewed and edited the final manuscript. All authors contributed to the final and version and approved the manuscript for publication.

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Data availability Anonymized data underlying the findings described in this manuscript will be made available upon request to the corresponding author(s).

Declarations

Ethics approval and consent to participate This study was approved by the Kenyatta National Hospital–University of Nairobi Ethics and Research Committee (Protocol #KNH-ERC/A/37) and formed part of a larger project approved by the Institutional Research Ethics Committee (IREC) of the International Livestock Research Institute (Approval #ILRI-IREC2021-31). The larger project was also authorized by the Kenyan Ministry of Agriculture, Livestock, Fisheries and Cooperatives (Ref: MOALF/SDL/DVS/DS/RES Vol.53/12) and the National Commission for Science, Technology and Innovation (Ref: NACOSTI/P/21/13249).

Consent for publication Not applicable.

Clinical trial number Not applicable.

Competing interests The authors declare no competing interests.

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References

1. Krishnamoorthy P, Goudar AL, Suresh KP, Roy P. Global and countrywide prevalence of subclinical and clinical mastitis in dairy cattle and buffaloes by systematic review and meta-analysis. *Res Vet Sci*. 2021;136:561–86. <https://doi.org/10.1016/j.rvsc.2021.04.021>.
2. De Buck J, Ha V, Naushad S, Nobrega DB, Luby C, Middleton JR, et al. Non-aureus Staphylococci and Bovine Udder Health: Current Understanding and Knowledge Gaps. *Front Veterinary Sci*. 2021;8. <https://doi.org/10.3389/fvets.2021.658031>.
3. Mbindyo CM, Gitao GC, Mulei CM. Prevalence, Etiology, and Risk Factors of Mastitis in Dairy Cattle in Embu and Kajiado Counties, Kenya. *Vet Med Int*. 2020;2020:1–12. <https://doi.org/10.1155/2020/8831172>.
4. Petzer I-M, Karzis J, Donkin EF, Webb EC, Etter EMC. Somatic cell count thresholds in composite and quarter milk samples as indicator of bovine intramammary infection status. *Onderstepoort J Vet Res*. 2017;84. <https://doi.org/10.4102/ojvr.v84i1.1269>.
5. Kashongwe OB, Bebe BO, Matofari JW, Huelsebusch CG. Associations between milking practices, somatic cell counts and milk postharvest losses in smallholder dairy and pastoral camel herds in Kenya. *Int J Vet Sci Med*. 2017;5:57–64. <https://doi.org/10.1016/j.ijvsm.2017.01.001>.
6. Haxhijaj K, Wishart DS, Ametaj BN, Mastitis. What It Is, Current Diagnostics, and the Potential of Metabolomics to Identify New Predictive Biomarkers. *Dairy*. 2022;3:722–46. <https://doi.org/10.3390/dairy3040050>.

7. Ruegg PL. A 100-Year Review: Mastitis detection, management, and prevention. *J Dairy Sci.* 2017;100:10381–97. <https://doi.org/10.3168/jds.2017-13023>.
8. Ashraf A, Imran M. Diagnosis of bovine mastitis: from laboratory to farm. *Trop Anim Health Prod.* 2018;50:1193–202. <https://doi.org/10.1007/s11250-018-1629-0>.
9. Blignaut D, Thompson P, Petzer I-M. Prevalence of mastitis pathogens in South African pasture-based and total mixed ration-based dairies during 2008 and 2013. *Onderstepoort J Vet Res.* 2018;85:1–7. <https://doi.org/10.4102/ojvr.v85i1.1482>.
10. Silva VM, Souza MT, Blagitiz MG, Souza FN, Batista CF, Alves AJ, et al. Milk lymphocyte profile and macrophage functions: new insights into the immunity of the mammary gland in quarters infected with *Corynebacterium bovis*. *BMC Vet Res.* 2021;17:282. <https://doi.org/10.1186/s12917-021-02989-5>.
11. Ashraf A, Imran M. Causes, types, etiological agents, prevalence, diagnosis, treatment, prevention, effects on human health and future aspects of bovine mastitis. *Anim Health Res Rev.* 2020;21:36–49. <https://doi.org/10.1017/S1466252319000094>.
12. Hoque MN, Istiaq A, Rahman MS, Islam MR, Anwar A, Siddiki AMAMZ, et al. Microbiome dynamics and genomic determinants of bovine mastitis. *Genomics.* 2020;112:5188–203. <https://doi.org/10.1016/j.ygeno.2020.09.039>.
13. Srednik ME, Grieben MA, Bentancor A, Gentilini ER. Molecular identification of coagulase-negative staphylococci isolated from bovine mastitis and detection of β -lactam resistance. *J Infect Developing Ctries.* 2015;9:1022–7. <https://doi.org/10.3855/jidc.5871>.
14. Rahman MH, Shahadat MN, Siddique AB, Ahmed S. Antimicrobial resistance profiles of non-aureus Staphylococci isolated from farm animals, farm environments and companion animals. *Sci Rep.* 2026;16:5564. <https://doi.org/10.1038/s41598-026-36455-9>.
15. Frey Y, Rodriguez JP, Thomann A, Schwendener S, Perreten V. Genetic characterization of antimicrobial resistance in coagulase-negative staphylococci from bovine mastitis milk. *J Dairy Sci.* 2013;96:2247–57. <https://doi.org/10.3168/jds.2012-6091>.
16. Phophi L, Petzer I-M, Qekwana DN. Antimicrobial resistance patterns and biofilm formation of coagulase-negative Staphylococcus species isolated from subclinical mastitis cow milk samples submitted to the Onderstepoort Milk Laboratory. *BMC Vet Res.* 2019;15:420. <https://doi.org/10.1186/s12917-019-2175-3>.
17. Mureithi DK, Njuguna MN. Prevalence of subclinical mastitis and associated risk factors in dairy farms in urban and peri-urban areas of Thika Sub County, Kenya. *Bullet Health Anim Health Prod Afr.* 2017;65:167–73.
18. Michira L, Kagira J, Maina N, Waititu K, Kiboi D, Ongera E, et al. Prevalence of subclinical mastitis, associated risk factors and antimicrobial susceptibility pattern of bacteria isolated from milk of dairy cattle in Kajiado Central sub-county, Kenya. *Vet Med Sci.* 2023;9:2885–92. <https://doi.org/10.1002/vms3.1291>.
19. FAO. Livestock Production Systems spotlight Kenya. 2018.
20. Arnott G, Ferris CP, O'Connell NE. Review: welfare of dairy cows in continuously housed and pasture-based production systems. *Animal.* 2017;11:261–73. <https://doi.org/10.1017/S1751731116001336>.
21. Abebe R, Hatiya H, Abera M, Megersa B, Asmare K. Bovine mastitis: prevalence, risk factors and isolation of Staphylococcus aureus in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Vet Res.* 2016;12:270. <https://doi.org/10.1186/s12917-016-0905-3>.
22. Mekonnen SA, Koop G, Melkie ST, Getahun CD, Hogeveen H, Lam TJGM. Prevalence of subclinical mastitis and associated risk factors at cow and herd level in dairy farms in North-West Ethiopia. *Prev Vet Med.* 2017;145:23–31. <https://doi.org/10.1016/j.prevetmed.2017.06.009>.
23. Kisoo L, Muloi DM, Oguta W, Ronoh D, Kirwa L, Akoko J, et al. Practices and drivers for antibiotic use in cattle production systems in Kenya. *One Health.* 2023;17:100646. <https://doi.org/10.1016/j.onehlt.2023.100646>.
24. Wieland B, Dione M, Alemu B, Fevre E, Grace D, Omoya L, Ström G, Roesel K, Wenemark M, Muneri C, Lindahl E, Magnusson U. AMUSE Livestock, version 2—Antimicrobial use in livestock production: A tool to harmonise data collection on knowledge, attitude and practices. Nairobi, Kenya: ILRI. ILRI. 2019; October:1–39.
25. NMC. Procedures for Collecting Milk Samples. 2004.
26. Mbindyo CM, Gitao GC, Plummer PJ, Kulohoma BW, Mulei CM, Bett R. Antimicrobial Resistance Profiles and Genes of Staphylococci Isolated from Mastitic Cow's Milk in Kenya. *Antibiotics.* 2021;10:772. <https://doi.org/10.3390/antibiotics10070772>.
27. Calderaro A, Chezzi C, MALDI-TOF MS. A Reliable Tool in the Real Life of the Clinical Microbiology Laboratory. *Microorganisms.* 2024;12. <https://doi.org/10.3390/microorganisms12020322>.
28. Rychert J. Benefits and Limitations of MALDI-TOF Mass Spectrometry for the Identification of Microorganisms. *J Infect (Grand Rapids).* 2019;2:1–5. <https://doi.org/10.29245/2689-9981/2019/4.1142>.
29. Singhal N, Kumar M, Kanaujia PK, Virdi JS. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Front Microbiol.* 2015;6 AUG:1–16. <https://doi.org/10.3389/fmicb.2015.00791>.
30. Croxatto A, Prod'homme G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. *FEMS Microbiol Rev.* 2012;36:380–407. <https://doi.org/10.1111/j.1574-6976.2011.00298.x>.

31. Li M, Gao Q, Yu T. Kappa statistic considerations in evaluating inter-rater reliability between two raters: which, when and context matters. *BMC Cancer*. 2023;23:799. <https://doi.org/10.1186/s12885-023-11325-z>.
32. Kim JH. Multicollinearity and misleading statistical results. *Korean J Anesthesiol*. 2019;72:558–69. <https://doi.org/10.4097/kja.19087>.
33. Schober P, Boer C, Schwarte LA. Correlation Coefficients: Appropriate Use and Interpretation. *Anesth Analg*. 2018;126:1763–8. <https://doi.org/10.1213/ANE.0000000000002864>.
34. Madhaiyan M, Wirth JS, Saravanan VS. Phylogenomic analyses of the Staphylococcaceae family suggest the reclassification of five species within the genus Staphylococcus as heterotypic synonyms, the promotion of five subspecies to novel species, the taxonomic reassignment of five Staphylococcus species to Mammaliicoccus gen. nov., and the formal assignment of Nosocomiicoccus to the family Staphylococcaceae. *Int J Syst Evol Microbiol*. 2020;70:5926–36. <https://doi.org/10.1099/ijsem.0.004498>.
35. Ndahetuye JB, Persson Y, Nyman A-K, Tukei M, Ongol MP, Båge R. Aetiology and prevalence of subclinical mastitis in dairy herds in peri-urban areas of Kigali in Rwanda. *Trop Anim Health Prod*. 2019;51:2037–44. <https://doi.org/10.1007/s11250-019-01905-2>.
36. Abrahmsén M, Persson Y, Kanyima BM, Båge R. Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda. *Trop Anim Health Prod*. 2014;46:99–105. <https://doi.org/10.1007/s11250-013-0455-7>.
37. Motaung TE, Petrovski KR, Petzer I-M, Thekisoe O, Tsilo TJ. Importance of bovine mastitis in Africa. *Anim Health Res Rev*. 2017;18:58–69. <https://doi.org/10.1017/S1466252317000032>.
38. Khasapane NG, Byaruhanga C, Thekisoe O, Nkhebenyane SJ, Khumalo ZTH. Prevalence of subclinical mastitis, its associated bacterial isolates and risk factors among cattle in Africa: a systematic review and meta-analysis. *BMC Vet Res*. 2023;19:123. <https://doi.org/10.1186/s12917-023-03673-6>.
39. Ndirangu PN, Kipronoh AK, Mungube EO, Ogali IN, Omwenga SG, Ndung'u DN, et al. Prevalence of bovine mastitis and antimicrobial sensitivities of the bacterial causes in smallholder farms of Kisumu County, Kenya. *J Agric Rural Dev Tropics Subtropics*. 2022;123:247–55. <https://doi.org/10.17170/kobra-202212057194>.
40. Katsande S, Matope G, Ndengu M, Pfukenyi DM. Prevalence of mastitis in dairy cows from smallholder farms in Zimbabwe. *Onderstepoort J Vet Res*. 2013;80:1–7. <https://doi.org/10.4102/ojvr.v80i1.523>.
41. Krishnamoorthy P, Suresh KP, Jayamma KS, Shome BR, Patil SS, Amachawadi RG. An Understanding of the Global Status of Major Bacterial Pathogens of Milk Concerning Bovine Mastitis: A Systematic Review and Meta-Analysis (Scientometrics). *Pathogens*. 2021;10:545. <https://doi.org/10.3390/pathogens10050545>.
42. Phiri BJ, Benschop J, French NP. Systematic review of causes and factors associated with morbidity and mortality on smallholder dairy farms in Eastern and Southern Africa. *Prev Vet Med*. 2010;94:1–8. <https://doi.org/10.1016/j.prevetmed.2010.01.012>.
43. Ekwem D, Enright J, Hopcraft JGC, Buza J, Shirima G, Shand M, et al. Local and wide-scale livestock movement networks inform disease control strategies in East Africa. *Sci Rep*. 2023;13:9666. <https://doi.org/10.1038/s41598-023-35968-x>.
44. Barkema HW, Schukken YH, Zadoks RN, Invited, Review. The Role of Cow, Pathogen, and Treatment Regimen in the Therapeutic Success of Bovine Staphylococcus aureus Mastitis. *J Dairy Sci*. 2006;89:1877–95. [https://doi.org/10.3168/jds.S0022-0302\(06\)72256-1](https://doi.org/10.3168/jds.S0022-0302(06)72256-1).
45. Sophorn N, Sambo N, Ohkura S, Nakamura S, Matsuyama S, Murase T, et al. Emerging of Uncommon Chronic Mastitis From *S. gallolyticus* and *S. chromogenes* in a Smallholder Dairy Farm in Cambodia. *Transbound Emerg Dis*. 2025;2025. <https://doi.org/10.1155/tbed/3621605>.
46. de Oliveira Veríssimo SA, Ruas YF, Pinheiro FA, Reis LC, Uscata BA, Goto H, et al. Host-pathogen interactions during early stages of bovine mastitis: divergent macrophage responses to distinct bovine-associated Staphylococci species and strains. *Brazilian J Microbiol*. 2026;57:57. <https://doi.org/10.1007/s42770-026-01888-x>.
47. Chuasakhonwilai A, Keeklangdon P, Chaisri W, Saipinta D, Photiboon K, Kaewmuangma D, et al. Different bacterial growth of major mastitis pathogens after coculturing with Staphylococcus chromogenes and Staphylococcus hominis in milk in vitro. *Front Microbiol*. 2025;16. <https://doi.org/10.3389/fmicb.2025.1707938>.
48. Angelopoulou A, Holohan R, Rea MC, Warda AK, Hill C, Ross RP. Bovine mastitis is a polymicrobial disease requiring a polydiagnostic approach. *Int Dairy J*. 2019;99:104539. <https://doi.org/10.1016/j.idairyj.2019.104539>.
49. Zemanova M, Langova L, Novotná I, Dvorakova P, Vrtkova I, Havlicek Z. Immune mechanisms, resistance genes, and their roles in the prevention of mastitis in dairy cows. *Arch Anim Breed*. 2022;65:371–84. <https://doi.org/10.5194/aab-65-371-2022>.
50. Deng Z, Koop G, Lam TJGM, van der Lans IA, Vernooij JCM, Hogeveen H. Farm-level risk factors for bovine mastitis in Dutch automatic milking dairy herds. *J Dairy Sci*. 2019;102:4522–35. <https://doi.org/10.3168/jds.2018-15327>.

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