

PREVALENCE, ANTIMICROBIAL RESISTANCE PATTERNS, AND ASSOCIATED RISK FACTORS OF *STAPHYLOCOCCUS AUREUS* IN RAW MILK FROM DAIRY CATTLE IN TILOTTAMA MUNICIPALITY, NEPAL

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ABSTRACT

Staphylococcus aureus (*S. aureus*)-associated bovine mastitis is a serious economic and animal health concern in Nepalese dairy production. Yet, antimicrobial resistance (AMR) data from commercial dairy herds remain scarce. A cross-sectional study was conducted from January to March 2020 in Tilottama Municipality, Rupandehi District, Nepal. A total of 207 lactating cows from 69 commercial dairy farms were enrolled by two-stage cluster sampling. Composite milk samples were pre-enriched in Buffered Peptone Water and cultured on Mannitol Salt Agar. *S. aureus* was considered positive by a five-step biochemical protocol with the tube coagulase test as the definitive criterion. Antimicrobial susceptibility to six agents was determined by the Kirby-Bauer disk diffusion method, and phenotypic methicillin-resistant *S. aureus* (MRSA) was identified by cefoxitin (30 µg) disk diffusion. Animal-level *S. aureus* prevalence was 33.3% (69/207) and herd-level prevalence was 53.6% (37/69). Cefoxitin non-susceptibility was detected in 52.2% of isolates; multidrug resistance (MDR) in 26.1% and a multiple antibiotic resistance (MAR) index ≥ 0.25 in 71.0% of isolates. Chloramphenicol showed the highest susceptibility rate (92.8%). After Bonferroni correction, animal age ≥ 5 years (OR=3.88) and early lactation stage ≤ 6 months post-calving (OR=3.30) were significantly associated with *S. aureus* positivity. Cefoxitin non-susceptibility and MDR were common among *S. aureus* isolates from commercial dairy farms of Tilottama Municipality. Older cows as well as early-lactating ones carried the highest infection risk. Culture-guided antimicrobial therapy, improved antibiotic stewardship, and sustained antimicrobial resistance (AMR) surveillance with molecular confirmation are recommended to prevent antimicrobial resistance.

Keywords: *S. aureus* prevalence, antimicrobial resistance, cefoxitin non-susceptibility dairy cattle

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INTRODUCTION

Dairy farming is a cornerstone of Nepal's rural economy, with the livestock sector contributing approximately 24.0% to agricultural GDP (MoALD, 2025). The country roughly maintains one million dairy cattle, producing 12,81,718 million metric tonnes of milk annually (MoALD, 2025). Bovine mastitis is the most costly infectious disease in lactating dairy cows worldwide, causing economic losses through reduced milk yield, poor milk quality, higher treatment costs, and premature culling of affected animals (Morales-Ubaldo *et al.*, 2023; Dego and Vidlund, 2024).

Staphylococcus aureus (*S. aureus*) is the primary mastitis pathogen in dairy cattle, responsible for both clinical and subclinical infections globally (Dego and Vidlund, 2024; Touaitia *et al.*, 2025). The organism is difficult to eliminate because it forms biofilms on teat surfaces, evades host immunity and readily acquires antibiotic resistance genes (Touaitia *et al.*, 2025). Animal-level *S. aureus* prevalence estimates from South Asia range from 29.7% in Nepal (Joshi *et al.*, 2014) to 46.7% in Bangladesh (Islam *et al.*, 2025), with Pakistan reporting 30.3 - 42.5% (Shahzad *et al.*, 2024) and India up to 57.5% (Brahma *et al.*, 2022).

There is a growing concern about the spread of antimicrobial resistance (AMR) in *S. aureus*. in Nepal, antibiotics are widely purchased without a veterinary prescription, creating sustained selection pressure for resistance (Acharya and Wilson, 2019; Rijal *et al.*, 2021). Globally, the

prevalence of methicillin-resistant *S. aureus* (MRSA) in dairy cattle milk is estimated at 3.9% by *mecA* polymerase chain reaction (PCR) confirmation, with higher rates in low- and middle-income countries (Khanal *et al.*, 2022). Within Nepal, MRSA was reported in 37.8% of *S. aureus* isolates from dairy farms of Pokhara, Kaski District, by phenotypic testing (Joshi *et al.*, 2014), while it was confirmed by *mecA*PCR in 6.9% of isolates in Chitwan (Shrestha *et al.*, 2021). Nepal adopted a National Action Plan on AMR for 2024-2028 (MoHFS, 2024), but sub-regional baseline data from commercial dairy herds remain limited.

Tilottama Municipality in Rupandehi District supports 8,718 cattle and 105 commercial dairy farms (VHLSEC Rupandehi, 2019), yet no published AMR data exist for bovine milk-associated *Staphylococcus aureus* from this area. This study therefore aimed to: (i) estimate animal- and herd-level prevalence of *S. aureus* in raw milk of dairy cattle from commercial farms in Tilottama Municipality; (ii) characterise the phenotypic AMR profile including phenotypic MRSA, MDR status, and MAR index; and (iii) identify animal- and herd-level risk factors associated with *S. aureus* positivity.

MATERIAL AND METHODS

Study design, area, and period

A cross-sectional observational study was conducted from January to March 2020 in Tilottama Municipality (27.700°N, 83.467°E), Rupandehi District,

Nepal. All bacteriological and antimicrobial susceptibility testing were performed at IAAS Paklihawa Campus, Tribhuvan University. The study was designed and reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (von Elm *et al.*, 2007).

Sample size and sampling strategy

The sample size was calculated at 95.0% confidence using the standard prevalence formula in Epitools, developed by Ausvet, Australia. An expected *S. aureus* prevalence of 11.0% was used, based on Joshi *et al.* (2014), the only Nepal-specific published estimate available at study inception. Absolute precision was set at 5.0% for animal-level and 8.0% for herd-level calculations, requiring minimum samples of 151 animals and 59 farms. After applying 15.0% and 13.0% non-response allowances, adjusted targets were 178 animals and 68 farms; 207 animals from 69 farms were ultimately enrolled.

The commercial farms in Tilottama Municipality recorded at VHLSEC Rupandehi (VHLSEC Rupandehi, 2019) and with ≥ 2 lactating cows were considered for the study. Individual cows were eligible if currently lactating, free from antimicrobial treatment for ≥ 14 days before sampling, and without severe clinical mastitis. Two-stage cluster sampling was used. In Stage 1, 69 of 105 eligible farms were selected by simple random sampling without replacement

(sampling fraction: 65.7%). In Stage 2, 2–5 cows per farm were enrolled by consecutive selection (mean: 3 per farm). A farm was classified as *S. aureus*-positive if at least one enrolled cow yielded a presumptive colony of *S. aureus* on culture.

California Mastitis Test (CMT) and milk sample collection

CMT was performed on fore-stripped milk from all four mammary quarters using a four-paddle plate and commercial CMT reagent (Qualitest, India). Milk and reagent were mixed in equal volumes (~2 mL each) by gentle circular agitation and read within 15 seconds. Results were scored on a five-point scale (N, T, 1+, 2+, 3+), with scores $\geq 1+$ recorded as CMT-positive. CMT results were recorded for all animals as a risk factor variable but did not determine sampling eligibility; all enrolled cows underwent bacteriological culture regardless of CMT outcome.

For aseptic milk collection, each teat orifice was disinfected with 70.0% isopropyl alcohol and air-dried for 30 seconds. The first three fore-streams were discarded, and approximately 2 mL of milk was collected aseptically from each of the four mammary quarters and pooled into a single sterile screw-capped universal tube (~8 mL composite per cow). Samples were maintained at $\leq 4^{\circ}\text{C}$ during transport, and pre-enrichment inoculation commenced within two hours of collection.

Bacteriological isolation and identification of *S. aureus*

S. aureus was isolated from composite milk samples following a protocol adapted and modified from ISO 6888-3:2003 (ISO, 2003). The protocol comprised five sequential steps:

(i) **Pre-enrichment:** 1 mL of composite milk was inoculated into 9 mL buffered peptone water (BPW; HiMediaM614, India) at a 1:10 (v/v) dilution, incubated at 37°C for 18–24 hours.

(ii) **Selective plating:** 10 µL of pre-enrichment broth was plated onto Mannitol Salt Agar (MSA; HiMediaM118, India), incubated aerobically at 37°C for 24–48 hours; colonies with yellow pigmentation and surrounding yellow zones were recorded as presumptive *S. aureus*.

(iii) **Gram staining:** only Gram-positive cocci in irregular grape-like clusters were advanced.

(iv) **Pure culture:** selected colonies were subcultured onto Nutrient Agar (HiMediaM001, India), incubated aerobically at 37°C for 18–24 hours.

(v) **Biochemical confirmation:** catalase test (3.0% H₂O₂, slide method) followed by tube coagulase test using 0.5 mL reconstituted rabbit plasma (HiMedia, India), read at 4 hours and 24 hours; any visible clot was interpreted as positive.

Only tube coagulase-positive isolates were considered as *S. aureus*. These isolates were stored at –20°C in Brain Heart Infusion broth with 20% (v/v) glycerol until susceptibility testing.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined by the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA; HiMediaM173, India). Bacterial suspensions were adjusted to 0.5 McFarland turbidity using a calibrated densitometer. MHA plates were inoculated by lawn streaking in three directions, dried for 3–5 minutes, and antibiotic discs were placed within 15 minutes of inoculation. Standard plates were incubated at 35°C ± 2°C in ambient air and read at 16–18 hours; cefoxitin plates at 24 hours following CLSI (2020a). Zone diameters were measured to the nearest mm with a digital Vernier calliper. Six antimicrobial agents, belonging to six different antimicrobial categories, were tested (Table 1). Cefoxitin non-susceptibility (zone diameter ≤21 mm) was used as the phenotypic MRSA surrogate as per CLSI (2020a): susceptible ≥22 mm; non-susceptible ≤21 mm. Break points for cefoxitin, gentamicin, trimethoprim-sulfamethoxazole, and chloramphenicol followed human staphylococcal criteria (CLSI, 2020a); for doxycycline and enrofloxacin, veterinary-specific breakpoints were applied (CLSI, 2020b).

Classification of multidrug resistance and MAR index

Multidrug resistance (MDR) was defined as acquired non-susceptibility to ≥ 1 agent in each of ≥ 3 distinct antimicrobial categories (Magiorakos *et al.*, 2012). The non-susceptibility was defined as an intermediate (I) or resistant (R) result, except for cefoxitin, where a zone diameter ≤ 21 mm was classified as non-susceptible. The MAR index was calculated as $MAR = a/b$, where a = number of agents to which the isolate was fully resistant (R only) and b = total number of agents tested (Krumperman, 1983). A MAR index ≥ 0.25 was used as the threshold indicating origin from a high-antibiotic-use environment.

Risk factor data collection

Animal- and herd-level data were collected through a structured, closed-ended questionnaire administered face-to-face by the lead investigator at each farm visit, pilot-tested on five farms outside the study area. Six animal-level variables were recorded: breed (Holstein Friesian vs Jersey); age (≥ 5 years vs < 5 years); parity (multiparous vs primiparous); lactation stage (early ≤ 6 months vs late > 6 months post-calving); daily milk yield (> 8 L/day vs ≤ 8 L/day) and CMT result at sampling (positive $\geq 1+$ vs negative). Seven herd-level variables were recorded: anthelmintic treatment frequency; haemorrhagic septicaemia (HS) and black quarter (BQ) vaccination status; barn disinfection practice; floor hygiene condition; milking order for CMT-positive

cows; teat dipping practice; and herd size (≥ 10 vs < 10 lactating cows).

Statistical analysis

Data were double-entered and verified in Microsoft Excel 2016 and analysed in IBM SPSS Statistics version 25.0. Animal and herd-level apparent prevalence was computed with 95% Wilson score confidence intervals (CIs; Newcombe, 1998) using EpiTools. Associations between candidate variables and *S. aureus* positivity were assessed by univariate binary logistic regression, expressed as unadjusted odds ratios (ORs) with 95% Wald CIs and two-tailed p-values. Bonferroni-corrected significance threshold of $\alpha/k = 0.05/13 = 0.0038$ was applied to control the family-wise Type I error rate across 13 simultaneous tests. Multivariate logistic regression was not attempted because the number of events per variable ($EPV = 69/13 = 5.3$) fell below the accepted minimum of 10 (Peduzzi *et al.*, 1996).

RESULTS AND DISCUSSION

Prevalence of *S. aureus*

S. aureus was detected in 69 of the 207 composite milk samples. Animal-level prevalence was 33.3% (69/207; 95% CI: 27.3–40.0%) and herd-level prevalence was 53.6% (37/69; 95% CI: 42.0–64.9%), indicating that more than half of enrolled commercial farms harboured at least one infected animal. Of the 69 culture-positive cows, 35 (50.7%) were CMT-negative across all four quarters, demonstrating that

the CMT, which detects elevated somatic cell counts rather than bacteria directly, missed half of infections identified by bacterial culture. This is consistent with the documented limitations of CMT for detecting chronic, low-grade, or early-stage intramammary infections (Sargeant *et al.*, 2001), and underscores the indispensability of bacteriological culture in AMR surveillance programs.

The animal-level prevalence of 33.3% is within the range reported across South Asia. Within Nepal, Joshi *et al.* (2014) reported 29.7% using quarter-level sampling in Pokhara, and Shrestha *et al.* (2021) isolated *S. aureus* from 15.2% of CMT-positive samples in Chitwan—a lower figure attributable to sampling only CMT-positive animals rather than all enrolled cows. In the broader region, Islam *et al.* (2025) reported 46.7% in northern Bangladesh and Shahzad *et al.* (2024) reported 25.2% in the Pothohar region of Pakistan. The higher prevalence observed here compared with earlier Nepalese studies likely reflects the combined effect of the commercial farm setting (higher animal density, increased pathogen circulation), composite cow-level sampling (detecting infection in any quarter), and universal culture irrespective of CMT status. The herd-level prevalence of 53.6% indicates that over half of enrolled farms were potential reservoirs for *S. aureus*, consistent with reports from commercial dairy settings in Nepal (Tiwari *et al.*, 2022) and South Asia, and highlights the need for herd-level mastitis control programs in this district.

Antimicrobial susceptibility profile

Antimicrobial susceptibility results are presented in Table 2. Chloramphenicol showed the highest susceptibility rate (92.8%), followed by gentamicin (84.1%), enrofloxacin (82.6%), doxycycline (79.7%), and trimethoprim - sulfamethoxazole (78.3%). The predominant resistance phenotype was cefoxitin non-susceptibility, recorded in 52.2% of isolates. These findings reflect disk diffusion category interpretations (susceptible/intermediate/resistant), which do not yield minimum inhibitory concentration (MIC) values. As such, treatment decisions in practice must be guided by individual culture results and, where feasible, confirmed by MIC testing in accordance with current CLSI standards. Notably, chloramphenicol — despite showing the highest susceptibility rate — has restricted use in food-producing animals in many countries owing to public health concerns regarding residues; its practical therapeutic role in dairy cattle therefore remains limited.

Cefoxitin non-susceptibility and phenotypic MRSA

Of 69 *S. aureus* isolates, 36 (52.2%) had cefoxitin zone diameters ≤ 21 mm. This corresponds to an animal-level phenotypic MRSA prevalence of 17.4% (36/207; 95% CI: 12.8–23.1%) and a herd-level phenotypic MRSA prevalence of 31.9% (22/69 farms; 95% CI: 22.1–43.6%). All estimates are based on phenotypic disk diffusion alone; mecAPCR confirmation was not performed. The phenotypic MRSA prevalence of 17.4%

is considerably higher than previously published Nepalese figures. Joshi *et al.* (2014) detected phenotypic MRSA in 37.8% of *S. aureus* isolates from dairy farms of Pokhara, while Shrestha *et al.* (2021) confirmed MRSA in 6.9% of the isolates in Chitwan by *mecA* PCR. Regionally, Shahzad *et al.* (2024) confirmed 21.0% *mecA*-mediated MRSA in Pakistan and Brahma *et al.* (2022) detected 25.0% MRSA (by oxacillin susceptibility) in India. The global pooled *mecA*-confirmed MRSA prevalence in dairy cattle milk is 3.9%, with higher rates in low- and middle-income countries (Khanal *et al.*, 2022). The present finding of 17.4% is consistent with the higher end of regional estimates, though direct comparison is constrained by differences in sampling method, farm type, and confirmation criteria. Cefoxitin disk diffusion (30 µg) is the CLSI-recommended phenotypic surrogate for *mecA*-mediated methicillin resistance in *S. aureus*, validated for high sensitivity and specificity (Anand *et al.*, 2009). Molecular confirmation by *mecA*PCR and SCCmec typing should be incorporated in future surveillance studies.

MAR index

Of 69 isolates, 49 (71.0%) had a MAR index ≥ 0.25 , with a median of 0.29 (IQR: 0.14–0.43; Table 3). According to Krumpelman (1983), a MAR index ≥ 0.25 identifies bacteria originating from environments with frequent antibiotic use and sustained selection pressure. 71.0% of isolates exceeding the threshold is consistent with the widespread non-prescription procurement and use of antimicrobials in

Nepalese livestock farming (Acharya and Wilson, 2019; Shrestha *et al.*, 2021).

MDR phenotype

18 of 69 isolates (26.1%) were classified as MDR using the Magiorakos *et al.* (2012) definition (Table 4). MDR was more prevalent among cefoxitin-non-susceptible isolates (15/36; 41.7%) than among cefoxitin-susceptible ones (3/33; 9.1%), suggesting that strains carrying methicillin resistance may co-accumulate resistance across multiple antibiotic classes. This observation is descriptive only; no formal statistical test was applied to compare these groups.

The MDR detection rate of 26.1% is lower than recent regional figures- 57.5% in India (Brahma *et al.*, 2022), 64.0% in northern Bangladesh (Islam *et al.*, 2025), and 69.2% in Bareilly, Uttar Pradesh (Das, *et al.*, 2025). Shahzad *et al.* (2024) also reported widespread co-resistance alongside 21.0% *mecA*-confirmed MRSA in Pakistan. This regional gradient likely reflects differences in antimicrobial use practices and farm management. Additionally, studies testing broader panels inherently detect higher MDR rates; the six-agent, six-category panel used here may yield a conservative MDR estimate relative to studies testing ≥ 10 agents.

Risk factor analysis

After Bonferroni correction ($\alpha=0.0038$), two animal-level variables were significantly associated with *S. aureus*

positivity: age ≥ 5 years (OR=3.88; 95% CI: 2.10–7.18; $p < 0.001$) and early lactation ≤ 6 months post-calving (OR=3.30; 95% CI: 1.70–6.40; $p < 0.001$; Table 5 and Table 6). No herd management variable reached the corrected threshold. Not milking CMT-positive cows last (OR=2.37; 95% CI: 1.30–4.31; $p = 0.004$) was nominally significant but did not satisfy the Bonferroni criterion; this result should be treated as exploratory.

Older age (≥ 5 years) was the strongest risk factor (OR=3.88). Over multiple lactations, older animals accumulate repeated intramammary pathogen exposures, and the physical integrity of the teat canal sphincter progressively deteriorates, reducing the mechanical barrier against ascending infection (Peeler *et al.*, 2000; Sordillo and Streicher, 2002). Similar age-related risk patterns have been reported in Nepal (Tiwari *et al.*, 2022) and Ethiopia (Abebe *et al.*, 2016), suggesting this is a consistent and generalisable finding across different production systems.

Early lactation (≤ 6 months post-calving; OR=3.30) was the second significant risk factor. The periparturient period is associated with the transient immunosuppression driven by hormonal shifts, metabolic stress, and the physiological demands of early milk synthesis, all of which reduce local mammary immune defences and increase susceptibility to new or reactivated intramammary infections (Sordillo and Streicher, 2002). Taken together, older cows in early lactation form the highest-risk subgroup, warranting

targeted preventive interventions including pre-calving teat dipping, hygienic milking practices, and routine CMT screening.

Not milking CMT-positive animals last (OR=2.37; $p=0.004$) is biologically plausible: milking infected cows before healthy ones could facilitate the transfer of *S. aureus* via teat cup liners and the milker's hands. Although this did not survive Bonferroni correction, milking CMT-positive animals last should be routinely implemented regardless of this study's statistical outcome.

Study limitations

Several limitations should be noted. First, MRSA classification relied on phenotypic cefoxitin disk diffusion; *mecA* PCR confirmation was not performed, so the true proportion of genotypic MRSA is unknown. Second, composite milk sampling may dilute low-shedding quarters below the pre-enrichment culture detection limit, potentially underestimating true prevalence. Third, the panel excluded penicillin and ampicillin — the commonly used antibiotics for staphylococcal mastitis — limiting the clinical relevance of resistance data. Fourth, the EPV of 5.3 precluded multivariate logistic regression (minimum EPV=10; Peduzzi *et al.* 1996); all risk factor findings are exploratory. Fifth, single-season data from the commercial farms limits seasonal and geographic generalisability. Sixth, molecular characterisation of resistance determinants (*mecA*, *blaZ*, *tetM*) was not performed.

CONCLUSION

Staphylococcus aureus was common in raw milk of commercial dairy cattle in Tilottama Municipality, Rupandehi District, Nepal, with an animal-level prevalence of 33.3% and herd-level prevalence of 53.6%. The study showed a higher rate of detection of MRSA (52.2%), MDR (26.1%) and high MAR index values (71.0%), showing evidence of substantial antibiotic selection pressure. Older cows and those in early lactation had higher odds of infection, making them priority targets for surveillance and prevention. These findings reinforce the need for culture-guided treatment, improved antibiotic stewardship and molecular-based AMR surveillance in the region.

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ETHICS APPROVAL

The study was ethically approved by the Internship Advisory Committee of IAAS, Paklihawa Campus. Verbal informed consent was obtained from the owners of the animals before sample collection.

Competing interests:

The authors declare no competing interests.

Data availability:

The datasets generated during this study are available from the corresponding author on reasonable request.

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ABBREVIATION

CLSI	:	Clinical and Laboratory Standards Institute
GDP	:	Gross Domestic Product
ISO	:	International Organisation for Standardisation
MoALD:		Ministry of Agriculture and Livestock Development
MoHFS:		Ministry of Health and Food Safety
SCC	:	Staphylococcal Cassette Chromosome
VHLSEC:		Veterinary Hospital and Livestock Service Expert Centre

Table 1. Antimicrobial categories and agents used for susceptibility testing

SN	Antimicrobial Category	Agent	Disc potency	Code	Interpretive Standard
1	Beta-lactams/ Cephamycins	Cefoxitin	30 µg	CX	CLSIM100 (2020a)
2	Aminoglycosides	Gentamicin	10 µg	GEN	CLSIM100 (2020a)
3	Tetracyclines	Doxycycline	30 µg	DO	CLSIVET01S (2020b)
4	Fluoroquinolones	Enrofloxacin	5 µg	ENR	CLSIVET01S (2020b)
5	Folate Pathway Antagonists	Trimethoprim-sulfamethoxazole	25 µg	SXT	CLSIM100 (2020a)
6	Phenicol	Chloramphenicol	30 µg	C	CLSIM100 (2020a)

Table.2. Antimicrobial susceptibility profile of *Staphylococcus aureus* isolates from composite raw milk of dairy cattle in Tilottama Municipality, Rupandehi District, Nepal (n=69 isolates)

SN	Antimicrobial Agent Code	Disc potency	S (n)	S (%)	I (n)	I (%)	R/NS (n)	R/NS (%)
1	Cefoxitin (CX)	30 µg	33	47.8	0	0.0	36	52.2
2	Gentamicin (GEN)	10 µg	58	84.1	3	4.3	8	11.6
3	Doxycycline (DO)	30 µg	55	79.7	8	11.6	6	8.7
4	Enrofloxacin (ENR)	5 µg	57	82.6	8	11.6	4	5.8
5	Trimethoprim-sulfamethoxazole (SXT)	25 µg	54	78.3	9	13.0	6	8.7
6	Chloramphenicol (C)	30 µg	64	92.8	1	1.4	4	5.8

S: susceptible; I: intermediate; R/NS: resistant or non-susceptible.

Table.3. Distribution of MAR index values among *S. aureus* isolates (n = 69; b = 6)

Agents Resisted (a)	MAR Index (a/6)	Isolates (n)	Isolates (%)	Cumulative (%)
0	0.00	3	4.3	4.3
1	0.17	17	24.6	29.0
2	0.33	18	26.1	55.1
3	0.50	14	20.3	75.4
4	0.67	8	11.6	87.0
5	0.83	5	7.2	94.2
6	1.00	4	5.8	100.0
Total	--	69	100.0	--

MAR = a/b; a = number of agents with full resistance (R category only); b = 6 (CX, GEN, DO, ENR, SXT, C). Threshold: MAR $\geq$ 0.25 (Krumperman, 1983). Isolates meeting threshold: 49/69 (71.0%).

Table.4. MDR phenotype distribution among *S. aureus* isolates (n = 69)

Non-susceptible Categories Met (of 6)	Isolates (n)	Isolates (%)	MDR Status
0	3	4.3	Non-MDR
1	27	39.1	Non-MDR
2	21	30.4	Non-MDR
3	8	11.6	MDR
4	5	7.2	MDR
5	3	4.3	MDR
6	2	2.9	MDR

MDR = non-susceptibility to $\geq$ 1 agent in $\geq$ 3 of 6 antimicrobial categories (Magiorakos et al., 2012). Total MDR: 18/69 (26.1%). Six categories: (1) beta-lactams/cephamycins (CX); (2) aminoglycosides (GEN); (3) tetracyclines (DO); (4) fluoroquinolones (ENR); (5) folate pathway antagonists (SXT); (6) phenicols (C).

Table.5. Univariate binary logistic regression — animal-level risk factors for *S. aureus* positivity (n = 207 animals from 69 herds)

Variable	Category	<i>S. aureus</i> +ve (n)	Prevalence (%)	OR (95% CI)	p-value
Breed	Holstein Friesian (n=134)	42	31.3	0.78 (0.43–1.42)	0.403
	Jersey (n=73) [Ref.]	27	37.0	—	—
Age	≥5 years (n=96)	47	49.0	3.88 (2.10–7.18)	<0.001 *†
	<5 years (n=111) [Ref.]	22	19.8	—	—
Parity	Multiparous (n=170)	61	35.9	2.03 (0.87–4.71)	0.095
	Primiparous (n=37) [Ref.]	8	21.6	—	—
Lactation Stage	Early ≤6 mo (n=126)	54	42.9	3.30 (1.70–6.40)	<0.001 *†
	Late >6 mo (n=81) [Ref.]	15	18.5	—	—
Daily Milk Yield	>8 L/day (n=144)	52	36.1	1.53 (0.80–2.94)	0.200
	≤8 L/day (n=63) [Ref.]	17	27.0	—	—
CMT Status	Positive ≥1+ (n=84)	34	40.5	1.71 (0.95–3.07)	0.072
	Negative (n=123) [Ref.]	35	28.5	—	—

*p < 0.05 (unadjusted). †Significant after Bonferroni correction ($\alpha = 0.0038$). [Ref.] = reference category.

All ORs are unadjusted univariate estimates. Intra-herd clustering was not accounted for in variance estimation. OR: odds ratio; CI: confidence interval.

Table 6. Univariate binary logistic regression — herd management risk factors for *S. aureus* positivity (n = 207 animals from 69 herds)

Management Variable	Category	<i>S. aureus</i> +ve (n)	Prevalence (%)	OR (95% CI)	p-value
Anthelmintic Treatment	Irregular (n=57)	19	33.3	1.00 (0.52–1.91)	1.000
	Regular (n=150) [Ref.]	50	33.3	—	—
HS and BQ Vaccination	Unvaccinated (n=32)	15	46.9	1.98 (0.92–4.25)	0.077
	Vaccinated (n=175) [Ref.]	54	30.9	—	—
Barn Disinfection	Not performed (n=152)	46	30.3	0.60 (0.32–1.14)	0.119
	Performed (n=55) [Ref.]	23	41.8	—	—
Floor Hygiene	Poor (n=139)	47	33.8	1.07 (0.58–1.98)	0.834
	Good (n=68) [Ref.]	22	32.4	—	—
Milking Order	CMT+ NOT milked last (n=106)	45	42.5	2.37 (1.30–4.31)	0.004*
	CMT+ milked last (n=101) [Ref.]	24	23.8	—	—
Teat Dipping	Not practised (n=168)	57	33.9	1.16 (0.55–2.45)	0.706
	Practised (n=39) [Ref.]	12	30.8	—	—
Herd Size	≥10 cows (n=104)	37	35.6	1.23 (0.69–2.19)	0.491
	<10 cows (n=103) [Ref.]	32	31.1	—	—

HS: haemorrhagic septicaemia; BQ: black quarter. * $p < 0.05$ (unadjusted); did not meet Bonferroni-corrected threshold ($\alpha = 0.0038$). [Ref.] = reference category. All ORs are unadjusted univariate estimates.

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