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Antimicrobial resistance profiles of bacterial isolates associated with subclinical mastitis in crossbred lactating goats in the Forest Zone of Cameroon

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ABSTRACT : This preliminary cross-sectional study aimed to determine the prevalence of subclinical mastitis (SCM), to identify the bacterial isolates associated with CMT-positive milk samples and to assess their susceptibility to antimicrobials in crossbred dairy goats in the forest zone of Cameroon. Screening using the California Mastitis Test (CMT) was carried out on 119 lactating goats from 30 farms and two markets in the Central Region. CMT-positive milk samples were subjected to bacteriological analysis, and the isolates were tested for antimicrobial susceptibility using the disc diffusion method (CLSI M100, 2021). The prevalence of SCM was 23.53% (95% CI: 16.8 - 31.9%), ranging from 6.67% to 38.46% depending on the sampling location (farms or markets). A total of 102 bacterial isolates belonging to six species were isolated, dominated by *Staphylococcus aureus* (21.57%) and Enterobacteriaceae (60.78% of isolates: *Klebsiella pneumoniae*, *Klebsiella aerogenes*, *Escherichia coli*, *Salmonella* spp.). Enterobacteria exhibited high rates of resistance to broad-spectrum cephalosporins, suggestive of an extended-spectrum β-lactamase mechanism, whilst staphylococci showed frequent resistance to cefoxitin (a phenotypic surrogate marker for mecA-mediated resistance) and complete resistance to tetracycline in *S. aureus*. Susceptibility to gentamicin and ciprofloxacin remained largely intact in *Klebsiella* spp. and staphylococci, but was markedly reduced in *E. coli* and *Salmonella* spp., particularly for ciprofloxacin (39% and 0% susceptible, respectively). These results indicate the presence of antimicrobial-resistant bacterial isolates of public health relevance, thereby warranting further epidemiological investigation, and call for larger-scale studies to assess the extent of antibiotic resistance in Cameroon's dairy goat sector.

Keywords: Antibiotic resistance, dairy goat, extended-spectrum β-lactamases, forest zone, subclinical mastitis.

INTRODUCTION

Small ruminant farming, particularly goat farming, plays a vital role in livestock production systems in sub-Saharan Africa because goats are hardy, adaptable to challenging environmental conditions, and capable of utilising low-quality forage resources (Kahi and Wasike, 2019). Goats are an important source of income, food security and nutrition for rural populations (Geldsetzer-Mendoza and Riveros, 2023; Tibebu *et al.,* 2026). Goat milk and its derivatives (yoghurt, cheese, milk powder) are valued for their nutritional qualities, including among consumers with cow's milk intolerance (Singh, 2024), yet production remains limited in sub-Saharan Africa, with yields often below 1 litre per goat per day under the predominantly extensive, small-herd systems that characterise the region Atoui *et al.,* 2023; Tibebu *et al.,* 2026). In order to improve zootechnical performance and meet the growing demand

(for dairy products, several countries in the region have undertaken crossbreeding programmes between local goat breeds and exotic dairy breeds with high milk potential (Kahi and Wasike, 2019; Oloo *et al.,* 2023). However, the gradual intensification of goat farming is accompanied by an increased risk of diseases affecting udder health, amongst which mastitis is the most prevalent. Mastitis is an inflammation of the mammary gland, most often of infectious origin. It manifests either in a clinical form, characterised by visible signs in the udder and milk, or in a subclinical form, which is generally asymptomatic but leads to a reduction in milk production, impaired milk quality and significant economic losses for farmers (Smistad *et al.,* 2024).

According to a recent meta-analysis conducted in accordance with the PRISMA guidelines, the global prevalence of subclinical mastitis in dairy goats is estimated at 31.6% (95% CI: 29.34 - 33.3%) (Tibebu *et al.,* 2026). In sub-Saharan Africa, particularly high prevalence rates have been reported, reaching 40.4% in Nigeria, of which 32.4% were subclinical cases (Oladele *et al.,* 2019), and as high as 84.7 % in Kenya (Kabui *et al.,* 2024). From an aetiological perspective, coagulase-negative staphylococci (CNS) are the most frequently isolated microorganisms in the milk of goats with subclinical mastitis, with proportions ranging from 44.7 to 95.9%. These are followed by *Staphylococcus aureus* (15.24%), *Escherichia coli* (11.43%) and *Streptococcus* spp. (10.95%) (Ma *et al.,* 2014; Virdis *et al*., 2010). At the same time, the emergence of antibiotic resistance is a growing concern in the management of mastitis. The frequent and sometimes inappropriate use of antimicrobials in livestock farming promotes the selection of resistant bacterial strains, compromising the effectiveness of treatments and increasing risks to public health (Tomanić *et al.,* 2025). In South Africa, a recent study showed that 78.9% of *S. aureus* isolates from mastitis cases were multidrug-resistant, particularly to amoxicillin–clavulanic acid, penicillin and tetracycline (Sizana *et al.,* 2026). In Kenya, 76.5% of bacterial isolates from subclinical mastitis cases exhibited resistance to at least one of the antibiotics tested (Kabui *et al.,* 2024).

Although mastitis cases have been reported in goats in other regions of Cameroon, notably in the North-West and Adamawa regions (Abegewi *et al.,* 2022), information on CMT-defined subclinical mastitis and on species-specific antimicrobial susceptibility profiles in crossbred dairy goats in the Centre Region remains limited. This lack of information constitutes a major obstacle to the implementation of effective prevention and control measures, as well as to the prudent management of antimicrobials in livestock farming. This preliminary study therefore aimed to: (i) determine the prevalence of CMT-defined subclinical mastitis; (ii) identify the bacterial isolates recovered from CMT-positive milk; and (iii) determine their antimicrobial susceptibility profiles, in crossbred lactating goats reared in forested areas of Cameroon.

MATERIALS AND METHODS

Study area and sample population

The study was carried out in the Centre Region, situated in Cameroon's humid forest zone. This region covers an area of approximately 68,953 km² and is characterised by an equatorial climate with four seasons, comprising two rainy seasons alternating with two dry seasons. Annual rainfall generally varies between 1,500 and 2,000 mm, whilst average annual temperatures are around 25 °C, against a backdrop of high humidity characteristic of Cameroon's humid forest zone (INS, 2017; Soh Wenda *et al.,* 2024). In this region, goat farming is predominantly extensive or semi-intensive, mostly organised at the family level. Herds are generally small and are managed within mixed systems combining livestock rearing with food crop cultivation. Production is primarily focused on meat, self-consumption and income generation. However, driven by growing demand for dairy products and genetic improvement programmes, the rearing of dairy goats resulting from crossbreeding between local and exotic breeds is gradually expanding (MINEPIA, 2023).

This preliminary cross-sectional study aimed to determine the bacterial aetiology of subclinical mastitis (SCM) and the antimicrobial resistance profiles of bacteria isolated from crossbred dairy goats reared in the forested region of Cameroon. Due to the exploratory nature of this study and the lack of prior epidemiological data to estimate the prevalence of the disease in the target population, no formal sample size calculation was carried out. The number of animals included was determined based on the availability of goats meeting the inclusion criteria on the farms visited, in accordance with generally accepted recommendations for pilot and exploratory studies (Thabane *et al.,* 2010).

The sampling was carried out in May and June, corresponding to the end of the short rainy season. It was carried out in four localities in the Central Region of Cameroon with significant goat farming activity: Nkolbisson (Nk), Obala (Ob), Ombessa (Om) and Mbalmayo (Mb), as well as at two major small ruminant markets in Yaoundé (M1 and M2), where animals from different rearing areas are traded (Figure 1). At the two markets, only lactating crossbred goats showing no clinical signs of mastitis were sampled, using the same inclusion criteria as on farms. These animals had been brought by farmers for sale and originated from several neighbouring localities, accounting for the diversity of rearing origins represented at these sites. Farms were selected on the recommendation of MINEPIA (Ministry of Livestock, Fisheries and Animal Industries) livestock agents as holdings known to keep crossbred goats; the two markets (M1 and M2) were selected because they are the main small ruminant markets in the study area. Within each selected farm or market, all lactating crossbred goats showing no clinical signs of mastitis were sampled (non-congested udder, macroscopically normal milk).

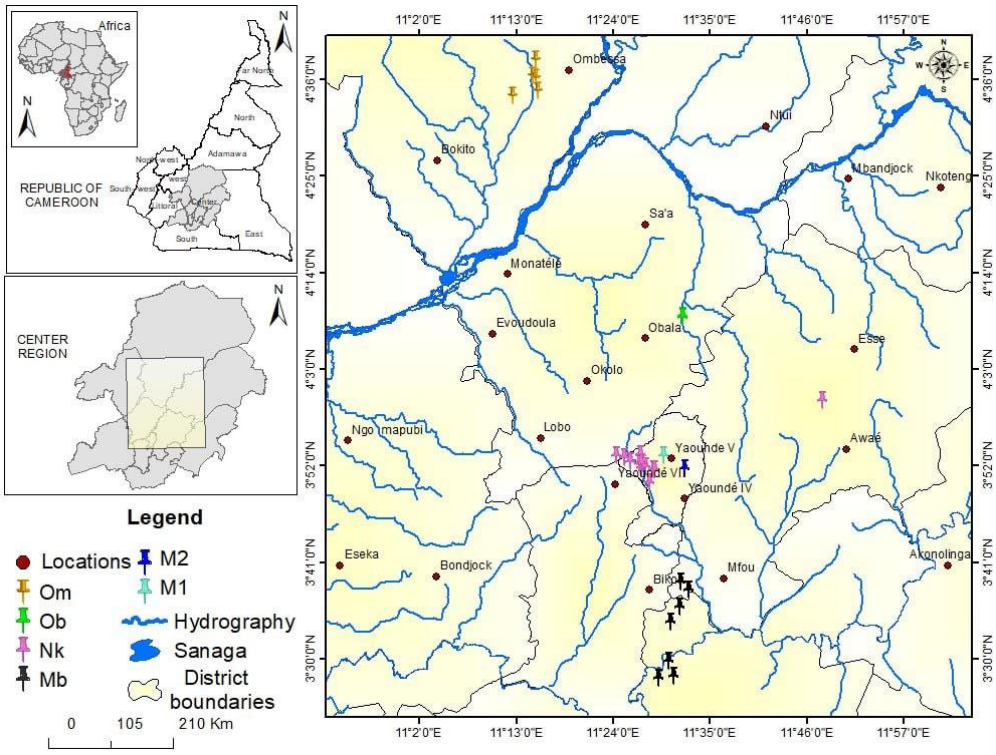


Figure 1. Map showing the areas where the study was carried out in the Central Region of Cameroon, indicated by pins.

Following a convenience-based sampling approach, a total of 119 lactating crossbred dairy goats (resulting from crossbreeding between local breeds - West African Dwarf and Sahel/Kirdi goats and exotic dairy breeds - Alpine, Saanen and Anglo-Nubian) were sampled from 30 small-scale family goat holdings (4 to 16 goats per herd) and two markets (Figure 1). A sample of 119 goats was retained for this preliminary study to obtain an initial estimate of the prevalence of subclinical mastitis (SCM) (pilot/descriptive estimate) and to identify the main associated bacteria.

Screening for subclinical mastitis using the California Mastitis Test (CMT)

Screening for subclinical mastitis was carried out using the California Mastitis Test (CMT) (ImmuCell® CMT, Portland, USA). Although the CMT was originally developed and calibrated for bovine milk, and its diagnostic performance in goats may be affected by the physiologically higher baseline somatic cell count associated with apocrine secretion in this species, it remains a rapid, low-cost and field-applicable screening tool widely used in caprine mastitis studies (Paape *et al.,* 2001; Contreras *et al.,* 2007). In this study, CMT-positive results were systematically confirmed by bacteriological culture, thereby limiting the impact of reduced specificity on the identification of true infection status. The CMT reagent, consisting mainly of an alkyl aryl sulphonate detergent, lyses the somatic cells present in the milk and releases their DNA. The released DNA then forms a viscous gel, the consistency of which is proportional to the somatic cell count. The higher the somatic cell count, the thicker and more viscous the gel formed (Verbeek *et al.,* 2008). It should be noted that, in goats, somatic cell counts are influenced not only by intramammary infection but also by several non-infectious factors, including parity, stage of lactation, season and milk yield; consequently, elevated somatic cell counts cannot be interpreted as a direct indicator of mastitis without accounting for these physiological factors (Paape *et al.,* 2001; Contreras *et al.,* 2007).

After cleaning and disinfecting the teats with 70° alcohol, the first two to three spurts of milk were discarded. Approximately 2 mL of milk was collected from each half-udder into each well of the CMT tray, followed by the addition of an equal volume of CMT reagent. The mixture was swirled in a circular motion for 10 to 30 seconds, and the result was independently interpreted visually by the same two trained observers throughout the study, using double independent reading, according to the scale set out by Quinn *et al.,* 2011. (Table 1).

For this study, SCM was operationally defined by the CMT criterion: a goat was considered to be positive for subclinical mastitis (SCM) if at least one of its teats tested CMT ≥ 1 (+). This CMT-based case definition reflects an

Table 1. Interpretation table for the California Mastitis Test (CMT) according to Quinn *et al.,* 2011.

CMT score	Reaction result	Reaction description	Interpretation
Negative (−)	Homogeneous, fluid mixture with no gel formation	No visible thickening or gel formation	No SCM
Trace (T)	Slight, transient viscosity	Slight viscosity that disappears rapidly	Doubtful
Positive 1 (+)	Slight gel formation	Light and discrete gel formation	Mild SCM
Positive 2 (++)	Clear gel formation	Distinct and persistent gel formation	Moderate SCM
Positive 3 (+++)	Thick, compact gel	Thick, compact gel that is difficult to move	Severe SCM

Note: Results corresponding to the "trace" score were considered negative and were not included in the calculation of the prevalence of subclinical mastitis (SCM). An animal was considered positive for SCM when at least one of the two udder halves had a positive 1 CMT score (≥1+). For animals showing different CMT scores between the two udder halves, the highest score was used for the animal-level classification. The prevalence of subclinical mastitis is calculated using the following formula: Prevalence (%) = (Number of infected samples / Number of samples tested) × 100 %.

elevated somatic cell count and does not by itself constitute a bacteriological diagnosis. The prevalence of subclinical mastitis is calculated using the following formula: Prevalence (%) = (Number of infected samples / Number of samples tested) × 100%

Collection of milk samples

For each animal with a positive CMT test result (≥ +), a single milk sample was collected per animal from the CMT-positive teat(s), pooled across teats where more than one tested positive. Milk samples were collected under strictly aseptic conditions. After re-disinfecting the teats, the initial milk flow was discarded, and approximately 10 mL of milk was collected into sterile, single-use vials that had been previously identified and labelled. The samples were stored at +4 °C in an insulated cool box and transported to the laboratory within 24 hours for bacteriological analysis, in accordance with current guidelines (Hogan *et al.,* 1999; Mishra *et al.,* 2018).

Isolation and identification of bacterial agents

In the laboratory, the milk samples were homogenised and then subjected to successive decimal dilutions in a sterile physiological solution (0.85% NaCl), in accordance with the general principles for the preparation of suspensions and dilutions for microbiological analyses (ISO 6887-1, 2017). Dilutions ranging from 10^{-2} to 10^{-4} were prepared in order to obtain plates with a countable number of colonies. One hundred microlitres (100 µL) of each dilution were then inoculated in triplicate using the surface spread technique onto the appropriate selective and differential agar media. The plates were incubated under conditions specific to each bacterial group. Milk samples were subjected to microbiological culture on appropriate media, with particular attention given to the search for the major bacterial agents reported in mammary infections of small ruminants. The microorganisms retained were *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes* and *Salmonella* spp.

Staphylococci, notably *S. aureus* and S*. epidermidis*, were isolated on Chapman's agar (Mannitol Salt Agar, MSA) and incubated at 37°C for 24. On this medium, *S. aureus*, which generally ferments mannitol, is characterised by yellow colonies accompanied by a yellow discolouration of the medium, whilst *S. epidermidis*, which generally does not ferment mannitol, produces whitish to pinkish colonies without any noticeable change in the colour of the medium. *Escherichia coli* was isolated on Endo agar and incubated at 37°C for 24 hours. The presumptive colonies were reddish, sometimes with a metallic sheen characteristic of lactose fermentation. *Klebsiella pneumoniae* and *Klebsiella aerogenes* were isolated on Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 hours. *K. pneumoniae* generally produces large, mucoid colonies, ranging from pink to purplish in colour, whilst *K. aerogenes* may form brownish to pinkish mucoid colonies, often with a darker centre. *Salmonella* spp. was isolated on Salmonella-Shigella (SS) agar and incubated at 37°C for 24 hours. Presumptive *Salmonella* colonies were generally colourless or translucent, with a black centre associated with the production of hydrogen sulphide (H_2S) (Holt *et al.,* 2000; Vyletělová *et al.,* 2011; Neyaz *et al.,* 2026).

All colonies exhibiting cultural characteristics compatible with the target bacteria were subcultured onto appropriate media to obtain pure cultures and were identified phenotypically at the species level using biochemical tests and API identification systems. The tests carried out included testing for catalase, coagulase and oxidase; glucose fermentation; gas production; motility; lactose and mannitol fermentation; as well as testing for H_2S production, urease, citrate utilisation, indole production and esculin hydrolysis. The test kits used were bioMérieux API 20E, 2010 and API Staph, 2009 for enterobacteria and staphylococci respectively, in accordance with the manufacturer's instructions. The procedures were carried out under aseptic conditions.

Antimicrobial susceptibility testing

After biochemical identification, the bacterial isolates were streaked onto slanted Plate Count Agar (PCA) and incubated at 37°C for 24 hours. Pure colonies were then picked using a sterile loop and resuspended in 10 mL of sterile saline solution (0.85% NaCl). The bacterial suspension was homogenised using a vortex mixer and then adjusted to a turbidity equivalent to the 0.5 McFarland standard, corresponding to a concen-tration of approximately 1 × 10^8 CFU/mL. Turbidity was verified by measuring the optical density at 625 nm using a DR/2800 spectrophotometer. The susceptibility of the isolates to antimicrobials was determined using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) M100. The antibiotics tested belonged to the following classes: β-lactams, fluoroquinolones/quinolones, aminoglycosides, tetracyclines and folate pathway antagonists: ciprofloxacin (CIP, 5 µg), meropenem (MEM, 10 µg), ceftazidime (CAZ, 30 µg), tetracycline (TE, 30 µg), amoxicillin/clavulanic acid (AMC, 20/10 µg), ceftriaxone (CRO, 30 µg), aztreonam (ATM, 30 µg), cefotaxime (CTX, 30 µg), gentamicin (CN, 10 µg), ertapenem (ETP, 10 µg), cefoxitin (FOX, 30 µg), nalidixic acid (NA, 30 µg), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg) and cefepime (FEP, 30 µg). The antibiotics tested were selected on the basis of their importance in veterinary medicine, their use in the treatment of mastitis in ruminants and their relevance to the monitoring of antibiotic resistance (Virdis *et al.,* 2010; CLSI M100, 2021; Kabui *et al.,* 2024).

After incubation at 37°C for 18 to 24 hours, the diameters of the inhibition zones were measured in millimetres and interpreted according to CLSI VET01S breakpoints (6th ed., 2023) as the primary veterinary reference, with CLSI M100 (2021) applied secondarily for antimicrobial agents without established veterinary breakpoints (Table 2).

The reference strain *Escherichia coli* ATCC 25922 was used as a quality control strain for antimicrobial susceptibility testing. The *Klebsiella pneumoniae* strain ATCC 700603 was used as a positive control for the phenotypic detection of extended-spectrum β-lactamase (ESBL) production.

Statistical analysis

The data were entered into Microsoft Excel 2016. The prevalence of subclinical mastitis and the frequencies of bacterial isolation were expressed as proportions with their 95% confidence intervals calculated using the Wilson score method (Wilson, 1927), implemented manually using the Wilson score formula in Microsoft Excel. Susceptibility profiles were interpreted as susceptible (S), intermediate (I) or resistant (R) according to CLSI VET01S breakpoints (CLSI VET01S, 2023), with CLSI M100 (2021) criteria applied for antimicrobial agents not covered by VET01S. Given the exploratory nature of the study and the limited sample size, the results were interpreted as descriptive findings specific to the sampled population.

RESULTS

Prevalence of subclinical mastitis

A total of 119 lactating crossbred goats underwent the California Mastitis Test (CMT). Of these, 91 (76.47%) tested negative, whilst 28 (23.53%) tested positive, indicating an overall prevalence of subclinical mastitis of 23.53% (95% CI: 16.8 - 31.9%). The prevalence varied according to the sampling sites. The Markets appeared to have the highest frequencies, with 37.50% at the Huitième market and 38.46% at the Mvog-Ada market. In contrast, the farms at Mbalmayo, Ombessa, Nkolbisson and Obala showed relatively low prevalences (6.67%, 9.09%, 18.18% and 20.00% respectively) (Table 3).

Isolated microorganisms

Biochemical identification

The bacterial isolates were identified phenotypically at the species level using culture characteristics and a battery of 13 biochemical tests. The results are presented in Table 4.

Frequency of bacterial isolate recovery

All 28 CMT-positive samples yielded bacterial growth (28/28); several isolates belonging to different species could be obtained from a single milk sample, each isolate being identified separately based on distinct morphological, cultural and/or biochemical characteristics. A total of 102 bacterial isolates belonging to 6 different species were isolated from the 28 milk samples that tested positive in the CMT (Table 5). The percentages reported below are calculated using the total number of bacterial isolates (n = 102) as the denominator, and therefore represent the proportion of each species among all isolates. *S. aureus* was the most common species isolated, with 22 isolates (21.57%), followed by *K. pneumoniae* (20 isolates; 19.61%), *S. epidermidis* and *E. coli* (18 isolates each; 17.65%), *K. aerogenes* (16 isolates; 15.69%), and *Salmonella* spp. (8 isolates, 7.84 %). Enterobacteria (Gram-negative bacteria) accounted for 60.78 % (62/102) of the isolates, whilst Gram-positive bacteria accounted for 39.22 % (40/102).

Antimicrobial resistance profiles of pathogens associated with subclinical mastitis

A total of 102 bacterial isolates collected from milk samples taken from goats with subclinical mastitis, belonging to six

Table 2. CLSI veterinary breakpoints for interpretation of antimicrobial susceptibility testing by disk diffusion in bacterial isolates from dairy goats, class, CLSI source and justification for inclusion.

Antimicrobial (disk content)	Class	Enterobacteriaceae (*Klebsiella*, *E. coli*, *Salmonella*)	*Staphylococcus aureus*	Coagulase-negative staphylococci (*S. epidermidis*)	CLSI source	Justification for inclusion
Ciprofloxacin (5 µg)	Fluoroquinolone	S ≥ 26; I = 22–25; R ≤ 21	S ≥ 21; I = 16–20; R ≤ 15	S ≥ 21; I = 16–20; R ≤ 15	VET01S	First-line fluoroquinolone; WHO critical antimicrobial for AMR surveillance
Meropenem (10 µg)	Carbapenem	S ≥ 23; I = 20–22; R ≤ 19	N/A	N/A	M100	Carbapenemase sentinel; not a veterinary treatment option (One Health surveillance)
Ceftazidime (30 µg)	3rd-generation cephalosporin	S ≥ 21; I = 18–20; R ≤ 17	N/A	N/A	VET01S	ESBL screening agent (CLSI)
Tetracycline (30 µg)	Tetracycline	S ≥ 15; I = 12–14; R ≤ 11	S ≥ 19; I = 15–18; R ≤ 14	S ≥ 19; I = 15–18; R ≤ 14	VET01S	First-line, low-cost antimicrobial in small ruminants
Amoxicillin–clavulanic acid (20/10 µg)	β-lactam/β-lactamase inhibitor combination	S ≥ 18; I = 14–17; R ≤ 13	N/A	N/A	VET01S	Common veterinary agent; loss of potentiation indicates ESBL/AmpC
Ceftriaxone (30 µg)	3rd-generation cephalosporin	S ≥ 23; I = 20–22; R ≤ 19	N/A	N/A	M100	ESBL screening agent; WHO highest-priority critical antimicrobial
Aztreonam (30 µg)	Monobactam	S ≥ 21; I = 18–20; R ≤ 17	N/A	N/A	VET01S	ESBL screening agent (AmpC-resistant, ESBL-hydrolysed)
Cefotaxime (30 µg)	3rd-generation cephalosporin	S ≥ 26; I = 23–25; R ≤ 22	N/A	N/A	VET01S	ESBL screening agent (CLSI), complements ceftazidime
Gentamicin (10 µg)	Aminoglycoside	S ≥ 15; I = 13–14; R ≤ 12	S ≥ 15; I = 13–14; R ≤ 12	S ≥ 15; I = 13–14; R ≤ 12	VET01S	First-line injectable for veterinary mastitis treatment
Ertapenem (10 µg)	Carbapenem	S ≥ 22; I = 19–21; R ≤ 18	N/A	N/A	M100	Carbapenemase sentinel; not a veterinary treatment option
Cefoxitin (30 µg)	Cephamycin	S ≥ 18; I = 15–17; R ≤ 14	S ≥ 22; R ≤ 21 *(no intermediate category)*	S ≥ 25; R ≤ 24 *(no intermediate category)*	VET01S	Surrogate marker for mecA and AmpC resistance
Nalidixic acid (30 µg)	Quinolone (1st generation)	S ≥ 19; I = 14–18; R ≤ 13	N/A	N/A	M100	Screening marker for reduced fluoroquinolone susceptibility
Trimethoprim–sulfamethoxazole (1.25/23.75 µg)	Folate pathway inhibitor combination	S ≥ 16; I = 11–15; R ≤ 10	S ≥ 16; R ≤ 10 *(no intermediate category)*	S ≥ 16; R ≤ 10 *(no intermediate category)*	VET01S	First-line, low-cost veterinary antimicrobial in Cameroon
Cefepime (30 µg)	4th-generation cephalosporin	S ≥ 25; I = 19–24; R ≤ 18	N/A	N/A	M100	Distinguishes ESBL from AmpC β-lactamase production

Note: breakpoints were drawn from CLSI VET01S (6th ed., 2023) where species/antimicrobial-specific veterinary breakpoints were available, and from CLSI M100 (2021) otherwise; the specific CLSI source document was verified for each antimicrobial-organism combination listed above. S = sensitivity threshold (≥); R = resistance threshold (≤); values between the two = Intermediate (I); N/A = Not applicable, as the antimicrobial was not tested/recommended for this organism; inhibition zone diameters (mm).

taxa (*Klebsiella aerogenes* (n = 16), *Klebsiella pneumoniae* (n = 20), *Escherichia coli* (n = 18), *Salmonella* spp. (n = 8), *Staphylococcus epidermidis* (n = 18) and *Staphylococcus aureus* (n = 22)) were tested for their susceptibility to antimicrobials. The Gram-negative isolates were tested against a panel of 14 antimicrobial agents, and the staphylococcal isolates were tested against a panel of five agents. The proportions of susceptible (S), intermediate (I) and resistant (R) strains for each species/genus are summarised in Tables 6 and 7.

Table 3. Results of the California Mastitis Test (CMT) and prevalence of subclinical mastitis in 119 lactating crossbred goats

Study site	Number of farms	Herd size	Number of lactating goats examined	CMT-negative n (%)	CMT-positive n (%)	95% CI
Nkolbisson	13	5–10	22	18 (81.82)	4 (18.18)	7.3–38.5
Ombessa	5	6–16	22	20 (90.91)	2 (9.09)	2.5–27.8
Obala	5	5–8	10	8 (80.00)	2 (20.00)	5.7–51.0
Mbalmayo	7	4–10	15	14 (93.33)	1 (6.67)	1.2–29.8
Huitième Market	–	–	24	15 (62.50)	9 (37.50)	21.2–57.3
Mvog-Ada Market	–	–	26	16 (61.54)	10 (38.46)	22.4–57.5
Total	30	–	119	91 (76.47)	28 (23.53)	16.8–31.9

Table 4. Biochemical profiles of the six isolated bacterial species.

Biochemical tests	*E. coli* (n=18)	*Salmonella* spp. (n=8)	*K. pneumoniae* (n=20)	*K. aerogenes* (n=16)	*S. aureus* (n=22)	*S. epidermidis* (n=18)
Catalase	+	+	+	+	+	+
Coagulase	−	−	−	−	+	−
Oxidase	−	−	−	−	−	−
Glucose	+	+	+	+	+	+
Lactose	+	−	+	+	−	−
H_2S	−	+	−	−	−	−
Gas production	+	+	+	+	−	−
Mannitol	+	+	+	+	+	−
Motility	+	+	−	+	−	−
Citrate	−	+	+	+	−	−
Urease	−	−	+	−	+	+
Indole	+	−	−	−	−	−
Esculin	−	−	+	+	−	−

(+: positive reaction in 90–100 per cent of strains; −: negative reaction in 90–100 per cent of strains).

Table 5. Frequency distribution of bacterial isolates identified from milk samples.

Bacterial species	Number of isolates	Frequency (%)	Gram stain
S. aureus	22	21.57	Gram-positive
S. epidermidis	18	17.65	Gram-positive
K. pneumoniae	20	19.61	Gram-negative
K. aerogenes	16	15.69	Gram-negative
E. coli	18	17.65	Gram-negative
Salmonella spp.	8	7.84	Gram-negative
Total	102	100.00	—

Both *Klebsiella* taxa exhibited a resistance profile consistent with the production of extended-spectrum β-lactamases (ESBLs): resistance to ceftazidime reached 100% in *K. aerogenes* and *K. pneumoniae*, as did resistance to cefepime in both species (10%), whilst resistance to cefotaxime and aztreonam was 81% in *K. aerogenes* and 100% in *K. pneumoniae*. Resistance to ceftriaxone was also high (87% and 100 %, respectively). The results also revealed resistance to amoxicillin-clavulanic acid in both species (75% and 80%), which is consistent with an ESBL-type mechanism. Furthermore, both species remained fully susceptible to gentamicin (100% S) and largely susceptible to tetracycline and ciprofloxacin (100 % S in *K. aerogenes*; 80 % S in *K. pneumoniae*). Only *K. aerogenes* was resistant to trimethoprim-sulfamethoxazole (100 % R). The *Escherichia coli* isolates exhibited the broadest cephalosporin/cephamycin resistance profile within the

Table 6. Antibiotic susceptibility profiles of Gram-negative isolates (Percentages (absolute numbers)) from the milk of crossbred dairy goats in the forest zone of Cameroon.

Antimicrobial agent	*Klebsiella aerogenes (n=16)*			*Klebsiella pneumoniae (n=20)*			*Escherichia coli (n=18)*			*Salmonella* spp. *(n=8)*		
	%S	%I	%R	%S	%I	%R	%S	%I	%R	%S	%I	%R
CIP	**100** (16)	0 (0)	0 (0)	80 (16)	20 (4)	0 (0)	39 (7)	39 (7)	22 (4)	0 (0)	75 (6)	25 (2)
MEM	**100** (16)	0 (0)	0 (0)	**100** (20)	0 (0)	0 (0)	**100** (18)	0 (0)	0 (0)	75 (6)	0 (0)	25 (2)
CAZ	0 (0)	0 (0)	**100** (16)	0 (0)	0 (0)	**100** (20)	0 (0)	0 (0)	**100** (18)	0 (0)	0 (0)	**100** (8)
TE	**100** (16)	0 (0)	0 (0)	80 (16)	20 (4)	0 (0)	78 (14)	22 (4)	0 (0)	25 (2)	37 (3)	38 (3)
AMC	25 (4)	0 (0)	**75** (12)	0 (0)	20 (4)	**80** (16)	0 (0)	22 (4)	**78** (14)	0 (0)	62 (5)	38 (3)
CRO	13 (2)	0 (0)	**87** (14)	0 (0)	0 (0)	**100** (20)	0 (0)	0 (0)	**100** (18)	0 (0)	0 (0)	**100** (8)
ATM	0 (0)	19 (3)	**81** (13)	0 (0)	0 (0)	**100** (20)	0 (0)	0 (0)	**100** (18)	25 (2)	0 (0)	**75** (6)
CTX	0 (0)	19 (3)	**81** (13)	0 (0)	0 (0)	**100** (20)	0 (0)	0 (0)	**100** (18)	0 (0)	0 (0)	**100** (8)
CN	**100** (16)	0 (0)	0 (0)	**100** (20)	0 (0)	0 (0)	**100** (18)	0 (0)	0 (0)	25 (2)	75 (6)	0 (0)
ETP	**100** (16)	0 (0)	0 (0)	80 (16)	20 (4)	0 (0)	72 (13)	28 (5)	0 (0)	75 (6)	25 (2)	0 (0)
FOX	25 (4)	50 (8)	25 (4)	20 (4)	80 (16)	0 (0)	0 (0)	0 (0)	**100** (18)	0 (0)	0 (0)	**100** (8)
NA	19 (3)	81 (13)	0 (0)	80 (16)	0 (0)	20 (4)	0 (0)	100 (18)	0 (0)	25 (2)	0 (0)	**75** (6)
SXT	0 (0)	0 (0)	**100** (16)	20 (4)	40 (8)	40 (8)	72 (13)	0 (0)	28 (5)	75 (6)	0 (0)	25 (2)
FEP	0 (0)	0 (0)	**100** (16)	0 (0)	0 (0)	**100** (20)	0 (0)	0 (0)	**100** (18)	0 (0)	0 (0)	**100** (8)

CIP: ciprofloxacin; MEM: meropenem; CAZ: ceftazidime; TE: tetracycline; AMC: amoxicillin–clavulanic acid; CRO: ceftriaxone; ATM: aztreonam; CTX: cefotaxime; CN: gentamicin; ETP: ertapenem; FOX: cefoxitin; NA: nalidixic acid; SXT: trimethoprim–sulfamethoxazole; FEP: cefepime; I: intermediate; S: susceptible; R: resistant.

Gram-negative group, with complete resistance (100%) to ceftazidime, ceftriaxone, aztreonam, cefotaxime, cefoxitin and cefepime. Furthermore, the susceptibility of *Escherichia coli* to meropenem and gentamicin remained complete (100% S) and decreased slightly for tetracycline, ertapenem and trimethoprim-sulfamethoxazole (78%, 72% and 72% S). Isolates of *Salmonella* spp. exhibited the same resistance profile to broad-spectrum cephalosporins/cephamycins, with 100% resistance to ceftazidime, ceftriaxone, cefotaxime, cefoxitin and cefepime, and 75% sensitivity to meropenem, ertapenem and trimethoprim-sulfamethoxazole (Table 6). Resistance to cefoxitin was detected at a high frequency in both staphylococcal taxa, reaching 100% in *S. epidermidis* and 77% in *S. aureus*. Resistance to tetracycline was complete in *S. aureus* (100% R). Susceptibility to ciprofloxacin and gentamicin (100% S) was complete for both species and relatively lower against trimethoprim-sulfamethoxazole (78% S and 68% S for *S. epidermidis* and *S. aureus*, respectively) (Table 7).

DISCUSSION

Prevalence of subclinical mastitis

The overall prevalence of subclinical mastitis (SCM) observed in this study (23.53%, 95 % CI: 16.8 - 31.9%) falls within the range reported in other African studies on dairy goats, where prevalence generally varies between 15% and 45% depending on the screening method (Contreras *et al.,* 2003; Tibebu *et al.,* 2026). These results are similar to the prevalence rates recorded in the Barbari and Jamunapari breeds in India (24.21% and 15.12%, respectively) (Mishra *et al.,* 2018), but lower than those documented in Nigeria (40.4%) (Oladele *et al.,* 2019) and in Kenya (84.7%) (Kabui *et al.,* 2024). The lower prevalence observed may be related to differences in management systems characterised by small herds and lower stocking densities, which are likely to limit contact between animals and, consequently, the transmission of certain pathogens responsible for mastitis (Tibebu *et al.,* 2026).

The marked spatial variation observed between rural farms (6.67 - 0.00%) and markets (37.50 - 38.46%) could be explained by a combination of several factors related to the environment. Firstly, the transport and grouping of animals from different

Table 7. Antibiotic susceptibility profiles of Staphylococcus epidermidis and Staphylococcus aureus (Percentages (absolute numbers) isolated from the milk of crossbred dairy goats in the forest zone of Cameroon.

Antimicrobial agent	*Staphylococcus epidermidis (n=18)*			*Staphylococcus aureus (n=22)*		
	%S	%I	%R	%S	%I	%R
Ciprofloxacin	**100** (18)	0 (0)	0 (0)	**100** (22)	0 (0)	0 (0)
Tetracycline	22 (4)	39 (7)	39 (7)	0 (0)	0 (0)	**100** (22)
Gentamicin	**100** (18)	0 (0)	0 (0)	**100** (22)	0 (0)	0 (0)
Cefoxitin (marker for mecA-mediated resistance)	0 (0)	0 (0)	**100** (18)	23 (5)	0 (0)	**77** (17)
Trimethoprim-sulfamethoxazole	78 (14)	0 (0)	22 (4)	68 (15)	0 (0)	32 (7)

I = intermediate, S = susceptible, R = resistant.

herds can be sources of stress and increase contact between individuals, thereby facilitating the spread of infectious agents (Ekwem *et al.,* 2021). Secondly, housing conditions, hygiene and milking practices are important factors in the occurrence of mastitis in small ruminants (Mekonnen *et al.,* 2006; Tibebu *et al.,* 2026). Indeed, available studies show that herd structure, housing conditions and husbandry practices are associated with the risk of mastitis (Mekonnen *et al.,* 2006; Jabbar *et al.,* 2020). Finally, the gathering of animals of different origins at marketing centres could increase the likelihood of contact with infected animals or asymptomatic carriers (Tibebu *et al.,* 2026; Jabbar *et al.,* 2020).

Frequency of bacterial species isolation

The predominance of *Staphylococcus aureus* (21.57%) and staphylococci (39.22% of isolates, comprising *S. aureus* and *S. epidermidis*) is consistent with several studies on subclinical mastitis in goats. Staphylococci, particularly S. aureus and coagulase-negative staphylococci (CNS), are among the main microorganisms responsible for intramammary infections in goats (Moroni *et al.,* 2005; Contreras *et al.,* 2007). This predominance of staphylococci has also been reported on African dairy farms, particularly in Rwanda (Ndahetuye *et al.,* 2019).
However, the high proportion of Enterobacteriaceae observed in our study (60.78% of isolates) is a significant finding. This high proportion could therefore be linked to significant exposure of the animals to environmental sources of contamination. These bacteria are generally associated with the environment, and their presence may be facilitated by poor hygiene conditions, particularly in terms of housing and milking practices. Studies carried out in Africa have shown that housing conditions and milking practices can influence the risk of mastitis (Abera *et al.,* 2012; Mahlangu *et al.,* 2018).

The detection of *Salmonella* spp. in the milk of clinically asymptomatic goats, although a minority (7.84%), is of particular public health significance given the zoonotic potential of this genus and the risk of contamination of the dairy food chain in the absence of pasteurisation – an issue already highlighted in similar contexts of informal milk trade in West and Central Africa (Grace, 2015; Farouk *et al.,* 2022).

Antimicrobial resistance profiles of pathogens associated with subclinical mastitis

Both *Klebsiella* species (*K. aerogenes* and *K. pneumoniae*) exhibited a phenotype strongly suggestive of extended-spectrum β-lactamase (ESBL) production, characterised by almost total resistance to ceftazidime (100%), cefepime (100%), cefotaxime (81 - 100%) and aztreonam (81 - 100%), combined with resistance to amoxicillin-clavulanic acid (75 - 80%). This co-phenotype (resistance to third- and fourth-generation cephalosporins + resistance to the amoxicillin-clavulanic acid combination) is consistent with the phenotypic screening criteria for ESBLs recommended by CLSI M100, 2021, and has been reported at comparable frequencies in *Klebsiella* isolates of animal origin in sub-Saharan Africa, particularly in cattle and goat farms in Nigeria and Ethiopia (Oyebanji *et al.,* 2023; Gebremeskel *et al.,* 2023).

The sensitivity to gentamicin (100% S) observed in both species (*K. aerogenes* and *K. pneumoniae*) is a favourable therapeutic outcome. This profile was also observed by Founou *et al.* in ESBL-producing isolates of porcine origin, in a multicentre comparative study between Cameroon and South Africa (Founou *et al.,* 2019). Nevertheless, this susceptibility to aminoglycosides should not be interpreted as long-lasting, as the co-selection of aminoglycoside resistance genes on the same IncF plasmids carrying the *bla*CTX-M genes is frequently reported (Carattoli, 2013; Altayb *et al.,* 2022). Isolates of *E. coli* and *Salmonella* spp. exhibited the broadest cephalosporin/cephamycin resistance profile in the study, with 100% resistance to ceftazidime, ceftriaxone, aztreonam, cefotaxime, cefoxitin and cefepime. The observed cefoxitin resistance may be compatible with mechanisms such as AmpC production; however, the specific resistance mechanism was not determined in this study. A similar association has been described in *E. coli* isolates from food of animal and plant origin (Njage and Buys, 2015; Boz *et al.,* 2026). This resistance phenotype, which may be consistent with ESBL- and/or AmpC-mediated mechanisms, warrants

molecular investigation and is, in the meantime, a cause for concern from a One Health perspective, as these strains may constitute a reservoir of resistance genes transferable to the human commensal flora through direct contact or the consumption of unpasteurised milk (Van Boeckel *et al.,* 2015).

In our study, no resistance to meropenem was observed in *E. coli* (100% S), whilst 75% of *Salmonella* spp. isolates were susceptible to this antibiotic. These results suggest that, in the isolates studied, phenotypic resistance to carbapenems remains limited. Resistance to carbapenems currently appears to be relatively limited among bacteria of animal origin in Africa, although specific data on small ruminants remain scarce. In Cameroon, a study conducted in Bamenda reported an overall susceptibility of 89.9% to imipenem and 88.9% to meropenem among the Enterobacteriaceae studied, whilst 6.7% of isolates exhibited resistance to carbapenems (Bissong *et al.,* 2023). Meropenem and ertapenem were included in the antimicrobial panel not as candidate veterinary treatment options but as sentinel agents for the phenotypic surveillance of carbapenemase-producing Enterobacteriaceae ; carbapenems are not authorised for use in veterinary medicine, and their use is explicitly discouraged under antimicrobial stewardship principles (EFSA and ECDC, 2023).The low levels of resistance observed in this study would therefore constitute a favourable outcome, given the clinical importance of carbapenems and the potential for the spread of resistance genes from a One Health perspective (Nordmann *et al.,* 2012).

Resistance to trimethoprim-sulfamethoxazole was particularly high in *K. aerogenes* (100% R), whilst lower proportions were observed in *E. coli* and *Salmonella* spp. (72% S and 75% S, respectively). Trimethoprim-sulfamethoxazole remains widely used in veterinary medicine on farms with limited resources due to its low cost (Ho and Juurlink, 2011), and increasing resistance to this compound has been linked to the spread of the *sul1/sul2* and *dfrA* genes carried by class 1 integrons, which are often co-localised with β-lactam resistance genes on the same mobile genetic elements (Ambrose and Hall, 2021).

In both staphylococcal taxa, resistance to cefoxitin – used as a phenotypic surrogate marker for *mecA*-mediated resistance in accordance with the CLSI M100 2021 guidelines - reached 100% in *S. epidermidis* and 77% in *S. aureus*. These proportions are high compared with data reported for staphylococcal isolates of caprine origin in East Africa and North Africa, where the prevalence of methicillin-resistant strains (MRSA/MRS) ranged from 15 to 45% (El-Ashker *et al.,* 2022; Dilek *et al.,* 2019). Furthermore, molecular confirmation (detection of the *mecA* or *mecC* gene by PCR) would be required before any definitive conclusions can be drawn regarding the actual prevalence of methicillin-resistant staphylococci in this herd, as cefoxitin may, in rare cases, produce false positives in the presence of other intrinsic resistance mechanisms (CLSI M100, 2021; Tluanpuii *et al.,* 2025).

Complete resistance to tetracycline in *S. aureus* (100% R) is also noteworthy and consistent with the widespread use of this compound in goat farming as a first-line antibiotic due to its low cost and availability (Muwonge *et al.,* 2025). The observed in vitro susceptibility to ciprofloxacin and gentamicin (100% S) in both species suggests that these agents could retain their activity against the isolates tested, offering a relevant alternative treatment option that should be based on veterinary clinical assessment, applicable regulations, withdrawal periods and antimicrobial stewardship principles, particularly given that fluoroquinolone use is already restricted under several regional regulatory frameworks (WHO, 2019).

Public health implications

Taken together, these results suggest the presence, within the limited dairy goat population sampled in this preliminary study (n = 119, 30 farms and 2 markets), of broad-spectrum resistance mechanisms in enterobacteria of zoonotic importance (*E. coli, Salmonella* spp., *Klebsiella* spp.) and potentially methicillin-resistant staphylococci. These findings should not be extrapolated to Cameroon's dairy goat production system as a whole. Although this pattern is consistent with the One Health conceptual framework, which identifies small ruminant dairy farms as potential interfaces between animal health, human health and food safety, particularly through the consumption of raw or inadequately processed milk (Robinson *et al.,* 2016; McEwen and Collignon, 2018). However, no epidemiological or molecular evidence of actual transmission between animals, the environment and humans was generated in this study; further investigations, including strain typing and cross-sectoral sampling, would be needed to confirm any such risk. These findings nonetheless support the need to strengthen surveillance of antibiotic resistance across goat production chains in Cameroon’s forested areas, in line with the priorities of Cameroon’s National Action Plan to Combat Antimicrobial Resistance (MINSANTE, 2024).

Limitations of the study

This study has several limitations. Firstly, its exploratory and cross-sectional nature, the absence of a formal sample size calculation, and the use of convenience sampling limit the representativeness of the results and their generalisation to the entire crossbred goat population of Cameroon's forest zone. In addition, the clustering of animals within farms was not accounted for in the precision of prevalence estimates, and the limited sample size did not allow for a robust multivariate analysis of risk factors. Secondly, bacterial identification relied primarily on phenotypic methods, and resistance mechanisms potentially associated with ESBL or mecA were not

confirmed by molecular analyses. Furthermore, the predefined bacterial panel did not allow full coverage of the agents potentially associated with subclinical mastitis. Thirdly, the use of the CMT as a screening tool should be interpreted with caution in goats, given the physiological variations in somatic cell count observed in this species. Finally, the relatively limited number of CMT-positive animals (n = 28) and isolates per taxon (8-22), together with the absence of detailed data on prior antimicrobial exposure, call for cautious interpretation of the bacterial distributions and resistance profiles, which should be regarded as indicative rather than definitive. Analytical studies with larger samples and molecular approaches will be needed to confirm these observations and to better characterise the factors associated with subclinical mastitis and antimicrobial resistance.

Conclusion

This exploratory study identified CMT-defined subclinical mastitis among crossbred dairy goats sampled in Cameroon's forest zone, with a higher observed prevalence at the two markets than on the sampled farms (23.53% overall). Staphylococci and Enterobacteriaceae dominate the bacterial aetiology, with concerning resistance profiles phenotypically suggestive of ESBL-associated resistance and methicillin-resistant staphylococcal phenotypes, as well as high resistance to tetracycline and trimethoprim-sulfamethoxazole. Although these results are exploratory, they highlight the importance of improving milking hygiene, rationalising the use of antimicrobials, and integrating the dairy goat sector into national antibiotic resistance surveillance programmes.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted in accordance with relevant institutional guidelines and regulations. All experimental procedures involving animals and farm data were reviewed and approved by the Research Ethics Committee of the Institute for Agricultural Research for Development (IRAD) in Cameroon (approval reference No.: 00242/IRAD.CRA-MB/CAS/SAAF/04/2026). Prior to sample collection and use of farm data, informed verbal consent was obtained from all farm owners, who were fully briefed on the objectives and procedures of the study.

COMPETING INTERESTS

The authors have no competing interests.

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