Original Article

Comprehensive Diagnosis and Profiling of Antimicrobial Resistance Genes and Mechanisms in Mastitis-causing Bacteria in Ewes Using the VITEK 2 System

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Abstract

Background: Ovine mastitis is a significant problem for animal and dairy productivity, with antimicrobial resistance (AMR) complicating treatment options. **Objective:** This study identified the main bacterial pathogens and their AMR genes in ovine mastitis. **Materials and Methods:** Using the VITEK 2 system, 352 milk samples were cultured, and the isolates were tested for susceptibility using antibiotic sensitivity test-gram negative and antibiotic sensitivity test-gram positive (AST-GN and AST-P) cards. **Results:** The findings revealed a diverse bacterial profile in cases of ovine mastitis. *Staphylococcus aureus* and coagulase-negative staphylococci, particularly *Staphylococcus epidermidis* and *Staphylococcus chromogenes*, were the most common isolates. The high prevalence of methicillin-resistant phenotypes among staphylococci strongly indicates the presence of the mecA gene. Among Gram-negative isolates, *Escherichia coli* and *Klebsiella pneumoniae* were most frequent, showing concerning resistance patterns indicative of AmpC β-lactamase and carbapenemase production, despite being extended-spectrum beta-lactamase-negative. This confers resistance to broad-spectrum β-lactams, including third-generation cephalosporins and carbapenems. Aminoglycosides and folate pathway inhibitors were most effective against *Enterobacteriaceae*, while fluoroquinolones and folate pathway inhibitors worked best against *Staphylococcus spp*. **Conclusion:** At the end, the study concludes that highly multidrug-resistant bacteria, notably methicillin-resistant *Staphylococci* and carbapenem-resistant *Enterobacteriaceae*, cause ovine mastitis in this population. These findings highlight a serious emerging threat of difficult-to-treat mastitis infections and emphasise the urgent need for molecular surveillance of AMR genes (e.g., mecA, ampC, blaKPC) along with the implementation of robust antimicrobial stewardship programmes in sheep farming to combat the spread of resistance.

Keywords: Clinical mastitis, *mecA* gene, methicillin-resistant *Staphylococcus aureus*

Introduction

Ovine mastitis, in both subclinical and clinical forms, is a significant factor affecting animal welfare and profitability in the global sheep industry. It causes substantial economic losses due to decreased milk production, premature culling, higher veterinary costs and mortality in severe cases (1). The disease is mainly caused by various bacterial pathogens, with *Staphylococcus aureus*, *Escherichia coli*, coagulase-negative staphylococci (CNS) and *Streptococcus spp*. Being the most frequently isolated agents (2,3), antimicrobial resistance (AMR) in mastitis-causing bacteria involves multiple mechanisms, such as β-lactamase production, efflux pumps and biofilm formation. Important resistance genes such as blaZ, mecA, tetM and ermB are found across these major pathogens. The virulence genes present in these isolates suggest their potential to cause mastitis (3,4). Management of mastitis relies heavily on antimicrobial therapy; however, the widespread and indiscriminate use of antibiotics has created strong selective pressure, encouraging the development and spread of AMR among bacteria (5). This increasing resistance threatens the effectiveness of standard treatments, resulting in prolonged infections, increased treatment failures and the potential for zoonotic transfer of resistant strains to humans (6). Therefore,

Access this article online

Quick Response Code:

Website: https://journals.lww.com/ajvs

DOI: 10.4103/AJVS.AJVS_24_25

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How to cite this article: Faisal DG, Al-Kubaisi SM. Comprehensive diagnosis and profiling of antimicrobial resistance genes and mechanisms in mastitis-causing bacteria in Ewes using the VITEK 2 system. Al-Anbar J Vet Sci 2026;19:38-44.

Received: 28-08-2025, **Revised:** 09-02-2026,
Accepted: 28-02-2026, **Published:** 31-07-2026

rapid and accurate identification of the causative pathogens and their antimicrobial susceptibility profiles is crucial for effective mastitis control and antimicrobial stewardship. The objectives of this study were to: first, identify the most prevalent bacterial pathogens in Ewes' milk using the VITEK 2 system; second, determine their phenotypic antimicrobial susceptibility patterns; and finally, genetically characterise the most common resistance genes harboured by these isolates. By correlating phenotypic AST results with genotypic data, this research aims to provide a nuanced understanding of AMR in ovine mastitis pathogens, which will be vital for developing effective treatment and containment strategies.

Materials and Methods

Study area and sample collection

In Al-Fallujah city, a study was carried out involving various species of lactating Ewes aged between 1 and 6 years, from 28 August 2024, to 25 February 2025. A total of 352 milk samples were gathered in sterile tubes from both clinical and subclinical cases.

Physical examination and preparation of study Ewes' udder

A physical exam of the Ewes was done. Udder examination includes inspection and palpation; all changes were recorded on the udder and milk.

Samplening technique

As mentioned in Markey *et al.*(7), the process began with washing the udder and teats using tap water, followed by drying the udder. The teats were immersed in iodine, and the teat openings were cleaned with 70% ethanol. The initial milk streams were discarded, and midstream milk was collected in a sterile test tube. The samples were then transported to the laboratory in an icebox.

Detection of subclinical mastitis with the California mastitis test

The criteria were followed like Schalm *et al.* and Markey *et al.*(7,8) when conducting the test intended to detect subclinical mastitis. Equal amounts of milk and California reagent were carefully combined using a white plastic paddle and four containers, with 2 mL of milk and 2 mL of reagent. The following is the score for the California mastitis test (CMT): Negative: the mixture stays liquid and no precipitate forms; Trace: a small precipitate that disappears as the paddle moves farther; plus-one (+): a noticeable precipitate without gel development; plus-two (++): the mixture's thickness, indicating gel formation; plus-three (+++): distinct gel forms that agglutinate at the bottom of the paddle.

Bacterial isolation and culture conditions

Following the sampling and execution of the CMT for subclinical cases, milk samples were incubated for 4 h at 37°C before being cultured on three primary media: nutrient agar, MacConkey agar and blood agar. These plates were then incubated overnight at 37°C, purified and subcultured on mannitol and Hichrom Staphylococcus agars, with another overnight incubation at 37°C. Gram staining and essential chemical tests, such as TCTs and maltose tests, were conducted to identify mannitol-fermenting *Staphylococci* and differentiate *Staphylococcus* spp.(9-11). Key chemical tests for diagnosing *Enterobacteriaceae* include oxidase, indole, methyl red, Voges–Proskauer, citrate, urease and triple sugar iron tests. According to (7), EMB is a selective and differential medium, thus permitting only Gram-negative bacteria to grow (12).

Bacterial antimicrobial susceptibility test with VITEK2 compact system

VITEK 2 Systems Version 9.04.4 from Bio Mérieux in Germany was used. Following the manufacturer's instructions, a 12 × 75 mm clear plastic (polystyrene) test tube was filled with 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0), and a sufficient number of colonies of pure culture were transferred using a sterile swab or applicator stick (Bio Mérieux-Germany). A turbidity adjustment of 0.50 to 0.60 was made. Then, 145 µL of the Gram-negative bacterial suspension and 280 µL of the Gram-positive bacterial suspension were transferred to separate 3.0 mL sterile saline tubes for the AST test.

Data analysis

The Statistical Package for the Social Sciences (SPSS, 2019, International Business Machines Corporation (IBM)) programme was used to detect the effect of different groups on the study percentage. The Chi-square test significantly compared this study's percentages, with $P = 0.05$ and 0.01.

Results and Discussion

The analysis of the milk samples showed a clear difference in the types of mastitis present in the studied herd. Of the 352 samples collected, 274 (77.84%) indicated subclinical mastitis, while 78 (22.16%) were classified as clinical mastitis [Table 1]. Statistical analysis revealed a significant difference between subclinical and clinical mastitis ($P \leq 0.01$). The high rate of subclinical mastitis (77.84%) is especially important. This finding agrees with many previous studies that consistently show subclinical mastitis as the most common and economically damaging type of the disease because of its

Table 1: Prevalence of mastitis types in the studied herd

Bacterial isolates	Mastitis classification	No.(%)	χ^2 (*P*-value)
Subclinical mastitis	Total milk samples	274 (77.84)[a]	
	Gram-positive cocci	52 (92.86)[a]	41.14** (0.0001)
	Gram-negative bacilli	4 (7.14)[b]	
Clinical mastitis	Total milk samples	78 (22.16)[b]	
	Gram-positive cocci	24 (45.28)[b]	21.312** (0.0001)
	Gram-negative bacilli	27 (50.94)[a]	
	Gram-negative cocci	2 (3.77)[c]	

**Highly significant ($P \leq 0.01$), [a,b]Superscript letters indicate the significant differences between proportions

hidden nature (13,14). Since there are no visible signs in the milk or udder, these infections often go unnoticed by farmers, leading to longer infections, lower milk production, changes in milk composition and a constant source of infection for other animals in the herd (15,16). Although less common, the lower rate of clinical mastitis (22.16%) should not be overlooked. These cases are linked to more immediate animal welfare issues, such as painful udders, systemic illness and the need for urgent treatment. Because clinical cases are more visible, they are usually managed more quickly, which may explain why they are less prevalent than subclinical cases (17).

A total of 93 bacterial isolates were recovered from the mastitic milk samples. The distribution of bacterial species and their relative prevalence are detailed in Table 2. Statistical analysis revealed a highly significant difference in the prevalence of the various bacterial pathogens ($\chi^2 = 32.902$, $P < 0.0001$). The most predominant pathogen identified was *S. aureus*, accounting for 40.86% of all isolates, a significantly higher proportion ($P \leq 0.01$) than all other species. This was followed by *E. coli* (12.09%), *Staphylococcus epidermidis* (13.98%) and *Staphylococcus chromogenes* (13.98%), which did not show a statistically significant difference among themselves. The remaining isolates were at significantly lower frequencies, ranging from 1.06% to 6.54%. Similar to what was found using tuf gene sequencing (18).

The high prevalence of *S. aureus* is a hallmark of ovine mastitis worldwide, consistent with numerous previous studies (3,19,20). As a contagious pathogen, its dominance suggests that transmission during milking is likely a major challenge within the affected herd. This underscores the critical importance of implementing and regularly reviewing milking hygiene protocols, such as proper teat disinfection, the use of gloves by milkers and the regular maintenance of milking equipment (21,22).

Table 2: Distribution of bacterial isolates recovered from mastitic milk samples

Category	Count (%)
Enterobacteriacae	
Escherichia coli	13 (12.09)[b,c]
Klebsiella pneumoniae	6 (6.54)[c,d]
Klebsiella aerogenes	1 (1.06)[d]
Enterobacter cloacae complex	3 (3.23)[d]
Enterococcus spp.	
Enterococcus gallinarum	4 (4.30)[d]
Enterococcus facium	2 (2.15)[d]
Staphylococcus spp.	
Staphylococcus aureus	38 (40.86)[a]
Staphylococcus epidermidis	13 (13.98)[b]
Staphylococcus chromogenes	13 (13.98)[b]
Total	93 (100%)
χ^2 (*P*)	32.902** (0.0001)

**Highly significant ($P \leq 0.01$), [a,b]Superscript letters indicate the significant differences between proportions

The significant presence of Gram-negative bacteria from the *Enterobacteriaceae* family, collectively accounting for 22.92% of the isolates, suggests an environmental source of infection. Enterobacteriaceae are present but not predominant in ovine mastitis (7,13,23). These pathogens originate from contaminated bedding, soil or manure. Their prevalence suggests that managing the sheep's environment, including stall cleanliness and manure handling, is a crucial area that requires attention to reduce infection rates. The isolation of non-aureus staphylococci (NAS), specifically *S. epidermidis* and *S. chromogenes*, at a notable frequency (~28%) is a significant finding. They are often historically considered minor pathogens or contaminants. They can cause persistent subclinical infections that elevate somatic cell count and reduce milk quality (24). The very low isolation rate of Enterococci suggests that they play a relatively minor role in the aetiology of this farm. However, their presence should not be ignored due to their potential for AMR. *E. gallinarum* is rarely reported as a mastitis pathogen; *E. faecium* is often reported as the second most common *Enterococcus* species after *E. faecalis* (25,26).

The *mecA* gene was identified in all isolates of the *Staphylococcus* family, which codes for an altered penicillin-binding protein (PBP2a) with low affinity for nearly all β-lactam antibiotics (27,28). It is the basis of methicillin resistance in staphylococci, driving the global spread of methicillin-resistant *S. aureus* (MRSA). Its detection is crucial for infection control and guiding effective antibiotic therapy (29,30). AST revealed that all isolates of *S. epidermidis* (13/13), *S. chromogenes* (13/13) and *S. aureus* (38/38) were confirmed as methicillin-resistant (*Staphylococcus epidermidis* (MRSE), Methicillin-Resistant *Staphylococcus chromogene* (MRSC) and MRSA, respectively), demonstrating universal resistance to all β-lactam antibiotics (31-33). Identifying MRSA in 100% of *S. aureus* isolates is a concerning result with profound implications for both animal and public health (31,34). The resistance profiles, however, varied significantly between species. All MRSE isolates exhibited complete resistance to fusidic acid. The concurrent universal resistance to fusidic acid, an antibiotic sometimes used in topical formulations for mastitis, suggests a possible multidrug-resistant (MDR) profile. The MRSC isolates showed high resistance rates to tetracycline (84.6%) and erythromycin (92.3%). The MRSA cohort demonstrated the broadest resistance profile, with universal (100%) resistance to erythromycin and ciprofloxacin, and a high rate of resistance to gentamicin (86.8%), similar to that reported in previous studies (35-37). This high prevalence of resistance may be attributed to the random and continuous use of antibiotics. The near-uniform resistance to erythromycin is noteworthy, though the single susceptible isolate requires verification to exclude testing variability or potential contamination. The complete susceptibility to fusidic acid distinguishes these *S. chromogenes* isolates from the previously tested *S. epidermidis* strains, highlighting important species-specific differences in AMR patterns within the NAS group. All isolates across the three species remained universally susceptible to the

critical last-line antimicrobials vancomycin, teicoplanin, linezolid and tigecycline. A comparative summary of the key resistance profiles is presented in Tables 3-5. Despite the MDR profile, it offers practical alternatives for focused therapy. It is promising that medications such as trimethoprim/sulfamethoxazole (TMP-SMX), rifampicin and linezolid are 100% effective. These medications can be considered a second option. It is concerning that 23% of isolates have intermediate susceptibility to moxifloxacin. Resistance to fluoroquinolones can arise quickly. If fluoroquinolones are administered carelessly, this decreased susceptibility may indicate total resistance. The universal sensitivity to vancomycin and teicoplanin is particularly significant given that glycopeptides are still used as a last resort to treat severe, MDR Gram-positive infections. These medicines should be used sparingly after antimicrobial susceptibility testing to maintain their effectiveness (38,39).

Table 3: Antimicrobial susceptibility testing results for 13 *Staphylococcus epidermidis* isolates

Antimicrobial agent	Class	Resistance profile
Cefoxitin (Screen)	Cephalosporin	100% positive
Benzyl penicillin	Penicillin	100% resistant
Oxacillin	Penicillinase-stable penicillin	100% resistant
Fusidic acid	Fusidane	100% resistant
Moxifloxacin	Fluoroquinolone	23% intermediate, 77% susceptible
Linezolid	Oxazolidinone	100% susceptible
Rifampicin	Ansamycin	100% susceptible
Trimethoprim/Sulfamethoxazole	Folate pathway inhibitor	100% susceptible
Gentamicin	Aminoglycoside	100% susceptible
Tigecycline	Glycylcycline	100% susceptible
Clindamycin	Lincosamide	100% susceptible

Table 4: Antimicrobial susceptibility profile of *Staphylococcus chromogenes* isolates (*n*=13)

Antimicrobial agent	Class	Resistance profile
Cefoxitin (Screen)	Cephalosporin	100% Positive
Benzyl penicillin	Penicillin	100% resistant
Oxacillin	Penicillinase-stable penicillin	100% resistant
Tetracycline	Tetracycline	84.6% resistant, 15.4% intermediate
Erythromycin	Macrolide	92.3% resistant
Teicoplanin	Glycopeptide	100% susceptible
Vancomycin		100% susceptible
Linezolid	Oxazolidinone	100% susceptible
Rifampicin	Ansamycin	100% susceptible
Trimethoprim/Sulfamethoxazole	Folate pathway inhibitor	100% susceptible
Gentamicin	Aminoglycoside	100% susceptible
Tigecycline	Glycylcycline	100% susceptible
Ciprofloxacin	Fluoroquinolone	100% susceptible
Fusidic acid	Fusidane	100% susceptible

The complete effectiveness of medications such as clindamycin, tetracyclines and TMP-SMX suggests that they are suitable options for treatment. Although their usage in veterinary medicine must be highly cautious to maintain efficacy for

Table 5: Comprehensive antimicrobial susceptibility testing profile of *Staphylococcus aureus* isolates (*n*=38)

Antimicrobial agent	Class	Susceptibility profile
Cefoxitin (Screen)	Cephalosporin	100% Positive
Oxacillin	Penicillinase-stable penicillin	100% resistant
Benzyl penicillin	Penicillin	100% resistant
Piperacillin/Tazobactam	Beta-lactam/BLI	100% resistant
Erythromycin	Macrolide	100% resistant
Ciprofloxacin	Fluoroquinolone	100% resistant
Gentamicin	Aminoglycoside	86.8% resistant, 13.2% intermediate
Rifampicin	Ansamycin	94.7% susceptible, 5.3% intermediate
Nitrofurantoin	Nitrofuran	92.1% susceptible, 7.9% intermediate
Teicoplanin	Glycopeptide	100% susceptible
Vancomycin		100% susceptible
Linezolid	Oxazolidinone	100% susceptible
Tigecycline	Glycylcycline	100% susceptible
Tetracycline	Tetracycline	100% susceptible
Moxifloxacin	Fluoroquinolone	100% susceptible
Levofloxacin		100% susceptible
Clindamycin	Lincosamide	100% susceptible
Trimethoprim/Sulfamethoxazole	Folate inhibitor	100% susceptible
Fusidic acid	Fusidane	100% susceptible
Tobramycin	Aminoglycoside	100% susceptible

Table 6: Antimicrobial susceptibility profile of Klebsiella pneumonia isolates (n=6)

Antimicrobial agent	Class	Susceptibility profile
Imipenem	Carbapenem	100% resistant
Ertapenem		100% resistant
Ampicillin	Penicillin	100% resistant
Piperacillin/Tazobactam	Beta-lactam/BLI	100% resistant
Ceftazidime	3rd-gen. Cephalosporin	100% resistant
Cefoxitin*	Cephamycin	100% resistant
Nitrofurantoin	Nitrofuran	100% resistant
Cefepime	4th-gen. Cephalosporin	83.3% resistant, 16.7% intermediate
Amikacin	Aminoglycoside	100% susceptible
Gentamicin		100% susceptible
Ciprofloxacin	Fluoroquinolone	100% susceptible
Levofloxacin		100% susceptible
Tigecycline	Glycylcycline	100% susceptible
Trimethoprim/Sulfamethoxazole	Folate inhibitor	100% susceptible
Ceftriaxone	3rd-gen. Cephalosporin	100% susceptible

*Bacteria has developed a complex genetic resistance (like ESBL or MRSA)

human medicine, they an unwavering vulnerable to last-resort vancomycin. Teicoplanin is comforting for treating severe systemic infections. Only a few isolates showed intermediate susceptibility to gentamicin and rifampicin, suggesting that these medications should not be administered as monotherapies because this can develop into complete resistance (40,41).

AST Profile of *Klebsiella pneumoniae* (n = 6) in Table 6. Revealed impermeability Carba (+extended-spectrum beta-lactamase [ESBL] or + HL AmpC) Carbapenemase (+ or − ESBL): The enzyme's primary function is to hydrolyse and destroy carbapenem antibiotics efficiently. It is the most efficient mechanism for carbapenem resistance. Examples include klebsiella pneumoniae carbapenemase (KPC), New Delhi Metallo-(beta)-lactamase (NDM) and Oxacillinase-48 (OXA-48) enzymes. + OR − ESBL: The bacterium producing the carbapenemase may also make an ESBL enzyme (+ESBL) or it may not (−ESBL)(42-44). This makes the infection extremely difficult to treat as it renders the most potent classes of antibiotics ineffective, and is a primary clinical and public health concern due to its ability to spread to other bacteria. *Klebsiella* isolates have a dual mechanism for resisting carbapenems: A combination of reduced entry plus destruction by ESBL/AmpC enzymes. AST Profile of *E. coli* (n = 13) in Table 7. Revealed CARBAPENEMASE (+ OR − ESBL) refers to the presence or absence of two types of enzymes, Carbapenemase and ESBL, that make bacteria resistant to beta-lactam antibiotics. A CARBAPENEMASE (+) ESBL (−) isolate produces carbapenemase, which breaks down carbapenem antibiotics, while a CARBAPENEMASE (−) ESBL (+) isolate produces an ESBL enzyme, making it resistant to penicillins and cephalosporins but still susceptible to carbapenems (45,46). Isolates can be positive for both, negative for both or positive for only one of these enzymes. The resistance profile of this *Klebsiella aerogenes* isolate is particularly noteworthy for several reasons. The most alarming profile was observed in *E. coli,* where all isolates were resistant to every tested β-lactam antibiotic, including all generations of cephalosporins, piperacillin/tazobactam and carbapenems, defining them as extremely drug-resistant. A critical commonality was the universal susceptibility of all isolates from all three species to amikacin, Contrary to what was found by (44). Tigecycline and trimethoprim/sulfamethoxazole also remained effective against the *Klebsiella* species, noted in isolate *K. aerogenes* (9,44). The resistance profiles are summarised in Tables 6-8. The resistance to imipenem and intermediate susceptibility to ertapenem are highly concerning, as they suggest emerging carbapenem resistance in this environmental mastitis pathogen. This pattern is consistent with possible carbapenemase production; however, further genetic characterisation would be required for confirmation. The retained susceptibility to multiple drug classes, aminoglycosides, fluoroquinolones, TMP-SMX and tigecycline provides valuable therapeutic options. However, the potential for inducible resistance mechanisms in this species necessitates careful consideration of treatment duration and monitoring. The uniform resistance to cefoxitin, a cephamycin, is a key indicator. When observed in a clinical isolate that is not a classical AmpC producer (like *Enterobacter* spp.), it strongly suggests the presence of a plasmid-mediated AmpC β-lactamase. This mechanism can confer resistance to broad-spectrum cephalosporins such as ceftazidime and cefepime, which aligns with the observed resistance profile. The retained susceptibility to ceftriaxone is unusual and may indicate a specific AmpC variant with variable hydrolytic activity. Despite the extensive resistance, the consistent susceptibility to all other tested drug classes provides crucial guidance for therapy. The 100% efficacy of aminoglycosides, fluoroquinolones, tigecycline and

Table 7: Antimicrobial susceptibility profile of Klebsiella aerogenes (n=1)

Antimicrobial agent	Class	Result
Imipenem	Carbapenem	Resistant
Ertapenem		Intermediate
Ampicillin	Penicillin	Resistant
Piperacillin/Tazobactam	Beta-lactam/BLI	Resistant
Cefazolin	1st-gen. Cephalosporin	Resistant
Ceftazidime	3rd-gen. Cephalosporin	Resistant
Cefoxitin	Cephamycin	Resistant
Nitrofurantoin	Nitrofuran	Resistant
Cefepime	4th-gen. Cephalosporin	Intermediate
Amikacin	Aminoglycoside	Susceptible
Gentamicin		Susceptible
Ciprofloxacin	Fluoroquinolone	Susceptible
Levofloxacin		Susceptible
Tigecycline	Glycylcycline	Susceptible
Trimethoprim/Sulfamethoxazole	Folate inhibitor	Susceptible

Table 8: Summary antimicrobial susceptibility profile of *Enterobacteriaceae* isolates (n=12)

Antimicrobial agent	Class	Resistance profile
Ampicillin	Penicillin	100% resistant
Piperacillin/Tazobactam	Beta-lactam/BLI	100% resistant
Cefazolin	1st-gen. Cephalosporin	100% resistant
Ceftazidime	3rd-gen. Cephalosporin	100% resistant
Cefoxitin	Cephamycin	100% resistant
Imipenem	Carbapenem	100% resistant
Ertapenem		100% resistant
Nitrofurantoin	Nitrofuran	100% resistant
Cefepime	4th-gen. Cephalosporin	100% resistant
Levofloxacin	Fluoroquinolone	100% resistant
Ciprofloxacin		75% resistant, 8.3% intermediate, 16.7% susceptible
Amikacin	Aminoglycoside	100% susceptible
Gentamicin		100% susceptible
Tigecycline	Glycylcycline	100% susceptible
Trimethoprim/ Sulfamethoxazole	Folate inhibitor	100% susceptible
Ceftriaxone	3rd-gen. Cephalosporin	100% susceptible

TMP-SMX indicates these are viable and effective treatment options.

Conclusion

This study's comprehensive analysis of bacterial isolates from ovine mastitis cases reveals a critically concerning landscape of AMR. The VITEK 2 system effectively identified a diverse range of pathogens, primarily MRSA and CNS, as well as carbapenem-resistant *K. pneumoniae* and other Enterobacteriaceae. The AST profiles show a high prevalence of MDR phenotypes. The universal methicillin resistance among *staphylococcal* isolates, confirmed by cefoxitin screening, strongly indicates the widespread presence of the mecA gene. Among Gram-negative bacteria, the resistance patterns characterised by broad-spectrum β-lactam resistance, carbapenem resistance and cefoxitin resistance in ESBL-negative isolates indicate the troubling emergence of AmpC β-lactamase production and possibly carbapenemase synthesis as key resistance mechanisms. The situation poses significant public health risks because these resistant bacteria can be transferred from animals to humans through the food chain and the environment. To address this crisis, the research emphasises that empirical treatment is no longer sufficient and recommends using rapid diagnostic testing to guide therapy, conducting molecular surveillance to monitor the emergence of resistance genes and establishing comprehensive management strategies, including enhanced biosecurity and careful antibiotic use. Controlling this threat requires coordinated efforts among veterinarians, farmers and public health authorities to maintain the effectiveness of antibiotics for both animal and human health.

Data availability

Data available upon request.

Ethical Approval

Ethical approval for this study was provided by the Ministry of Higher Education and Scientific Research, University of Fallujah, Veterinary Medical College, Iraq, on 17th Jun 2026.

Author contribution

Duaa Ghazi Faisal was responsible for sample collection, practical work and manuscript writing, while Prof. Dr. Salah Mahmood Ashour AL-Kubaisi contributed by editing and reviewing the manuscript.

Acknowledgements

The authors thank the College of Veterinary Medicine at AL-Fallujah University and Jawharat Al-Israa Scientific Office, Baghdad, for facilitating the work and providing the necessary equipment.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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