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RESEARCH ARTICLE

Antimicrobial Resistance and Virulence Genes of *Escherichia coli* Isolated in a Large-Scale Dairy Farm from Clinical Mastitis in Southern Xinjiang, China

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ABSTRACT

Mastitis causes significant economic losses to the dairy industry. *Escherichia coli (E. coli)* is considered a primary cause. The study aimed to investigate the antibiotic resistance profiles and presence of virulence genes in *E. coli* isolated from milk samples collected from cows with clinical mastitis on a large dairy farm in Hetian, southern Xinjiang, China. *E. coli* was detected in 26.9% of milk samples from 130 cows with clinical mastitis. Multilocus sequence typing (MLST) revealed that the dominant sequence lines were ST1121, ST88, and ST58. A total of 34.3% of the isolates were multidrug-resistant; all isolates were completely resistant to penicillin and lincomycin, and highly resistant to ampicillin (80.0%). All isolates carried the transcription-repair coupling factor (*Mfd*) and various efflux pump resistance genes. Aminoglycoside resistance genes were fully consistent with the corresponding resistance phenotypes. Except one isolate, all the remaining 34 isolates carried multiple virulence genes, including 33 carrying the curli gene. Notably, the *fecI* gene, associated with reduced virulence in bovine mastitis, was detected in 94.3% of isolates. This study shows that the *E. coli* strains responsible for clinical mastitis in this dairy farm are dominated by the ST1121 lineage, which exhibits pronounced multidrug resistance (MDR) and distinctive virulence gene profiles, suggesting multiple sources of infection. This provides a molecular basis for targeted antibiotic treatment and prevention of *E. coli* mastitis in the dairy farm in southern Xinjiang. This study offers a reference for the control of mastitis in dairy cattle in northwestern China.

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INTRODUCTION

Mastitis in dairy cattle is an inflammatory disease caused by pathogenic microorganisms that invade the mammary tissue and result in significant economic losses for dairy industry (He *et al.*, 2020; Guo *et al.*, 2021; Chen *et al.*, 2023). *Escherichia coli* is a widespread Gram-negative bacterium that occurs naturally in various environments and is considered one of the main causative agents of mastitis in dairy cattle (Denamur *et al.*, 2021). Mastitis-causing *E. coli* (MPEC) possesses several virulence genes, particularly adhesion factors, molecules associated with invasion, and iron uptake systems (Shoaib *et al.*, 2023). Mastitis caused by MPEC does not depend on a single specific virulence factor, but rather on the synergistic interaction of several bacterial virulence factors that colonize and survive in the host cells and ultimately trigger an inflammatory response (Cheng and Han, 2020). The Use of antibiotics is one of the major methods for treating bovine mastitis, but long-term or overuse of antibiotics may promote the emergence of drug-resistant bacteria (Gohar *et al.*, 2017; Amoafo *et al.*, 2021). These pathogens generally harbor a variety of resistance genes, which are often located in mobile genetic elements and possibly undergo horizontal gene transfer among strains resulting in rapid spread of multi-drug-resistant strains (Rossolini *et al.*, 2014). MPEC strains often exhibit multidrug resistance (MDR) (Tahar *et al.*, 2020; Yu *et al.*, 2020). The mechanisms of multidrug resistance (MDR) are associated with β-lactamase genes, such as those encoding extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases, which confer

resistance to multiple β-lactam antibiotics like penicillin and ampicillin (Poirel *et al.*, 2018; Pinheiro *et al*., 2020). MPEC also carries antibiotic resistance genes such as *tet* and *aadA*, which confer resistance to antibiotics such as tetracyclines and aminoglycosides (Kazemzadeh *et al.*, 2025).

In the last 15 years, few studies focused on the molecular epidemiological characteristics of different species of mastitis-causing bacteria in dairy cattle in Xinjiang province, China. However, only multilocus sequence typing (MLST) analyses of *Streptococcus uberis*, antibiotic resistance and virulence genes in *Staphylococcus aureus* were reported in southern Xinjiang (Wang *et al.*, 2013; Ren *et al.*, 2020; Cai *et al.*, 2025). Furthermore, molecular epidemiological characterization of MPEC in dairy cattle in southern Xinjiang has not yet been pursued. Large-scale dairy farms have significant advantages including stable milk supply, improved production efficiency, and systematic disease monitoring due to their concentrated production, standardized management, and usage of advanced technology, yet increased livestock density poses more serious issues for the prevention and treatment of infectious diseases, such as bovine mastitis (Aubé *et al.*, 2022). This study isolated and identified *E. coli* strains from milk samples collected from cows with clinical mastitis at a large-scale dairy farm in Hetian, Xinjiang. It aims to analyze the presence of antibiotic resistance and virulence genes in MPEC strains from this dairy farm. The functional annotation of such genes provides valuable molecular information in the design of mastitis prevention and control strategies for the dairy farm. Meanwhile, the study provides a reference for the prevention and control of bovine mastitis in southern Xinjiang and the surrounding areas of China.

MATERIALS AND METHODS

Sample collection and handling: Between June and September 2025, a total of 130 milk samples were aseptically collected from dairy cows with clinical mastitis at a large-scale dairy farm in Luopu County, Hetian, Xinjiang. These cows were preliminarily diagnosed with clinical mastitis based on clinical signs such as udder redness and swelling, fever, and the presence of flocculent matter or discoloration in the milk (Goulart and Mellata, 2022). The specific collection methods followed those described previously (Ren *et al.*, 2020).

Isolation and biological characterization of *E. coli*: These samples were centrifuged at 3,000 RPM for 10 minutes. The precipitate was collected under sterile conditions, followed by bacterial isolation and culture. Then, staining was performed using the Gram stain method, followed by microscopic examination (My *et al.*, 2023). Single bacterial colonies were isolated from the MacConkey agar and subjected to biochemical testing, including indole production, urease activity, and citrate utilization (Adkins and Middleton, 2017).

16S rDNA Sequence Analysis: Bacterial strains were molecularly identified with 16S rDNA sequencing. Amplification by polymerase chain reaction (PCR) was performed using universal primers synthesized by Sangon Biotech (Shanghai) Co., Ltd. (515F: 5'-CCTAYGGGRBGCASCAG-3'; 806R: 5'-GGACTACNNGGGTATCTAAT-3') (Caporaso *et al.*, 2011; Caporaso *et al.*, 2012). The PCR reaction mixture was prepared according to the kit instructions (2×TSINGKE® Master Mix (Blue)). Annealing was performed at 55°C for 30 seconds. PCR products were sent for sequencing (Beijing Novogene Co., Ltd) and analyzed using NCBI BLAST (http://www.ncbi.nlm.nih.gov).

Antibiotic susceptibility testing: In accordance with the CLSI VET01/VET01S standards and with reference to previous studies, disk diffusion testing was performed on confirmed *E. coli* isolates to determine their susceptibility to 12 antibiotics, using *E. coli* ATCC 25922 as the control strain (Clinical and Laboratory Standards Institute (CLSI), 2013; Li *et al.*, 2022). Based on the criteria (Table 1), the MDR patterns of the isolates to each antimicrobial agent and the multiple antibiotic resistance index (MARI) were calculated. MDR was defined as resistance to three or more classes of antibiotics (Rafailidis and Kofteridis, 2022). The MARI analysis was conducted in accordance with previous studies (Kakooza *et al.*, 2025).

Table 1: Criteria for determining antibiotic susceptibility in *E. coli*

Classification	Antibiotics	Antibacterial zone diameter / mm		
		Resistant (R)	Intermediate (I)	Sensitive (S)
β-lactams	Penicillin	≤14	15-21	≥22
	Ampicillin	≤13	14-16	≥17
	Ceftriaxone	≤19	20-22	≥23
	Cefepime	≤18	19-20	≥21
Quinolones	Ciprofloxacin	≤20	21-24	≥25
Aminoglycosides	Gentamicin	≤12	13-14	≥15
Tetracyclines	Tetracycline	≤11	12-14	≥15
Amide alcohols	Chloramphenicol	≤12	13-17	≥18
	Florfenicol	≤12	13-17	≥18
Macrolides	Erythromycin	≤13	14-22	≥23
Lincosamide	Lincomycin	-	-	-
Sulfonamides	Co-trimoxazole	≤10	11-15	≥16

Note: Since the CLSI has not established susceptibility criteria for lincomycin against *E.coli*, this study only records the diameter of the inhibition zone and does not perform susceptibility analysis.

Whole-genome sequencing: Single colonies purified from MacConkey agar were inoculated into Mueller-Hinton broth and incubated at 37°C with continuous shaking for 16 hours to propagate the culture. Then, bacterial suspensions were sequenced (Beijing Novogene Co., Ltd) for whole-genome sequencing.

Multilocus typing (MLST) analysis: After obtaining the complete genome sequences of all isolates, analysis was performed according to the method described earlier (Li *et al.*, 2022).

Analysis of gene functional annotations: The sequences obtained as a result of whole-genome sequencing were compared with the CARD (https://card.mcmaster.ca/), VFDB-202303 (http://www.mgc.ac.cn/VFs/), and PHI (https://www.phi-base.org) databases. To ensure the accuracy of the results, this study analyzed only those resistance and virulence genes that showed 100% consistency with the reference database.

RESULTS

Isolation and biochemical identification results of *E. coli*: Observation of colony morphology revealed that the isolated strain formed smooth pink colonies on MacConkey agar, with some colonies exhibiting a bile salt precipitation ring at the edge (Fig. 1A). Gram staining and microscopic examination confirmed the presence of red Gram-negative short rods (Fig. 1B). Biochemical identification results indicated that the isolates tested positive for the indole test and the urease test, while negative for the citrate utilization test. These results are consistent with the biochemical characteristics of *E. coli*.

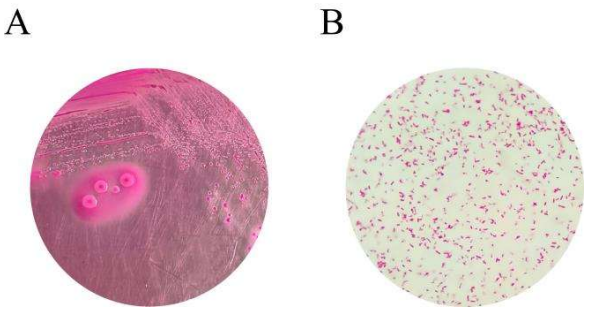

Fig. 1: 1A: Colony morphology of the isolate on MacConkey agar plates; 1B: Gram staining microscopic examination results (1000X).

Results of 16S rDNA Sequence, PCR amplification, and MLST: PCR amplification of suspected *E. coli* isolates yielded amplification bands at 500 base pairs (bp) (Fig. 2A). Sequence alignment of the obtained 16S rDNA sequences against the NCBI-BLAST database revealed that all 35 isolates shared > 99.9% sequence similarity with *E. coli* reference strains, such as *E. coli* ATCC 25922, confirming their molecular identification as *E. coli*. A total of 35 *E. coli* strains were isolated from 130 milk samples, designated D1–D35, yielding an isolation rate of 26.9%. MLST results for all isolates revealed 20 belonging to ST1121, 6 isolates to ST88, 4 isolates to ST58, and 3 isolates to ST448; all corresponding to known MPEC ST types. However, 2 isolates (ST1848 and ST10022) showed no match to known MPEC-associated STs, representing the first detection of these clonal sequences in this region. (Fig. 2B).

Results of *E. coli* antibiotic susceptibility test: Results showed that the *E. coli* isolates exhibited high resistance to ampicillin, with a resistance rate of 80.0%, lower resistance rate to cefepime and ciprofloxacin, 8.6% and 11.4%, respectively (Fig. 3A). A total of 12 isolates were multidrug-resistant, with 4 resistant to 6 classes of antibiotics and 3 to 5 (Fig. 3B). In addition, 54.3% of the isolates had a MARI ≥ 0.2, and 25.7% of the isolates had a MARI ≥ 0.75 (Table 2).

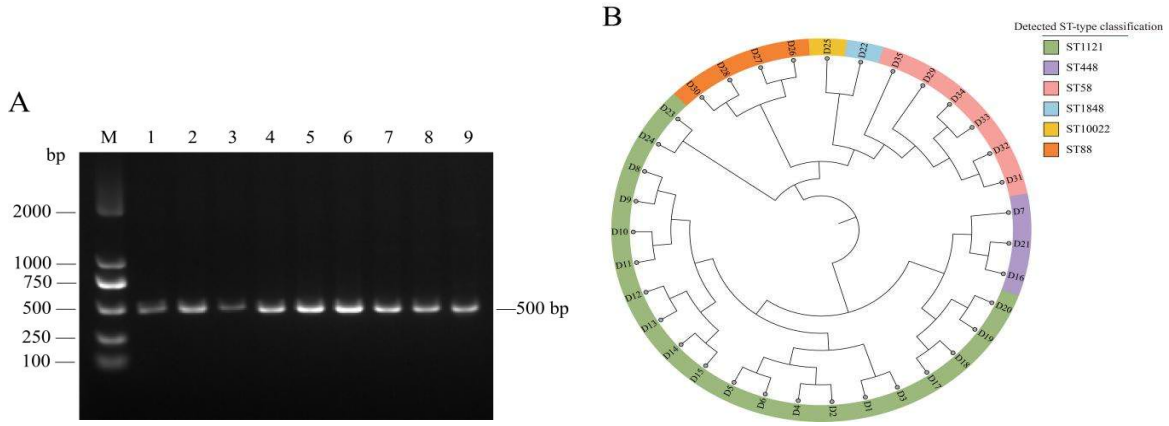

Fig. 2: 2A: PCR amplification results for 16S rDNA from selected isolates; Lane M: 2000 bp Marker DNA; Lane (1-9) reaction amplified products of 16S rDNA from the isolated strain; 2B: Phylogenetic tree of 35 *E. coli* isolates.

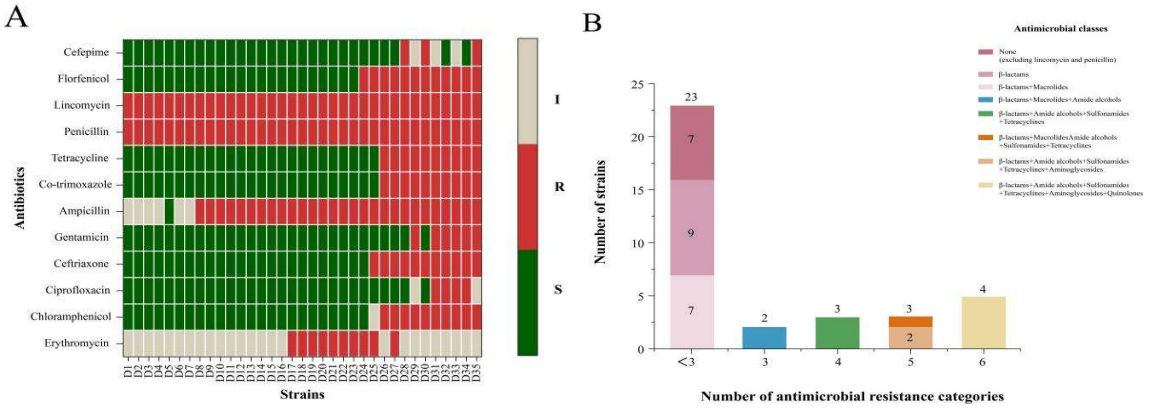

Fig. 3: 3A: Antimicrobial resistance profiles of the isolates to 12 antibiotics (based on the disk diffusion method); 3B: Number of strains in each combination of antimicrobial drug classes. Note: Since *E. coli* is generally resistant to penicillin and lincomycin, the results of susceptibility testing for these two agents are not included in the analysis and discussion of MDR and MARI results; they are included solely to ensure the completeness of the study.

Results of antibiotic resistance genes in *E. coli*: A total of 74 antibiotic resistance genes across 7 categories were detected in the isolates. Among these, the *Mfd* gene exhibited the highest detection rate at 100%, while *tuF*, *lmrC*, *rpoB*, *efrA*, *mprF*, *emeA*, *lsaA*, *efrB*, *lmrD*, and *taeA* showed the lowest detection rates, each at only 2.9% (Table 3). Further selection of efflux pump system resistance genes, gene mutation-type resistance genes, antibiotic target protection proteins, and antibiotic inactivation enzyme resistance genes were conducted as directly related resistance genes for MPEC. The results showed that all strains harbored quinolone resistance genes and efflux pump MDR genes, while only one isolate carried a lincosamide resistance gene (Fig. 4A). The results further indicated that MPEC from this dairy farm commonly carried multiple direct resistance genes (Fig. 4B). The correspondence between antibiotic resistance genes and resistance phenotypes in *E. coli* showed complete concordance for aminoglycosides and high concordance for β-lactams (97.1%), while lincosamide resistance genes showed the lowest correspondence rate at only 2.9% (Table 4).

Table 2: Distribution of MARI among *E. coli*

MARI	Number of isolates	Percentage (%)
<0.2	16	45.7
0.2	7	20.0
0.3	1	2.9
0.4	1	2.9
0.6	1	2.9
0.7	4	11.4
0.8	5	14.3

MARI=the ratio of the number of antibiotics to which the isolate was resistant / the total number of antibiotics tested.

Table 3: Detection of antimicrobial resistance genes in *E. coli* isolates (by function)

Genes Classified by Function	Drug resistance gene	Detection rate/%
External drainage pump system	*mdtL*, *mdtF*, *mdtK*, *mdtH*, *mdtG*, *mdtA*, *tolC*, *rob*, *patA*, *macB*, *msbA*, *emrA*, *emrY*, *mdfA*, *acrE*, *acrA*, *baeS*, *yojI*, *mexN*, *rosB*	97.1
	mdtE, *mdtO*, *mdtM*, *mdtP*, *rosA*, *emrK*, *emrD*, *emrB*, *acrB*, *gadX*, *cpxA*	94.3
	mdtN	77.1
	mdtB	17.1
	evgS	91.4
	muxB	80.0
	mexK, *oqxA*, *mdsC*, *lmrC*, *efrA*, *emeA*, *lsaA*, *efrB*, *lmrD*, *taeA*	2.9
	floR	28.6
	cmlA5	11.4
Antibiotic resistance determining clusters in glycopeptide antibiotics	*bacA*	97.1
	mprF	2.9
	leuO	94.3
Gene mutation-type resistance gene	*gyrA*, *katG*	97.1
	GlpT	94.3
	uhpt, *tuF*, *rpoB*	2.9
Antibiotic target protective protein	*Mfd*	100.0
	sul2	28.6
	sul3	11.4
Regulating antibiotic permeability proteins	*lamB*, *ompK37*	97.1
Gene for altering cell wall charge	*pmrC*, *pmrF*	94.3
	PmrE	97.1
	arnA	91.4
Antibiotic inactivating enzyme	blaCMY-63	97.1
	blaCTX-M-55, blaTEM-1	28.6
	APH(3')-IIa, *aadA*, *aadA25*	11.4
	APH(3")-Ib, APH(6)-Id, AAC(3)-IIc	17.1

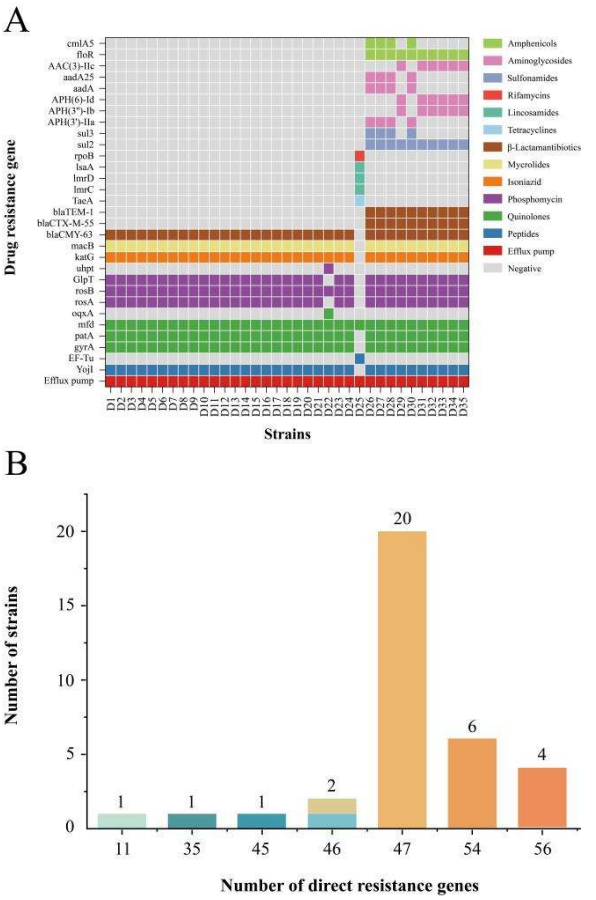

Fig. 4: 4A: Detection of antimicrobial resistance genes in *E. coli* isolates (by antimicrobial category); 4B: Distribution of strains with different numbers and combinations of resistance genes (different colors represent different combinations of drug-resistance genes).

Results of *E. coli* virulence gene detection: A total of 25 core virulence genes associated with mastitis pathogenicity across 8 categories were detected in the isolates. Among these, the curli genes *csgB*, *csgD*, *csgE*, *csgF*, and *csgG* exhibited the highest detection rates at 94.3% each, while the adhesin genes *strc* and *efaA*, along with the capsular and surface structure genes *cpsA* and *cpsB*, showed the lowest detection rates at 2.9% each. Except for D22, all 34 isolates carried multiple core virulence genes: one isolate carried 4, 18 isolates carried 8, 2 isolates carried 9, 3 isolates carried 10, and 10 isolates carried 15 (Fig. 5A, 5B).

Results of other gene functional annotation: Genomic analysis of proteins secreted from the isolates showed that 35 MPEC sequences contained detectable proteins for the type III secretion system (T3SS), with a maximum detection frequency of 82.9% in one isolate. It should be noted that none of the isolates contained T5SS proteins (Fig. 6A). Comparison of the genomes of the isolates with the TCDB database showed that primary transporters had the highest detection rate, ranging from 29.9% to 48.5% in one isolate, while transport cofactors had the lowest detection rate, ranging from 1.5% to 3.0% (Fig. 6B). Comparison of the genomic sequences of the isolates with the PHI database allowed individual *E. coli* isolates to be matched with an annotated number of genes ranging from

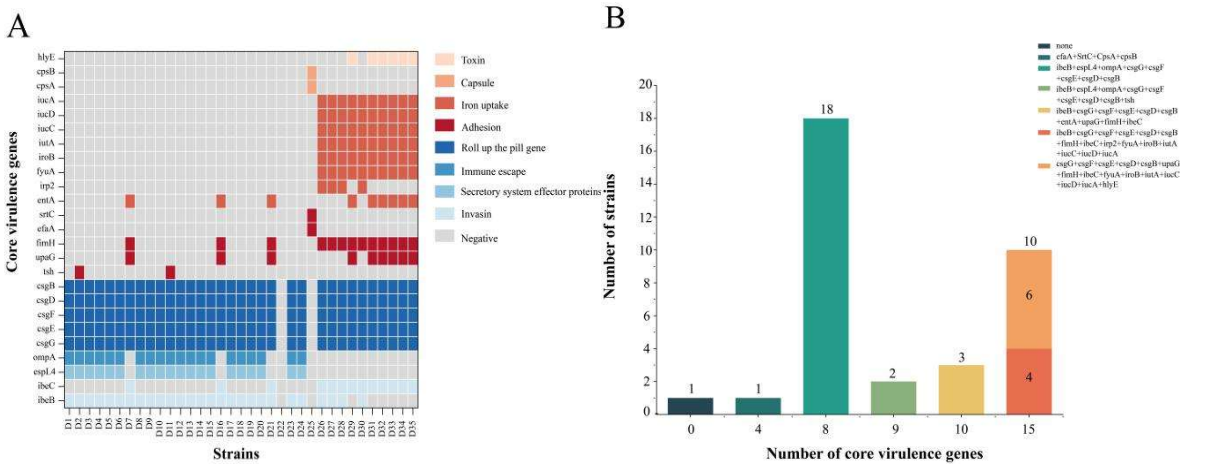

Fig. 5: 5A: Detection of core virulence genes in *E. coli* isolates (through whole-genome sequencing); 5B: Core virulence gene combinations carried by *E. coli* isolates.

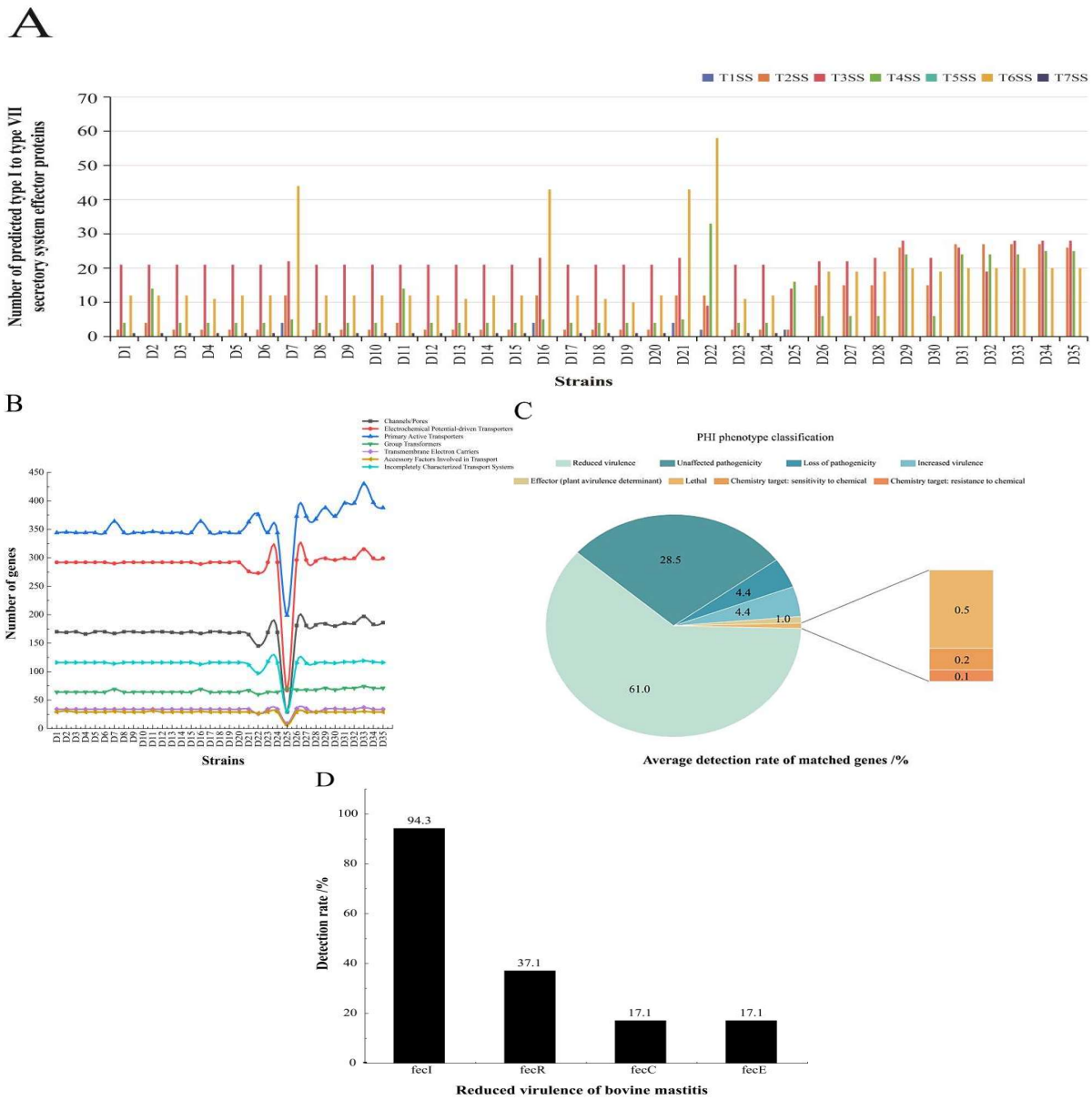

Fig. 6: 6A: Detection status of proteins in the *E. coli* secretion system; 6B: *E. coli* isolates TCDB detection results; 6C: *E. coli* isolates PHI detection results; 6D: Detection results of mutations associated with reduced virulence in bovine mastitis.

416 to 973. Classification based on known phenotypic variants showed that among the eight categories of phenotypic variants, phenotypic variations in virulence-reducing genes had the highest detection rate, with an average of 61.0%, while chemical target genes and chemical resistance phenotype genes had the lowest detection rate, with an average of 0.1% (Fig. 6C). Results indicated that the detection rates of *fecI*, *fecR*, *fecC*, and *fecE* genes, which reduce *E. coli* pathogenicity in bovine mastitis after mutation, were 94.3%, 37.1%, 17.1%, and 17.1%, respectively, across 35 isolates (Fig. 6D).

Table 4: Correlation between antimicrobial resistance genes and phenotypes in *E. coli* isolates

Types of Antibacterial Agents	Number of drug-resistant strains	Number of strains harboring drug-resistant genes	Phenotypic resistance rate/%	Positive rate of strains harboring drug-resistant genes/%	Match Rate/%
β-lactams	35	34	100.0	97.1	97.1
Quinolones	4	35	11.4	100.0	11.4
Aminoglycosides	6	6	17.1	17.1	100.0
Tetracyclines	10	34	28.6	97.1	29.4
Amide alcohols	12	34	34.3	97.1	35.3
Macrolides	11	34	31.4	97.1	32.4
Lincosamide	35	1	100.0	2.9	2.9
Sulfonamides	10	34	28.6	97.1	29.4

Correspondence rate = Positive rate of strains containing relevant resistance genes / Phenotypic resistance rate

DISCUSSION

Mastitis is a frequent disease in dairy cattle farming that causes huge financial losses for the livestock industry (Shehata *et al.*, 2024). *E. coli* plays a crucial part in the occurrence of mastitis (Gao *et al.*, 2017). A total of 35 strains of *E. coli* were isolated and identified in this study from 130 milk samples collected from cows with clinical mastitis at a large-scale dairy farm in Hetian, Xinjiang, China, yielding an isolation rate of 26.9%. Zhao *et al.* isolated 105 strains of *E. coli* from 252 milk samples from clinically mastitic dairy cattle in Hohhot, Inner Mongolia, China, representing an isolation rate of 41.7% (Zhao *et al.*, 2024). Li *et al.* isolated and identified 40 strains of *E. coli* from 196 samples of milk collected from mastitis-infected cows in northern China, representing an isolation rate of 20.4% (Li *et al.*, 2022). This study's findings are varied from those of the above-mentioned studies. It may be attributed to sample size, geographic distribution, and analytical methods. Furthermore, 94.3% of isolates belonged to the ST type, which can cause mastitis, consistent with previous research findings (Nüesch-Inderbinen *et al.*, 2019; Lacher *et al.*, 2020). Further analysis revealed 6 ST types among the 35 isolates, while ST58 and ST88 were common MPEC clonal types, ST1121 was the predominant clonal type in this study (57.1%). ST1121 was reported by Deng *et al.* (2015) to carry the IncHI2 plasmid and resistance genes, which undergo clonal transmission in pig populations, indicating that this sequence type has strong adaptive potential in livestock and poultry farming environments (Deng *et al.*, 2015). Currently, ST1121 is not considered a common MPEC clonal type, with a reported detection rate of only 4.3%, significantly lower than that of established types like ST10 (Laterza *et al.*, 2025). Compared, the high detection rate of ST1121 in this study exhibits significant regional specificity, suggesting that the clonal type may have developed a regional transmission advantage in the local farming environment and may continue to spread within dairy farms through specific transmission routes such as cross-contamination during milking and environmental vector transmission.

Antibiotics are widely used to treat and prevent bovine mastitis (Wilm *et al.*, 2024). Milk borne pathogens may exhibit high resistance to antibiotics, posing serious challenges for prevention, clinical treatment and public health (Goulart and Mellata, 2022; Yaseen *et al*., 2025). The results of this study indicated that *E. coli* isolates from this dairy farm exhibited significant resistance to ampicillin and florfenicol, with resistance rates of 77.8% and 26.7%, respectively. A study by Zuo *et al*. showed that *E. coli* strains associated with bovine mastitis had significant resistance to ampicillin (36.8%), while florfenicol was only 12.6% (Zuo *et al.*, 2025). Yu e*t al.* collected milk samples from cows with mastitis across multiple regions of China, including Xinjiang, and found that the isolated *E. coli* exhibited complete resistance to penicillin and a resistance rate of 98.8% to lincomycin (Yu *et al.*, 2020). The findings of the present study differ from those of the above-mentioned researches, which may originate from differences in antibiotic usage patterns and treatment strategies in dairy cattle farming. Moreover, 34.3% of the isolates from the dairy farm were multidrug-resistant, with MARI values ranging from 0.3 to 0.8. Notably, 14.3% of the isolates had a high MARI (≥ 0.75) and were resistant to 8 antimicrobial agents. Based on previous studies, this study demonstrates that *E. coli* isolates associated with mastitis in dairy herds exhibit severe MDR, with resistance being widespread (Chowdhury *et al.*, 2025; Moradi *et al.*, 2025). These isolates likely originate from environments with high antibiotic exposure, a phenomenon that may be closely linked to the long-term, low-dose use of antibiotics in livestock farming as growth promoters or for prophylactic purposes (Arbab *et al.*, 2025). Thus, the mastitis treatment strategy for this dairy herd requires an approach distinct from those in northeast or southern China (Gao *et al.*, 2025).

Efflux pump systems mediated by primary active transporters are one of the main factors reducing the effectiveness of mastitis treatment in MDR mechanisms of bacterial (Nikaido and Pagès, 2012). It can actively transport a variety of antimicrobial drugs out of the cell and reduce drug accumulation in the bacterial cell (Kazemzadeh *et al.*, 2025). The detection rate of primary active transport proteins in this study was the highest, ranging from 29.9% to 48.5% across 35 isolates, and the efflux pump resistance genes were identified in all isolates. It indicates that the MDR observed in these MPEC strains is closely associated with efflux pump systems. The transcription-repair coupling factor *Mfd* promotes bacterial genome stability and environmental adaptability by coupling deoxyribonucleic acid (DNA) repair with transcription, thereby protecting bacterial ribonucleic acid (RNA) polymerase from damage, and this factor further facilitates the development of drug resistance (Martin *et al.*, 2025). Additionally, the *Mfd* gene promotes an increase in the bacterial mutation rate,

thereby accelerating the development of antibiotic resistance (Ragheb *et al.*, 2018). Studies found that deletion of the *Mfd* gene reduces the frequency of fluoroquinolone-resistant mutations in bacteria by approximately 100-fold (Han *et al.*, 2008). Lee *et al.* demonstrated that *Mfd*-deficient mutants displayed markedly increased sensitivity to a range of antibiotics (Lee *et al.*, 2009). This underscores the crucial role of this gene in the development of bacterial antibiotic resistance. All *E. coli* isolates in the present study carried the *Mfd* gene, demonstrating that this gene may represent a critical factor in the MDR of *E. coli* strains causing mastitis at this dairy farm. Regrettably, previous research in this area has been limited, and further in-depth exploration of the mechanisms involved is still required.

Antibiotic resistance genes determined corresponding resistance phenotypes (Liu *et al.*, 2021). Our results showed that aminoglycoside resistance genes in MPEC were fully associated with phenotypic resistance, which suggested that aminoglycoside resistance was mainly mediated by the AAC(3)-IIc, aadA, and APH(6)-Id target genes in the strains. Poirel *et al.* reported that extended-spectrum β-lactamase-producing *E. coli* (ESBL-Ec) and AmpC-producing *E. coli* (AMPC-Ec) were resistant to a variety of frequently used antimicrobial drugs, and the resistance genes could transfer between the same or different species (Poirel *et al.*, 2018). Our results also showed that ESBL genes (blaCTX-M55, blaTEM-1) and AMPC genes (blaCMY-63) were detected in MPEC isolates with detection rates of 28.6% and 97.1%, respectively. These results were similar to the results of Fazel *et al.* (2019) and Yu *et al.* (2020) who reported an association between MDR in MPEC from dairy farms and the existence of ESBL and AMPC genes. Moreover, our results showed that the detection rates of the quinolone resistance genes *Mfd*, *gyrA*, and *oqxa* were 100%, 97.1%, and 2.9%, respectively, but the match rate between these resistance genes and phenotypic resistance was only 11.4%. It suggests that the quinolone resistance phenotype in the isolated strains may originate mainly from efflux mechanisms mediated by plasmids such as *oqxa* rather than from mutations in *gyrA*. *Mfd* may act as a potentially fitness-enhancing factor, indirectly involved in *E. coli* resistance to quinolones by affecting the accumulation of the mutation (Han *et al.*, 2008; Poirel *et al.*, 2018). Though the isolates were totally resistant to lincomycin, the concordance rate between their resistance genes and phenotypes was only 2.9% in this study; and there is a possibility that the *E. coli* isolates in this study carry unknown lincomycin resistance genes or mechanisms, requiring further investigation.

The pathogenicity of MPEC is mainly influenced by the combination of specific virulence gene groups, which mediate critical virulence processes (Shoaib *et al.*, 2023). In the mechanism of bovine mastitis, the curli fimbriae of MPEC mediate the primary attachment of bacteria to host cells, laying the foundation for biofilm formation and creating the necessary conditions for invasion genes to ensure bacterial survival and parasitism inside cells (Xu *et al.*, 2024). The detection rates of curli genes (*csgG*, *csgF*, *csgE*, *csgD*, *csgB*) and invasion genes (*ibeB*, *ibeC*) in MPEC at this dairy farm were both as high as 94.3%, suggesting that biofilm formation and mammary barrier invasion capacity may represent the core virulence mechanisms underlying the occurrence and progression of *E. coli* mastitis in this dairy farm. T3SS of Gram-negative bacteria, as a highly specialized protein secretion apparatus, functions as a transmembrane injector, which directly injects a series of effector proteins and virulence factors from the bacterial cytoplasm into the cytoplasm of eukaryotic host cells, thereby disrupting the normal functions of the host cell (Guerra *et al.*, 2020). This study identified the effector protein virulence gene *espL4* in 20 of 35 MPEC isolates, with all strains harboring the T3SS gene cluster. This study also found that MPEC isolates from this dairy farm harbored diverse combinations of core virulence genes, with the *ibeB+espL4+ompA+csgG+csgF+csgE+csgD+csgB* combination exhibiting the highest carriage rate at 51.4%, indicating that the T3SS plays a significant role in the pathogenesis of *E. coli* mastitis at this dairy farm. However, genome-wide analysis of the D22 isolate in this study did not identify any known virulence genes, indicating the MPEC in this dairy farm may contain unknown virulence mechanisms.

The Fec system is an important iron acquisition mechanism in *E. coli* (Olson *et al.*, 2018). Studies have shown that this system detects iron-citrate in the external environment and induces the expression of related genes, thereby enhancing bacterial colonization and survival within the host (Yokoyama *et al.*, 2021). Recent studies have linked the Fec system to bacterial virulence. In uropathogenic *E. coli*, the Fec system is significantly upregulated under iron-limiting conditions, conferring a competitive advantage to the bacteria in the host environment; the same study revealed that deletion of ferric citrate uptake genes not only markedly reduced the strain's ability to utilize ferric citrate as an iron source but also significantly diminished its adaptability in the host environment. (Frick-Cheng *et al.*, 2022). Indicating the critical role of this system in the pathogenesis of *E. coli* infection. This study demonstrated that the *fecI* gene, associated with reduced virulence in bovine mastitis, had the highest detection rate among all isolates (94.3%). The results are in agreement with the *in vivo* experimental findings by Blum *et al*. (2018) which indicate that the MPEC-P4 variant lacking the Fec system has lost its ability to induce mastitis in dairy cattle (Blum *et al.*, 2018). Therefore, the high detection rate of *fecI* reflects the significant clinical utility of the Fec system in the MEPC of this study.

In the present study, 34.3% isolated MPEC strains displayed MDR, with especially high rates of resistance to ampicillin, and all isolates manifested multiple genes conferring resistance to efflux pumps. On the basis of antimicrobial sensitivity test results, it is suggested that this dairy farm should better avoid using the antibiotics listed previously when treating *E. coli* mastitis. Instead, antibiotics with high sensitivity to the pathogen should be selected, such as cefepime or ciprofloxacin. Antibiotics that are easily tested and eliminated by efflux pumps should also be avoided to prevent reduced efficacy due to inadequate accumulation of the drug within bacteria. High detection rates of biofilm-associated virulence genes (such as curli pili and invasins) were detected across all MPEC

isolates, with 94.3% carrying the *fecI* gene, which reduces virulence by inducing mutations. During treatment, following antibiotic susceptibility testing, priority should be given to antibiotics that can prevent biofilm formation and effectively target intracellular bacteria. Dairy farms may also consider using iron chelating agents, which reduce the iron available to bacteria, thereby reducing bacterial colonization and reproduction within the mammary gland (Lippolis *et al.*, 2016). Additionally, a dominant MPEC clone with a superior serotype was identified at this dairy farm. Alongside rational antibiotic use, it is recommended to strengthen farm hygiene management, standardize milking procedures, and improve udder health monitoring systems to control infection and antimicrobial resistance transmission risks at the source (Masarikova *et al.*, 2025).

Conclusions: This study investigated the antimicrobial resistance and virulence gene characteristics of *E. coli* isolates causing clinical mastitis in a large-scale dairy farm in southern Xinjiang, China. Over 15 antibiotic resistance and virulence genes were detected in MPEC from this dairy farm, exhibiting a diverse profile. These findings provide a basis for the precise prevention and control of *E. coli* mastitis in this large-scale dairy farm and offer a reference for formulating mastitis control strategies in the surrounding areas of southern Xinjiang, China.

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Authors contribution: JC and DY: investigation, data analysis and writing--original draft and editing. SQ and FC: writing--review and editing, project design and supervision.

REFERENCES

Adkins PRF and Middleton JR, 2017. Laboratory Handbook on Bovine Mastitis. 3rd Ed. National Mastitis Council: Madison, USA, pp:147.

Amoafo OY, Malekar V, Jones E, *et al.*, 2021. Antibiotic resistance and phylogenetic profiling of *Escherichia coli* from dairy farm soils; organic versus conventional systems. Current Research in Microbial Sciences 3:100088.

Arbab S, Ullah H, Wang W, *et al.*, 2025. Prevalence and antimicrobial drug resistance of Gram-negative bacteria in dairy feed and water: a One Health concern. Frontiers in Veterinary Science 12: 1654200.

Aubé L, Mialon MM, Mollaret E, *et al.*, 2022. Review: Assessment of dairy cow welfare at pasture: measures available, gaps to address, and pathways to development of ad-hoc protocols. Animal 16(8): 100597.

Blum SE, Goldstone RJ, Connolly JPR, *et al.*, 2018. Postgenomics characterization of an essential genetic determinant of mammary pathogenic *Escherichia coli*. mBio 9(2): e00423-e00518.

Cai K, Xu M, Liu L, *et al.*, 2025. Molecular epidemiology and antimicrobial resistance of *Klebsiella pneumoniae* strains isolated from dairy cows in Xinjiang, China. Veterinary Medicine and Science 11(1): e70120.

Caporaso JG, Lauber CL, Walters WA, *et al.*, 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences 108 (Suppl 1): 4516-22.

Caporaso JG, Lauber CL, Walters WA, *et al.*, 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal, 6(8): 1621-4.

Chen S, Zhang H, Zhai J, *et al.*, 2023. Prevalence of clinical mastitis and its associated risk factors among dairy cattle in mainland China during 1982-2022: a systematic review and meta-analysis. Frontiers in Veterinary Science 10: 1185995.

Cheng WN and Han SG, 2020. Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments - A review. Asian-Australasian Journal of Animal Sciences 33(11): 1699-713.

Chowdhury MSR, Hossain H, Rahman MN, *et al.*, 2025. Emergence of highly virulent multidrug and extensively drug-resistant *Escherichia coli* and *Klebsiella pneumoniae* in buffalo subclinical mastitis cases. Scientific Reports 15(1): 11704.

Clinical and Laboratory Standards Institute (CLSI) 2013. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. CLSI document VET01-A4, 4th Ed. Wayne, PA, USA.

Denamur E, Clermont O, Bonacorsi S, *et al.*, 2021. The population genetics of pathogenic *Escherichia coli*. Nature Reviews Microbiology 19(1): 37-54.

Deng H, Si HB, Zeng SY, *et al.*, 2015. Prevalence of extended-spectrum cephalosporin-resistant *Escherichia coli* in a farrowing farm: ST1121 clone harboring IncHI2 plasmid contributes to the dissemination of blaCMY-2. Frontiers in Microbiology 6: 1210.

Fazel F, Jamshidi A and Khoramian B, 2019. Phenotypic and genotypic study on antimicrobial resistance patterns of *E. coli* isolates from bovine mastitis. Microbial Pathogenesis, 132: 355-61.

Frick-Cheng AE, Sintsova A, Smith SN, *et al.*, 2022. Ferric citrate uptake is a virulence factor in uropathogenic *Escherichia coli*. mBio 13(3): e0103522.

Gao J, Barkema HW, Zhang L, *et al.*, 2017. Incidence of clinical mastitis and distribution of pathogens on large Chinese dairy farms. Journal of Dairy Science 100(6): 4797-806.

Gao J, Wu Y, Ma X, *et al.*, 2025. Prevalent and drug-resistant phenotypes and genotypes of *Escherichia coli* isolated from healthy cow's milk of large-scale dairy farms in China. International Journal of Molecular Sciences 26(2): 454.

Gohar S, Abbas G, Sajid S, *et al.*, 2017. Prevalence and antimicrobial resistance of *Listeria monocytogenes* isolated from raw milk and dairy products. Matrix Science Medica 1(1): 10-14.

Goulart DB and Mellata M, 2022. *Escherichia coli* mastitis in dairy cattle: Etiology, diagnosis, and treatment challenges. Frontiers in Microbiology 13: 928346.

Guerra ST, Orsi H, Joaquim SF, *et al.*, 2020. Short communication: Investigation of extra-intestinal pathogenic *Escherichia coli* virulence genes, bacterial motility, and multidrug resistance pattern of strains isolated from dairy cows with different severity scores of clinical mastitis. Journal of Dairy Science 103(4): 3606-14.

Guo Y, Qiu P, Su H, *et al.*, 2021. Antimicrobial resistance and virulence genes distribution in *Trueperella pyogenes* isolated from dairy cows with clinical mastitis in Liaoning of China. Pakistan Veterinary Journal 41(3): 329-34.

Han J, Sahin O, Barton YW, *et al.*, 2008. Key role of *Mfd* in the development of fluoroquinolone resistance in *Campylobacter jejuni*. PLoS Pathogens 4(6): e1000083.

He W, Ma S, Lei L, *et al.*, 2020. Prevalence, etiology, and economic impact of clinical mastitis on large dairy farms in China. Veterinary Microbiology 242: 108570.

Kakooza S, Mahero M, Munyiirwa DFN, *et al.*, 2025. Multidrug resistance in bacterial isolates from clinical samples submitted to a national veterinary diagnostic facility in Uganda (2014-2020): A retrospective analysis. Antibiotics 14(12): 1276.

Kazemzadeh S, Korneeva O, Shabunin S, *et al.*, 2025. Antibiotic resistance in mastitis-causing bacteria: Exploring antibiotic-resistance genes, underlying mechanisms, and their implications for dairy animal and public health. Open Veterinary Journal 15(9): 3980-4006.

Lacher DW, Mammel MK, Gangiredla J, *et al.*, 2020. Draft genome sequences of isolates of diverse host origin from the *E. coli* reference center at Penn State University. Microbiology Resource Announcements 9(39): 10-1128.

Laterza G, Meroni G, Soggiu A, *et al.*, 2025. Bovine clinical *E. coli* mastitis in Italian dairy herds is not associated with a specific pathotype. Pathogens 14(11): 1181.

Lee GH, Jeong JY, Chung JW, *et al.*, 2009. The *Helicobacter pylori Mfd* protein is important for antibiotic resistance and DNA repair. Diagnostic Microbiology and Infectious Disease 65(4): 454-6.

Li K, Hou M, Zhang L, *et al*., 2022. Analysis of antimicrobial resistance and genetic correlations of *Escherichia Coli* in dairy cow mastitis. Journal of Veterinary Research 66(4): 571-9.

Lippolis JD, Brunelle BW, Reinhardt TA, *et al*., 2016. Differential gene expression of three mastitis-causing *Escherichia coli* strains grown under planktonic, swimming, and swarming culture conditions. mSystems 1(4): e00064-16.

Liu Y, Chen Y, Feng M, *et al*., 2021. Occurrence of antibiotics and antibiotic resistance genes and their correlations in river-type drinking water source, China. Environmental Science and Pollution Research 28(31): 42339-52.

Martin HA, Heron R, Leyva-Sanchez H, *et al*., 2025. *Mfd* deficiency decreases the abundance of complete transcripts of sporulation genes and alters sporogenesis and the structure of dormant *Bacillus subtilis* spores. Frontiers in Microbiology 16: 1680580.

Masarikova M, Papouskova A, Sukkar I, *et al*., 2025. Genomic analysis of extended-spectrum beta-lactamase-producing *E. coli* from Czech dairy calves and their caretakers. Frontiers in Veterinary Science 12: 1552297.

Moradi A, Bidarian B, Mohammadian F, *et al*., 2025. Trends in antimicrobial resistance of major mastitis-causing pathogens: A nine-year study. Veterinary Medicine and Science 11(4): e70417.

My TT, Thien LV, Manh VD, *et al*., 2023. Antimicrobial resistance and molecular characterization of *Escherichia coli* isolated from bovine mastitis samples in Nghe An province, Vietnam. Veterinary World 16(4): 743-51.

Nikaido H and Pagès JM, 2012. Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. FEMS Microbiology Reviews 36(2): 340-63.

Nüesch-Inderbinen M, Käppeli N, Morach M, *et al*., 2019. Molecular types, virulence profiles and antimicrobial resistance of *Escherichia coli* causing bovine mastitis. Veterinary Record Open 6(1): e000369.

Olson MA, Siebach TW, Griffitts JS, *et al*., 2018. Genome-wide identification of fitness factors in mastitis-associated *Escherichia coli*. Applied and Environmental Microbiology 84(2): e02190-17.

Pinheiro REE, Chaves TP, Melo ES, *et al*., 2020. Modulatory-antibiotic activity of the essential oil from *Eucalyptus citriodora* against MDR bacterial strains. Cellular and Molecular Biology 66(4): 60-64.

Poirel L, Madec JY, Lupo A, *et al*., 2018. Antimicrobial resistance in *Escherichia coli*. Microbiology Spectrum 6(4): 10-1128.

Rafailidis PI and Kofteridis D, 2022. Proposed amendments regarding the definitions of multidrug-resistant and extensively drug-resistant bacteria. Expert Review of Anti-Infective Therapy 20(2): 139-46.

Ragheb MN, Thomason MK, Hsu C, *et al*., 2018. Inhibiting the evolution of antibiotic resistance. Molecular Cell 73(1): 157-65.

Ren Q, Liao G, Wu Z, *et al*., 2020. Prevalence and characterization of *Staphylococcus aureus* isolates from subclinical bovine mastitis in southern Xinjiang, China. Journal of Dairy Science 103(4): 3368-80.

Rossolini GM, Arena F, Pecile P, *et al*., 2014. Update on the antibiotic resistance crisis. Current Opinion in Pharmacology 18: 56-60.

Shehata AA, Abd-Elfatah EB, Elsheikh HEM, *et al*., 2024. Epidemiological features, biochemical indices, antibiogram susceptibility profile, and biofilm factor genes of *Klebsiella pneumoniae* isolated from bovine clinical mastitis cases. Pakistan Veterinary Journal 44(1): 141-7.

Shoaib M, He Z, Geng X, *et al*., 2023. The emergence of multi-drug resistant and virulence gene carrying *Escherichia coli* strains in the dairy environment: A rising threat to the environment, animal, and public health. Frontiers in Microbiology 14: 1197579.

Tahar S, Nabil MM, Safia T, *et al*., 2020. Molecular characterization of multidrug-resistant *Escherichia coli* isolated from milk of dairy cows with clinical mastitis in Algeria. Journal of Food Protection 83(12): 2173-78.

Wang L, Chen W, Zhang L, *et al*., 2013. Genetic diversity of *Streptococcus uberis* isolates from dairy cows with subclinical mastitis in Southern Xinjiang Province, China. Journal of General And Applied Microbiology 59(4): 287-93.

Wilm J, Svennesen L, Kirkeby C, *et al*., 2024. Treatment of clinically severe bovine mastitis - a scoping review. Frontiers in Veterinary Science 11: 1286461.

Xu L, Wang W, Zhang X, *et al*., 2024. Role of *LsrR* in the regulation of biofilm formation in mammary pathogenic *Escherichia coli*. BMC Veterinary Research, 20(1): 220.

Yaseen K, Ali S, Rahman SU, and Sajid MS, 2025. Comparative molecular virulence typing and antibiotic resistance of *Campylobacter* species at the human–animal–environment interface. Foodborne pathogens and disease 22(2): 109-117.

Yokoyama T, Niinae T, Tsumagari K, *et al*., 2021. The *Escherichia coli* S2P intramembrane protease RseP regulates ferric citrate uptake by cleaving the sigma factor regulator FecR. Journal of Biological Chemistry 296: 100673.

Yu ZN, Wang J, Ho H, *et al*., 2020. Prevalence and antimicrobial-resistance phenotypes and genotypes of *Escherichia coli* isolated from raw milk samples from mastitis cases in four regions of China. Journal of Global Antimicrobial Resistance 22: 94-101.

Zhao H, Ma H, Song C, *et al*., 2024. Prevalence and molecular characterization of multi-resistant *Escherichia coli* isolates from clinical bovine mastitis in China. Animal Biotechnology 35(1): 2322541.

Zuo J, Lv Z, Lian L, *et al*., 2025. Difference analysis on virulence genes, biofilms and antimicrobial susceptibility of *Escherichia coli* from clinical and subclinical bovine mastitis. Veterinary Sciences 12(2): 132.