

Analysis of the transmission of *Staphylococcus aureus* drug resistance and genetic diversity in the 'animal-environment/food-human' dairy production chain

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Research Article

Keywords: *Staphylococcus aureus*, One Health, Bovine, AMR, Transmission

Posted Date: September 15th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10427364/v1>

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Additional Declarations: No competing interests reported.

1 **Analysis of the transmission of *Staphylococcus aureus* drug resistance**
2 **and genetic diversity in the ‘animal-environment/food-human’ dairy**
3 **production chain**

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Abstract: *Staphylococcus aureus* (*S. aureus*) causes mastitis in dairy bovine and spreads along the food chain, affecting human health. To effectively prevent and control the transmission of *S. aureus* from dairy bovine, this study was based on the concept of 'One Health', and 2236 samples were collected from large-scale farms in Jiaodong region of China, from the breeding and milking session, and the downstream process such as markets and hospitals. These samples were analyzed by mass spectrometry identification, and complemented by whole gene sequencing, in order to explore the molecular genetic characteristics of *S. aureus* from various sources and factors affecting its transmission. It was found that the overall isolation rate of *S. aureus* in 2236 samples was 17.12%, and the isolation rates in the four sampling sessions were farming (11.66%), milking (28.82%), market (1.33%), and hospital (18.71%). The most contaminated samples in the milking session, with high separation rates, were samples from the tank walls, air ducts, pens, towels, towel washing water and human sources (20% to 40.74%). The results of drug sensitivity showed that the multi-resistance rate of the 171 representative strains was 37.71% (66/175), and the multi-resistance rate in the farm sector was 36.91% (55/149), which was much higher than that in the hospital sector (28.57%) ($P < 0.01$). Except for penicillin (86.86%), the drug resistance rate of all strains to other antibiotics was below 35.43%. The 121 representative strains covered 7 clonal groups and 12 ST types, the dominant ST types being ST59 (33.05%), ST97 (20.66%), and ST81 (9.9%). SNP analysis showed that 97.5% of the MRSA-ST59-t437 strains were situated within Clade I, and 89.74% of these strains sourced from four sampling sessions. This study reveals that the implicit

1 carriage of *S. aureus* by bovine in the farming session is the main source of *S.*
2 *aureus* in milk, and that the dominant type MRSA-ST59-t437 are widely clonally
3 transmitted between animals-environment-workers in dairy farms, which has the
4 risk of clonally transmitting to the consumer along the food chain. The milking
5 session is a key risk point for *S. aureus* transmission in the production chain, with
6 contaminated towels and their washing water, poor milking worker practices being
7 the main influencing factors. It is suggested to establish the concept of 'One health'
8 in production practice, strengthen the biosafety management of dairy farms, and
9 effectively reduce the spread risk of *S. aureus*, especially multidrug-resistant
10 strains, along the production chain between 'animal-environment/food-human'.

11 **Keywords:** *Staphylococcus aureus*; One Health; Bovine;AMR; Transmission

1 Introduction

Staphylococcus aureus (*S. aureus*) is one of the main pathogens causing bovine mastitis, which is characterized by its persistence and recurrence. Antibiotic treatment is a crucial measure for controlling bovine mastitis ^[1, 2]. The use of antimicrobial agents to treat bovine mastitis can increase the selection pressure on *S. aureus*, leading to the emergence and spread of drug-resistant *S. aureus* strains. Because of the low probability of being cured by antibiotics, animals that fail to be treated are usually slaughtered, which leads to huge losses in dairy industry ^[3, 4]. *S. aureus* is also the third major cause of foodborne diseases in human beings ^[5, 6] and can be transmitted from livestock through the food chain, leading to human food poisoning ^[7], posing a serious threat to public health. The report of the US Centres for Disease Control and Prevention (CDC) on antibiotic resistance has listed methicillin-resistant *S. aureus* (MRSA) as a serious public health threat^[8]. It is predicted that by 2050, antibiotic resistance will lead to 10 million deaths per year and 100 trillion dollars in global economic loss.

Raw milk goes through many stages of production before reaching consumers, including farming, milking, processing and distribution. As a significant foodborne pathogen, *S. aureus* can proliferate and disseminate throughout these stages when present in bovine. Richardson found that bovines are the main host source of *S. aureus* infections in humans, and multiple host interactions and switching events between bovine and humans have occurred during the past 3000 years ^[9]. In recent years, many studies have found that there was a close relationship between human carrying LA-MRSA and farm occupational exposure ^[10, 11], but there

1 were relatively few studies on the risk factors for *S. aureus* transmission throughout
2 the whole farm-to-table production chain. Utilizing the 'One Health' concept and
3 whole genome sequencing technology, this study investigates the molecular
4 genetic characteristics of *S. aureus* in dairy farming, milking, marketing, and
5 hospital sessions, as well as the key transmission risk points and influencing
6 factors. The aim is to establish the foundation for promoting healthy dairy farming
7 practices and ensuring public health and safety.

8 **2 Material and methods**

9 **2.1 Sample collection**

10 Between July and September 2024, a total of 2,236 samples were collected from
11 various sessions, including dairy farming, milking, markets, and hospitals in the
12 Jiaodong region of China. Specifically, 1,312 samples of 15 different types were
13 obtained from five dairy farms, 694 samples of 12 types were collected from milking
14 operations, 75 samples of pasteurized milk were sourced from supermarkets and
15 milk bars, and 155 samples of patient feces were gathered from hospitals. The
16 distribution of sample types and their respective quantities is detailed in Table 1.

17 **Table 1: Isolation rate of *Staphylococcus aureus* at different sessions.**

Session	Type	Number of samples	Number of isolates	Isolation rate
Farm (breeding)	Milk	300	48	16.00%
	Nasal swab(Bovine)	305	64	20.98%
	Udder swab(Bovine)	65	7	10.77%
	Pen	75	5	6.67%
	Air(cowshed)	3	0	0.00%
	Bedding	75	1	1.33%
	Feed	75	0	0.00%
	Cow dung	75	2	2.67%
	Fly	54	0	0.00%

	Water	20	0	15.56%
	Feed(barns)	45	7	0.00%
	Dust(warehouse)	35	1	2.86%
	Air(warehouse)	5	0	0.00%
	Transportation	60	6	10.00%
	Pen(outside the milking parlor)	65	3	4.62%
	Floor(outside the milking parlor)	55	9	16.36%
	Subtotal	1312	153	11.66%
Farm (milking)	tank milk	50	12	24.00%
	air duct	50	17	34.00%
	Air(milking parlor)	5	0	0.00%
	Pen	80	31	38.75%
	Milking unit attachment	75	14	18.67%
	Towel	75	27	36.00%
	Effluent	27	11	40.74%
	Floor	70	9	12.86%
	Fly	17	0	0.00%
	Tank wall	55	11	20.00%
	Nasal swab(Worker)	40	15	37.50%
	Hand swab(Worker)	50	19	38.00%
	Clothes swab(Worker)	50	18	36.00%
	Sole swab(Worker)	50	16	32.00%
	Subtotal	694	200	28.82%
Market	Milk bar	15	1	6.67%
	Supermarket	60	0	0.00%
	Subtotal	75	1	1.33%
Hospital	Faeces	155	29	18.71%
Total		2236	383	17.12%

2.2 Bacterial isolation and identification

The strains were isolated and identified according to the reference ^[12], and the quality control strain (ATCC 29213) was provided by the China Animal Health And Epidemiology Center.

2.3 Antimicrobial Sensitivity Test

A total of 139 representative strains were selected according to different sources and sample types, and the minimum inhibitory concentrations of the representative

1 strains against 18 antimicrobial drugs of 12 classes, including penicillin (PEN),
2 amoxicillin/clavulanic acid (AMC), erythromycin (ERY), clindamycin (CLI),
3 enrofloxacin (ENR), ofloxacin (OFL), ceftiofur (CEF), ceftiofur (CEF), ceftiofur (CEF), ceftiofur (CEF), sulfisoxazole
4 (SIS), oxacillin (OXA), vancomycin (VAN), sulfamethoxazole/trimethoprim (SXT),
5 doxycycline (DOX), fosfomic acid (FFC), tiamulin (TIA), tiludronate (TIL), gentamicin
6 (GEN), linezolid (LZD), tetracycline (TET). The results were interpreted as valid if
7 the quality control strain ATCC 29213 was used simultaneously, and if the quality
8 control strain was within the quality control range and the positive control had
9 bacterial growth, and the negative control had no bacterial growth. The
10 experimental strains were simultaneously resistant to 3 or more classes of
11 antimicrobials and were multidrug-resistant.

12 **2.4 Whole gene sequencing analysis**

13 One hundred and twenty-one representative strains from different sessions (31
14 bovine-source strains, 59 environmental-source strains and 24 human-source
15 strains, 1 market-source strain and 6 hospital-source strains) were selected, and
16 the strains were screened for carrying the *mecA* gene by using PCR to confirm
17 MRSA strains with reference to the literature ^[13]. At the same time, 121 strains were
18 sent for whole genome sequencing and splicing to Bioengineering (Shanghai) Co.
19 The whole genome sequence information was submitted to the Centre for Genome
20 Epidemiology (CGE) website (<https://www.genomicepidemiology.org/>), and typing
21 analysis was carried out by MLST and Sccmec modules, and Bionumerics
22 software was used to construct the MLST of *S. aureus* isolates, the cgMLST
23 minimum spanning tree, and strains were typed for staphylococcal A protein (spa)

1 using the spa (<https://cge.food.dtu.dk/services/spaTyper/>) website. Core genome
2 evolutionary tree construction was performed on whole genome sequencing data
3 using BioNumerics v7.6 software and beautified by the ChiPlot online website
4 (<https://www.chiplot.online/#>). Inter-strain SNPS locus differences were analysed
5 by the BacWGSTdb database using strain N315_BA000018_ST5 as the reference
6 sequence.

7 **2.5 Statistical Analysis**

8 Using the software SPSS 26.0 chi-square test for statistical and significance of
9 difference analysis, $p < 0.01$, the difference is highly significant, indicated by ‘***’;
10 $0.01 < p < 0.05$, the difference is significant, indicated by ‘**’; $p > 0.05$, the difference
11 is not significant, indicated by ‘ns’. then the difference is not significant, indicated
12 by ‘ns’.

13 **3 Results**

14 **3.1 Results of *S. aureus* isolation at different sessions**

15 The overall isolation rate of *S. aureus* was 17.12% (383/2236), of which the
16 isolation rate of target organisms in the farm and hospital sessions was 17.59%
17 (353/2006) and 18.71% (29/155), respectively, which was much higher than that in
18 the market sessions which was 1.33% (1/75) ($p < 0.01$), and the specific results
19 were shown in Table 1.

20 The isolation rate of the target bacteria in the on-farm breeding session was 11.66%
21 (153/1312). Among them, the isolation rate of strains of animal source was 18.51%
22 (112/605), which was much higher than the isolation rate of strains of
23 environmental source (5.79%, 41/707) ($p < 0.01$). The sample types with higher

isolation rates in samples from environmental sources were floor sameples (16.36%), water sameples (15.56%), udder swabs (10.77%) and vehicle swabs (10%). No target bacteria were isolated from air, feed and fly samples (Table 1).

The isolation rate of *S. aureus* in the farm milking session was 28.82% (200/694), which was much higher than that in the farming session ($p < 0.01$). The milk isolation rate was 24% (12/50), which was increased compared with that of the farming session, but the difference was not significant ($p > 0.05$). The isolation rate of strains from environmental sources was 26.43% (120/454), and the samples with higher isolation rate were effluent (40.74%), pen swabs (38.75%), towel swabs (36% (27/75)), air duct swabs (34%), tank milk for (24%) and tank wall swabs (20%), flies were not isolated with the target strain. The isolation rate of strains from human sources was 35.78% (68/190), and the isolation rate of strains from all four human samples collected (nasal swab, hand swab, clothes swab and sole swab) was above 28% (Table 1).

3.2 Drug sensitivity results of *S. aureus* at different sessions

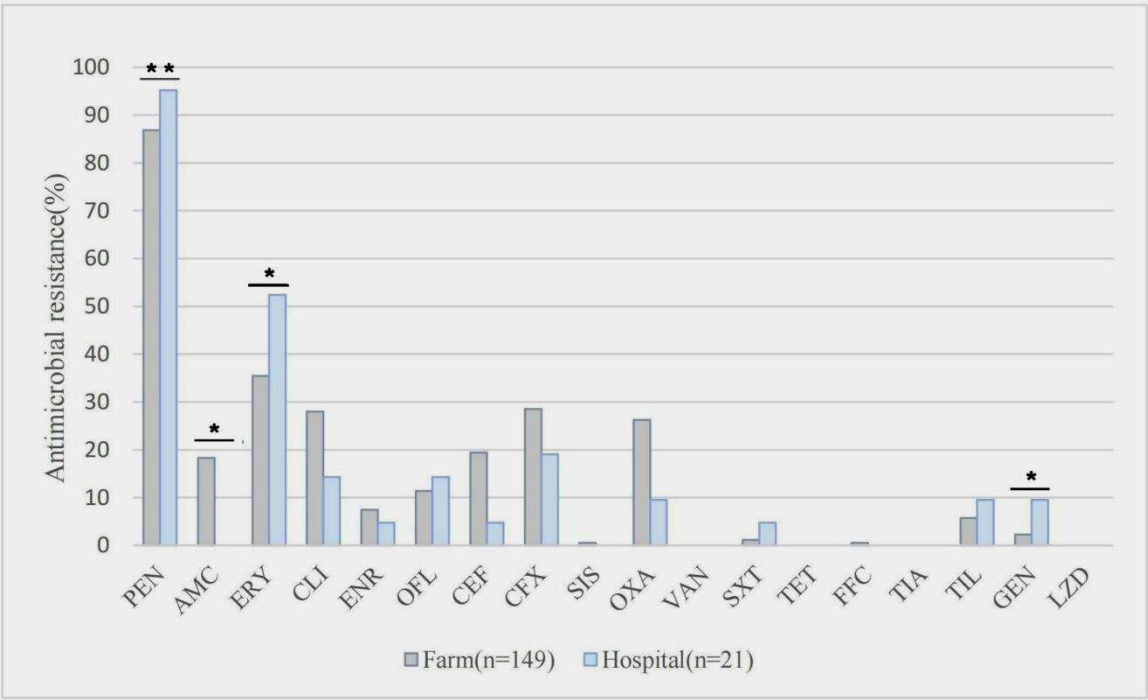
The multi-resistance rate of 171 representative strains was 37.71% (66/175), with resistance weights distributed from 0 to 7 resistance, of which the resistance rate to penicillin was as high as 86.86%, erythromycin was 35.43%, cefoxitin was 28.57%, clindamycin was 28%, oxacillin was 26.29%, ceftiofur was 19.43%, amoxicillin/clavulanic acid was 18.29%, ofloxacin 11.43%, and no resistance to vancomycin, tetracycline, tiamulin, or linezolid (Fig. 1A).

The multi-drug resistance (MDR) rate was 36.91% (55/149) in the farm link, and the MDR was mainly distributed in 7 resistance, and there was no resistance to

vancomycin, tetracycline, tiamulin, and linezolid (Fig. 1B and Fig. 1C). The MDR rate was 34.21% (13/38) for animal source strains, 35.71% (30/84) for environmental-derived strains, and 44.44% (12/27) for human source strains, and the MDR of animal-derived strains was mainly in the range of 5-7 resistance, that of environmental source strains was mainly in the range of 7 resistance, and that of human source strains was mainly in the range of 3-7 resistance (Fig. 1C). Animal-source MRSA strains were 100% resistant to three drugs: penicillin, amoxicillin/clavulanic acid and oxacillin, 85.71% resistant to ceftiofur, and 71.43% resistant to both erythromycin and clindamycin. The MRSA strain of environmental source showed a resistance rate of up to 100% to the three drugs penicillin, erythromycin and clindamycin, 95.65% to oxacillin, and 86.21% and 78.26% to amoxicillin/clavulanic acid and ceftiofur, respectively. The MRSA strains of human source showed 100% resistance to penicillin, 81.82% and 63.64% to erythromycin and oxacillin, 54.55% to both clindamycin and ceftiofur, and 36.36% to amoxicillin/clavulanic acid. None of the three source strains were resistant to any of the four drugs, vancomycin, tetracycline, tiamulin, or linezolid (Fig. 1B) .

The rate of multi-resistance in the hospital session was 28.57% (6/21), and the distribution of MDR was in the range of 3-7 resistance (Fig. 1C). The resistance rate to penicillin was 95.24% (20/21), erythromycin was 52.38% (11/21), clindamycin and enrofloxacin were 14.29% (3/21), and there was no resistance to amoxicillin/clavulanic acid, sulfamethoxazole/trimethoprim, vancomycin, tetracycline, tiamulin, fosfomicol, and linezolid (Fig. 1B).

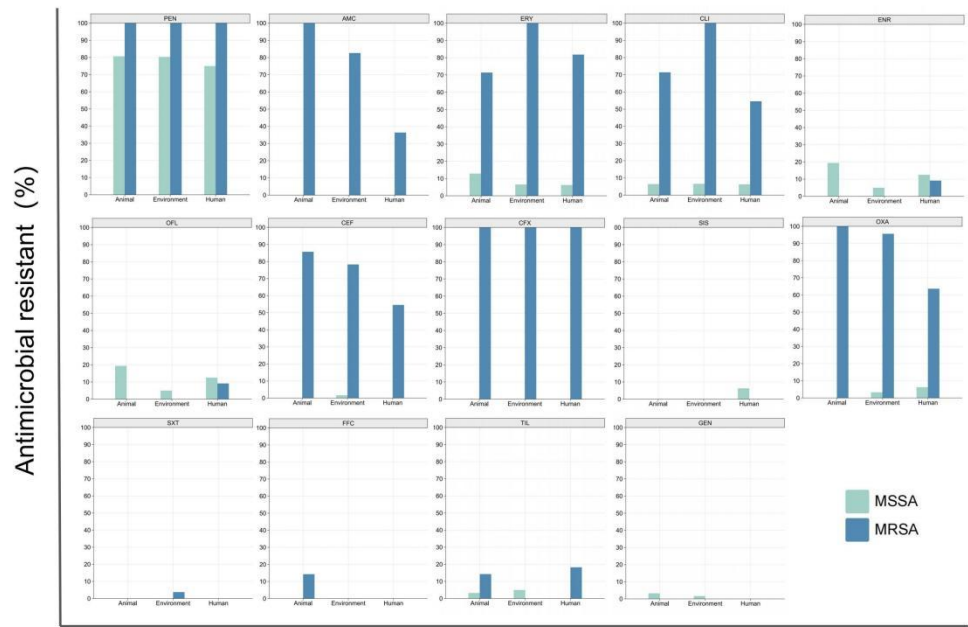
1 (A)



2

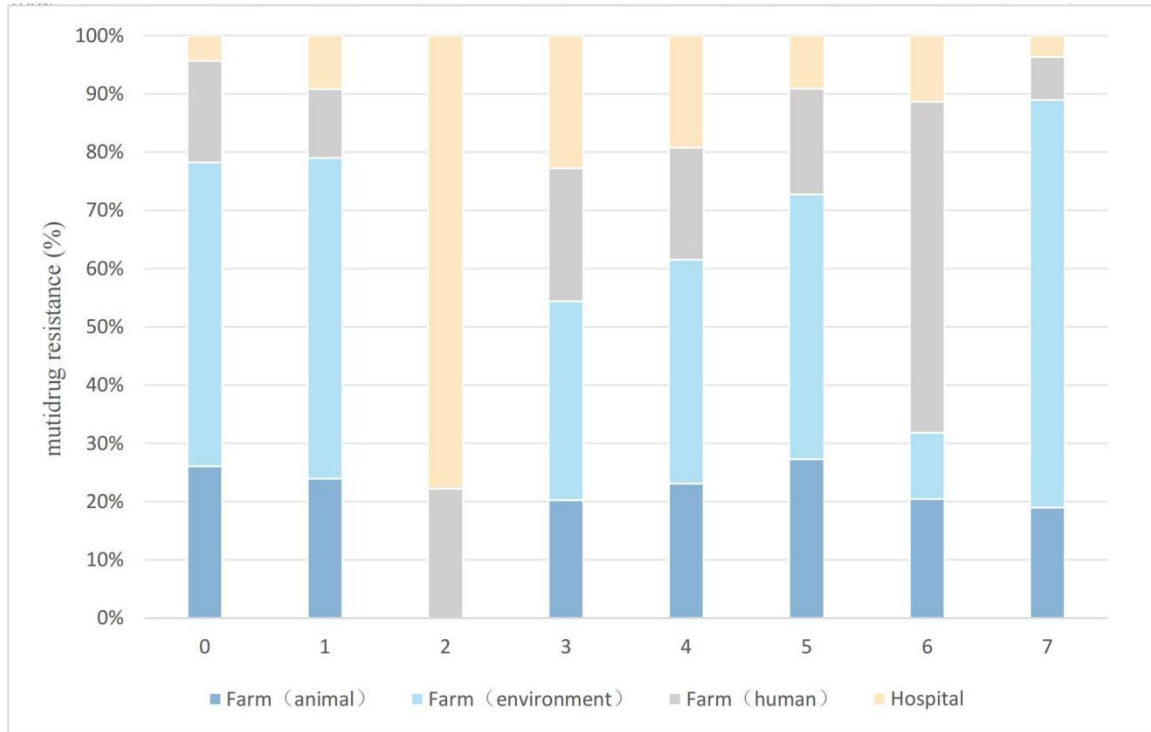
3

4 (B)



5

1 (C)



2

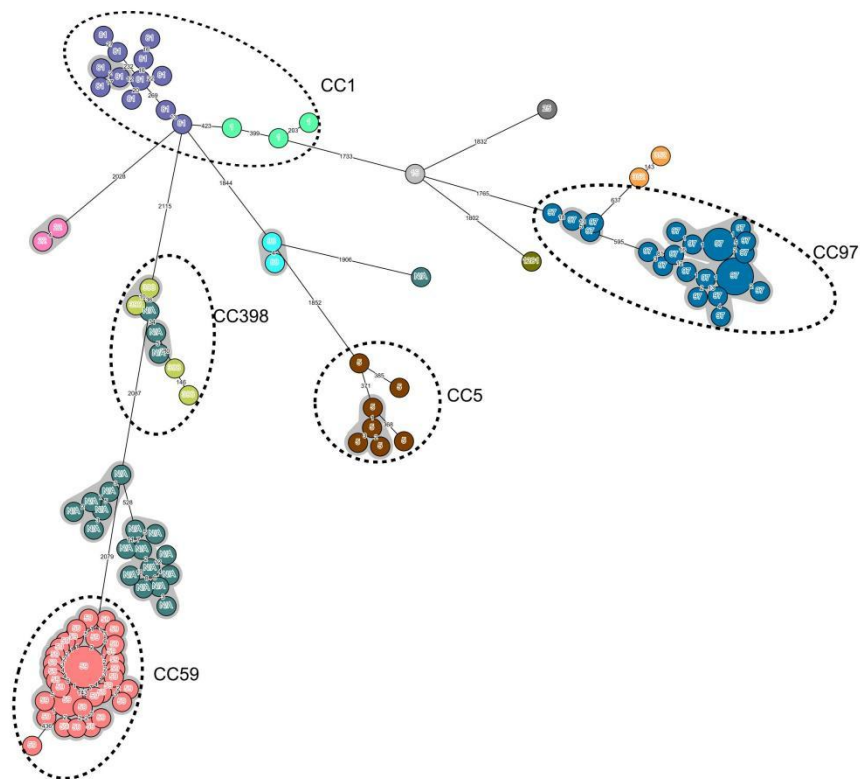
3 **Fig. 1.** (A) Antibiotic susceptibility results of *S. aureus* in different sessions. (B)
 4 Antibiotic susceptibility results of *S. aureus* from animal、environment, and human
 5 sources in the farm section. (C) Distribution of MDR *S. aureus* in different sessions.

6 **3.3 Distribution of *S. aureus* sequence types**

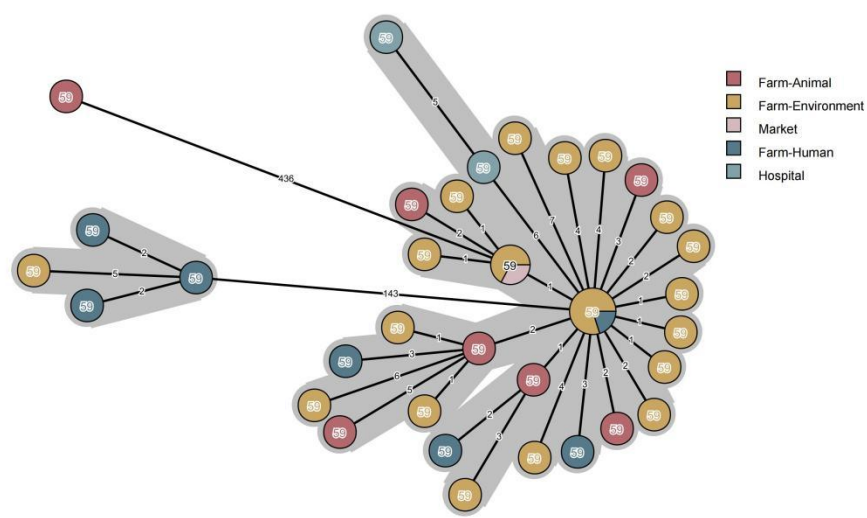
7 121 strains of *S. aureus* were found in 7 clonal groups and 12 ST types. The clonal
 8 groups included CC59, CC97, CC1, CC5, CC398, CC22 and CC15, of which the
 9 dominant types were ST59 (33.05%), ST97 (20.66%) and ST81 (9.92%) (Fig.
 10 2A). The 40 strains of ST59 were the most widely distributed. They were all MRSA
 11 strains from 15 types of samples, including animals (nasal swabs, individual milk),
 12 environment (pens, dust, bedding, water, floors, air ducts, towels, effluent, tank
 13 walls, and vehicles), and humans (nasal swabs, hand swabs, and sole swabs) (Fig.
 14 2B); followed by ST97 strains from animals (nasal swabs, milk, and tank milk),

1 environment (udder swabs, floors, and tank walls), and human (nasal swabs, hand
2 swabs, and sole swabs) (Fig. 2C). ST81 came from 9 types of samples from
3 animals (milk, tank milk), the environment (floors, pens, towels, tank walls), and
4 human (clothes swabs, hand swabs, sole swabs). The hospital session contained
5 four types of typing (ST59, ST398, ST1281, and ST15), the market session
6 contained only ST59 typing, and the cross-trait typing of the three sessions was
7 ST59. No CC97 or CC1 clones were found in the hospital and market sessions.
8 (Fig. 2B, 2C, and 2D).

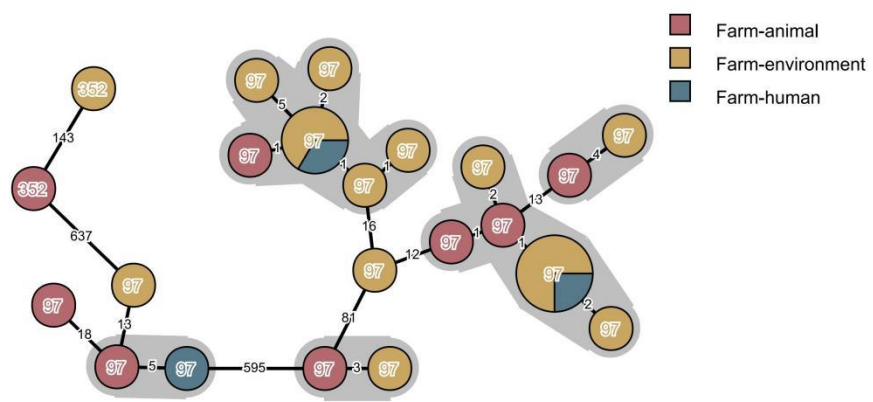
9 (A)



1 (B)



2



3

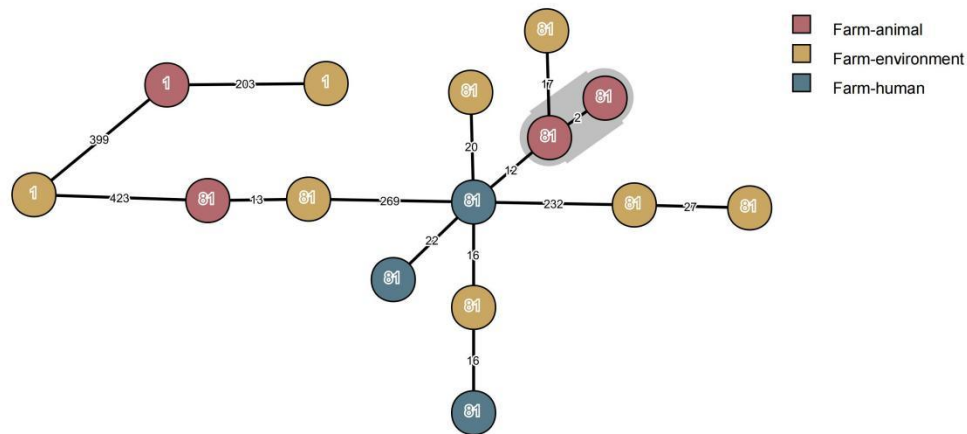


Fig. 2. (A) Minimum spanning tree calculated for 121 *S. aureus* isolates. The tree was created using 2079 core genome multilocus sequence typing loci, with the sequence type (ST) of each isolate shown within a circle. Clonal complexes CC59, CC97, CC1, CC398 and CC5 isolates were surrounded by circles, and isolates were shown with interconnecting lines and numbers indicating allelic distances between nodes (Strains with inter-strain differences of 10 alleles or less use grey shading for background). (B) Distribution of the 40 CC59 clones (MRSA). (C) Distribution of the 27 CC97 clones. (D) Distribution of 15 CC1 clones.

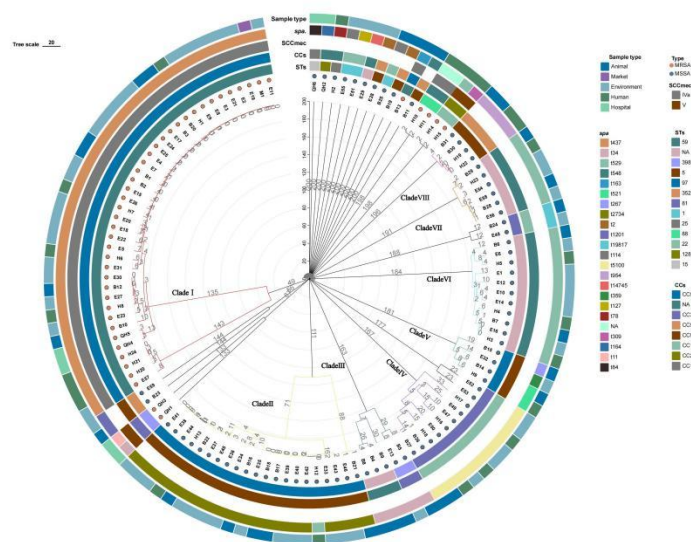
3.4 Analysis of the core genome evolutionary tree of *S. aureus* at different sessions

An evolutionary tree was constructed for 121 representative strains, all containing eight major branches with SNP differential sites ranging from 0 to 18923. Among

1 them, Clade I (red) was the furthest from the root node, and all of the strains
 2 were of ST59 type, containing only 0 to 151 differential sites. The composition of
 3 the major sub-branches was derived from 19.35% (6/39) strains of animal source
 4 (nasal swabs, milk), 37.29% (22/59) strains of environmental source (pens, dust,
 5 bedding, water, floors, air ducts, towels, effluent, tank walls, vehicles), 16.66%
 6 (4/24) strains of human source (nasal swabs, hand swabs, sole swabs), 33.33%
 7 (2/6) hospital session strains, and one market session strain. All three source
 8 strains from the farm link had less than 10 differential sites, and included 0-15
 9 differential sites with the hospital (QH4, QH5) and market session strains (M1).
 10 The secondary sub-branch contained three strains of human source (nasal swabs,
 11 sole swabs) and one strain of environmental source (ground), which included 0-8
 12 differential sites among the four strains, and 138-151 differential sites compared to
 13 the primary sub-branch strains in residence (Fig. 3).
 14 Clade II (yellow) contained only ST97 strains, 21 strains containing 0-89 differential
 15 loci from 16.13% (5/31) of animal source (nasal swabs, individual milk, vat milk).
 16 23.73% (14/59) of environmental source (cow udder swabs, floors, pens, drinking
 17 water, ducts, milk liners, towels, towel wash water, vehicles), and 8.33% (2/24)
 18 strains of human source (hand swabs, sole swabs). The major sub-branch
 19 comprising the three source strains from the farm link containing 0-18 differential
 20 loci, and the minor sub-branch from animals (tank milk) and the environment
 21 (vehicles), which differed by only one differential locus, and 82-89 differential loci
 22 from the other strains in the major sub-branch. The remaining ST97 strains were
 23 distributed in the Clade V branch and came from three different types of samples

(tank milk, floor and human sole swabs) in the farm session, and included 6-19 differential sites between strains (Fig. 3).

Clade IV contained only ST81-type strains, and all strains included 1 to 249 differential loci from animal source (milk, tank milk), environmental source (pen, towel, tank wall), and human source (clothes swabs, hand swabs, and sole swabs) from the farm session, with strains B26 (vat milk) and H16 (human hand swabs) including 1 and 2 differential loci, respectively, and strains B27 (tank milk) and E50 (towel swabs) containing 2 differential loci, and strains E47 (pen swab) and H16 (human hand swab) containing 1 and 2 differential loci. B27 (tank milk) and E50 (towel swabs) contained 2 differential loci. Environmental source strain E47 (pen) with human source strains H16 (hand swabs) and H17 (clothes swabs) contained 1 and 3 differential loci, respectively (Fig. 3).



1 **Fig. 3.** Phylogenetic tree of the core genome of 121 *S. aureus* strains.

2 **3.5 Distribution of drug-resistant genes carried by *S. aureus* in different** 3 **sessions**

4 The 121 representative strains contained a total of 14 resistance genes in 7
5 classes, namely aminoglycosides (5), β -amides (2), macrolides (2), folate pathway
6 antagonists (2), lincosamide (1), tetracyclines (1), and peptides (1). Among them,
7 the β -amide resistance genes *blaZ* and *mecA* were carried 52.89% (64/121) and
8 38.01% (46/121), respectively; aminoglycoside resistance genes *ant(6)-Ia* and
9 *aph(3')-III* accounted for 33.88% (41/121) and 31.4% (38/121), respectively;
10 macrolide resistance genes *erm(B)* accounted for 33.88% (41/121). The farm link
11 contained 14 resistance genes, and both the hospital and market strains contained
12 5 resistance genes, with the farm having a wider diversity of resistance genes
13 compared to the hospital and market strains (Fig. 4A). Farm animal source carried
14 10 drug-resistant genes, while both environmental and human source strains
15 carried 11 drug-resistant genes.

16 There were significant differences in the number of resistance genes carried by
17 different types of strains. All ST59 strains carried *mecA* resistance genes (40/40),
18 95.12% carried *blaZ* (39/41), and 92.68% carried three resistance genes, *ant(6)-*
19 *Ia*, *aph(3')-III* and *erm(B)* (38/41). The high positive rates of the above five drug-
20 resistant genes were consistent with the resistance results of the drug sensitivity
21 assay. Strain ST97 contained only two drug-resistant genes, *blaZ* and *str*, with
22 detection rates of 16% (4/25) and 8% (2/25), respectively. Strain ST81 carried only
23 one resistance gene, *str*, with a detection rate of 8.3% (1/12). There was a

1 significant difference in the number of resistance genes carried by the three
2 dominant clonal groups, with strain CC59 carrying a much higher number of
3 resistance genes than strains CC97 and CC1 ($p < 0.01$), while there was no
4 significant difference between strains CC97 and CC1 ($p > 0.05$) (Fig. 4B). Some
5 of the resistance genes were specific to certain ST types and source strains. *Inu(G)*
6 was found only in animal source strains (ST59), *bleO* was found only in human
7 source strains (ST88), and *tet(K)* was found only in environmental and human
8 source strains (ST59).

9 The 121 representative strains contained a total of four drug-resistant mutations,
10 *griB* (p.E422D), *griA* (p.S80Y and p.S80F), *dfrB* (p.H31N), and *gyrA* (p.S84L),
11 which were detected in 54.54% (66/121), 49.58% (64/121), 9.09% (11/121) and
12 8.26% (10/121). Among them, three mutations, *griA*, *griB* and *gyrA*, were
13 associated with fluoroquinolone resistance, and the *dfrB* mutation phenotyped as
14 methicarbamazepine. *griB* was carried by more than 50% of the three source
15 strains on the farm, whereas the *dfrB* and *gyrA* mutations were found only in the
16 farm session (Fig. 4A).

4

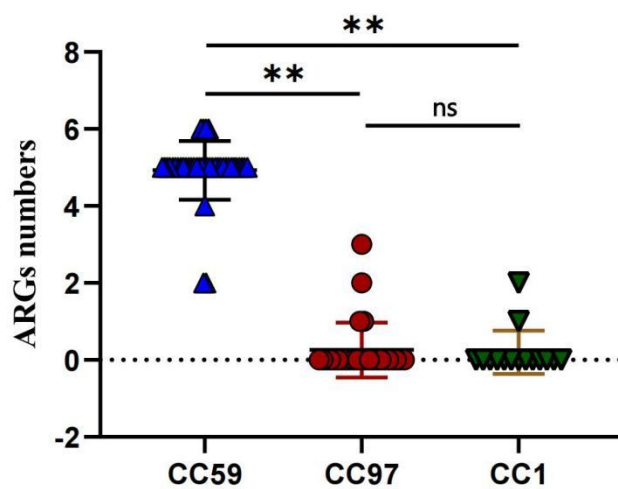


Fig. 4. (A) Distribution of drug resistance in 121 strains of *S. aureus*. (B) Distribution chart of the number of drug resistance genes carried by different clones.

4 Discussion

The results of isolation of *S. aureus* from each session of 'animal-food-human'showed that the isolation rate of target bacterial from pasteurised milk samples collected from the market (1.33%) was much lower than that of the other two sessions (17.59% and 18.71%), indicating that dairy farms were the main source of *S. aureus* contamination in fresh milk. Pasteurisation of fresh milk basically kills the bacteria in milk, but one strain was still detected in the pasteurised milk samples collected from the market, posing a risk of transmission to humans along the food chain. Further analysis of the isolation results of strains from 'animal-environment-human' in the farm chain showed that the isolation rate in the milking chain (28.82%) was much higher than that in the farming chain (11.66%) ($p < 0.01$), and the isolation rate of milk from tank (24%) was higher than that of individual milk in the farming chain (16%), indicating that the milking chain of dairy farms may be the cause of the bacteria in raw milk. Except for air and flies, the isolation rate of strains in the milking session was more than 12.86% in all 12 types of samples, and the isolation rates of strains in three types of environmental samples (effluent, pens, and towels) and four types of human samples (nasal swabs, hand swabs, clothes swabs, and shoe swabs) were all more than 30%, which suggests that contamination of different environmental samples and the mobility of personnel may be the key risk points for the spread of *S. aureus* in the

1 milking session. *S. aureus* to spread to each other in the milking process,
2 especially the towel used to wipe the udder, the water used to clean the towel and
3 the irregular operation of the milking workers, which could easily lead to cross-
4 contamination. The use of the same towel, the same bucket of washing water by
5 multiple bovine and irregular hand washing and disinfection by milking workers
6 during the first three milking were also found in the sampling, which may be the
7 key influencing factors leading to the inter-transmission of *S. aureus* among the
8 milk, environment and humans. In addition, the isolation rate of tank wall swab
9 samples (20%) was also high, indicating that the cleaning and disinfection of milk
10 tanks were not thorough. The isolation rate of air duct swab samples was as high
11 as 34%, which may be due to the high location of the air ducts in the disinfection
12 blind area. Combined with the result that no target organisms were isolated from
13 the air samples of the parlour, it shows that it has not yet played an important role
14 in the transmission of the target organisms in the parlour. The results suggested
15 that dairy farms should pay attention to this issue by strengthening the disinfection
16 of parlour facilities and expanding the disinfection area. The isolation rate of *S.*
17 *aureus* from milk samples in the farming session in this study was 16%, which is
18 much lower than that in California (40%) and Pakistan (30.32%) [14, 15]. This may
19 be due to the fact that the latter were collected from mastitis and occult mastitis
20 cows screened by mastitis detection method (CMT) and somatic cell count (SCC),
21 whereas the present study included healthy individual cows, which also suggests
22 that screening of occult carrier cows should be strengthened during the breeding
23 process, to prevent them from entering the milking process at the source and

1 leading to bacterial transmission. In addition, the isolation rate of 20.98% from
2 nasal swabs of cows in this study was higher than that from nasal swabs of
3 Algerian cows (16%) (Titouche et al., 2024), but lower than that of *S. aureus* from
4 domestic bovine sources (25.7%-36.8%) [16-18]. This may be due to the differences
5 in the isolation rate of the target organisms due to different feeding management
6 levels in different countries and regions.

7 The cgMLST and SNP analysis based on the isolated strains showed that cgMLST
8 was mainly divided into three branches, the dominant clonal groups included CC59,
9 CC97 and CC1, and the dominant phenotypes were ST59 (33.05%), ST97
10 (20.66%), and ST81 (9.9%) SNP evolutionary tree showed that the differently
11 typed strains were clustered in the same cluster of branches, respectively. The
12 cross typing of strains from three sources, animal, environmental and human, in
13 the farm session included ST59, ST97, ST81 and ST5, Clade I was the largest
14 branch, all strains were MRSA-ST59-t437 type, and its main branches were from
15 strains from three source, farm, market and hospital, and the differential loci of
16 strains between the farm sessions were all less than 10, especially the individual
17 milk (B20) with only 1 differential locus between strains sourceating from airway
18 swabs (E8, E9) and human nasal swabs (H1), suggesting extensive clonal
19 transmission between strains from the three farm source. The cross typing of the
20 farm session with the market and hospital sessions was ST59, and the differential
21 loci between the strains of the three sessions were also distributed in 0-15,
22 containing only 2 differential loci between individual milk (B12) and the market
23 source strain (M1), and 1 differential locus with the patient source strain (QH4).

1 This confirmed that bovine-derived *S. aureus* did pose a risk of transmission along
2 the food chain through the market. In addition, it was also found that the differential
3 loci of hand swabs (H8) and towel swabs (E30), towel washing water (E16, E31),
4 and air duct swabs (E24, E25, E26) were less than 10, and the isolation rate of the
5 strains in the four types of samples in the milking process was as high as 30% or
6 more, which confirmed that unhygienic towels and effluent, irregular operation of
7 the milkers and incomplete cleaning and disinfection of the environment were the
8 main causes of *S. aureus* transmission through the market chain. Clade II and
9 Clade IV consisted of ST97-t2734 and ST81-t5100, respectively, and all strains
10 came from farms, and multiple strains from the three sources had differential loci
11 less than 10, which indicated that the strains in the farms were closely related to
12 each other. In this study, ST59 MRSA strains, which are the main community-
13 associated MRSA strains (HA-MRSA) in China [19, 20], were detected in samples
14 from animals, the environment, and workers on dairy farms, suggesting that the
15 monitoring and control of ST59 MRSA strains on dairy farms should be
16 strengthened to avoid their transmission to humans, especially practitioners, and
17 to trigger public health safety. practitioners, which may cause public health and
18 safety problems.

19 The results of drug sensitivity showed that the multi-resistance rate in the farm
20 session was 36.91% (55/149), much higher than the multi-resistance rate in the
21 hospital session, which was 28.57% (6/21) ($p < 0.01$). The resistance rate of *S.*
22 *aureus* to penicillin in milk source was as high as 100%, which was higher than the
23 resistance rate of *S. aureus* in milk sources in Hunan Province and Xinjiang

1 Autonomous Prefecture by Ning Qiang and Ren Keming et al. [21, 22], and the
2 resistance rate to erythromycin, clindamycin, and benzathine was 25%, which was
3 basically the same as that in the study of Ning Qiang and Ren Keming et al.
4 Therefore, it is necessary to strengthen the promotion of raw milk testing,
5 alternative therapies and biological control, and strengthen monitoring.
6 The highest isolation rate of β -amide resistance gene *blaZ* was 52.89% (64/121),
7 followed by aminoglycoside resistance genes *ant(6)-Ia* (33.88%), *aph(3')-III*
8 (31.4%) and macrolide resistance gene *erm(B)* (33.88%) in the present study. The
9 detection rate of strains of human and environmental source carrying *ant(6)-Ia*,
10 *aph(3')-III* and *erm(B)* resistance genes was as high as 100%, and that of strains
11 of animal source was 83.33%, and ST59-MRSA strains from hospital and farm
12 session were also found to carry three resistance genes at the same time, which
13 is in line with the results that the multi-drug resistance rates of strains of human
14 and environmental source were higher than strains of animal source. In addition,
15 strains carrying *tet(K)* resistance genes (E57, H20, H21, H24) were found only in
16 environmental and human ST59-MRSA strains, suggesting that the environmental
17 and human strains have a wider diversity of resistance genes compared to animal
18 source strains. It has been shown [18, 23] that a serine to leucine amino acid
19 substitution at position 84 of the *gryA* gene and a mutation from serine (TCA) to
20 tyrosine at position 80 of the *griA* gene are genetic variations capable of affecting
21 the spatial conformation of the DNA in the transcription, auxiliary, and
22 recombination processes. This ultimately led to bacterial resistance to
23 fluoroquinolone. In this study, the detection rate of *griA* gene was as high as

49.58%, which indicated that the mutation at the above locus might be the main mechanism for the resistance of the strains to oxfloxacin and enrofloxacin. *S. aureus* can control the expression of virulence factors and resistance genes through a complex regulatory gene network, so it can survive in different animals, humans and environments [22, 24, 25]. In the present study, we found that human-source strains (H7) and environmental-source strains (E20) on farms carried the same resistance gene profile and contained only four SNP difference loci between them, suggesting that there is a risk of transmission of multi-drug-resistant bacteria to animals by human carriage and environmental contamination. The strains of animal source (B20, B34, B36) as well as the strains of environmental source from drinking water (E15), tank wall swabs (E19) and air duct swabs (E24) also shared the same resistance gene profiles with the strains of market (M1) and hospital (QH28, QH29), and the SNPs of these strains were less than 10, which further suggests that animals can transmit multidrug-resistant bacteria to animals via This further suggests that animals can transmit multidrug-resistant bacteria to humans through direct or indirect environmental factors.

Ethical statements

The China Animal Health and Epidemiology Centre Laboratory Animal Welfare and Ethics Review Committee provided ethical approval, approval number DWFL-2022-05.

Data and model availability statement

All data supporting this study are included in the article.

1 **Declaration of generative AI and AI-assisted technologies in the writing**
2 **process**

3 No Generative AI was used in the preparation of this manuscript.

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6 **Declaration of Competing Interest**

7 The authors declare that they have no known financial conflicts of interest or
8 personal relationships that could influence the work reported in this paper.

9 **Acknowledgements**

10 The authors would like to thank the Chinese Animal Health and Epidemiology
11 Center and the College of Veterinary Medicine, Shanxi Agricultural University.

12 **Data availability statement**

13 The datasets generated during this study are fully available within the article.

14 **Research Funding**

15 This work has been supported by the National Key Research and Development
16 Plan (2022YFC2303900).

References

- [1] F. Gomes, M. Henriques, Control of Bovine Mastitis: Old and Recent Therapeutic Approaches, *Curr Microbiol.* 72 (2016) 377-382. <https://doi.org/10.1007/s00284-015-0958-8>.
- [2] Z. Kovačević, N. Kladar, I. Čabarkapa, et al., New Perspective of *Origanum vulgare* L. and *Satureja montana* L. Essential Oils as Bovine Mastitis Treatment Alternatives, *Antibiotics* (Basel). 10 (2021) <https://doi.org/10.3390/antibiotics10121460>.
- [3] H. Derakhshani, K.B. Fehr, S. Sepehri, et al., Invited review: Microbiota of the bovine udder: Contributing factors and potential implications for udder health and mastitis susceptibility, *J Dairy Sci.* 101 (2018) 10605-10625. <https://doi.org/10.3168/jds.2018-14860>.
- [4] Y.H. Schukken, J. Günther, J. Fitzpatrick, et al., Host-response patterns of intramammary infections in dairy cows, *Vet Immunol Immunopathol.* 144 (2011) 270-289. <https://doi.org/10.1016/j.vetimm.2011.08.022>.
- [5] Y. Cha, B. Son, S. Ryu, Effective removal of staphylococcal biofilms on various food contact surfaces by *Staphylococcus aureus* phage endolysin LysCSA13, *Food Microbiol.* 84 (2019) 103245. <https://doi.org/10.1016/j.fm.2019.103245>.
- [6] G. Lv, R. Jiang, H. Zhang, et al., Molecular Characteristics of *Staphylococcus aureus* From Food Samples and Food Poisoning Outbreaks in Shijiazhuang, China, *Front Microbiol.* 12 (2021) 652276. <https://doi.org/10.3389/fmicb.2021.652276>.
- [7] A.F. Mechesso, D.C. Moon, G.S. Ryoo, et al., Resistance profiling and molecular characterization of *Staphylococcus aureus* isolated from goats in Korea, *Int J Food Microbiol.* 336 (2021) 108901. <https://doi.org/10.1016/j.ijfoodmicro.2020.108901>.
- [8] Centers for Disease Control. 2019. Antibiotic resistance threats in the United States.(2019) <https://doi.org/10.15620/cdc:82532>.
- [9] E.J. Richardson, R. Bacigalupe, E.M. Harrison, et al., Gene exchange drives the ecological success of a multi-host bacterial pathogen, *Nat Ecol Evol.* 2 (2018) 1468-1478. <https://doi.org/10.1038/s41559-018-0617-0>.
- [10] S. Abraham, S. Jagoe, S. Pang, et al., Reverse zoonotic transmission of community-associated MRSA ST1-IV to a dairy cow, *Int J Antimicrob Agents.* 50 (2017) 125-126. <https://doi.org/10.1016/j.ijantimicag.2017.05.001>.
- [11] S. Sahibzada, M. Hernández-Jover, D. Jordan, et al., Emergence of highly prevalent CA-MRSA ST93 as an occupational risk in people working on a pig farm in Australia, *PLoS One.* 13 (2018) e0195510. <https://doi.org/10.1371/journal.pone.0195510>.
- [12] F. Hu, Y. Liu, L. Wang, et al., Study on the multidrug resistance and transmission factors of *Staphylococcus aureus* at the 'animal-environment-human' interface in the broiler feeding cycle, *Front Microbiol.* 16 (2025) 1495676. <https://doi.org/10.3389/fmicb.2025.1495676>.
- [13] N. Ullah, H.A. Dar, K. Naz, et al., Genomic Investigation of Methicillin-Resistant

- Staphylococcus aureus ST113 Strains Isolated from Tertiary Care Hospitals in Pakistan, *Antibiotics* (Basel). 10 (2021) <https://doi.org/10.3390/antibiotics10091121>.
- [14] I.U. Haq, M. Kamal, A.A. Swelum, et al., Alarming multidrug resistance in *Staphylococcus aureus* isolated from raw milk of cows with subclinical mastitis: Antibiotic resistance patterns and occurrence of selected resistance genes, *PLoS One*. 19 (2024) e0301200. <https://doi.org/10.1371/journal.pone.0301200>.
- [15] M. Shahid, R. Hussain, Z. Nawaz, et al., Occurrence of Virulence Genes among Methicillin-Resistant *Staphylococcus aureus* Isolated from Subclinical Bovine Mastitis, *ACS Omega*. 8 (2023) 38111-38117. <https://doi.org/10.1021/acsomega.3c04206>.
- [16] H. Liu, S. Li, L. Meng, et al., Prevalence, antimicrobial susceptibility, and molecular characterization of *Staphylococcus aureus* isolated from dairy herds in northern China, *J Dairy Sci*. 100 (2017) 8796-8803. <https://doi.org/10.3168/jds.2017-13370>.
- [17] C. Shi, Z. Yu, H. Ho, et al., Occurrence, Antimicrobial Resistance Patterns, and Genetic Characterization of *Staphylococcus aureus* Isolated from Raw Milk in the Dairy Farms over Two Seasons in China, *Microb Drug Resist*. 27 (2021) 99-110. <https://doi.org/10.1089/mdr.2019.0358>.
- [18] X. Wang, J. Meng, T. Zhou, et al., Antimicrobial susceptibility testing and genotypic characterization of *Staphylococcus aureus* from food and food animals, *Foodborne Pathog Dis*. 9 (2012) 95-101. <https://doi.org/10.1089/fpd.2011.0987>.
- [19] Y. Chen, L. Sun, X. Ba, et al., Epidemiology, evolution and cryptic susceptibility of methicillin-resistant *Staphylococcus aureus* in China: a whole-genome-based survey, *Clin Microbiol Infect*. 28 (2022) 85-92. <https://doi.org/10.1016/j.cmi.2021.05.024>.
- [20] S. Li, S. Sun, C. Yang, et al., The Changing Pattern of Population Structure of *Staphylococcus aureus* from Bacteremia in China from 2013 to 2016: ST239-030-MRSA Replaced by ST59-t437, *Front Microbiol*. 9 (2018) 332. <https://doi.org/10.3389/fmicb.2018.00332>.
- [21] K. Ning, R. Zhou, M. Li, Antimicrobial resistance and molecular typing of *Staphylococcus aureus* isolates from raw milk in Hunan Province, *PeerJ*. 11 (2023) e15847. <https://doi.org/10.7717/peerj.15847>.
- [22] Q. Ren, G. Liao, Z. Wu, et al., Prevalence and characterization of *Staphylococcus aureus* isolates from subclinical bovine mastitis in southern Xinjiang, China, *J Dairy Sci*. 103 (2020) 3368-3380. <https://doi.org/10.3168/jds.2019-17420>.
- [23] J. Yamagishi, T. Kojima, Y. Oyamada, et al., Alterations in the DNA topoisomerase IV *grlA* gene responsible for quinolone resistance in *Staphylococcus aureus*, *Antimicrob Agents Chemother*. 40 (1996) 1157-1163. <https://doi.org/10.1128/aac.40.5.1157>.
- [24] F.I. Antók, R. Mayrhofer, H. Marbach, et al., Characterization of Antibiotic and Biocide Resistance Genes and Virulence Factors of *Staphylococcus* Species

Associated with Bovine Mastitis in Rwanda, *Antibiotics* (Basel). 9 (2019) <https://doi.org/10.3390/antibiotics9010001>.

- [25] M. El-Ashker, S. Monecke, M. Gwida, et al., Molecular characterisation of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* clones isolated from healthy dairy animals and their caretakers in Egypt, *Vet Microbiol.* 267 (2022) 109374. <https://doi.org/10.1016/j.vetmic.2022.109374>.