



Antimicrobial resistance, virulence gene profile and molecular typing of *Staphylococcus aureus* isolates from dairy cows in Xinjiang Province, northwest China



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ABSTRACT

Objectives: *Staphylococcus aureus* (SA) is a major pathogen causing dairy cow mastitis and endometritis. Recently, animal-derived SA strains pose a serious public-health threat. However, little is known about antimicrobial resistance and virulence factors of SA isolated from dairy cows in Xinjiang, China. In this study, antimicrobial resistance, virulence gene profiles and genotypes of SA from clinical mastitis and endometritis in dairy cows were investigated.

Methods: A total of 337 clinical samples (186 milk samples from clinical mastitis cases and 151 endometritis swab samples) were collected from 15 large-scale dairy farms and were screened for SA. All SA isolates were subjected to antimicrobial susceptibility testing, detection of virulence genes and molecular typing.

Results: A total of 155 SA strains were isolated; 22 (14.2%) were methicillin-resistant *S. aureus* (MRSA). Resistance of MRSA isolates was significantly higher than that of methicillin-susceptible *S. aureus* (MSSA). The percentage of virulence genes varied between MSSA and MRSA. The strains could be divided into two SCCmec types (I and IVa), three *agr* types (I, II and III) and four *spa* types (t779, t2883, t13751 and t1939). MLST identified 14 sequence types, among which ST1 and ST9 had relatively high detection rates.

Conclusions: These findings revealed that ST9-t1939-*agr*I was the main genotype of MSSA, whilst ST1-SCCmecI-t1939-*agr*I was the main genotype of MRSA from dairy cows. More significantly, a novel ST (STX) was identified for the first time. The majority of SA strains from dairy cows were multidrug-resistant and carried multiple virulence genes, posing a potential public-health risk.

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1. Introduction

Dairy cow mastitis and endometritis are two diseases in the dairy industry that have a high occurrence and are most difficult to treat [1]. According to an investigation of China's dairy cow diseases, the occurrence of mastitis and endometritis can reach 20–32% and 35–45%, respectively, in large-scale dairy farms, causing huge economic losses to the dairy industry [2]. An aetiological investigation revealed that *Staphylococcus aureus* (SA)

is one of the most common pathogens causing these two diseases. The infection rate of SA is ca. 20–50% [3]. As a result of the extensive use of antibiotics in dairy farms, drug residues in milk have increased and new resistant strains continue to occur, bringing greater difficulties for the prevention and treatment of dairy cow diseases [4].

Methicillin-resistant *S. aureus* (MRSA) is an important pathogen of hospital- and community-acquired infections [5,6]. Recently, animal-derived MRSA is widely present in Chinese livestock, causing not only animal diseases but also cross-infection between animals and humans. As a result, MRSA is a serious threat to public health and has been extensively studied [7,8]. It is currently known that MRSA has a complex resistance mechanism and is usually

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associated with multidrug resistance. Staphylococcal cassette chromosome *mec* (*SCCmec*) carrying resistance genes for methicillin, heavy metals and other drugs can be integrated into the genome. The *mecA* gene encodes penicillin-binding protein 2a (PBP2a), leading to high resistance to methicillin [9–11]. In addition, virulence genes carried by MRSA can also enhance its pathogenicity and the spread of antimicrobial-resistant bacteria [12].

Xinjiang Province, located in northwest China, is the most important dairy farming base, possessing 3.6 million dairy cows. Most dairy farms are large-scale farms and the average milk yield is ca. 6500 kg/cow per year. However, mastitis and endometritis occur frequently in these large-scale farms. In order to treat diseased dairy cows, long-term use or abuse of antibiotics in dairy farms has led to the emergence of drug-resistant strains. However, antimicrobial resistance and molecular characteristics of SA isolates from dairy cows in Xinjiang Province are not completely understood. The objective of the present study was to explore the antimicrobial resistance, virulence gene profile and molecular characteristics of clinical SA isolates from dairy cows in Xinjiang, northwest China.

2. Materials and methods

2.1. Sample collection

A total of 337 clinical samples (including 186 milk samples from cases of clinical mastitis and 151 swab samples of endometritis) were collected from 15 large-scale dairy farms in Wujiaqu, Shihezi, Tacheng, Yili, Urumqi, Aksu and Kashi areas of Xinjiang Province (see Supplementary Fig. S1 in the online version at DOI: [10.1016/j.jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)). Udders were washed with warm water and the teats were sterilised with 75% alcohol. Milk after the third squeeze was collected in a sterile tube that was placed in an icebox and delivered to the Xinjiang Key Laboratory of Animal Disease Prevention and Control (Xinjiang, China) for bacterial isolation and cultivation.

2.2. Isolation and identification of *S. aureus*

Samples were inoculated in Baird–Parker medium (AMRESCO Inc., Solon, OH) and were incubated at 37 °C for 12–48 h. A single colony with typical characteristics was picked and was purified on BD™ Baird–Parker nutrient agar medium (Beckton Dickinson, Franklin Lakes, NJ). Purified bacteria were subjected to smearing, Gram staining and microscopic examination. Biochemical reactions were performed using a microbial biochemical identification kit (Thermo Fisher, Waltham, MA). Universal primers were designed according to the sequence of the 16S rRNA gene of SA [13] and were used to verify the identity of SA isolates. SA isolates

were stored at –20 °C for subsequent antimicrobial resistance characterisation, virulence gene detection and molecular typing.

2.3. Determination of antimicrobial susceptibility of *S. aureus* isolates

A single colony was picked with a sterile inoculation loop and was cultured in Luria–Bertani (LB) medium at 37 °C for 20–24 h. The bacterial concentration was adjusted to an optical density at 600 nm of 0.5 using sterile normal saline and then 200 μL was evenly spread on Mueller–Hinton agar (MHA) medium (AMRESCO Inc.). Antimicrobial disks for 17 antimicrobial agents were placed on the MHA agar medium with SA. Following 48 h of incubation at 37 °C, the diameter of the zone of inhibition was measured and was interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. According to the CLSI, SA with a cefoxitin zone of inhibition of ≤21 mm were analysed by amplifying the *mecA* gene [15] to confirm MRSA strains [16].

2.4. Extraction of bacterial genomic DNA

Briefly, a single colony was inoculated into LB medium and was incubated at 37 °C overnight. Then, 1.5 mL of the bacterial culture was centrifuged at 12 000 rpm and the pellet was processed for genomic DNA extraction using a commercial kit (TaKaRa, Tokyo, Japan). Purified DNA was stored at –20 °C for virulence gene detection and molecular typing.

2.5. Detection of antimicrobial resistance genes in *S. aureus* isolates

Multiplex PCR was used to detect the presence of macrolide resistance genes (*msrA*, *msrB*), erythromycin resistance genes (*ermA*, *ermC*), streptogramin acetyltransferase genes (*vata*, *vataB*, *vataC*), aminoglycoside resistance genes (*aacA–D*), tetracycline resistance genes (*tetK*, *tetM*), lincosamide resistance gene (*linA*), methicillin resistance gene (*mecA*) [17], chloramphenicol resistance genes (*fexA*, *fexB*), oxazolidinone ketone resistance genes (*cfp*, *optrA*) and vancomycin resistance genes (*vgaA*, *vgaC*) [18] (see Supplementary Table S1 in the online version at DOI: [10.1016/j.jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)).

2.6. Detection of virulence genes in *S. aureus* isolates

Multiplex PCR was used to detect the presence of the leukotoxin genes (*PVL*, *lukED*, *lukM*), haemolysin genes (*hla*, *hly*, *hld*) [19], epidermal desensitising genes (*eta*, *etb*) [20], the toxic shock syndrome toxin gene (*tst*), the adhesion gene (*edin*) [21] and a novel virulence gene encoding cell wall-anchored protein (*sasX*) [22] (see Supplementary Table S1 in the online version at DOI: [10.1016/j.jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)).

Table 1

Isolation of *Staphylococcus aureus* in milk samples of clinical mastitis and swab samples of endometritis from dairy cows in seven regions of Xinjiang Province, China.

Region	Milk samples of clinical mastitis				Swab samples of endometritis			
	No. examined	No. positive (%)	MSSA (%)	MRSA (%)	No. examined	No. positive (%)	MSSA (%)	MRSA (%)
Wujiaqu	13	5 (38.5)	4 (30.8)	1 (7.7)	10	4 (40.0)	3 (30.0)	1 (10.0)
Shihezi	14	7 (50.0)	5 (35.7)	2 (14.3)	16	7 (43.8)	6 (37.5)	1 (6.3)
Tacheng	25	18 (72.0)	13 (52.0)	5 (20.0)	18	9 (50.0)	7 (38.9)	2 (11.1)
Yili	40	20 (50.0)	18 (45.0)	2 (5.0)	27	6 (22.2)	6 (22.2)	0
Urumqi	10	6 (60.0)	5 (50.0)	1 (10.0)	12	6 (50.0)	5 (41.7)	1 (8.3)
Aksu	52	24 (46.2)	22 (42.3)	2 (3.8)	38	13 (34.2)	13 (34.2)	0
Kashi	32	18 (56.3)	15 (46.9)	3 (9.4)	30	12 (40.0)	11 (36.7)	1 (3.3)
Total	186	98 (52.7)	82 (44.1)	16 (8.6)	151	57 (37.7)	51 (33.8)	6 (4.0)

MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

2.7. Molecular typing of *S. aureus* isolates based on SCCmec, accessory gene regulator (*agr*) and staphylococcal protein A (*spa*) typing and multilocus sequence typing (MLST)

Multiplex PCR was used to determine the types and subtypes of SCCmec including I, II, III, IVa, IVb, IVc, IVd and V [8] (see Supplementary Table S1 in the online version at DOI: [10.1016/j.jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)). Amplified PCR products were sequenced and the genotypes were determined according to the sequences. Primers for the *agr* allele (I–IV) were designed and PCR products were sequenced to determine the *agr* subtypes [20]. MLST was performed as described previously [23]. The *spa* gene [24] was amplified and sequenced to determine *spa* subtypes based on the Ridom SpaServer database (<https://www.spaserver.ridom.de/>). Alleles and sequence types (STs) for each strain were obtained from the following database (<http://saureus.beta.mlst.net>).

3. Results

3.1. Isolation and identification of *S. aureus* strains

A total of 155 SA strains were isolated from 337 clinical samples collected from 15 large-scale cattle farms in Xinjiang Province, among which 22 (14.2%) were confirmed as MRSA by PCR. Thus, MRSA strains accounted for 6.5% (22/337) of the total samples (Table 1).

3.2. Antimicrobial resistance in *S. aureus* strains

Of the 155 SA strains, 133 (85.8%) were methicillin-susceptible *S. aureus* (MSSA) and 22 (14.2%) were MRSA. Vancomycin and linezolid resistance was not detected in any of the isolates. High levels of resistance to penicillin (91.7%), erythromycin (42.9%) and trimethoprim (37.6%) were observed in the MSSA strains, whereas resistance to rifampicin (2.3%) and teicoplanin (5.3%) was relatively low. MRSA strains had high levels of resistance to penicillin (100%), cefoxitin (86.4%), tetracycline (77.3%) and clindamycin (77.3%), but low resistance to rifampicin (0%) and nitrofurantoin (18.2%) (Table 2). Antimicrobial resistance of MSSA was generally lower than that of MRSA. Furthermore, the distribution of resistant strains to different antibiotics varied between MRSA and MSSA (see Supplementary Fig. S2 in the online version at DOI: [10.1016/j.jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)).

3.3. Percentage of antimicrobial resistance genes in *S. aureus* strains

The oxazolidinone resistance genes *cfp* and *optrA* and the vancomycin resistance genes *vgaA* and *vgaC* were not detected in any strains. The resistance genes *linA*, *tetM* and *ermC* were detected in 42.1%, 40.6% and 30.8% of MSSA strains, respectively. The resistance genes *mecA*, *linA*, *ermC* and *vatC* were detected in 100%, 90.9%, 72.7% and 72.7% of MRSA strains, respectively (Table 3). Multiplex PCR could detect characteristic bands for nine antimicrobial resistance genes (see Supplementary Fig. S3 in the online version at DOI: [10.1016/j.jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)). The percentage of antimicrobial resistance genes in MRSA was significantly higher than that in MSSA, except for *tetK* that was only detected in MSSA (Table 3).

3.4. Percentage of virulence genes in *S. aureus* strains

The main virulence genes detected in SA strains included *lukED*, *hla*, *hly*, *hld* and *tst*. The percentage of *hla*, *hly* and *hld* was high in MSSA, whilst *lukED* and *tst* were widely present in MRSA strains. These results indicated that different virulence genes are distributed in MSSA and MRSA strains (Table 4).

3.5. Molecular typing of *S. aureus* strains based on SCCmec, *agr* and *spa* typing and MLST

The main SCCmec types detected in MRSA strains were SCCmec I and IVa as exhibited by two characteristic bands in multiplex PCR analysis (see Supplementary Fig. S4A in the online version at DOI: [10.1016/j.jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)). None of SCCmec genotypes were detected in MSSA strains. SCCmec I was the main genotype of MRSA isolates. SCCmec I and IVa were detected in 77.3% (17/22) and 22.7% (5/22) of MRSA strains, respectively. Three *agr* molecular types (I, II and III) were detected as exhibited by three different characteristic bands in multiplex PCR analysis (see Supplementary Fig. S4B in the online version at DOI: [10.1016/j.jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)). The proportions of *agr* I, II and III were 51.0%, 19.4% and 13.5%, respectively. The distribution of SCCmec and *agr* types in SA strains was different (Fig. 1A). The percentage of these two genotypes was also different in MRSA types (Fig. 1B). *spa* molecular typing detected four subtypes (t779, t2883, t13751 and t1939) as exhibited by four characteristic bands in PCR analysis (see Supplementary Fig. S5 in the online version at DOI: [10.1016/j.jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)).

Table 2

Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates from dairy cows in Xinjiang Province, China, to 17 antimicrobial agents tested.

Antimicrobial agent (concentration tested)	No. (%) resistant		
	Overall (n = 155)	MSSA (n = 133)	MRSA (n = 22)
Ciprofloxacin (5 µg)	38 (24.5)	34 (25.6)	4 (18.2)
Oxacillin (1 µg)	20 (12.9)	11 (8.3)	9 (40.9)
Chloramphenicol (30 µg)	39 (25.2)	32 (24.1)	7 (31.8)
Trimethoprim (5 µg)	64 (41.3)	50 (37.6)	14 (63.6)
Levofloxacin (5 µg)	27 (17.4)	23 (17.3)	4 (18.2)
Penicillin (10 U)	144 (92.9)	122 (91.7)	22 (100)
Erythromycin (15 µg)	70 (45.2)	57 (42.9)	13 (59.1)
Tetracycline (30 µg)	61 (39.4)	44 (33.1)	17 (77.3)
Cefoxitin (30 µg)	33 (21.3)	14 (10.5)	19 (86.4)
Nitrofurantoin (300 µg)	25 (16.1)	21 (15.8)	4 (18.2)
Rifampicin (5 µg)	3 (1.9)	3 (2.3)	0
Teicoplanin (30 µg)	12 (7.7)	7 (5.3)	5 (22.7)
Clindamycin (2 µg)	48 (31.0)	31 (23.3)	17 (77.3)
Gentamicin (10 µg)	25 (16.1)	13 (9.8)	12 (54.5)
Vancomycin (30 µg)	0	0	0
Florfenicol (30 µg)	21 (13.5)	15 (11.3)	6 (27.3)
Linezolid (30 µg)	0	0	0

MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

Table 3
Detection of antimicrobial resistance genes of *Staphylococcus aureus* isolates from dairy cows in Xinjiang Province, China.

Antimicrobial class/resistance gene	No. (%) of positive isolates		
	Total isolates (n = 155)	MSSA (n = 133)	MRSA (n = 22)
Macrolides			
<i>msrA</i>	0	0	0
<i>msrB</i>	42 (27.1)	34 (25.6)	8 (36.4)
Erythromycin			
<i>ermA</i>	0	0	0
<i>ermC</i>	57 (36.8)	41 (30.8)	16 (72.7)
Aminoglycosides			
<i>aacA–D</i>	24 (15.5)	16 (12.0)	8 (36.4)
Tetracyclines			
<i>tetK</i>	5 (3.2)	5 (3.8)	0
<i>tetM</i>	64 (41.3)	54 (40.6)	10 (45.5)
Lincosamides			
<i>linA</i>	76 (49.0)	56 (42.1)	20 (90.9)
Chloramphenicol			
<i>fexA</i>	11 (7.1)	9 (6.8)	2 (9.1)
<i>fexB</i>	0	0	0
Oxazolidinones and phenicols			
<i>cfr</i>	0	0	0
<i>optrA</i>	0	0	0
Vancomycin			
<i>vgaA</i>	0	0	0
<i>vgaC</i>	0	0	0
Streptogramins			
<i>vatA</i>	0	0	0
<i>vatB</i>	0	0	0
<i>vatC</i>	28 (18.1)	12 (9.0)	16 (72.7)
β-Lactams			
<i>mecA</i>	22 (14.2)	0	22 (100)

MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

Table 4
Percentage of virulence genes in *Staphylococcus aureus* isolates from dairy cows of Xinjiang Province, China.

Virulence factor/ gene ^a	No. (%) of positive isolates		
	Total isolates (n = 155)	MSSA (n = 133)	MRSA (n = 22)
Panton–Valentine leukocidin			
<i>PVL</i>	0	0	0
<i>lukED</i>	27 (17.4)	11 (8.3)	16 (72.7)
<i>lukM</i>	0	0	0
Haemolysin			
<i>hla</i>	57 (36.8)	54 (40.6)	3 (13.6)
<i>hlb</i>	36 (23.2)	34 (25.6)	2 (9.1)
<i>hld</i>	49 (31.6)	43 (32.3)	6 (27.3)
Exfoliative toxins A and B			
<i>eta</i>	0	0	0
<i>etb</i>	0	0	0
Toxic shock syndrome toxin-1			
<i>tst</i>	41 (26.5)	23 (17.3)	18 (81.8)
Invasive toxin			
<i>edin</i>	0	0	0
Mobile genetic element			
<i>sasX</i>	0	0	0

MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

[jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)). Antimicrobial resistance and virulence genes were also different among different subtypes (see Supplementary Table S2 and Fig. S6 in the online version at DOI: [10.1016/j.jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)). A total of 14 STs were detected among the 155 SA strains (ST188, ST584, ST9, ST805, ST2139, ST1, ST2700, ST903, ST2454, ST2990, ST63, ST968, ST2373 and STX), with ST9 being the predominant type. A corresponding ST was not identified for one strain when all the alleles of housekeeping genes were imported into the MLST database and this ST was designated as STX. A total of seven characteristic bands were obtained for the seven housekeeping genes (see Supplementary Fig. S7 in the online version at DOI: [10.1016/j.jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)). ST1 was the main genotype of MRSA. In addition, different STs carried different virulence and antimicrobial resistance genes (see Supplementary Table S3 and Fig. S8 in the online version at DOI: [10.1016/j.jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)). Different STs also correspond to different types of SCCmec, agr and spa (Table 5). Different STs were also distributed to different strains (see Supplementary Fig. S9 in the online version at DOI: [10.1016/j.jgar.2018.08.024](https://doi.org/10.1016/j.jgar.2018.08.024)).

4. Discussion

In this study, antimicrobial resistance, virulence gene profiles and molecular typing of SA isolates from clinical mastitis and endometritis in dairy cows in Xinjiang Province, northwest China, were characterised. MSSA and MRSA accounted for 85.8% and 14.2% of the 155 SA isolates, respectively. Thus, MSSA was the

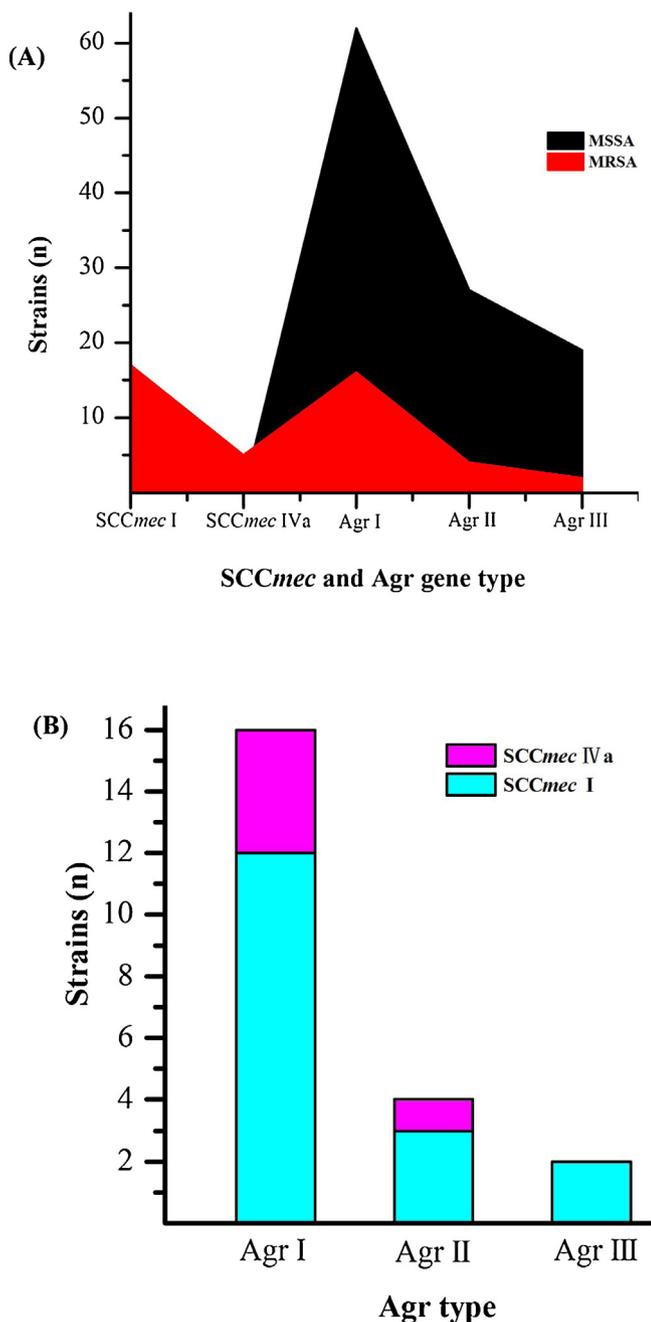


Fig. 1. Distribution of staphylococcal cassette chromosome *mec* (SCC*mec*) and accessory gene regulator (*agr*) types in *Staphylococcus aureus* strains from dairy cows in Xinjiang Province, China, for: (A) methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) strains; and (B) among different MRSA strains.

predominant strain in dairy cows. However, compared with MSSA, MRSA had a high percentage of multidrug-resistant (MDR) strains. Moreover, the presence of a particular antimicrobial resistance gene was not fully consistent with resistance to this particular antibiotic, suggesting that although these strains carry this particular antimicrobial resistance genes, they may not be expressed to confer a resistant phenotype. Furthermore, these strains may be converted into resistant strains under certain conditions and thus may have important epidemiological significance in the transmission of SA resistance.

Antimicrobial resistance and genetic diversity are responsible for wide epidemics and transmission of MRSA [25,26]. Recently,

Panton–Valentine leukocidin (PVL) has been detected in animal and foodborne SA isolates [27], in which *lukED* belongs to a leukotoxin and is an important marker of SA pathogenicity [19]. The results also showed that MRSA and MSSA isolates could carry a variety of virulence genes and that these virulence genes were distributed differently in different genotypes, suggesting that SA virulence is not only manifested at the strain level but is also associated with the strain's origin and genetic background. Studies have reported that acquired antimicrobial resistance is accompanied by deletion mutations in virulence factors in SA, leading to a low detection rate of virulence genes [28,29]. In the current study, the detection rate of the haemolysin virulence factor in MDR MRSA was lower than that in MSSA, which is consistent with previous studies [30]. This could be due to compensatory mutation during the process of acquiring multidrug resistance by MRSA, thus offsetting its haemolysin gene and reducing its detection rate.

The recent emergence of different genotypes in SA isolates has posed a significant threat to veterinary clinical and public health. Studies have shown that hospital-associated SCC*mec* I and community-associated SCC*mec* IV [31,32] genotypes are also present in cattle-derived MRSA [33]. It is speculated that MRSA transmission between humans and animals may be related to the molecular mechanism of SCC*mec* epidemics. A high proportion of SCC*mec* I may be due to its transmission between humans and animals by farmers carrying SCC*mec* I. SCC*mec* IVa SA may be transmitted between humans and animals through the environment. Thus, preventing the spread of pathogens between humans and animals is essential for reducing MRSA epidemics. *agr* typing is one of the important markers to study SA epidemiology. It has been reported that *agr* I is highly prevalent in hospital- and cattle-derived SA [13,34]. Among the SA isolates from dairy cows, the highest proportion in cattle-derived SA is *agr* I, followed by *agr* II.

In this study, *spa* types t779 and t1939 were isolated that are rarely epidemic [24,35]. In the past years, these genotypes of SA strains were isolated from animals and slaughterhouse workers. Furthermore, *spa* genotype t1939 was the predominant genotype in SA isolates from dairy cows in Xinjiang. *spa* types were different between human and animal SA, but there are also similarities. Studies have reported that ST188 is one of the major STs in milk SA. ST188 can be isolated not only from workers who are associated with meat products and dairy processing, but also from humans who are not in contact with animals [36,37]. In addition, pig-derived ST63 was also isolated from cattle in this study [38]. In North Dakota, studies have reported the isolation of human disease-associated ST9 [39], which was also isolated from cattle in this study. Therefore, these four genotypes from a cattle source have been reported in animal and humans. More importantly, the genotypes isolated from different sources (cattle, pig, human) are identical, indicating that some genotypes of MRSA can not only be transmitted in the same species but can also spread among humans, animals and the environment. Moreover, a new genotype (STX) was also identified, which belonged to the genotype of t1939-*agr*I in MSSA. STX was resistant to penicillin, tetracycline and clindamycin and carried *ermC* and *linA* resistance genes and the *hld* virulence gene. Identification of a new genotype (STX) for the first time suggests that SA isolates can undergo significant genetic variation under the pressure of antibiotics and the human immune system. However, the pathogenicity of STX genotype and its transmission between cattle and humans remain unclear. The public-health implications of STX genotype should be further investigated and explored.

In summary, the present study demonstrated that the main genotypes of SA isolates from dairy cows in Xinjiang were ST9-t1939-*agr*I in MSSA and ST1-SCC*mec* I-t1939-*agr*I in MRSA. Also, a new STX genotype was identified for the first time, which enriches the MLST genotyping database and lays the foundation for

Table 5

Distribution of sequence types (STs) by multilocus sequence typing (MLST), staphylococcal protein A (*spa*) types, accessory gene regulator (*agr*) types and staphylococcal cassette chromosome *mec* (SCC*mec*) types in *Staphylococcus aureus* strains from dairy cows in Xinjiang Province, China.

MLST	No. (%) of isolates		<i>spa</i> type (n)		<i>agr</i> type (n)		SCC <i>mec</i> type (n)	
	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA
ST188	13 (9.7)	4 (18.2)	t1939 (6), t13751 (7)	t1939 (3), t13751 (1)	I (5), II (3), III (3)/(2)	I (3), III (1) /	/	I (3), Iva (1)
ST584	1 (0.8)	0	t1939 (1)	/	I (1)	/	/	/
ST9	27 (20.3)	3 (13.6)	t1939 (10), t13751 (8), t779 (9)	t1939 (1), t779 (2)	I (13), II (9), III (3)/(2)	I (1), II (2) /	/	I (2), Iva (1)
ST805	5 (3.8)	0	t1939 (5)	/	I (2), II (2)/(1)	/	/	/
ST968	0	2 (9.1)	/	t1939 (1), t13751 (1)	/	I (2)	/	I (2)
ST2373	0	1 (4.5)	/	t1939 (1)	/	I (1)	/	I (1)
ST2139	17 (12.8)	1 (4.5)	t1939 (9), t2883 (3), t13751 (5)	t1939 (1)	I (10), II (2), III (5)	I (1)	/	I (1)
ST1	23 (17.3)	6 (27.3)	t1939 (4), t2883 (8), t779 (5), t13751 (5)/(1)	t1939 (1), t2883 (3), t779 (2)	I (14), II (6), III (2)/(1)	I (5), III (1) /	/	I (4), Iva (2)
ST2700	6 (4.5)	2 (9.1)	t1939 (2), t2883 (3), t779 (1)	t1939 (1), t2883 (1)	I (4), III (1)/(1)	I (1), II (1) /	/	I (2)
ST903	9 (6.8)	0	t1939 (4), t779 (2), t13751 (3)	/	I (6), III (3)	/	/	/
ST2454	7 (5.3)	0	t779 (5), t13751 (2)	/	I (3), II (2), III (2)	/	/	/
ST2990	3 (2.3)	0	t1939 (3)	/	I (1), II (2)	/	/	/
ST63	1 (0.8)	2 (9.1)	t1939 (1)	t1939 (1), t2883 (1)	I (1)	I (2)	/	I (2)
STX	1 (0.8)	0	t1939 (1)	/	II (1)	/	/	/
NT	20 (15.0)	1 (4.5)	t779 (1), t2883 (1), t13751 (2), t1939 (1)/(15)	t1939 (1)	I (2)/(18)	I (1)	/	Iva (1)

MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; /, not applicable; NT, not typed.

subsequent studying and monitoring the spread and prevalence of this genotype. The results of this study suggest that there is a potential risk of SA transmission between dairy cows and humans.

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Competing interests

None declared.

Ethical approval

Ethical approval for this study was received from the Research and Ethical Committee of Shihezi University (Shihezi, China).

References

- Chao G, Zhang X, Zhang X, Huang Y, Xu L, Zhou L, et al. Phenotypic and genotypic characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA) from different sources in China. *Foodborne Pathog Dis* 2013;10:214–21.
- Xiao YH, Giske CG, Wei ZQ, Shen P, Heddiini A, Li LJ. Epidemiology and characteristics of antimicrobial resistance in China. *Drug Resist Updat* 2011;14:236–50.
- Weese JS, van Duijkeren E. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. *Vet Microbiol* 2010;140:418–29.
- Zehra A, Singh R, Kaur S, Gill JPS. Molecular characterization of antibiotic-resistant *Staphylococcus aureus* from livestock (bovine and swine). *Vet World* 2017;10:598–604.
- Sahibzada S, Abraham S, Coombs GW, Pang S, Hernández-Jover M, Jordan D, et al. Transmission of highly virulent community-associated MRSA ST93 and livestock-associated MRSA ST398 between humans and pigs in Australia. *Sci Rep* 2017;7:5273.
- Harkins CP, Pichon B, Doumith M, Parkhill J, Westh H, Tomasz A, et al. Methicillin-resistant *Staphylococcus aureus* emerged long before the introduction of methicillin into clinical practice. *Genome Biol* 2017;18:130.
- Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. *Int J Antimicrob Agents* 2012;39:273–82.
- Khemiri M, Akrouf Alhusain A, Abbassi MS, El Ghaieb H, Santos Costa S, Belas A, et al. Clonal spread of methicillin-resistant *Staphylococcus aureus*-t6065–CC5–SCC*mecV*-*agr*II in a Libyan hospital. *J Glob Antimicrob Resist* 2017;10:101–5.
- Li T, Lu H, Wang X, Gao Q, Dai Y, Shang J, et al. Molecular characteristics of *Staphylococcus aureus* causing bovine mastitis between 2014 and 2015. *Front Cell Infect Microbiol* 2017;7:127.
- Houri H, Kazemian H, Sedigh Ebrahim-Saraie H, Tajji A, Tayebi Z, Heidari H. Linezolid activity against clinical Gram-positive cocci with advanced antimicrobial drug resistance in Iran. *J Glob Antimicrob Resist* 2017;10:200–3.
- Reichmann NT, Pinho MG. Role of SCC*mec* type in resistance to the synergistic activity of oxacillin and cefoxitin in MRSA. *Sci Rep* 2017;7:6154.
- Pomorska-Wesołowska M, Chmielarczyk A, Chlebowski M, Ziółkowski G, Szczypta A, Natkaniec J, et al. Virulence and antibiotic resistance of *Staphylococcus aureus* isolated from bloodstream infections and pneumonia in Southern Poland. *J Glob Antimicrob Resist* 2017;11:100–4.
- Bardiau M, Caplin J, Detilleux J, Graber H, Moroni P, Taminiau B, et al. Existence of two groups of *Staphylococcus aureus* strains isolated from bovine mastitis based on biofilm formation, intracellular survival, capsular profile and *agr*-typing. *Vet Microbiol* 2016;185:1–6.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests. 13th ed. Wayne, PA: CLSI; 2018 CLSI standard M02.
- Conceição T, Coelho C, de Lencastre H, Aires-de-Sousa M. Frequent occurrence of oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* (OS-MRSA) strains in two African countries. *J Antimicrob Chemother* 2015;70:3200–4.
- Vandendriessche S, Vanderhaeghen W, Larsen J, de Mendonça R, Hallin M, Butaye P, et al. High genetic diversity of methicillin-susceptible *Staphylococcus aureus* (MSSA) from humans and animals on livestock farms and presence of SCC*mec* remnant DNA in MSSA CC398. *J Antimicrob Chemother* 2014;69:355–62.
- Dormanesh B, Siroosbakhat S, Khodaverdi Darian E, Afsharkhas L. Methicillin-resistant *Staphylococcus aureus* isolated from various types of hospital infections in pediatrics: Pantón–Valentine leukocidin, staphylococcal chromosomal cassette *mec* SCC*mec* phenotypes and antibiotic resistance properties. *Jundishapur J Microbiol* 2015;8:e11341.
- Fan R, Li D, Wang Y, He T, Feßler AT, Schwarz S, et al. Presence of the *optrA* gene in methicillin-resistant *Staphylococcus sciuri* of porcine origin. *Antimicrob Agents Chemother* 2016;60:7200–5.
- Stephan R, Annemüller C, Hassan AA, Lammler C. Characterization of enterotoxigenic *Staphylococcus aureus* strains isolated from bovine mastitis in north-east Switzerland. *Vet Microbiol* 2001;78:373–82.
- Cheraghi S, Pourgholi L, Shafaati M, Fesharaki SH, Jalali A, Nosrati R, et al. Molecular analysis of virulence genes and the accessory gene regulator (*agr*) types among methicillin resistant *Staphylococcus aureus* (MRSA) strains. *J Glob Antimicrob Resist* 2017;10:315–20.
- Xie Y, He Y, Gehring A, Hu Y, Li Q, Tu SJ, et al. Genotypes and toxin gene profiles of *Staphylococcus aureus* clinical isolates from China. *PLoS One* 2011;6:e28276.
- Li M, Du X, Villaruz AE, Diep BA, Wang D, Song Y, et al. MRSA epidemic linked to a quickly spreading colonization and virulence determinant. *Nat Med* 2012;18:816–9.

- [23] Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000;38:1008–15.
- [24] Buntenkoetter V, Blaha T, Tegeler R, Fetsch A, Hartmann M, Kreienbrock L, et al. Comparison of the phenotypic antimicrobial resistances and *spa*-types of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates derived from pigs in conventional and in organic husbandry systems. *Berl Munch Tierarztl Wochenschr* 2014;127:135–43.
- [25] Azara E, Piras MG, Parisi A, Tola S. Antimicrobial susceptibility and genotyping of *Staphylococcus aureus* isolates collected between 1986 and 2015 from ovine mastitis. *Vet Microbiol* 2017;205:53–6.
- [26] Gostev V, Kruglov A, Kalinogorskaya O, Dmitrenko O, Khokhlova O, Yamamoto T, et al. Molecular epidemiology and antibiotic resistance of methicillin-resistant *Staphylococcus aureus* circulating in the Russian Federation. *Infect Genet Evol* 2017;53:189–94.
- [27] Ahmed MO, Baptiste KE, Daw MA, Elramalli AK, Abouzeed YM, Petersen A. *spa* typing and identification of *pvl* genes of methicillin-resistant *Staphylococcus aureus* isolated from a Libyan hospital in Tripoli. *J Glob Antimicrob Resist* 2017;10:179–81.
- [28] Tong J, Zhan ZP. Study of the virulence genes and molecular characteristics of *Staphylococcus aureus* strains [in Chinese]. *Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zhi* 2015;35:46–50.
- [29] Moreno-Flores A, Potel-Alvarellos C, Otero-Fernández S, Álvarez-Fernández M. Phenotypic and genetic characteristics of fluoroquinolone- and methicillin-resistant *Staphylococcus aureus* [in Spanish]. *Enferm Infecc Microbiol Clin* 2018;36:403–8.
- [30] Collins J, Rudkin J, Recker M, Pozzi C, O’Gara JP, Massey RC. Offsetting virulence and antibiotic resistance costs by MRSA. *ISME J* 2010;4:577–84.
- [31] Samutela MT, Kalonda A, Mwansa J, Lukwesa-Musyani C, Mwaba J, Mumbula EM, et al. Molecular characterisation of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated at a large referral hospital in Zambia. *Pan Afr Med J* 2017;26:108.
- [32] Zhong YM, Yuan R, Ding JS, Yang F, Liu WE. Emergence of methicillin-resistant *Staphylococcus aureus* SCCmec type IV/V epidemic clones in a large teaching hospital in China [in Chinese]. *Nan Fang Yi Ke Da Xue Xue Bao* 2017;37:861–5.
- [33] Mistry H, Sharma P, Mahato S, Saravanan R, Kumar PA, Bhandari V. Prevalence and characterization of oxacillin susceptible *mecA*-positive clinical isolates of *Staphylococcus aureus* causing bovine mastitis in India. *PLoS One* 2016;11:e0162256.
- [34] Khan S, Rasheed F, Zahra R. Genetic polymorphism of *agr* locus and antibiotic resistance of *Staphylococcus aureus* at two hospitals in Pakistan. *Pak J Med Sci* 2014;30:172–6.
- [35] Normanno G, Dambrosio A, Lorusso V, Samoilis G, Di Taranto P, Parisi A. Methicillin-resistant *Staphylococcus aureus* (MRSA) in slaughtered pigs and abattoir workers in Italy. *Food Microbiol* 2015;51:51–6.
- [36] Fan Y, Wang X, Li L, Yao Z, Chen S, Ye X. Potential relationship between phenotypic and molecular characteristics in revealing livestock-associated *Staphylococcus aureus* in Chinese humans without occupational livestock contact. *Front Microbiol* 2016;7:1517.
- [37] Sobhanipoor MH, Ahmadrabji R, Karmostaji A, Saffari F. Molecular characterization of nasal methicillin resistant *Staphylococcus aureus* isolates from workers of an automaker company in southeast Iran. *APMIS* 2017;125:921–6.
- [38] Li D, Wu C, Wang Y, Fan R, Schwarz S, Zhang S. Identification of multiresistance gene *cfr* in methicillin-resistant *Staphylococcus aureus* from pigs: plasmid location and integration into a staphylococcal cassette chromosome *mec* complex. *Antimicrob Agents Chemother* 2015;59:3641–4.
- [39] Velasco V, Buyukcangaz E, Sherwood JS, Stepan RM, Koslofsky RJ, Logue CM. Characterization of *Staphylococcus aureus* from humans and a comparison with isolates of animal origin, in North Dakota, United States. *PLoS One* 2015;10:e0140497.