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Prevalence, antimicrobial resistance, and staphylococcal toxin genes of *bla*$_{TEM-1a}$-producing *Staphylococcus aureus* isolated from animals in Chongqing, China

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Abstract

Background: *Staphylococcus aureus* infection of livestock animals and humans is a major public health issue. There are reports of antimicrobial resistance and multiple staphylococcal superantigen genes in many countries and several provinces of China, but the status in Chongqing, China is uncertain.

Objectives: The aim of this study was to determine the prevalence, antimicrobial susceptibility, and other molecular characteristics of *S. aureus* isolates from livestock animals in Chongqing.

Methods: *Staphylococcus aureus* was isolated and identified by selective enrichment and amplification of the *nuc* gene from 1371 samples collected at farms in Chongqing. The agar dilution method was used to determine the resistant phenotype, and extended spectrum β-lactamase genes were amplified by PCR. Methicillin-resistant *S. aureus* was verified by the presence of the *mecA* gene, and the presence or absence of SE, SEI, and TSST-1 genes was detected in the isolates.

Results: We cultured 89 *S. aureus* isolates from 1371 samples between March 2014 and December 2017. These isolates were from pigs, cattle, goats, rabbits, and chickens. There were four methicillin-resistant *S. aureus* strains (three from pigs and one from a chicken). The 89 isolates had high resistance to penicillin (93.3%) and ampicillin (92.1%), but most were susceptible to amikacin and ofloxacin, with resistance rates below 10%. A total of 62.9% of the isolates had varying degrees of multidrug resistance. Almost all strains, except for three isolates from chickens, were positive for *bla*$_{TEM-1a}$. There were 19 of 20 tested staphylococcal SE/SEI/TSST-1 genes present (all except for *seq*), and the predominant genes were *sei* (58.4%), *tst-1* (56.2%), and *seg* (51.7%).

Conclusions: The high antimicrobial resistance and prevalence of *bla*$_{TEM-1a}$ reinforce the need to reduce the usage of antimicrobials in livestock. The universal existence of staphylococcal toxin genes implies a potential threat to public health by animal-to-human transmission via the food chain.

KEYWORDS
ESBL, MRSA, resistance, SE, Staphylococcus aureus

1 | INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen that commonly infects humans and multiple livestock species, such as pigs, poultry, ruminants, and rabbits (Liu et al., 2018; Portillo et al., 2013). *Staphylococcus aureus* is responsible for many human diseases, including superficial skin and soft tissue infections, pneumonia, septicemia, endocarditis, and other severe or even fatal diseases (David and Daum, 2017). Staphylococcus *aureus* also causes some animal diseases (mastitis, omphalitis, arthritis, septicemia, and enteritis), and these infections are responsible for severe economic losses to businesses in animal husbandry (Iwata et al., 2014; Kim et al., 2015; Li et al., 2017a; Terzolo & Shimizu, 1979).

Staphylococcus aureus can acquire resistance to antimicrobial agents, which has led to the emergence of multidrug resistant strains (Rybak & LaPlante, 2005). Methicillin-resistant *S. aureus* (MRSA) carries the *mecA* or *mecC* genes, which encode the enzymes penicillin-binding protein 2a (PBP2a) or PBP2c, respectively, and provides resistance to methicillin and other β-lactam antibiotics (Fishovitz et al., 2014). MRSA was initially discovered in an inpatient in 1961 (Eriksen, 1961), and it has become an important public threat to human health. Nowadays, hospital-associated MRSA was considered the main reservoir (Deurenberg & Stobberingh, 2009). In addition, since the 1990s, community-associated MRSA has also become one of the serious health problem worldwide (DeLeo et al., 2010; Deurenberg & Stobberingh, 2009). In 1972, livestock-associated MRSA (LA-MRSA) was first reported in mastitic cows (Devriese et al., 1972). Since then, there has been increasing concern about the prevalence of LA-MRSA, and LA-MRSA has been detected in many different animals and countries (Aires-de-Sousa, 2017; Hanley et al., 2015; Liu et al., 2018; Monaco et al., 2013; Siiriken et al., 2016). Human MRSA infections might be transmitted from pigs, as documented for MRSA ST398 (Huss et al., 2016), indicating that pigs may be a major reservoir and source of human MRSA infections. Recent studies also isolated MRSA ST398 from goats (Loncaric et al., 2013), rabbits (Loncaric & Künzel, 2013), cattle (Fessler et al., 2011), and other animals. In addition, isolates of methicillin-susceptible *S. aureus* (MSSA) from humans and animals exhibited multidrug resistance (MDR), defined as resistance to three or more antimicrobial classes (Chao et al., 2013; Vandendriessche et al., 2014).

The use of antimicrobials is important for the control and treatment of bacterial disease at farms, although "national action plan for reducing the use of veterinary antimicrobials (2021–2025)" have been launched and the use of antimicrobials is decreasing year by year in China. Some antimicrobials are often used by veterinarians, such as penicillin, ampicillin, amikacin, erythromycin, tetracycline, doxycycline, florfenicol, ciprofloxacin, enrofloxacin, and trimethoprim.

Extended spectrum β-lactamases (ESBLs) are a class of enzymes usually produced by certain bacteria that are able to hydrolyze extended-spectrum cephalosporins and aztreonam but are inhibited by β-lactamase inhibitors, such as clavulanic acid and tazobactam (Ghafourian et al., 2015). ESBLs have spread threateningly in many regions of the world in human beings and many species of animals (Ghafourian et al., 2015; Paterson & Bonomo, 2005), but there is no evidence of ESBLs in *S. aureus*. Additionally, most strains of *S. aureus* produce a variety of superantigens, including staphylococcal enterotoxins (SEs), SE-like toxins (SEls), and toxic shock syndrome toxin 1 (TSST-1) (Ono et al., 2015). These toxins are responsible for food poisoning following the consumption of *S. aureus*-contaminated foods, such as milk and chicken (Hyeon et al., 2013; Johler et al., 2015).

Many recent epidemiological studies in China reported *S. aureus* infections from food-producing animals (Dan et al., 2019; Guo et al., 2018; Liu et al., 2018). However, little is known about the prevalence and occurrence of this organism in Chongqing, China. The aim of this study was to investigate the prevalence of *S. aureus* isolated from livestock animals in Chongqing and to characterize their antimicrobial susceptibility. Furthermore, we assessed the MRSA strains, ESBL genes, and staphylococcal toxin genes from *S. aureus* isolates.

2 | MATERIALS AND METHODS

2.1 | Sample collection

From March 2014 to December 2017, 1371 samples were collected from healthy animals in Chongqing (Table 1). Samples were collected randomly from nasal swabs of pigs at five farms and one abattoir (n = 343) and rabbits at three farms (n = 105), from the feces of beef cattle at four farms (n = 165) and goats at two farms (n = 88), from anal swabs of chickens at nine farms (n = 480), and from the milk of dairy cattle at three farms (n = 190). Not more than 60 samples were collected from each farm, except dairy farm three. The sampling was conducted from different pens of the farm, to avoid clones of isolates from the same farm. Each farmer gave permission for sample collection. None of the animals suffered from any disease, and none was receiving any antimicrobials or other drugs during the period of sample collection. All samples were stored on ice and returned to the laboratory within 6 h.

2.2 | Isolation and identification of *S. aureus*

Isolation and identification of *S. aureus* were performed immediately after samples arrived at the laboratory. Briefly, each nasal swab, anal swab, or fecal sample (1 g) was mixed with 2 ml of PBS for 2 h to release

TABLE 1 Prevalence of *S. aureus* and methicillin-resistant *S. aureus* (MRSA) isolated from different animals

Animal	Farm/Abattoir	No. of samples	No. of *S. aureus*	No. of MRSA
Pig (nasal swab)	Pig farm 1	50	5 (10.0%)	3 (6.0%)
	Pig farm 2	50	1 (2.0%)	
	Pig farm 3	50	0	
	Pig farm 4	50	1 (2.0%)	
	Pig farm 5	53	12 (22.6%)	
	Abattoir	90	6 (6.7%)	
	Total	343	25 (7.3%[a,b])	3 (0.9%)
Beef cattle (fece)	Beef cattle farm 1	48	1 (2.1%)	
	Beef cattle farm 2	32	0	
	Beef cattle farm 3	45	0	
	Beef cattle farm 4	40	0	
	Total	165	1 (0.6%[b])	
Dairy cattle (milk)	Dairy farm 1	50	1 (2.0%)	
	Dairy farm 2	50	1 (2.0%)	
	Dairy farm 3	90	3 (3.3%)	
	Total	190	5 (2.6%[b])	
Goat (fece)	Goat farm 1	30	6 (20.0%)	
	Goat farm 2	58	4 (6.9%)	
	Total	88	10 (11.4%[a,b])	
Rabbit (nasal swab)	Rabbit warren 1	5	3 (60.0%)	
	Rabbit warren 2	50	8 (16.0%)	
	Rabbit warren 3	50	5 (10.0%)	
	Total	105	16 (15.2%[a])	
Chicken (anal swab)	Chicken farm 1	50	4 (8.0%)	
	Chicken farm 2	50	7 (14.0%)	
	Chicken farm 3	50	2 (4.0%)	
	Chicken farm 4	50	2 (4.0%)	
	Chicken farm 5	50	1 (2.0%)	1 (2.0%)
	Chicken farm 6	50	0	
	Chicken farm 7	60	5 (8.3%)	
	Chicken farm 8	60	3 (5.0%)	
	Chicken farm 9	60	8 (13.3%)	
	Total	480	32 (6.7%[a,b])	1 (0.2%)
Total		1371	89 (6.5%)	4 (0.3%)

[a,b]Values in the same line with different letter superscripts indicate significant differences ($p < 0.05$).

the bacteria. Approximately 0.2 ml of milk or PBS mixture was added to 10 ml of Mueller-Hinton broth that contained 10% NaCl and was cultured at 37°C for 10 h. The medium was streaked onto a mannitol salt plate and incubated at 37°C for 20 h. Presumptive colonies were transferred into Luria-Bertani medium for enrichment at 37°C for 8 h on a rotary incubator. The culture was centrifuged at 5000 r/min for 5 min. Then, the pellet was resuspended in 1 ml of sterile water and centrifuged again, followed by removal of the supernatant. The resulting pellet was resuspended in 0.1 ml of sterile water, subjected to three rounds of heating (105°C for 10 min) followed by freezing (−20°C for 30 min), and then centrifuged again at 10000 r/min for 10 min. The supernatant was removed without disturbing the pellet, and the extracted genomic DNA was stored at −20°C as the template for subsequent PCR procedures. *Staphylococcus aureus* isolates were confirmed by amplification of the *nuc* gene using primers and annealing temperatures listed in Table S1 with *Taq* PCR Master Mix (Sangon Biotech, Shanghai, China). Primers of *nuc* gene were designed according to the nucleotide sequence of *nuc* of *S. aureus* strain 1913 (GenBank no: EF529607.1) with Primer Premier 5.0. The specificity of the primers was checked by aligning the sequences of primers with

BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Amplification procedures were performed as follows: predenaturation at 94°C for 5 min followed by 30 cycles of 30 s at 94°C, 30 s at 58°C, and 1 min at 72°C and then a final extension of 5 min at 72°C. *Staphylococcus aureus* ATCC 25923 was used as a positive control. All confirmed strains were cultured at 37°C in Luria-Bertani medium until the OD_{600} (Optical density) reach to 0.7–0.9 which means the bacteria reached the exponential growth phase (Bogue et al., 2020), and were then stored in 40% glycerol at −80°C.

2.3 | Antimicrobial susceptibility testing

Antimicrobial susceptibility of all *S. aureus* isolates was performed using the disk diffusion method on Mueller–Hinton agar plates and interpreted according to the Clinical and Laboratory Standards Institute guidelines VET01-S4 (CLSI, 2015a) and M100-S25 (CLSI, 2015b). The antimicrobials tested were penicillin (10 units), ampicillin (10 μg), cephalothin (30 μg), cefazolin (30 μg), cefoxitin (30 μg), imipenem (10 μg), kanamycin (30 μg), gentamicin (30 μg), amikacin (30 μg), tobramycin (10 μg), erythromycin (15 μg), azithromycin (15 μg), clarithromycin (15 μg), tetracycline (30 μg), doxycycline (30 μg), chloramphenicol (30 μg), clindamycin (2 μg), norfloxacin (10 μg), ciprofloxacin (5 μg), enrofloxacin (5 μg), ofloxacin (5 μg), enoxacin (10 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), and trimethoprim (5 μg). *Staphylococcus aureus* ATCC 25923 was used as a quality control strain. Isolates were classified as susceptible, intermediate, or resistant to each antimicrobial on the basis of the zone diameter interpretive criteria using the breakpoint values (mm) in accordance with the CLSI guidelines (CLSI, 2015a, 2015b).

2.4 | Detection of *mecA* and ESBL genes and nucleotide sequencing

To verify MRSA and the presence of ESBL genes, PCR was performed for the detection of *mecA* (Murakami et al., 1991), bla_{TEM} (Stürenburg et al., 2004), $bla_{CTX\text{-}M}$ (Monstein et al., 2007), bla_{SHV} (Chen et al., 2004), $bla_{OXA\text{-}1}$ (Oliver et al., 2002), $bla_{OXA\text{-}2}$ (Oliver et al., 2002), $bla_{OXA\text{-}10}$ (Oliver et al., 2002), bla_{PSE} (Qiao et al., 2017), bla_{PER} (Qiao et al., 2017), bla_{GES} (Dallenne et al., 2010), and bla_{VEB} (Dallenne et al., 2010) using specific primers and other parameters (Table S1). The amplified products of the ESBL genes were sequenced from both directions by the BGI Group. Nucleotide sequences were analyzed by searching GenBank using BLAST (http://www.ncbi.nlm.nih.gov/blast/). The acceptance criterion was based on the query cover and identity.

2.5 | Determination of SE/SEI/TSST-1 genes

The presence of SE, SEI, and TSST-1 genes in the *S. aureus* isolates was confirmed by PCR using specific primers (Table S1). Except for the annealing temperature, the other PCR conditions were the same as those used for *nuc* gene amplification. Twenty genes were identified using amplification conditions as previously described (Becker et al., 1998; Kano et al., 2009; Omoe et al., 2005; 2002; Smyth et al., 2005).

2.6 | Statistical analysis

Data analyses were performed using SAS 8.2 statistical software (SAS Institute, Inc., Cary, NC, USA). The data were subjected to one-way analysis of variance using the general linear model procedure in SAS 8.2 statistical software. p Values of ≤ 0.05 were considered to be statistically significant.

3 | RESULTS

3.1 | Prevalence of *S. aureus* in livestock animals

We recovered 89 *S. aureus* isolates from 1371 samples collected from March 2014 to December 2017 in Chongqing, corresponding to an isolation rate of 6.5% (Table 1). The percentage of positive samples varied among different animals. There were 25 (7.3%) from 343 pig samples, 1 (0.6%) from 165 beef cattle samples, 5 (2.6%) from 190 dairy cattle samples, 10 (11.4%) from 88 goat samples, 16 (15.2%) from 105 rabbit samples, and 32 (6.7%) from 480 chicken samples. Thus, the isolation rates of strains from pigs, chickens, goats, and rabbits were significantly higher than those from cattle ($p < 0.05$).

Four isolates had the *mecA* gene, and we classified them as MRSA, corresponding to an isolation rate of 0.3% for all samples (4/1371) and 4.5% among *S. aureus* isolates (4/89). Three MRSA isolates were from pigs, all from the same farm. Thus, the isolation rate of MRSA in pigs was 0.9% (3/343) for all pig samples and 12% among *S. aureus* isolates from pigs (3/25). One MRSA was isolated from a chicken, corresponding to an isolation rate of 0.2% (1/480) for all chicken samples and 3.1% among *S. aureus* isolates from chickens (1/32).

3.2 | Susceptibility of *S. aureus* isolates to different antimicrobials

Table 2 summarizes the results of antimicrobial susceptibility testing. Overall, the 89 *S. aureus* isolates had high resistance to many antimicrobials, especially penicillin (93.3%), ampicillin (92.1%), and tetracycline (57.3%). Notably, there was low resistance to amikacin (4.5%), ofloxacin (9.0%), cephalothin (10.1%), gentamicin (12.4%), and imipenem (15.7%). We also separately examined the resistance patterns of isolates from different types of animals. All 25 isolates from pigs had resistance to penicillin and ampicillin, but most of them were susceptible or had intermediate susceptibility to amikacin (100.0%) and cephalothin (96.0%). Strains isolated from cattle and goats were resistant to penicillin (100.0%) and ampicillin (100.0%). Chicken-associated isolates had resistance to penicillin (100.0%), and many of

TABLE 2 Antimicrobial resistance of *S. aureus* isolates from different animals

Antimicrobial agents	Pig (%)	Cattle (%)	Goat (%)	Rabbit (%)	Chicken (%)	Total (%)
Penicillin	25 (100.0)	6 (100.0)	10 (100.0)	10 (62.5)	32 (100.0)	83 (93.3)
Ampicillin	25 (100.0)	6 (100.0)	10 (100.0)	10 (62.5)	31 (96.9)	82 (92.1)
Cephalothin	1 (4.0)	0	1 (10.0)	5 (31.3)	2 (6.3)	9 (10.1)
Cefazolin	12 (48.0)	0	5 (50.0)	8 (50.0)	17 (53.1)	42 (47.2)
Cefoxitin	9 (36.0)	0	0	5 (31.3)	11 (34.4)	25 (28.1)
Imipenem	5 (20.0)	1 (16.7)	0	5 (31.3)	3 (9.4)	14 (15.7)
Kanamycin	12 (48.0)	1 (16.7)	6 (60.0)	0	12 (37.5)	31 (34.8)
Gentamicin	8 (32.0)	0	0	0	3 (9.4)	11 (12.4)
Amikacin	0	0	1 (10.0)	0	3 (9.4)	4 (4.5)
Tobramycin	11 (44.0)	1 (16.7)	5 (50.0)	0	10 (31.3)	27 (30.3)
Erythromycin	14 (56.0)	1 (16.7)	3 (30.0)	7 (43.8)	12 (37.5)	37 (41.6)
Azithromycin	15 (60.0)	2 (33.3)	3 (30.0)	3 (18.8)	13 (40.6)	36 (40.4)
Clarithromycin	12 (48.0)	2 (33.3)	2 (20.0)	5 (31.3)	14 (43.8)	35 (39.3)
Tetracycline	14 (56.0)	1 (16.7)	7 (70.0)	8 (50.0)	21 (65.6)	51 (57.3)
Doxycycline	15 (60.0)	1 (16.7)	5 (50.0)	0	19 (59.4)	40 (44.9)
Chloramphenicol	13 (52.0)	0	2 (20.0)	0	13 (40.6)	28 (31.5)
Clindamycin	15 (60.0)	1 (16.7)	0	5 (31.3)	13 (40.6)	34 (38.2)
Norfloxacin	13 (52.0)	1 (16.7)	0	0	10 (31.3)	24 (27.0)
Ciprofloxacin	12 (48.0)	1 (16.7)	2 (20.0)	0	11 (34.4)	26 (29.2)
Enrofloxacin	13 (52.0)	0	0	0	11 (34.4)	24 (27.0)
Ofloxacin	4 (16.0)	0	1 (10.0)	0	3 (9.4)	8 (9.0)
Enoxacin	12 (48.0)	1 (16.7)	0	0	10 (31.3)	23 (25.8)
Trimethprim-sulfamethoxazole	11 (44.0)	1 (16.7)	0	2 (12.5)	4 (12.5)	18 (20.2)
Trimethprim	13 (52.0)	1 (16.7)	1 (10.0)	3 (18.8)	7 (21.9)	25 (28.1)

TABLE 3 Number of antimicrobial categories to which *S. aureus* isolates from different animals were resistant

No. of antimicrobial categories	Pig (%)	Cattle (%)	Goat (%)	Rabbit (%)	Chicken (%)	Total (%)
1	4 (16.0)	2 (33.3)	3 (30.0)	5 (31.3)	4 (12.5)	18 (20.2)
2	4 (16.0)	2 (33.3)	0	4 (25.0)	5 (15.6)	15 (16.9)
3	2 (8.0)	1 (16.7)	0	6 (37.5)	4 (12.5)	13 (14.6)
4	1 (4.0)	0	3 (30.0)	1 (6.3)	6 (18.8)	11 (12.4)
5	1 (4.0)	1 (16.7)	4 (40.0)	0	3 (9.4)	9 (10.1)
6	1 (4.0)	0	0	0	8 (25.0)	9 (10.1)
7	2 (8.0)	0	0	0	1 (3.1)	3 (3.4)
8	10 (40.0)	0	0	0	1 (3.1)	11 (12.4)
Total	25	6	10	16	32	89

them also had resistance to ampicillin (96.9%), tetracycline (65.6%), doxycycline (59.4%), and cefazolin (53.1%).

Among 89 *S. aureus* isolates, 56 (62.9%, 56/89) had varying degrees of MDR (Table 3, Figure 1). There were 17 multidrug resistant isolates from pigs (68.0%, 17/25), 2 from cattle (33.3%, 2/6), 7 from goats (70.0%, 7/10), 7 from rabbits (43.8%, 7/16), and 23 from chickens (71.9%, 23/32). Notably, the isolates from pigs and chickens (70.2%, 40/57) had substantially greater multidrug resistant rates than those

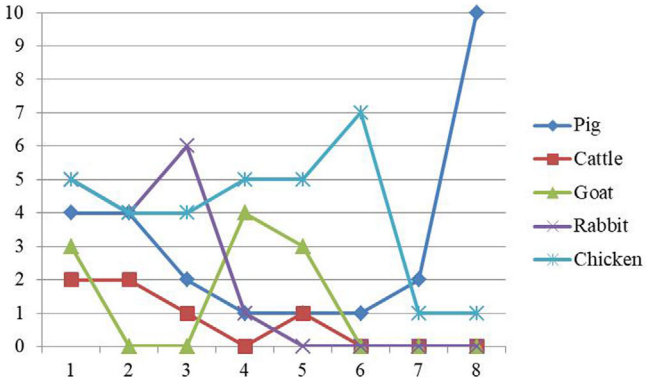

FIGURE 1 Number of antimicrobial categories in which different livestock-originated *S. aureus* were resistant

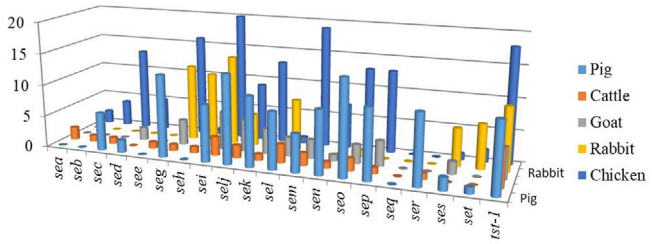

FIGURE 2 Percentage of positive staphylococcal toxin genes in *S. aureus* isolates from animals. The horizontal axis shows each toxin gene, and the vertical axis shows the percentage.

from herbivores (50.0%, 16/32), although there was no significant difference ($p > 0.05$). Overall, 23 isolates (25.8%, 23/89) were resistant to 6 or more classes of antimicrobials, 13 from pigs and 10 from chickens. Ten isolates from pigs and one isolate from chicken were resistant to eight classes of drugs.

The MRSA isolates were resistant to penicillin, ampicillin, erythromycin, azithromycin, clarithromycin, tetracycline, doxycycline, chloramphenicol, clindamycin, trimethoprim-sulfamethoxazole, and trimethoprim. In addition, three MRSA isolates from pigs (CQP1, CQP2, and CQP3) had resistance to kanamycin, tobramycin, norfloxacin, ciprofloxacin, enrofloxacin, and enoxacin (Table S2). The four MRSA isolates all had high MDR. Chicken-associated MRSA (CQC1) was resistant to six classes of drugs. However, all pig-associated isolates were resistant to eight classes of drugs.

3.3 | Prevalence of ESBL genes

Among 89 isolates of *S. aureus*, 86 (96.6%) harbored bla_{TEM} genes (with three chicken-associated strains being the exceptions) and all bla_{TEM} genes were $bla_{TEM\text{-}1a}$. No other ESBL gene was detected using the primers listed in Table S1. Interestingly, bla_{TEM}-negative isolates were multidrug resistant, and MRSA isolates from a chicken did not harbor bla_{TEM} gene.

3.4 | Profile of staphylococcal SE/SEl/TSST-1 genes

We investigated the occurrence of 20 staphylococcal toxin genes in all *S. aureus* isolates (Figure 2 and Table S3) and detected the presence of 19 (all except *seq*). The most prevalent were *sei* (58.4%), *tst-1* (56.2%), *seg* (51.7%), *sej* (39.3%), *seo* (38.2%), *sek* (36.0%), *sep* (32.6%), *sel* (27.0%), *sec* (23.4%), *sem* (22.5%), and *sen* (21.3%). The prevalence of five genes was between 10% and 20% (*seh*, *ser*, *ses*, *set*, and *sed*), the prevalence of two genes was below 10% (*sea* and *seb*), and only one isolate (from dairy cattle) was positive for *see*.

The number of toxin genes ranged from 1 to 10 among the different isolates, except for one rabbit-associated strain in which we did not detect any toxin gene (Table 4, Table S3). Four isolates had only one toxin gene, most isolates (76) had two to seven toxin genes, five isolates had eight toxin genes, three had nine toxin genes, and one isolate from a pig had ten toxin genes.

4 | DISCUSSION

In this study, we investigated the prevalence of *S. aureus* infections in five livestock animals (pigs, cattle, goats, rabbits, and chickens) in Chongqing. As far as we know, this is the first comprehensive survey of livestock-associated *S. aureus* infections in Chongqing, although there were numerous epidemiological studies of this organism elsewhere in China (Dan et al., 2019; Li et al., 2017b; Liu et al., 2018), and there was a report of *S. aureus* isolated from goats in Chongqing (Zhou et al., 2017). It is preferable to collect samples from diseased animals. However, the animals in these farms were healthy. Therefore, we collected fecal samples from healthy animals. There are two main reasons why these animals do not get sick. First, many farms have taken good biosecurity precautions. Second, some farms use antimicrobials to prevent the occurrence of bacterial diseases. The isolation rate of *S. aureus* in our study (6.5%) was similar to that reported for Jiangmen (Guo et al., 2018) but less than that reported for other areas of China and elsewhere in the world. For instance, a study in Henan Province showed that 23.7% of samples from cows, swine, chickens, and ducks were positive for *S. aureus* (Liu et al., 2018). Dan et al. (2019) reported that the rate of isolation of *S. aureus* from cattle farms in Xinjiang Province was 46.0%. A longitudinal study in Ireland reported that the average isolation rate of *S. aureus* in pigs ranged from 26% to 73% (Burns et al., 2014). A previous study in Chongqing (Zhou et al., 2017) reported a higher isolation rate of *S. aureus* isolates from goats (46%) than that in our study (11.4%). Some of these differences can be attributed to differences in culture media and methods, sampling, geographic region, animal species, and farm hygiene. Notably, our isolation rate varied among different animals and ranged from 0.6% for beef cattle samples and 15.2% for rabbit samples, which may be due to different farm environments, animal species, and types of samples.

We identified four MRSA isolates, three from pigs and one from a chicken. Thus, the isolation rate (0.3%) of MRSA in our study was

TABLE 4 Number of staphylococcal toxin genes in *S. aureus* isolates from different animals

No. of toxin gene pattern	Pig	Cattle	Goat	Rabbit	Chicken	Total
1	2			1	1	4
2	1	1	1		5	8
3	5	4	1	1	2	13
4	4	1	2	4	7	18
5	4	1		3	4	12
6	1	1	1	1	5	9
7	1	2		4	8	15
8	4			1		5
9	2		1			3
10	1					1

lower than those reported by previous studies from dairy cows in Xinjiang (14.2%), bovine mastitis in Shanghai and Zhejiang (6.7%), and from healthy animals in Henan (5.59%), China (Dan et al., 2019; Li et al., 2017b; Liu et al., 2018) and from milk and dairy products in Greece (3.6%) (Papadopoulos et al., 2019). However, a previous study in Chongqing did not detect the *mecA* gene among any *S. aureus* isolates from goats (Zhou et al., 2017), in agreement with our finding for goats. Although the isolation rate of MRSA was low in our isolates, other isolates showed significant resistance to penicillins. Therefore, the prevalence of LA-MRSA might be much higher in the future because of selective pressures (Matuszewska et al., 2022). Because LA-MRSA might function as a reservoir for MRSA that can infect people (Fessler et al., 2011; Li et al., 2017b; Loncaric et al., 2013; Loncaric & Künzel, 2013), we suggest regular longitudinal surveillance of MRSA in livestock animals, especially pigs and chickens. Although three MRSA strains were isolated from the same pig farm, their different antimicrobial phenotypes and different staphylococcal toxin genes indicate that they did not come from the same original clone.

Most of the isolates in this study, particularly those from pigs and chickens, had high resistance to antimicrobials and were multidrug resistant, similar to previous reports of livestock animals (Dan et al., 2019; Li et al., 2017b; Liu et al., 2018; Pirolo et al., 2019). Antimicrobial agents are often used to prevent and treat infectious diseases, and their use is common in livestock animals that are in intensive production (Kuang et al., 2015). Based on private communications with farmers, overuse of antimicrobials to prevent the occurrence of bacterial diseases is a possible explanation for extensive drug resistance (Butaye, 2013; Xiong et al., 2018). Most of our isolates were resistant to penicillins but were susceptible to amikacin, ofloxacin, cephalothin, gentamicin, and imipenem. On the other hand, the resistance rate of most *S. aureus* isolated from herbivores was lower than that of *S. aureus* isolated from pigs and chickens. In particular, the ratio of multidrug resistant isolates from pigs and chickens was higher than those from herbivores. This might be related to the more frequent bacterial diseases and greater use of antimicrobials in pigs and chickens according to private communication with farm owners (Butaye, 2013; Xiong et al., 2018). More importantly, some isolates were resistant to antimicrobials whose use is prohibited in animals (imipenem, clarithromycin, chloramphenicol, clindamycin, and enoxacin), probably because of cross-resistance. For example, florfenicol (a type of amphenicol) is widely used to treat veterinary infectious diseases. Although chloramphenicol (also an amphenicol) is prohibited for the treatment of animal diseases, our isolates had a resistance rate of 31.5% to this antimicrobial. It is possible that resistance to both of these drugs is due to the production of the same chloramphenicol/florfenicol exporter gene (Schwarz et al., 2004).

A large proportion of our isolates were resistant to cephalosporins. This suggests that these isolates could have one or more ESBL genes. We found that almost all isolates (except for three from chickens) were positive for $bla_{\text{TEM-1a}}$ but did not harbor other ESBL genes, even though we tested for ESBL genes using more than one pair of primers based on previous studies (Dierikx et al., 2010; Qiao et al., 2017). In the majority of cases, ESBL genes were localized on the mobile genetic elements, such as plasmid (Naas et al., 2008). The mobile genetic elements can be transferred between different bacteria. ESBL genes are prevalent in gram-negative bacteria, especially in Enterobacteriaceae and *Pseudomonas aeruginosa* (Ghafourian et al., 2015). ESBL genes can be transferred in different gram-negative bacteria through mobile genetic elements. Nevertheless, it cannot be ruled out that ESBL genes can be transferred from gram-negative bacteria to gram-positive bacteria through mobile genetic elements, regardless of the species of bacteria. To the best of authors' knowledge, however, no ESBL genes have been reported in *S. aureus* before. The most prevalent beta-lactamase is TEM-1. Most ampicillin-resistant *E. coli* are related to TEM-1 (Ghafourian et al., 2015). Because of the widespread existence of $bla_{\text{TEM-1}}$, it could be transfer $bla_{\text{TEM-1}}$ in plasmids from gram-negative bacteria to *S. aureus* (Mlynarczyk-Bonikowska et al., 2022). In a future study, we plan to isolate more *S. aureus* strains from livestock animals in an effort to identify more ESBL genes and to examine the conjugation mechanism by which the TEM-1a gene moves from Enterobacteriaceae into *S. aureus* and between different *S. aureus* strains.

Most *S. aureus* strains often harbor superantigens, such as SEs, SEls and TSST-1. In order to explore the existence of superantigens in *S. aureus*, we detected the superantigen genes in *S. aureus* isolates. The prevalence of staphylococcal toxin genes in our isolates was different from that in other reports. The major toxin genes in isolates were *sei* (56.2%), *tst-1* (56.2%), *seg* (51.7%), *sej* (39.3%), *seo* (38.2%), *sek* (36.0%), and *sep* (32.6%). However, two SE genes were rare (*sea*: four isolates and *seb*: five isolates). In contrast, a previous study of livestock animals by Liu et al. (2018) reported that the predominant toxin genes were *sed* (20.28%), *sej* (20.98%), and *set* (37.76%). In addition, *sea*, *seb*, and *sec* were more prevalent in *S. aureus* strains isolated from Xinjiang (Dan et al., 2019) than in our study. The detection rates of toxin genes in goat-associated *S. aureus* isolates from Chongqing (Zhou et al., 2017) were very different from our results for goats. We isolated 10 *S. aureus* strains from two farms in southeastern Chongqing, while 32 *S. aureus* strains isolated in another study (Zhou et al., 2017) mainly came from western Chongqing. Therefore, the staphylococcal toxin genes carried by the strains may be different due to the different isolation sites. In future research, we should isolate more strains from farms in different geographical locations in Chongqing to more comprehensively understand the situation of *S. aureus* strains from livestocks and poultry carrying staphylococcal toxin genes. However, some studies reported that the *sec* gene was the most prevalent and widespread SE gene, especially in Europe (Papadopoulos et al., 2019; Riva et al., 2015; Vitale et al., 2015). Although we did not detect the *seq* gene, it was previously reported in livestock from other provinces in China (Liu et al., 2018; Wang et al., 2017; Wu et al., 2018).

In this study, we found that the livestock-associated *S. aureus* isolated from Chongqing were high resistant to many antimicrobials and many of them harbor multiple staphylococcal SE/SEl/TSST-1 genes. As a zoonosis pathogen, *S. aureus* may be transmitted from animals to humans, which has been reported before (Huss et al., 2016). If these high antimicrobial resistant and staphylococcal SE/SEl/TSST-1-rich *S. aureus* isolates are transmitted to humans through the food chain, it will be a great threat to health. In future research, we will collect samples from both animals and workers of *S. aureus*-positive farms to detect whether workers carry *S. aureus* and whether the *S. aureus* isolates originated from animals.

5 | CONCLUSION

In conclusion, this was the first investigation of the prevalence, antimicrobial susceptibility, and molecular characteristics of *S. aureus* isolates from multiple livestock animals in Chongqing. The high antimicrobial resistance and prevalence of $bla_{\text{TEM-1a}}$ in our *S. aureus* isolates indicate the urgent need to reduce the use of antimicrobials. The nearly universal presence of staphylococcal SE/SEl/TSST-1 genes in our *S. aureus* isolates implies a possible high virulence and toxins of livestock-associated *S. aureus* in Chongqing.

AUTHOR CONTRIBUTIONS

Methodology: Qingshuang Dong and Yun Zhang.

Investigation: Qing Wang.

Conceptualization and methodology: Yao Chen.

Data curation and formal analysis: Haoju Wang.

Conceptualization, *data curation*, *formal analysis*, *supervision*, *and writing*: Honglei Ding.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Ministry of Health, China. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Southwest University (Approval no. IACUC-20140110-01) and performed accordingly. The objectives, protocols, and potential risks were clearly explained to all participating farm owners. Written informed consent was obtained from all participating farm owners.

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PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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