Prevalence, antimicrobial resistance and staphyloccocal toxin gene of blaTEM-1a-producing Staphylococcus aureus isolated from animals in Chongqing, China

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Abstract
Background
Livestock-associated Staphylococcus aureus is one of the most important etiological agents in both human and animals. It has been reported with high antimicrobial resistance and multiple staphylococcal superantigen genes in many countries and several provinces of China. However, large-scale investigation of this organism has not been documented in Chongqing, China. The aim of this study is to demonstrate the prevalence, antimicrobial susceptibility and some molecular characteristics of S. aureus acquired from animals in Chongqing.

Results
A total of 89 S. aureus isolates were cultured from 1371 samples picked up from March 2014 to December 2017. The isolates were originated from pigs (25), cattle (6), goats (10), rabbits (16) and chicken (32). Four MRSA strains were identified from 3 pig samples and 1 chicken sample. The isolates showed high resistance to penicillin (93.3%) and ampicillin (92.1%), but were more susceptible to amikacin and ofloxacin, since the resistance rates of these two drugs were less than 10%. Meanwhile, 74.2% isolates exhibited varying degree of MDR. Almost all strains, except for 3 chicken-originated isolates, were positive for blaTEM-1a, but did not harbor other ESBL genes. Nineteen staphylococcal SE/SEI/TSSST-1 genes, except seq, were detected in isolates. The predominant genes were sei (58.4%), tst-1 (56.2%) and seg (51.7%).

Conclusions
The high antimicrobial resistance and prevalence of blaTEM-1a seriously reminded that it was urgent to standardize and cut down the usage of antimicrobials. The universal existence of staphylococcal toxin genes in isolated strains implied a potential threat of public health from animals to human through the food chain.

Background
Staphylococcus aureus (S. aureus) is a versatile opportunistic pathogen exiting widely in humans and food producing animals, such as pigs, poultry, ruminants and rabbits [1, 2]. Many human diseases, ranging from superficial skin and soft tissue infection, pneumonia, septicemia, to endocarditis and
other severe or even fatal diseases, are associated with *S. aureus* [3]. Some animal infectious diseases, such as mastitis, omphalitis, arthritis, septicemia, enteritis, are also mainly caused by *S. aureus*, and responsible for severe economic losses to animal husbandry [4-7].

*S. aureus* has the ability to gain antibiotic resistance determinants, and subsequently, displays resistance to corresponding antimicrobial agents and leads to the emergence of multi-drug resistant strains [8]. Methicillin-resistant *S. aureus* (MRSA) is *S. aureus* strain, that has *mecA* gene which encodes the penicillin-binding protein 2a (PBP2a), mediating resistance to methicillin and all other β-lactam antibiotics [9]. MRSA was initially discovered in an inpatient [10] and hospital-associated MRSA (HA-MRSA) were considered as the main reservoir of this etiological agent [11]. Since 1990s, community-associated MRSA (CA-MRSA) has been concerned as a serious health problem worldwide [11, 12]. In 1972, livestock-associated MRSA (LA-MRSA) was first discovered from mastitic cows [13]. Since then, the prevalence of LA-MRSA has been concerned and detected by many scientists in different animals and countries [1, 14-17]. Human infection might be transmitted from pig-associated MRSA, such as ST398 [18]. This indicated that pigs were important reservoir for human-infected MRSA. Recent studies also found that MRSA ST398 could be isolated from other animals, such as goats [19], rabbits [20], and cattle [21], etc. In addition, methicillin-susceptible *S. aureus* (MSSA) isolated from both human and animals also exhibited multi-drug resistance (MDR) to various classes of antimicrobials [22, 23].

Extended spectrum β-lactamases (ESBLs) are a class of enzymes produced usually by Gram-negative bacteria, especially in enterobacteriaceae and *Pseudomonas aeruginosa*, that are able to hydrolyze extended spectrum cephalosporin and aztreonam while being inhibited by β-lactamase inhibitors, like clavulanic acid and tazobactam [24]. ESBLs have spread threateningly in many regions of the world in human beings and many species of animals [24, 25]. To date, no ESBL gene identified from *S. aureus* has been reported. Additionally, most of *S. aureus* produce a variety of superantigens, including staphylococcal enterotoxins (SEs), SE-like toxins (SEls) and toxic shock syndrome toxin 1 (TSST-1) [26]. These toxins are the causes of food poisoning resulting from the consumption of *S. aureus* contaminated food, such as milk and chicken [27, 28].
Although there have been a great deal of reports on the epidemiological studies of *S. aureus* originated from food producing animals in China in recent years [1, 29, 30], little is known about the prevalence and occurrence of this organism in Chongqing, China. In this study, we investigated the prevalence of *S. aureus* isolated from food animals in Chongqing. Furthermore, antimicrobial susceptibility, MRSA strains, ESBL genes and staphylococcal toxin genes were also characterized.

**Results**

**Prevalence of *S. aureus* from animals**

A total of 89 *S. aureus* isolates were recovered from 1371 samples during March 2014 and December 2017 in Chongqing, and the overall prevalence was 6.5% (Table 1). The isolation frequency varied in different animals. Of these, 25 (7.3%) were isolated from 343 pig samples, 1 (0.6%) were isolated from 165 beef cattle samples, 5 (2.6%) were isolated from 190 dairy cattle samples, 10 (11.4%) were isolated from 88 goat samples, 16 (15.2%) were obtained from 105 rabbit samples, and 32 (6.7%) were obtained from 480 chicken samples. The prevalence rate of *S. aureus* among rabbits was the highest. However, the prevalence rate in cattle was low, and the rate in beef cattle was the lowest and no more than 1%.

Four strains carrying *mecA* gene were classified as MRSA and the prevalence was 0.3% from samples. The positive rate of MRSA in *S. aureus* isolates was 4.5%. Three MRSA were pig-originated and all these strains were obtained from the same farm. The prevalence of MRSA in pigs was 0.9% and the rate in pig-associated isolates was 12.0%. Additionally, 1 was isolated from a chicken farm. Therefore, the prevalence of MRSA in chicken was 0.2% and the rate in chicken-associated isolates was 3.1%. The MRSA isolation rate was higher in pigs than that in chicken. Conversely, MRSA was not detected in other animal samples.

**Susceptibility of *S. aureus* to antimicrobials**

The result of the antimicrobial susceptibility of all 89 *S. aureus* isolates were summarized in Table 2. Overall, *S. aureus* isolates showed high resistance rates against multiple antimicrobials, including penicillin (93.3%, 83/89), ampicillin (92.1%, 82/89), followed by tetracycline (57.3%, 51/89), cefazolin (47.2%, 42/89), doxycycline (44.9%, 40/89), erythromycin (41.6%, 37/89), azithromycin (40.4%,
36/89), clarithromycin (38.3%, 35/89), clindamycin (38.2%, 34/89), kanamycin (34.8%, 31/89), chloramphenicol (31.5%, 28/89), tobramycin (30.3%, 27/89). Moderate resistance can be found from ciprofloxacin (29.2%, 26/89), ceftoxitin (28.1%, 25/89), trimethoprim (28.1%, 25/89), norfloxacin (27.0%, 24/89), enoxacin (27.0%, 24/89), enoxacin (25.8%, 23/89) and trimethoprim-sulfamethoxazole (20.2%, 18/89). On the contrary, the isolates indicated significantly low resistance to amikacin (4.5%, 4/89), ofloxacin (9.0%, 8/89), cephalothin (10.1%, 9/89), gentamicin (12.4%, 11/89) and imipenem (15.7%, 14/89).

All pig-associated isolates showed resistance to penicillin and ampicillin, and majority of them was resistant to azithromycin (60.0%, 15/25), doxycycline (60.0%, 15/25), clindamycin (60.0%, 15/25), erythromycin (56.0%, 14/25), tetracycline (56.0%, 14/25), chloramphenicol (52.0%, 13/25), norfloxacin (52.0%, 13/25), enoxacin (52.0%, 13/25) and trimethoprim (52.0%, 13/25). Meanwhile, most of them were susceptible or intermediately susceptible to amikacin (100.0%, 25/25) and cephalothin (96.0%, 24/25). All cattle-associated isolates showed resistance to penicillin, ampicillin, and susceptibility or intermediate susceptibility to cefoxitin, amikacin, ofloxacin, cephalothin, gentamicin, chloramphenicol, enoxacin and cefazolin. Meanwhile, most isolates were susceptible to other antimicrobials. All goat-associated isolates showed resistance to penicillin and ampicillin and (intermediate) susceptibility to cefoxitin, imipenem, enoxacin, gentamicin, clindamycin, norfloxacin, enoxacin, enoxacin, and trimethoprim-sulfamethoxazole. More than 80% isolates were (intermediately) susceptible to cephalothin, amikacin, clarithromycin, chloramphenical, ciprofloxacin, ofloxacin and trimethoprim. All rabbit-associated isolates were susceptible or intermediately susceptible to 4 tested aminoglycosides (kanamycin, gentamicin, amikacin and tobramycin), 5 fluoroquinolones (norfloxacin, ciprofloxacin, enoxacin, ofloxacin, enoxacin), chloramphenicol and doxycycline. Most strains were (intermediate) susceptibility to azithromycin (81.3%, 13/16), trimethoprim-sulfamethoxazole (87.5%, 14/16) and trimethoprim (81.3%, 13/16). Nevertheless, more than half of them were resistant to penicillin (62.5%, 10/16) and ampicillin (62.5%, 10/16). All chicken-associated isolates showed resistance to penicillin and more than half of them was resistant to ampicillin (96.9%, 31/32), tetracycline (65.6%, 21/32), doxycycline (59.4%, 19/32) and cefazolin
Meanwhile, most of them were more (intermediately) susceptible to cephalothin (93.8%, 30/32), imipenem (90.6%, 29/32), gentamicin (90.6%, 29/32), amikacin (90.6%, 29/32), ofloxacin (90.6%, 29/32) and trimethoprim-sulfamethoxazole (87.5%, 28/32).

All MRSA strains were resistant to penicillin, ampicillin, erythromycin, azithromycin, clarithromycin, tetracycline, doxycycline, chloramphenicol, clindamycin, trimethoprim-sulfamethoxazole and trimethoprim. Three pig-associated MRSA indicated resistance to kanamycin, tobramycin, norfloxacain, ciprofloxacin, enrofloxacin and enoxacin (Table S2).

Among the 89 organisms, 66 (74.2%) isolates exhibited varying degree of MDR. The S. aureus isolates that exhibited MDR were composed of 19 (76.0%) strains from pigs, 3 (50.0%) strains from cattle, 7 (70.0%) strains from goats, 10 (62.5%) strains from rabbits, and 27 (84.4%) strains from chicken. Notably, the isolates (80.7%) from pigs and chicken exhibited substantially higher MDR rate than those (62.5%) from herbivores. Twenty-two (24.7%) isolates were resistant to 7 or more classes of antimicrobials; among them, 13 were isolated from pigs and 9 were acquired from chicken, the ratios in their total quantities were 52.0% and 28.1%, respectively. Three isolates from pigs were found to be resistance to 10 classes of drugs.

Four MRSA strains had high MDR. The chicken-associated MRSA was resistant to 8 classes of drugs. However, 2 pig-associated isolates were resistant to 9, and 1 was resistant to 10 classes of antimicrobials.

**Prevalence of ESBL genes**
In all, 86 (96.6%) isolates harbored \( \text{bla}_{\text{TEM}} \) genes with the exception of 3 chicken-associated strains, and all \( \text{bla}_{\text{TEM}} \) belonged to TEM-1a. No other ESBL gene was detected with listed primers in Table S1. Interestingly, 1 MRSA strain isolated from chicken sample did not contain \( \text{bla}_{\text{TEM}} \) gene.

**Profile of staphylococcal SE/SEI/TSST–1 genes**
A total of 20 staphylococcal toxin genes were amplified in S. aureus isolates. The results were shown in Table 4, Table S3 and Fig. 1. Nineteen genes, except seq, were detected in this study. The most prevalent gene was sei (52, 58.4%), followed by tst–1 (50, 56.2%), seg (46, 51.7%), selj (35, 39.3%),
seo (34, 38.2%), sem (32, 36.0%), sek (29, 32.6%), sel (24, 27.0%), sec (21, 23.4%) and sen (19, 21.3%). The prevalence rates of 5 genes were between 10% and 20%, namely, seh (13, 14.6%), ser (12, 13.5%), ses (11, 12.4%), set (10, 11.2%) and sed (9, 10.1%). However, sea and seb were amplified from 4 (4.5%) and 5 (5.6%) strains, respectively. Only 1 strain obtained from dairy cattle was positive for gene see.

The toxin patterns were variable, from 1 to 10 except for one rabbit-associated strain that no toxin gene was amplified (Table 5, Table S3). Four isolates only had 1 gene. More than 1 gene were detect in 8, 13, 18, 12, 9, 15 and 5 isolates, and they were positive for 2, 3, 4, 5, 6, 7 and 8 toxin genes. Two pig-associated isolates and 1 rabbit-associated isolate harbored 9 toxin genes. Ten toxin genes were observed from 1 strain isolated from a pig.

Discussion

In this study, we investigated the prevalence of S. aureus in different food producing animals, including pigs, cattle, goats, rabbits and chicken, in Chongqing, China. To our understanding, this is the first comprehensive survey on livestock-associated S. aureus in Chongqing, although numerous epidemiological studies of this organism have been carried out in other regions of China [1, 29, 30], and a report about S. aureus isolated from goats in Chongqing [31] has been published. The average prevalence of S. aureus in our study was similar with those obtained from Jiangmen, China [30], but differed from other areas. For instance, a study carried out in Henan showed that 23.7% of animal samples collected from cows, swine, chicken and ducks, were positive for S. aureus [1]. Dan M et al. showed that a high isolation rate of S. aureus (46.0%) was obtained from cattle farms in Xinjiang Province, China [29]. A longitudinal study in Ireland indicated the average prevalence of S. aureus colonization in pigs varied between 26% and 73% [32]. The study [31] carried out in Chongqing indicated that the prevalence (46%) of goat-associated S. aureus was higher than isolation rate (11.4%) of S. aureus obtained from goats in our study. The differences of isolation rates can be interpreted because of the culture media and methods, samples types, different regions, animal species, farm hygienic conditions, and so on. In our study, the isolation rate varied significantly between different animals, from 0.6% of beef cattle samples to 15.2% of rabbit samples.
In the present study, 4 MRSA were isolated from 3 pig samples and 1 chicken sample, but not other animals. The prevalence of MRSA in our study was lower than most researches, both in China [1, 29, 33] and foreign countries [14, 34]. However, the study developed in Chongqing did not detect \textit{mecA} gene in all 32 identified goat-originated \textit{S. aureus} [31]. This study was identical to our result about the goat-associated isolates. Although the prevalence of MRSA was at a low level, the resistance of \textit{S. aureus} isolates to penicillins was serious. Therefore, LA-MRSA might become very popular and the prevalence rate might be much higher in animals in the future under the selective pressure of \(\beta\)-lactam antibacterial drugs. As the LA-MRSA is a reservoir of health-threatening organism for people [19–21, 33], longitudinal surveillance of MRSA in animals, especially in pigs and chicken, should be performed periodically.

Most of isolates, particularly obtained from pigs and chicken, in this study, were high resistance and multi-drug resistant. The high prevalence of resistance to antimicrobials in our study was similar to that reported in previous studies [1, 29, 33, 35]. Antimicrobial agents are often used for the prevention and treatment of infectious diseases and growing promotion of food producing animals in intensive production. Based on records of antimicrobial usage at each farm, overuse of antimicrobials is the possible explanation of extensive drug resistance. The isolates were most resistant to penicillins, but seemed susceptible to amikacin, ofloxacin, cephalothin, gentamicin and imipenem. On the other hand, most antimicrobials showed less resistance in herbivores than in pigs and chicken.

The trend was also observed in MDR strains in different animals. For example, the ratio of MDR isolates obtained from pigs and chicken was higher than herbivore-originated strains. This might be related to the more frequencies of bacterial infectious diseases, thereby leading to more usage of antimicrobials, in pigs and chicken than in herbivores. More importantly, some isolates were resistant to animal-prohibited antimicrobials, such as imipenem, clarithromycin, chloramphenicol, clindamycin and enoxacin, for the selective pressure from the corresponding same class of antimicrobials, which were widely used in animal production. For example, florfenicol was applied in veterinary infectious disease treatment universally. Although chloramphenicol was inhibited in application on animal diseases, the resistance rate was 31.5% in our study. The reason might be that chloramphenicol and
florfenicol were both resistant to the productions of chloramphenicol/florfenicol exporter genes [36]. We found a great proportion of isolates were resistant to cephalosporins. This suggested that these strains have one or more ESBL genes, although no similar assay has been reported. In this study, we discovered that almost all strains, except for 3 chicken-originated isolates, were positive for \( \text{bla}_{\text{TEM-1a}} \), but did not harbor other ESBL genes, even though more than one pair primers published in articles were attempted (Data not shown). In the further study, we will isolate more \( \text{S. aureus} \) clinical strains and try to find more ESBL genes. Meanwhile, we will explore the conjugation machanism of TEM-1a gene from Enterobacteriaceae to \( \text{S. aureus} \) and from \( \text{S. aureus} \) to \( \text{S. aureus} \).

The prevalence of staphylococcal toxin genes in this study was different from other reports. The prevailing toxin genes was \( \text{sei} \) (56.2%), \( \text{tst-1} \) (56.2%) and \( \text{seg} \) (51.7%), which were more than 50% in isolated strains, followed by \( \text{selj} \) (35, 39.3%), \( \text{seo} \) (34, 38.2%) and \( \text{sem} \) (32, 36.0%), that the prevalence was between 30% and 60%. The SE genes, \( \text{sea} \) and \( \text{seb} \), existed only in 4 and 5 strains, respectively. The most predominant toxin genes were \( \text{sed} \) (20.28%), \( \text{selj} \) (20.98%) and \( \text{set} \) (37.76%) in the report from Liu et al. [1]. Dan et al. showed that \( \text{sea}, \text{seb} \) and \( \text{sec} \) were more prevalent in \( \text{S. aureus} \) isolated from Xinjiang [29] than in our study. The detection rates of \( \text{sea} \) to \( \text{selj} \) and \( \text{tst-1} \) performed in goat-associated \( \text{S. aureus} \) isolated from Chongqing [31] were very different from our result. The reason might be different sampling time and different counties. However, some studies reported that \( \text{sec} \) gene was the most prevalent and widespread in SEs [34, 37, 38], especially in Europe [34, 37, 38]. Though we did not detect \( \text{sep} \) gene, it could be found in other provinces in China [1, 39, 40], ever much higher.

Conclusions
In conclusion, this was the first investigation of prevalence, antimicrobial susceptibility and molecular characterization of \( \text{S. aureus} \) isolates from food producing animals in Chongqing. The high antimicrobial resistance and prevalence of \( \text{bla}_{\text{TEM-1a}} \) seriously reminded that it was urgent to standardize the use of antimicrobials. Meanwhile, optimization of management practices and housing conditions should be applied to animals, such that reduction of veterinary infectious diseases in herds will be effective and the usage of antimicrobials can be cut down. The universal existence of
staphylococcal SE/SEl/TSST-1 genes in isolated strains implied a risk of staphylococcal food poisoning that livestock-associated *S. aureus* were transmitted from animals to human through the food chain.

**Methods**

**Samples collection**

From March 2014 to December 2017, a total of 1371 samples were collected from healthy animals in Chongqing, China. Samples were collected in a random manner from pigs (n = 343) and rabbits (n = 105) by nasal swabs, beef cattle (n = 165) and goats (n = 88) by feces, chicken (n = 480) by anal swabs, and dairy cattle (n = 190) by milk. The farmers of each farm gave permission for sample collection. The animal did not suffer from any diseases and administer any drugs. All samples were stored in low temperature with ice and were taken back to laboratory within 6 h.

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

Isolation and identification of *S. aureus* were performed after the samples arrived at laboratory immediately. Briefly, each fecal sample was mixed with 2 mL PBS for 2 h in order to release bacteria. About 0.2 mL milk or PBS mixture was added to 10 mL Mueller-Hinton broth containing 10% NaCl and cultured at 37°C for 10 h. The medium was streaked onto 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. DNA was extracted from culture, and *S. aureus* isolate was confirmed by amplifying its specific gene *nuc*. The primers and annealing temperature were listed in Table S1. *S. aureus* ATCC 25923 was used as positive control. All confirmed *S. aureus* clinical strains were cultured in Luria-Bertani medium until reached to exponential grown phase and stored with 40% glycerol at -80°C.

**Bacterial DNA extraction**

Culture was centrifuged at 5 000 r/min for 5 min, and the pellet was resuspended in 1 mL sterile water. Suspension was centrifuged and the supernatant was discarded. The pellet resuspended with 0.1 mL sterile water was treated in a thermostat at 105°C for 10 min and immediately frozen at -20°C for 30 min three times, followed by centrifugation at 10 000 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
**Antimicrobial susceptibility testing**

Antimicrobial susceptibility of all *S. aureus* isolates were performed by disk diffusion method on Mueller-Hinton agar plates and interpreted according to the Clinical and Laboratory Standards Institute guidelines VET01-S4 [41] and M100-S25 [42]. The antimicrobials included 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). *S. aureus* ATCC 25923, *Escherichia coli* ATCC 25922 were used as quality control strains. MDR was defined as resistance to at least three or more different classes of antimicrobials.

**Detection of mecA and ESBL genes, and nucleotide sequencing**

To verify MRSA and the presence of ESBL genes in *S. aureus*, PCR were carried out to detect mecA [43], *bla*TEM [44], *bla*CTX-M [45], *bla*SHV [46], *bla*OXA-1 [47], *bla*OXA-2 [47], *bla*OXA-10 [47], *bla*PSE [48], *bla*PER [48], *bla*GES [49] and *bla*VEB [49] genes. Primers and related parameters were summarized in Table S1. The amplified products of ESBL genes were sequenced from both directions by BGI company. Nucleotide sequences were analyzed by searching GenBank using BLAST (http://www.ncbi.nlm.nih.gov/blast/).

**Determination of SE/SEl/TSSST-1 genes**

The presence of SE, SEl and TSST-1 genes was confirmed by PCR using specific primers described in Table S1. Twenty genes were determined using amplification conditions described in corresponding literatures [50–54].

**Abbreviations**

ESBL: extended-spectrum β-lactamases; MRSA: methicillin-resistant *Staphylococcus aureus*; SEs: staphylococcal enterotoxins; SEls: SE-like toxins; TSST-1: toxic shock syndrome toxin-1.
Declarations

Ethics approval and consent to participate

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 Ethics Committee of Southwest University and performed accordingly.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

HD conceived and designed the study and analyzed the data. HW, YC and XJ performed the experiments, interpreted the results. HD wrote the manuscript. All authors reviewed the results and approved the final version of manuscript.

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Tables

Table 1 Prevalence of S. aureus and MRSA isolated from animals
| Animal                     | No. of samples | No. of S. aureus | No. of MRSA |
|---------------------------|----------------|------------------|-------------|
| Pig (nasal swabs)         | 343            | 25 (7.3%)        | 3 (0.9%)    |
| Beef cattle (feces)       | 165            | 1 (0.6%)         | 0           |
| Dairy cattle (milk)       | 190            | 5 (2.6%)         | 0           |
| Goat (feces)              | 88             | 10 (11.4%)       | 0           |
| Rabbit (nasal swabs)      | 105            | 16 (15.2%)       | 0           |
| Chicken (anal swabs)      | 480            | 32 (6.7%)        | 1 (0.2%)    |
| Total                     | 1371           | 89 (6.5%)        | 4 (0.3%)    |

Table 2 Antimicrobial susceptibility of S. aureus isolated from animals to different antimicrobials

| Antimicrobial agents | Pig | Cattle | Goat   | Rabbit | Chicken | Total |
|---------------------|-----|--------|--------|--------|---------|-------|
| Penicillin          |     |        |        |        |         |       |
| R (%)/S (%)/I (%)   | 25  | 6 (100.0)/0/0 | 10 (100.0)/0/0 | 10 (62.5)/6 (37.5)/0 | 32 (100.0)/0/0 | 83 (93.3)/6 (6.7)/0 |
| Ampicillin          |     |        |        |        |         |       |
| R (%)/S (%)/I (%)   | 25  | 6 (100.0)/0/0 | 10 (100.0)/0/0 | 10 (62.5)/6 (37.5)/0 | 31 (96.9)/1 (3.1)/0 | 82 (92.1)/7 (7.9)/0 |
| Cephalothin         |     |        |        |        |         |       |
| R (%)/S (%)/I (%)   | 1 (4.0)/20 (80.0)/4 (16.0) | 0/5 (83.3)/1 (16.7) | 1 (10.0)/9 (90.0)/0 | 5 (31.2)/10 (62.5)/1(6.3) | 2 (6.3)/25 (78.1)/5 (15.6) | 9 (10.1)/69 (77.5)/11 (12.4) |
| Cefazolin           |     |        |        |        |         |       |
| R (%)/S (%)/I (%)   | 12 (48.0)/10 (40.0)/3 (12.0) | 0/2 (33.3)/4 (66.7) | 5 (50.0)/4 (40.0)/1 (10.0) | 8 (50.0)/8 (50.0)/0 | 17 (53.1)/10 (31.3)/5 (15.6) | 42 (47.2)/34 (38.2)/13 (14.6) |
| Cefoxitin           |     |        |        |        |         |       |
| R (%)/S (%)/I (%)   | 9 (36.0)/16 (64.0)/0 | 0/6 (100.0)/0 | 0/10 (100.0)/0 | 5 (31.3)/11 (68.7)/0 | 11 (34.4)/21 (65.6)/0 | 25 (28.1)/64 (71.9)/0 |
| Imipenem            |     |        |        |        |         |       |
| R (%)/S (%)/I (%)   | 5 (20.0)/16 (64.0)/4 (16.0) | 1 (16.7)/2 (33.3)/3 (50.0) | 0/10 (100.0)/0 | 5 (31.2)/8 (50.0)/3 (18.8) | 3 (9.4)/23 (71.9)/6 (18.7) | 14 (15.7)/59 (66.3)/16(18.0) |
| Kanamycin           |     |        |        |        |         |       |
| R (%)/S (%)/I (%)   | 12 (48.0)/9 (36.0)/4 (16.0) | 1 (16.7)/2 (33.3)/3 (50.0) | 6 (60.0)/2 (20.0)/2 (20.0) | 0/16 (100.0)/0 | 12 (37.5)/13 (40.6)/7 (21.9) | 31 (34.8)/42 (47.2)/16 (18.0) |
| Gentamicin          |     |        |        |        |         |       |
| R (%)/S (%)/I (%)   | 8 (32.0)/14 (56.0)/3 (12.0) | 0/5 (83.3)/1 (16.7) | 0/9 (90.0)/1 (10.0) | 0/16 (100.0)/0 | 3 (9.4)/27 (84.4)/2 (6.2) | 11 (12.4)/71 (79.8)/7 (7.8) |
| Amikacin            |     |        |        |        |         |       |
| R (%)/S (%)/I (%)   | 0/24 (96.0)/1 (4.0) | 0/6 (100.0)/0 | 1 (10.0)/8 (80.0)/1 | 0/16 (100.0)/0 | 3 (9.4)/25 (78.1)/4 | 4 (4.5)/79 (88.8)/6 (6.7) |
| Antibiotic        | Resistance (%) | Sensitivity (%) |
|-------------------|----------------|-----------------|
| Tobramycin        | 11 (44.0)/11 (12.0) | 1 (16.7)/5 (83.3)/0 |
|                   | 5 (50.0)/5 (50.0)/0 | 0/16 (100.0)/0 |
|                   | 10 (31.2)/22 (68.8)/0 | 27 (30.3)/59 (66.3)/3 (3.4) |
| Erythromycin      | 14 (56.0)/2 (8.0)/9 (36.0) | 1 (16.7)/0/5 (83.3) |
|                   | 3 (30.0)/4 (40.0)/3 (30.0)/0 | 3 (18.7)/12 (75.0)/1 (6.3) |
|                   | 12 (37.5)/2 (6.2)/18 (56.3) | 37 (41.6)/7 (7.9)/45 (50.5) |
| Azithromycin      | 15 (60.0)/8 (32.0)/2 (8.0) | 2 (33.3)/3 (50.0)/1 (16.7) |
|                   | 2 (20.0)/6 (60.0)/2 (20.0) | 5 (31.2)/9 (56.2)/2 (12.6) |
|                   | 13 (40.6)/9 (28.1)/10 (31.3) | 36 (40.4)/37 (41.6)/16 (18.0) |
| Clarithromycin    | 12 (48.0)/12 (48.0)/1 (4.0) | 2 (33.3)/3 (50.0)/1 (16.7) |
|                   | 2 (20.0)/6 (60.0)/2 (20.0) | 5 (31.2)/9 (56.2)/2 (12.6) |
|                   | 14 (43.7)/13 (40.6)/5 (15.7) | 35 (39.3)/43 (48.3)/11 (12.4) |
| Tetracycline      | 14 (56.0)/4 (16.0)/7 (28.0) | 1 (16.7)/1 (16.7)/4 (66.6) |
|                   | 7 (70.0)/0/3 (30.0) | 8 (50.0)/7 (43.7)/1 (6.3) |
|                   | 21 (65.6)/6/18.8/5 (15.6) | 51 (57.3)/18 (20.2)/20 (22.5) |
| Doxycycline       | 15 (60.0)/10 (40.0)/0 | 1 (16.7)/5 (83.3)/0 |
|                   | 5 (50.0)/3 (30.0)/2 (20.0) | 0/9 (56.3)/7 (43.7) |
|                   | 19 (59.4)/11 (34.4)/2 (6.2) | 40 (44.9)/38 (42.7)/11 (12.4) |
| Chloramphenicol   | 13 (52.0)/8 (32.0)/4 (16.0) | 0/3 (50.0)/3 (50.0) |
|                   | 2 (20.0)/4 (40.0)/4 (40.0) | 0/15 (93.8)/1 (6.2) |
|                   | 13 (40.6)/10 (31.2)/9 (28.2) | 28 (31.5)/40 (44.9)/21 (23.6) |
| Clindamycin       | 15 (60.0)/0/10 (40.0) | 1 (16.7)/1 (16.7)/4 (66.6) |
|                   | 0/0/10 (100.0) | 5 (31.3)/9 (56.2)/2 (12.5) |
|                   | 13 (40.6)/1 (3.1)/18 (56.3) | 34 (38.2)/11 (12.4)/44 (49.4) |
| Norfloxacin       | 13 (52.0)/12 (48.0)/0 | 1 (16.7)/4 (66.7)/1 (16.6) |
|                   | 0/8 (80.0)/2 (20.0) | 0/16 (100.0)/0 |
|                   | 10 (31.3)/21 (65.6)/1 (3.1) | 24 (27.0)/61 (68.5)/4 (4.5) |
| Ciprofloxacin     | 12 (48.0)/11 (44.0)/2 (8.0) | 1 (16.7)/4 (66.7)/1 (16.6) |
|                   | 2 (20.0)/4 (40.0)/4 (40.0) | 0/16 (100.0)/0 |
|                   | 11 (34.4)/12 (37.5)/9 (28.1) | 26 (29.2)/47 (52.8)/16 (18.0) |
| Enrofloxacin      | 13 (52.0)/12 (48.0)/0 | 0/3 (50.0)/3 (50.0) |
|                   | 0/6 (60.0)/4 (40.0) | 0/14 (87.5)/2 (12.5) |
|                   | 11 (34.4)/10 (31.2)/11 (34.4) | 24 (27.0)/45 (50.5)/20 (22.5) |
| Ofloxacin         | 4 (16.0)/15 (60.0)/6 (24.0) | 0/6 (100.0)/0 |
|                   | 1 (10.0)/8 (80.0)/1 (10.0) | 0/16 (100.0)/0 |
|                   | 3 (9.4)/22 (68.7)/7 (21.9) | 8 (9.0)/67 (75.3)/14 (15.7) |
| Enoxacin          | 12 (48.0)/12 (48.0)/1 (4.0) | 1 (16.7)/5 (83.3)/0 |
|                   | 0/10 (100.0)/0 | 0/16 (100.0)/0 |
|                   | 10 (31.3)/21 (65.6)/1 (3.1) | 23 (25.8)/64 (71.9)/2 (2.3) |
| Trimethprim-       | 11 (44.0)/13 (52.0)/1 (4.0) | 1 (16.7)/5 (83.3)/0 |
| sulfamethoxazole  | 0/8 (80.0)/2 (20.0) | 2 (12.5)/14 (87.5)/0 |
|                   | 4 (12.5)/25 (78.1)/3 (9.4) | 18 (20.2)/65 (73.0)/6 (6.8) |
| Trimethprim       | 13 (52.0)/11 (44.0)/1 (4.0) | 1 (16.7)/5 (83.3)/0 |
|                   | 1 (10.0)/9 (90.0)/0 | 3 (18.8)/13 (81.2)/0 |
|                   | 7 (21.9)/22 (68.8)/3 (9.3) | 25 (28.1)/60 (67.4)/4 (4.5) |
Table 3 Resistant pattern and MDR of S. aureus isolates

| Resistant pattern | Pig (%) | Cattle (%) | Goat (%) | Rabbit (%) | Chicken (%) | Total (%) |
|-------------------|---------|------------|----------|------------|-------------|-----------|
| 1                 | 1 (4.0) | 1 (16.7)  | 3 (30.0) | 5 (31.3)  | 1 (3.1)     | 11 (12.3) |
| 2                 | 5 (20.0)| 2 (33.3)  | 0        | 1 (6.3)   | 4 (12.5)    | 12 (13.5) |
| 3                 | 2 (8.0) | 2 (33.3)  | 0        | 3 (18.7)  | 4 (12.5)    | 11 (12.3) |
| 4                 | 3 (12.0)| 0         | 0        | 3 (18.7)  | 4 (12.5)    | 10 (11.2) |
| 5                 | 1 (4.0) | 1 (16.7)  | 5 (50.0) | 3 (18.7)  | 5 (15.6)    | 15 (16.9) |
| 6                 | 0       | 0         | 2 (20.0) | 1 (6.3)   | 5 (15.6)    | 8 (9.0)   |
| 7                 | 2 (8.0) | 0         | 0        | 0         | 7 (22.0)    | 9 (10.1)  |
| 8                 | 2 (8.0) | 0         | 0        | 0         | 1 (3.1)     | 3 (3.4)   |
| 9                 | 6 (24.0)| 0         | 0        | 0         | 1 (3.1)     | 7 (7.9)   |
| 10                | 3 (12.0)| 0         | 0        | 0         | 0           | 3 (3.4)   |
| Total             | 25      | 6          | 10       | 16        | 32          | 89        |

Table 4 Prevalence of staphylococcal toxin genes in S. aureus isolates
| Toxin gene | Pig (%) | Cattle (%) | Goat (%) | Rabbit (%) | Chicken (%) | Total (%) |
|------------|---------|------------|----------|------------|-------------|-----------|
| sea        | 0       | 2 (33.3)   | 0        | 0          | 2 (6.3)     | 4 (4.5)   |
| seb        | 0       | 1 (16.7)   | 0        | 0          | 4 (12.5)    | 5 (5.6)   |
| sec        | 6 (24.0)| 1 (16.7)   | 0        | 1 (6.3)    | 13 (40.6)   | 21 (23.4) |
| sed        | 2 (8.0) | 0          | 2 (20.0) | 0          | 5 (15.6)    | 9 (10.1)  |
| see        | 0       | 1 (16.7)   | 0        | 0          | 0           | 1 (1.1)   |
| seg        | 13 (52.0)| 1 (16.7) | 4 (40.0) | 12 (75.0)  | 16 (50.0)   | 46 (51.7) |
| seh        | 0       | 1 (16.7)   | 0        | 11 (68.8)  | 1 (3.1)     | 13 (14.6) |
| sei        | 9 (36.0)| 3 (50.0)   | 6 (60.0) | 14 (87.5)  | 20 (62.5)   | 52 (58.4) |
| selj       | 14 (56.0)| 2 (33.3)| 5 (50.0) | 5 (31.3)   | 9 (28.1)    | 35 (39.3) |
| sek        | 11 (44.0)| 1 (16.7)| 4 (40.0) | 0          | 13 (40.6)   | 29 (32.6) |
| sel        | 9 (36.0)| 3 (50.0)   | 3 (30.0) | 8 (50.0)   | 1 (3.1)     | 24 (27.0) |
| sem        | 6 (24.0)| 2 (33.3)   | 3 (30.0) | 2 (12.5)   | 19 (59.4)   | 32 (36.0) |
| sen        | 10 (40.0)| 1 (16.7)| 1 (10.0) | 0          | 7 (21.9)    | 19 (21.3) |
| seo        | 15 (60.0)| 2 (33.3)| 3 (30.0) | 1 (6.3)    | 13 (40.6)   | 34 (38.2) |
| sep        | 11 (44.0)| 1 (16.7)| 4 (40.0) | 0          | 13 (40.6)   | 29 (32.6) |
| seq        | 0       | 0          | 0        | 0          | 0           | 0         |
| ser        | 11 (44.0)| 1 (16.7)| 0        | 0          | 0           | 12 (13.5) |
| ses        | 2 (8.0) | 0          | 2 (20.0) | 6 (37.5)   | 1 (3.1)     | 11 (12.4) |
| set        | 1 (4.0) | 0          | 0        | 7 (43.8)   | 2 (6.3)     | 10 (11.2) |
| tst-1      | 11 (44.0)| 6 (100.0)| 5 (50.0) | 10 (62.5)  | 18 (56.3)   | 50 (56.2) |

Table 5 Staphylococcal toxin gene pattern of *S. aureus* isolates
| 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     |

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