Prevalence and molecular characterization of *Staphylococcus aureus* isolated from goats in Chongqing, China

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**Abstract**

**Background:** *Staphylococcus aureus* is an important zoonotic pathogen which not only causes significant economic loss in livestock production but also poses a potential threat to public health. Compared with bovine and swine, the information on the colonization of *S. aureus* in goats is very limited. To understand the prevalence and characteristics of *S. aureus* in goats, we used the nasal swabs collected from apparently healthy goats to isolate *S. aureus*, and tested their antimicrobial susceptibility, virulence gene carrying levels, and multilocus sequence typing (MLST).

**Results:** In 74 nasal swabs of apparently healthy goats, 32 (43.24%) *S. aureus* strains were isolated and identified, most of which were susceptible to many antibiotics, except for trimethoprim, furazolidone, amoxicillin, lincomycin and roxithromycin, and the resistance incidence of which were 50%, 40.63%, 37.5%, 28.13%, and 21.88% respectively. All the isolates were methicillin-susceptible *S. aureus* (MSSA) and meca-negative. Enterotoxin genes were found in 53.13% of the strains. Of which, sej was the most prevalent (21.88%), followed by seb, sec, and see with the same level (18.75%). The most prevalent combination were seb + see and seb + tst. None of the *S. aureus* isolates harbored sea, sed, seh, eta and etb. Multilocus sequence typing (MLST) revealed 6 new alleles (aroE-552, aroE-553, glpF-500, pta-440, yqil-482 and yqil-496) and 5 new sequence types (STs) (3431, 3440, 3444, 3445 and 3461). Using eBURST, the 5 STs were assigned to clonal complex 522 (CC522) and a further CC with no predicted ancestor. Phylogenetic analysis of seven concatenated MLST alleles revealed that the 5 STs were grouped into cluster I composed of *S. aureus* mainly from goats and sheep.

**Conclusion:** We provide the data for prevalence of *S. aureus* in goats in Chongqing municipality and their characterization which will help in tracking evolution of epidemic strains and their control methods.

**Keywords:** *Staphylococcus aureus*, Goats, Antimicrobial resistance, Virulence genes, MLST

**Background**

*Staphylococcus aureus* is an important opportunistic pathogen and the cause of infection among human, domestic and wild animals [1–3]. Due to its broad spectrum of inherent virulence factors, the infection of *S. aureus* usually plays an important role for the causing of abscesses, mastitis, pneumonia and meningitis in mammals [4–6]. The invasion of *S. aureus* in domestic animals not only causes significant economic loss in livestock production but also poses a potential threat to public health since these animals can act as the reservoir of methicillin-resistant *S. aureus* (MRSA) [7, 8]. The livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) represented by clonal complex 398 (CC 398) have been shown to be able to colonize and cause serious infections in people having close contact with animals such as veterinarians, farmers and their family members [9–11]. In addition, both handling and consumption of products of these animals colonized by MRSA may provide a potential transmission to humans.
Thus, it is of importance to understand the prevalence and characterization of *S. aureus* colonizing the livestock. In contrast to the studies of *S. aureus* infections in bovine and human, less is known on these bacteria in goats or their relevant products. In 2008, Chongqing municipality was incorporated into the national "Advantage of agricultural products regional planning" in China and recognized as the key areas for beef cattle and goats breeding. The number of live sheep and goats in Chongqing was about 2.26 million and 2.74 million slaughtered was provided by the end of 2015 (Chongqing Statistical Yearbook, 2016). However, there is no investigation on the incidences of colonization in goats by *S. aureus* in Chongqing. To understand the prevalence and characteristics of *S. aureus* in goats, we used the nasal swabs from apparently healthy goats to isolate *S. aureus*, test their antimicrobial susceptibility and virulence gene carrying levels, and defined multilocus sequence typing (MLST).

### Methods

#### Sample collection and *S. aureus* isolation

The nasal swabs were collected from 74 apparently healthy goats from 10 herds in 4 counties (Rongchang, Jiangjing, Zhongxian and Dazu) of Chongqing municipality, transported to the laboratory and stored at 4 °C prior to isolation. The bacteria were enriched in a common broth at 37 °C for 18 h, and then inoculated on 7.5% NaCl agar plates for cultivation at 37 °C for 24 h. The colonies suspected to be *S. aureus* were identified by Gram staining, colony morphology, and coagulase

| Gene | Primer sequence (5′ to 3′) | Amplification size (bp) | Reference |
|------|---------------------------|-------------------------|-----------|
| nuc  | SF:GCGATTGATGTGATACGGTT   | 279                     | [13]      |
|      | SR:AGGACCACCTTTAGCGAACCTAAGC |                        |           |
| mecA | SF: AAAATCGATGTTAAAGTTGTC  | 533                     | [15]      |
|      | SR: AGTTCGACTGACCTCGGATTGC |                        |           |
| sea  | SF: GTTTATCAATGTCGGGTTG    | 102                     | [16]      |
|      | SR: CGGCACCTTTTCTCTTGGG    |                        |           |
| seb  | SF: GTATGCTGGTGAACGAGC     | 164                     | [16]      |
|      | SR: CAAATATGGAAGCTACCGG    |                        |           |
| sed  | SF: CCAATAATAGAGAAATAAAG   | 278                     | [16]      |
|      | SR: ATGGTATTCTTCTTCTTCAC   |                        |           |
| see  | SF: AGGTTTTCTCAGACGGTATCC  | 209                     | [16]      |
|      | SR: CTTTTCTTCTCGTTCAATC    |                        |           |
| seg  | SF: TGCTACGACATCAACACC     | 704                     | [17]      |
|      | SR: CCAGATCTCAATGAGAACC    |                        |           |
| seh  | SF: CGAAGCAGAGATTACACCG    | 495                     | [17]      |
|      | SR: GA CCTTTTATATTTTGCTCTG |                        |           |
| sei  | SF: CATCAGACTTTGTTTGGCTAG  | 142                     | [18]      |
|      | SR: CTGATTCTCATACTGAAGAT   |                        |           |
| tst  | SF: ACCCGTGCTCTTTCTAC     | 326                     | [16]      |
|      | SR: TTTCGATGAATTGCTAAGCC   |                        |           |
| eta  | SF: ATATCAACGAGGCTCTGATAC  | 1155                    | [20]      |
|      | SR: ATGCAGTCGTCTCTGCTA     |                        |           |
| etb  | SF: CACACATTACGGATAATGCAAG | 604                     | [20]      |
|      | SR: TCAACCGAATGAGCTTTTATCT |                        |           |
| pvl  | SF: GTGCCAGACAATTGATACCC   | 255                     | [19]      |
|      | SR: TTCAGAATTGCTTACCTTTCTC |                        |           |

#### Table 1 Primers used for *nuc*, *mecA* and virulence genes amplification

![Fig. 1 Gel electrophoresis of amplified *nuc* in *S. aureus* isolated from goats in Chongqing. M: DL-2000 DNA marker; 1: Staphylococcus sciuri; 2–5: the *nuc* gene of some *S. aureus* isolates by amplified at 297 bp](image)
testing. All the presumptive S. aureus colonies were then subcultured and confirmed by the PCR amplification of nuc (a thermonuclease gene characteristic of S. aureus) [13] (Table 1) with the following conditions: denaturation for 4 min at 94 °C, followed by 30 cycles for 1 min at 94 °C, 30 s at 58 °C and 90 s at 72 °C, and a final extension at 72 °C for 3.5 min. S. sciuri was used as nuc-negative control strain.

Antimicrobial resistance profile

The S. aureus isolates were used for antimicrobial susceptibility test using disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. Following antibiotics with stated concentrations (μg/disc) were used: cefotaxime (30), ceftriaxone (30), streptomycin (10), kanamycin (30), gentamicin (10), tetracycline (30), chloramphenicol (30), lincomycin (2), macrodantin (300), furazolidone (300), vancomycin (30), norfloxacin (10), minocycline (30), amoxicillin (10), cefoxitin (30), clarithromycin (15), levofloxacin (5), roxithromycin (15), trimethoprim (5) and cefepime (30). The presence of mecA (staphylococci methicillin resistance gene) [15] (Table 1) was analyzed by PCR method under the same amplification conditions as stated earlier for nuc, ATCC 43300 (mecA positive) and ATCC 29213 (mecA negative) were used as control strains.

Detection of virulence genes

DNA extraction was done using a commercial DNA extraction kit (Dalian TaKaRa Biotechnology Co., Ltd.) following the manufacturer’s instructions. Thirteen S. aureus virulence genes including enterotoxins (SEs), sea, sec, sed, see, seg, seh, sei, sej [16–18]; cytotoxin pvl [19]; exfoliative toxins (ETs), eta, etb [20]; and a toxic shock syndrome toxin 1 (tst) [16] (Table 1) were selected.

Table 2

| Antibiotics      | No. of S. aureus | Percentage of resistance or sensitive |
|------------------|------------------|---------------------------------------|
|                  | R    | I    | S    | Resistance rate (%) | Sensitive rate (%) |
| Macrodamtin      | 2    | 4    | 26   | 6.25              | 81.25             |
| Streptomycin     | 2    | 2    | 28   | 6.25              | 87.50             |
| Gentamicin       | 1    | 1    | 30   | 3.13              | 93.75             |
| Norfloxacin      | 4    | 0    | 28   | 12.50             | 87.50             |
| Kanamycin        | 2    | 4    | 26   | 6.25              | 81.25             |
| Vancomycin       | 3    | 1    | 28   | 9.38              | 87.50             |
| Ceftriaxone      | 3    | 17   | 12   | 9.38              | 37.50             |
| Tetracycline     | 2    | 8    | 22   | 6.25              | 68.75             |
| Cefotaxime       | 1    | 5    | 26   | 3.13              | 81.25             |
| Chloramphenicol  | 5    | 6    | 21   | 15.63             | 65.63             |
| Trimethoprim     | 16   | 5    | 11   | 50.00             | 34.38             |
| Cefepime         | 1    | 1    | 30   | 3.13              | 93.75             |
| Roxithromycin    | 7    | 14   | 11   | 21.88             | 34.38             |
| Levofloxacin     | 5    | 0    | 27   | 15.63             | 84.38             |
| Lincomycin       | 9    | 10   | 13   | 28.13             | 40.63             |
| Minocycline      | 0    | 4    | 28   | 0                 | 87.50             |
| Cefoxitin        | 0    | 1    | 31   | 0                 | 96.88             |
| Clarithromycin   | 5    | 2    | 25   | 15.63             | 78.13             |
| Amoxicillin      | 12   | 4    | 16   | 37.50             | 50.00             |
| Furazolidone     | 13   | 8    | 11   | 40.63             | 34.38             |

*R* represents resistance; *I* represents intermediate; *S* represents susceptible.

Table 3

| Gene | Number of isolates | Detection rate(%) | Genes | Number of isolates | Detection rate(%) |
|------|--------------------|-------------------|------|--------------------|-------------------|
| seb  | 6                  | 18.75             | seb + see + pvl | 2                 | 6.25             |
| sec  | 6                  | 18.75             | seb + tst + pvl | 2                 | 6.25             |
| see  | 6                  | 18.75             | seb + see + sej| 2                 | 6.25             |
| seg  | 1                  | 3.13              | seb + see      | 4                 | 12.50            |
| sei  | 3                  | 9.38              | see + sej      | 3                 | 9.38             |
| sej  | 7                  | 21.88             | seb + tst     | 4                 | 12.50            |
| tst  | 6                  | 18.75             | seb + pvl     | 3                 | 9.38             |
| pvl  | 6                  | 18.75             | sec + pvl      | 2                 | 6.25             |
|      |                    |                   | sec + tst      | 2                 | 6.25             |
for PCR with the following amplification conditions: initial denaturation for 5 min at 94 °C, followed by 30 cycles of 94 °C for 1 min, 50 °C for 1 min (55 °C for seg and pvl), and 72 °C for 1 min, with a final elongation of 72 °C for 10 min. PCR products were sequenced both in forward and reverse directions by Shanghai Invitrogen Biotechnology Co., Ltd. The sequences for each locus were compared to the allele sequences through the MLST website (http://www.mlst.net). Isolates were defined by their alleles at the seven loci (allelic profile), and each allelic profile was submitted to the database to obtain a sequence type (ST) number.

### Multilocus sequence typing (MLST) of S. aureus

A random selection of 13 S. aureus isolates were analyzed by MLST. The 7 housekeeping genes, carbamate kinase (arcc), shikimate dehydrogenase (aroE), glycerol kinase (glf), guanylate kinase (gmk), phosphate acetyltransferase (pta), triosephosphate isomerase (tpi), and acetyl coenzyme A acetyltransferase (yqil) (http://saureus.mlst.net/misc/info.asp), were amplified by PCR method consisting of initial denaturation at 95 °C for 5 min, followed by a total of 35 cycles of 30 s denaturation at 94 °C, 30 s annealing at 55 °C, and 1 min elongation at 72 °C, with a final elongation step of 10 min at 72 °C. PCR products were sequenced by Shanghai Invitrogen Biotechnology Co., Ltd.

### Clonal complexes (CCs) clustering and phylogenetic analysis of S. aureus STs

The clustering of STs was performed using eBURST algorithm (http://eburst.mlst.net/) [21] by comparing the present dataset to that of the MLST database of 4066 STs by the end of May 2017, and ran with the default settings. Clonal complexes (CCs) were composed of STs that shared at least six alleles in common and a predicted ancestral ST and its associated single locus variants (SLVs: the variants differ at one of the seven MLST alleles from the ancestor) and double locus variants (DLVs: the variants differ at two of the seven MLST alleles from the ancestor) [3]. Along with the STs obtained in this research, 23 STs from human (ST7, 12, 13, 15, 27, 88, 101 and 240), goats and sheep (ST130, 133, 398, 425, 522, 1595, 1740, 1742, 1743, 1758, 1780, 1781, 2011, 2305, 2328) [2, 9, 22, 23] were collected for phylogenetic analyses. The concatenated sequences of all seven MLST alleles for each ST were aligned using the CLUSTAL X program with default parameters followed by manual inspection. MEGA 4.0 was used to construct neighbour-
joining trees [24]. Bootstrapping was performed with 1000 replicates.

Results
Isolation and identification of S. aureus
Of 74 nasal swabs of goats collected from 10 herds, 32 (43.24%) were identified as S. aureus through nuc amplification (Fig. 1), and all the S. aureus isolates were found to be coagulase positive.

Antimicrobial resistance profile
The antimicrobial susceptibility test showed most of the S. aureus isolates susceptible to a majority of antibiotics and the resistance rates below 20%, with the exceptions of following: trimethoprim (50% resistant), furazolidone (40.63%), amoxicillin (37.5%), lincomycin (28.13%), and roxithromycin (21.88%) (Table 2). All the isolates (n = 32) were methicillin-susceptible S. aureus (MSSA) and cefoxitin-susceptible.

Distribution of virulence genes among S. aureus isolates
Since every S. aureus carries several virulence genes, but here 19/32 strains carried at least one of the small chosen set of virulence genes (Fig. 2). Enterotoxin genes were detected in majority (53.13%) of the strains, and sej gene was found in 7 (21.88%), followed by seb, sec and see in 18.75% isolates each, sei (9.38%), seg (3.13%). In addition, both tst gene and pvl gene were detected in 6 (18.75%) isolates. The most prevalent combination was determined to be seb + see and seb + tst in 12.5% of all isolates (Table 3). There were 8 isolates which only encode a virulence gene.

MLST analysis
After MLST analysis, 6 new alleles (aroE-552, aroe-553, glpf-500, pta-440, yqil-482 and yqil-496) and 5 new STs (ST 3431, ST 3440, ST 3444, ST 3445 and ST 3461) were identified and added to the S. aureus MLST database (Table 4). The dominant type of aroE was aroE-552, which was present in 92.31% (12/13) of S. aureus isolates, followed by pta-440 and yqil-496, which
accounted for 69.23% (9/13) and 61.54% (8/13) respectively. The ST3444 was the most common ST type which accounted for 61.54% (8/13) of the *S. aureus* isolates, followed by ST3445 (2 strains), and ST 3431, ST 3440, ST 3461 which just had one strain.

**Clonal complexes (CCs) clustering and phylogenetic analysis of *S. aureus* STs**

eBURST generated 103 groups and 483 singletons. The *S. aureus* isolates tested in this study were clustered into CC522 containing ST3440 and ST3445, and a further CC with no predicted ancestor containing ST3431 and ST3444, while ST3461 was a singleton (Fig. 3). The phylogenetic relationship of 28 STs, based on concatenated sequences of seven MLST alleles, resulted in two main clusters: cluster I (mainly from goats and sheep) and cluster II (from humans). In cluster I, there were three sub-clusters (SCs) (SC1 containing ST130, 522, 1740, 1742, 1758, 2011, 3440, 3445 and 3461; SC2 containing ST133, 425, 1743, 1780, 1781, 2305 and 2328; SC3 containing ST 398, 1595, 3431 and 3444) (Fig. 4).

**Discussion**

This is the first study of the prevalence of *S. aureus* from the nose swabs of apparently healthy goats in Chongqing. Our results were comparable to the carriage frequency of *S. aureus* in healthy sheep in Tunisia [25] approaching to ~ 45% level, which were higher than those reported by other investigators in dairy sheep and goats [26–28], but lower than that in goats in Norway and Denmark [22, 29].

It is interesting that all the *S. aureus* isolates in this study were methicillin-susceptible *S. aureus* (MSSA) since they were sensitive or medium sensitivity to cefoxitin and meca tested negative. The majority of *S. aureus* showed relatively low resistance to vancomycin, ceftriaxone, macrodantin, streptomycin, kanamycin, tetracycline, gentamicin, cefotaxime and cefepime, which is similar to the resistance of *S. aureus* isolated from sheep and goat in Spain [23], but lower than the resistance of *S. aureus* from goats in Taiwan [26]. The relatively high sensitivity spectrum of *S. aureus* from Chongqing is likely due to a limited use of antimicrobials for goats [28].

The infections caused by *S. aureus* is associated with its virulence factors which allow it to adhere to surface, invade or avoid the immune system, and cause harmful toxic effects to the host [30, 31]. More than half of isolates in this study were tested positive for SE genes, which is similar to other reports in *S. aureus* from dairy goats [29] and their milk products [32], and higher than the result in goats and cows affected with mastitis [33]. Of the SE genes tested, *sej* was the most predominantly occurring gene which is different from the detection rates in goats from Taiwan or sheep in Tunisia where there were no *sej* occurrence [25, 26]. Except for *sec*, the *seb*, *see*, and *tst* and *pvl* carriage rate of 18.75% is similar to the other reports in *S. aureus* from goats and their milk products [26, 32, 34, 35] but lower than *sec* and *tst* found in *S. aureus* from dairy goats [29], or in MSSA from sheep [25]. In contrast to the other report [29], although *sec* and *tst* showed the same detection rates, they were not always co-detected. In the previous study, *sec* was identified only in *S. aureus* associated with mastitis in goat [26], the carry level of *seb* and *see* in this study are inconsistent with other reports [32, 35] where there were no *S. aureus* strain harboured *seb* and *see*. *Pvl* is associated with *S. aureus* causing goat subclinical mastitis [36]. Different from the previous report [25], we found 18.75% isolates *pvl*-positive.

To understand the molecular characteristics of *S. aureus* isolates from Chongqing goats, we used MLST typing and found 6 new alleles (*aroE*-552, *aroE*-553, *glpf*-500, *pta*-440, *yqil*-482 and *yqil*-496) and 5 new STs (3431, 3440, 3444, 3445 and 3461). The dominant alleles belonged to *aroE* was *aroE*-552, which occurred in most of *S. aureus* strains, followed by *pta*-440 and *yqil*-496. In the newly found STs, ST3444 was the most common, which occurred in approximately 60% of the *S. aureus* isolates. It is relatively normal for *S. aureus* from goats to possess novel alleles or STs in contrast to human or bovine strains, which may be simply due to the investigation of a new population, since most reports have focused on human clinical isolates or bovine mastitis [37].

The eBURST analyses assigned *S. aureus* isolates to CC522 containing ST3440 and ST3445, and a further CC with no predicted ancestor. The results from phylogenetic analyses showed that STs from goats and sheep were separated from that of human, thus formed two main clusters. Three new STs (ST3440, 3445 and 3461) were sub-grouped in the branch of SC1 containing ST522, the primary founder ST of CC522, which was reported previously from cases of goat mastitis or goat’s milk samples [2, 3, 23]. ST3444, the predominate ST in this study, and ST3431 were sub-clustered into the branch SC3 containing ST398, which is associated with animals infection [9]. In SC2, ST133 was found in variety of animals including cows, goats and sheep [2, 3, 22, 23], and was found to be the most common animal-associated MLST type [3]. Our results confirmed the relative close relationship of ST 3440, ST3445 and ST3461 with ST522, and ST3444, ST3431 with ST398.

**Conclusion**

This is the first study to report the prevalence rate, antimicrobial resistance profile, virulence genes association and MLST characteristics of *S. aureus* from goats in Chongqing municipality. This study will help in tracking evolution of *S. aureus* epidemic strains and proving the methods to control *S. aureus* in goats in China.
The authors declare that they have no competing interests.

Competing interests

Consent for publication

Availability of data and materials

All datasets supporting our findings are available from the corresponding author on reasonable request.

Authors’ contributions

ZYZ and MSZ performed the experiments, analyzed the data, and drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval

All animal procedures were reviewed and approved by the Ethics Committee of Southwest University. Consent was verbally approved by owners of the goats to take the nasal swabs.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 4 July 2017 Accepted: 15 November 2017

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