Association between agr group, genetic background, virulence factors and disease types of Staphylococcus aureus isolated from Chinese children

Yan Xu
Beijing Children's Hospital, Capital Medical University, National Center for Children's Health
https://orcid.org/0000-0003-0261-8376
Suyun Qian (syqian1211@163.com)
Beijing Children's Hospital https://orcid.org/0000-0002-5974-8254
Kaihu Yao
Beijing Children's Hospital
Fang Dong
Beijing Children's Hospital
Wenqi Song
Beijing Children's Hospital
Chen Sun
Beijing Children's Hospital
Xin Yang
Beijing Children's Hospital
Jinghui Zhen
Beijing Children's Hospital
Xiqing Liu
Beijing Children's Hospital
Zhiyong Lv
Beijing Children's Hospital
Xi Yang
Beijing Children's Hospital

Research article

Keywords: Staphylococcus aureus, Accessory gene regulator, Genetic background, Virulence genes, Disease types, Children, China

Posted Date: March 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-19189/v1

Abstract

Background *Staphylococcus aureus* (*S. aureus*) accessory gene regulator (*agr*) system plays a critical role in staphylococcal pathogenesis. Our study aimed to investigate the relationship between *agr* group, the genetic background, virulence genes and disease types distribution of *S. aureus* isolated from different clinical sources among Chinese children.

Methods *S. aureus* strains were isolated from Beijing Children's hospital from October 2017 to October 2019. Isolates were typed by multilocus sequence typing (MLST), staphylococcal protein A (*spa*), *agr,* and staphylococcal cassette chromosome *mec* (SCC*mec*) typing (for methicillin-resistant *S. aureus* [MRSA] isolates). Furthermore, all isolates were tested for the presence of 19 selected virulence genes.

Results A total of 191 non-repetitive *S. aureus* clinical isolates were collected and the *agr* type I was the most prevalent (84.8%). *S. aureus* isolates were divided into 33 sequence types (STs), 20 clonal complexes (CCs) and 59 *spa* types, ST59 (39.8%) and t437 (37.7%) were the predominant types. CC59, CC25, CC22, CC951, CC8, and CC398 isolates possessed *agr* group I; CC15 isolates harbored *agr* group II; CC30 strains were characterized as *agr* group III, and CC121 harbored *agr* group IV. Of the 19 virulence genes, the *tst* gene was more prevalent among *agr* group III compared to other groups ($p = 0.006$); *eta* and *etb* genes were more prevalent among *agr* group IV than other groups ($p = 0.003$ and 0.001, respectively); nearly all strains that harbored the *lukS/F-PV* gene (98.3%) belonged to *agr* group I ($p = 0.004$); the frequencies of *bbp* and *ebpS* genes belonged to *agr* group I were statistically lower than that of other groups ($p < 0.001$). Among 161 diagnoses, the frequency of strains from cellulitis patient harbored *agr* group III was higher than that of other groups ($p = 0.046$), and one strain isolated from staphylococcal scalded skin syndrome (SSSS) patient, which was identified as *agr* type IV ($p = 0.021$).

Conclusions The results indicated that the *S. aureus agr* type was linked to the genetic background. Besides, a possible relationship between the *agr* group, several virulence determinants, and specific disease types was observed.

Background

Staphylococcus aureus (S. aureus) is one of the most common pathogens of community and hospital acquired infections with high morbidity and mortality. The increase of methicillin-resistance S. aureus (MRSA), the emergence of vancomycin-resistant S. aureus (VRSA) strains, and the ability to form biofilm on indwelling medical devices make the infections associated with these microorganisms an important public health issue [1]. Also, the continuous and heavy burden of S. aureus infections poses a threat to Chinese children [2]. Although the prevalence of MRSA among Chinese hospitals has been decreased markedly within recent years [3], according to the report from China Antimicrobial Resistance Surveillance System (CARSS) [4], S. aureus strains were the top one isolates of gram-positive bacteria among Chinese children, and the prevalence of MRSA still increased from 27.5% in 2014 to 29.5% in 2017. Meanwhile, the

contribution of methicillin-susceptible S. aureus (MSSA) to the burden of S. aureus disease has been underestimated [5].

The agr quorum-sensing (QS) system is a dominant regulator of pathogenesis as well as biofilm development in S. aureus, which represses the transcription of many cell wall-associated proteins (protein A and microbial surface components recognizing adhesive matrix molecules [MSCRAMMs]) and activates several exoproteins (hemolysins, exfoliative toxins [ETs], toxic shock syndrome toxins [TSSTs], etc.) in a cell density-dependent manner [6]. Totally, Bronner et al. [7] identified 104 genes that are up-regulated and 34 that are down-regulated by the agr system. Four polymorphisms in the agr locus of S. aureus have been found and were classified into agr groups I, II, III, and IV [8]. Many studies have reported the association between certain agr classes, specific clonal complexes, types of diseases and corresponding virulence factors. For example, Holtfreter et al. [9] found specific S. aureus lineages correlate with different agr types. Jarraud et al. [10] reported agr group IV strains are closely related to ETs caused generalized exfoliative syndromes and bullous impetigo; endocarditis is mainly caused by agr groups I and II strains; toxic shock syndrome (TSS) belongs to agr group III and associated with the expression of genes encoding for specific exotoxins such as TSST-1. However, as far as we know, limited data of these associations were available among Chinese children and the results were not consistent across studies.

In the present study, we analyzed 191 S. aureus isolates from Beijing children's hospital, to determine their agr types and their genetic background (by MLST, spa, and SCCmec element typing), as well as to determine the distribution of 19 virulence genes, in order to investigate the possible relationship between these characteristics among Chinese children.

Methods

Bacterial Strains

From October 2017 to October 2019, 196 samples were collected from Beijing children's hospital. Only one strain was included from each patient. Of the remaining 191 isolates, 46 (19.7%) were identified as colonization, 26 (11.2%) isolated from outpatients (lack of medical records), and 119 (62.3%) from inpatients. This study was reviewed and approved by the Ethics Committee of Beijing Children's Hospital affiliated to Capital Medical University (No. 2016–93, 23/06/2016), and obtained clearance from the Institutional Biosafety Committee (IBSC) ([2017] No.43).

Patient's age and gender, as well as the clinical sources were recorded when these 191 strains were collected from the bacteriology laboratory of this hospital. Meanwhile, the electronic medical records of the 119 inpatient's were reviewed and the following variables of data were collected: types of diseases; the length of hospital stay; pediatric intensive care unit (PICU) transfer; the length of PICU stay; antimicrobial agents therapy; the presence of an invasive device; and a history of surgery, hospitalization, dialysis in the 12 months preceding the culture. *S. aureus* clinical infections were categorized as hospital associated (HA) or community associated (CA) according to the previous definition [11].

These 191 *S. aureus* strains were isolated from several clinical sources: 94 (49.2%) isolated from respiratory tract (58 from sputum, 23 from bronchial alveolar lavage fluid, and 13 from throat swab); 62 (33.5%) isolated from skin and soft tissue (23 from pus, 11 from secretions of omphalitis, 9 from skin secretions, 8 from wound surface, 7 from ear secretions, and 4 from eye secretions); 28 (13.6%) isolated from sterile sites (18 from blood, 3 from bone marrow, 3 from seroperitoneum, 1 from cerebrospinal fluid, 1 from joint effusion, 1 from lymph node, and 1 from pleura tissue); 5 isolated from vaginal secretions and 2 from feces.

The identification of *S. aureus* isolates was performed by colony morphological characteristics and identified by VITEK® MS system (BioMérieux, France). The coagulase test and detection of *nuc* gene were employed to identify *S. aureus* as described previously [12, 13]. The MRSA isolates were screened by cefoxitin disc (30 mg, Oxoid) diffusion test (≤22 mm) according to the Clinical and Laboratory Standards Institute (CLSI, 2019) guidelines [14]. Then *mecA* gene was detected by polymerase chain reaction (PCR) as previously described [15]. All strains were stored at −80 °C until further use.

Extraction of genomic DNA

A typical colony was grown on blood agar at 37 °C overnight. Bacteria genomic DNA was extracted by using Nucleic Acids Isolation & Purification kit (Saibaisheng gene technology Ltd., China) according to the manufacturer's protocol.

agr genotyping

The *agr* typing was done by a multiplex-PCR to determine the *agr* allele types I to IV, using the *agr* group specific primers and amplification conditions as described by Gilot et al. [16]. In brief, multiplex PCR was performed with the following primers: Pan (5'-ATG CAC ATG GTG CAC ATG C-3'), *agr*I (5' -GTC ACA AGT ACT ATA AGC TGC GAT-3'), *agr*II (5'-TAT TAC TAA TTG AAA AGT GGC CAT AGC-3'), *agr*III (5'-GTA ATG TAA TAG CTT GTA TAA TAA TAC CCA G-3') and *agr*IV (5' -CGA TAA TGC CGT AAT ACC CG-3'). The *agr* groups were identified by amplicon size: 441 bp for *agr* I; 575 bp for *agr* II; 323 bp for *agr* III; 569 bp for *agr* IV. *S. aureus* strains col (*agr* group I), N315 (*agr* group II), TY114 (*agr* group III), A920210 (*agr* group IV) were used as positive controls for *agr* group identification.

Molecular genotyping analysis

MLST

MLST was performed as described previously [17]. The seven housekeeping gene (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) sequences were compared with known alleles, and isolates were assigned STs and CCs according to the MLST database (http://saureus.mlst.net/). STs were used to generate a global optimal eBURST (goeBURST) diagram using the PHYLOViZ 2.0 program (http://www.phyloviz.net).

spa typing

The spa typing was based on variations in the polymorphic X region of the *spa* gene [18], and determined by submitting the sequencing data to the *S. aureus spa* type database (http://www.genomicepidemiology.org).

SCC*mec* typing

For MRSA isolates, the SCC*mec* types were determined by multiplex PCR and the detailed protocol was published previously [19]. The following MRSA strains were used as positive controls for SCC*mec* types: NCTC10442 (SCC*mec* I), N315 (SCC*mec* II), 85/2082 (SCC*mec* III), JCSC4744 (SCC*mec* IV), IMVS 67(SCC*mec* V).

Detection of virulence genes

Simplex and multiplex PCRs were used to detect the following genes:

Amplification of *tst* (encoding toxic shock syndrome toxin-1), *hla, hlb* and *hld* (encoding alpha, beta, and delta-haemolysin precursor), *eta* and *etb* (encoding exfoliative toxin A and B) and *lukS/F-PV* (encoding Panton-Valentine leukocidin) genes was performed using simplex PCR as previously described [20].

The distribution of four biofilm-forming genes was detected using simplex PCR as described by Pereyra et al. [21]: *ica A*, *ica C* and *ica D* (encoding intercellular adhesion protein A, C and D) and *bap* (encoding biofilm-associated protein).

The distribution of nine MSCRAMM genes was detected using multiplex PCR as previously described [22]: *ebpS* (encoding elastin binding protein), *eno* (encoding laminin binding protein), *cna* (encoding collagen binding protein), *fnbB* (encoding fibronectin binding proteins B), *fib* (encoding fibrinogen binding protein), *clfA* and *clfB* (encoding clumping factors A and B), and *bbp* (encoding bone sialoprotein binding protein).

Statistical analysis

Statistical analysis was performed with SPSS v.23.0 (SPSS Inc., Chicago, IL, United States). Chi-squared ($\chi 2$) test or Fisher's exact test was used to analyze the categorical variables. Kruskal-Wallis test and a Dunn-Bonferroni test for post hoc comparisons was used to compare the differences in age, hospital and PICU stay length among different *agr* groups. $P < 0.05$ was considered to be statistically significant.

Results

Clinical characteristics and corresponding disease syndromes

A total of 191 non-repetitive *S. aureus* isolates were collected from Beijing Children's Hospital, including 120 MRSA (62.8%) and 71 MSSA (37.2%). Seventy-nine (41.4%) strains were CA, 40 (20.9%) were HA, 46 (24.1%) isolated from asymptomatic carriers, and 26 (13.6%) from outpatients. Table 1 presented patient's age, gender, infection characteristics, clinical sources, and the *agr* genotyping of the 191 *S. aureus* isolates, as well as the clinical characteristics (hospital length of stay, PICU transfer, PICU length

of stay, antibiotics usage) of the 119 inpatients. The median age was 12.0 months (ranged from 2.0 to 72.0 months; 30 were ≤ 28 days old) and 102 (53.4%) were male; the hospital length of stay ranged from 7 to 18 days, with a median of 11 days; twenty-four (12.6%) were transferred to PICU, with a median of 18.5 days (6.5-28.0); all of them received intravenous antibiotics while in the hospital, with 54 (45.4%) received vancomycin (24, 20.2%), linezolid (29, 24.4%), meropenem (18, 15.1%) and ertapenem (4, 3.4%) either alone or as part of combination treatment. In addition to systemic antibiotics, mupirocin ointment (8, 6.7%), levofloxacin ear drops (1, 0.8%) and eye drops (1, 0.8%) were prescribed. To rule out selection bias among available isolates, we analyzed our data and found no significant differences by age (p = 0.915), gender (p = 0.442), hospital length of stay (p = 0.514), PICU length of stay (p = 0.502), or vancomycin (p = 0.288) and linezolid usage (p = 0.901) among different *agr* groups in patients whose isolates were available for laboratory testing. While the PICU transfer rate of *agr* group III was significantly higher than that of other groups (p = 0.009). There was no difference between strains from diverse clinical sources among four *agr* groups (p = 0.495) (Fig. 1a). Whereas the number of MRSA isolates belonged to *agr* group I was significantly higher than that of MSSA isolates (p < 0.001) (Fig. 1b).

Upon discharge, there were a total of 161 clinical diagnoses for these 119 inpatients (Table 2), including 66 (28.3%) cases of respiratory tract infections, 38 (16.3%) cases of skin and soft tissue infections (SSTIs), 52 (22.3%) cases of invasive infections, and 5 (2.1%) cases of exfoliative toxin-mediated diseases. The distribution of different diagnoses according to *agr* genotypes was shown in Table 2. The frequency of strains isolated from cellulitis patients harbored *agr* group III was significantly higher than that of other *agr* groups (p = 0.046). There was only one strain isolated from SSSS patient, which was identified as *agr* type IV (p = 0.021). Besides, neonatal diseases (including neonatal pneumonia, omphalitis, and bullous impetigo) were all community acquired and 95% of which belonged to *agr* group I (except the strain which isolated from a cellulitis patient was identified as *agr* group III). Among medical device-related infections, 50% of isolates were *agr* group II, and necrotizing pneumonia isolates were more likely to be *agr* group III. However, there was no statistical difference between *agr* groups according to these diagnoses (p > 0.05).

agr genotyping

By multiplex PCR, the *agr* types were successfully identified in all of the isolates. As shown in Table 1, the *agr* type I was the most prevalent (162, 84.8%), followed by *agr* type II, which was found in 16 (8.4%) isolates, whereas *agr* types III and IV possessed by only 9 (4.7%) and 4 (2.1%) isolates, respectively.

Molecular characteristics

The genotypic diversity was high, 191 *S. aureus* isolates were divided into 33 STs, ST59 (76, 39.8%) was the predominant type, followed by ST22 (24, 12.6%), ST398 (15, 7.9%), and ST25 (10, 5.2%). The frequencies of the remaining STs were ranging from 0.5% to 3.7%. The 33 STs belonged to 20 CCs, CC59 (81, 42.4%), CC22 (29, 15.2%), CC398 (15, 7.9%), CC5 (14, 7.3%), and CC25 (10, 5.2%) were the most prevalent CCs.

A total of 59 distinct *spa* strain types were seen. The most common type was t437 (72, 37.7%), followed by t309 (20, 10.5%), t571 (10, 5.2%), and t078 (7, 3.7%). The prevalence rates of the remaining *spa* types were ranging from 0.5% to 2.1%. Besides, three strains could not be determined by *spa* typing.

Of the 120 MRSA strains, all of the five different SCC*mec* types were detected. Most of which carried SCC*mec* type IV (77, 65.0%), followed by SCC*mec* type V (25, 20.8%), SCC*mec* type I (14, 11.7%), SCC*mec* type III (2, 1.7%), and SCC*mec* type II (1, 0.8%). Also, 0.8% (1) of the isolates could not be typed by multiplex PCR.

The genotypic characteristics of 191 *S. aureus* stratified by *agr* types were shown in Fig. 2 and Additional file 1. Except that 3 CCs (CC1, CC5, and CC45) which mostly belonged to one specific *agr* group partly superimposed on another *agr* group, all of the CC59, CC25, CC22, CC951, CC8 and CC398 strains had *agr* group I, CC15 isolates harbored *agr* group II, CC30 possessed *agr* group III, and CC121 harbored *agr* group IV. Furthermore, the distribution of prevalent *spa* types across the patients reflected the CC distribution. Among strains that belonged to *agr* group I, the most common types were CC59-t437, CC25-t078, CC22-t309, CC951-t437, and CC398-t571. While CC5 and CC15 which mostly belonged to *agr* group II had greater *spa* type diversity compared to other prominent CCs. In *agr* groups III and IV, the two dominant types were CC1-t114 and CC121-t2092, respectively.

Prevalence and Distribution of Virulence Genes

As illustrated in Table 3, all isolates harbored the *hla* (191, 100%), *hlb* (191, 100%) and *hld* (191, 100%) genes. The prevalence of the *lukS/F-PV* gene was 31.4% (60), while the frequencies of *tst, eta* and *etb* genes were 7.9% (15), 3.7% (7) and 1.0% (2), respectively.

All isolates possessed the *icaA* (191, 100%) and *icaD* (191, 100%) genes, the frequency of the *icaC* gene was also high at 93.7% (179). Notably, all of the ST25 (10) strains were *icaC* negative. In contrast, only 1 (0.5%) isolate harbored the *bap* gene, which isolated from the bronchial lavage of a cystic fibrosis patient.

All isolates harbored the *eno* (100%), *clfA* (100%) and *clfB* (100%) genes, followed by high frequency of *fib* gene (143, 74.9%). The prevalence of *bbp, ebpS, cna* and *fnbB* genes were 3.1% (6), 19.9% (38), 11.5% (22) and 16.8% (32), respectively.

Of these 19 virulence genes, the distribution of the following 6 genes differed among the isolates according to the *agr* genotyping: *tst* ($p = 0.006$), *eta* ($p = 0.003$), *etb* ($p = 0.001$), *lukS/F-PV* ($p = 0.004$), *bbp* ($p < 0.001$), and *ebpS* ($p < 0.001$) genes (Table 3). The *tst* gene was more prevalent among *agr* group III compared to other groups. Among 7 strains that harbored the *eta* gene, 2 of which also had *etb* gene and belonged to *agr* group IV. It is noted that nearly all of the strains that harbored the *lukS/F-PV* gene belonged to *agr* group I (except 1 strain belonged to *agr* group III, which isolated from a necrotizing pneumonia patient). Additionally, the frequencies of *bbp* and *ebpS* genes that belonged to *agr* group I were statistically lower than that of other groups.

The average number of virulence genes was examined based on *agr* genotyping, which were 10.5, 11.1, 11.7 and 13.3 for *agr* groups I, II, III, and IV, respectively (Fig. 3). The coexistence of the studied virulence genes was also investigated. Most of strains had 10 (81, 42.4%) and 11 (82, 42.9%) genes investigated. The number of strains had 12, 13 and 14 genes were 14 (7.3%), 3 (1.6%) and 3 (1.6%), respectively. Besides, there were 1 (0.5%) and 7 (3.7%) strains had only 8 and 9 genes investigated (Fig. 4).

Discussion

An association between agr groups and the genetic background of S. aureus isolates from Chinese children was observed in the present study, as each ST belonged to a specific agr type, which was consistent with both domestic and international studies reported previously [9, 23–25]. However, different STs were included in the same agr group among these studies, and there were 3 CCs belonged to more than one specific agr group in our study, questioning the hypothesis that S. aureus strains are subdivided in a manner that corresponds to agr groups, whereas supporting the theory proposed by Monecke et al. [26] and Robinson et al. [27], there was recombination between agr groups, since agr influences the expression of many virulence genes, small phenotypic differences encoded by different agr alleles might be selectable into larger evolutionary differences.

The frequency of agr I was higher than previous reports in Chinese children [25, 28]. To explain, clonal differences of strains should be regarded. MRSA strains are known to be highly clonal, and the number of MRSA strains outweighed that of MSSA in our study. We found although agr I was the dominant type in both MRSA and MSSA isolates, the number of MRSA isolates harbored agr type I was significantly higher than that of MSSA. Similar to our study, Willem et al. [29] found agr type I was the most prevalent (92.2%) among 192 carriers and disease isolates, and 71% of the isolates were MRSA strains. In contrast, among Jarraud et al. [10] carefully selected collection of S. aureus clinical isolates, the four agr types were relatively evenly distributed throughout France. Furthermore, the genetic background of strains isolated from different regions should also be considered, since studies from different countries showed disparate results [30–32].

Many studies have demonstrated that certain SCCmec elements seem to have a strong predilection for particular S. aureus genotypes defined by MLST. Such as Enright et al. [33] found STs 5, 8, 45, and 254 included MRSA isolates with different SCCmec types, whereas the other STs were uniform in SCCmec type. Wright and colleagues [34] reported SCCmec elements are found in agr groups I, II, and III rather than in group IV. However, in the current study, except that SCCmec IV was the dominant type in ST59 and ST951, there was no clear correlation between other STs and specific agr groups. Meanwhile, similar to a previous Chinese study [35], we found SCCmec IV was the most dominant type in all isolates. Researchers [36] suggested the reason is that the combination of smaller size and lower cost on fitness may make SCCmec type IV the selectively favored element for transfer among all S. aureus isolates.

Our results highlighted the potential importance of adhesion genes (icaA, icaD, eno, clfA, and clfB) and hemolysin genes (hla, hlb, and hld) among S. aureus infections in children, as these genes were present in

all isolates irrespective of clinical sources. Suggesting that these ubiquitous virulence genes carried by S. aureus play significant roles in staphylococcal pathogenicity.

The association of agr group, specific virulence genes and disease types has been discussed. Our data were consistent with a previous study, which demonstrated that phages and plasmids frequently appear in agr-IV strains that carry eta or etb, whereas the TSST-1 is preferentially carried by agr-III strains [34]. However, unlike prior studies that demonstrated Panton-Valentine leukocidin (PVL) is linked to a broad array of necrotizing diseases such as pneumonia and SSTIs [37–39], there was no significant association between the lukS/F-PV gene and these specific disease syndromes in this study. An article also reported that the importance of PVL as a determinant of CA-MRSA virulence was controversial and initially overestimated [38]. Our data were in agreement with the finding [39] that most CA-MRSA strains have the lukS/F-PV gene while their frequency in MSSA is much lower. Meanwhile, agr I was the most prevalent type, especially among MRSA strains, so it is reasonable that most of the lukS/F-PV gene positive strains belonged to agr group I in this study. We also found the two predominant STs, ST59 and ST22 harbored most of the lukS/F-PV gene positive strains, suggesting that although the lukS/F-PV gene is encoded by a bacteriophage, it is likely that the bacteriophage has spread easily among the same genetic background.

According to Jarraud et al. [10], some suppurative diseases did not specifically relate to particular agr groups. Since the virulence factors specifically involved in these infections did not clear, they speculate MSCRAMMs likely play a role in these diseases. Montanaro et al. [40] reported that specific adhesion gene patterns emerged in association with agr groups, for agr I and agr II associated with the presence of the adhesion genes fib and fnbB, agr III associated with the presence of bbp, and the eno and ebpS genes form a common background transversally crossing all agr polymorphisms. However, except that bbp and ebpS genes showed much lower frequencies in agr group I, most of the MSCRAMM and biofilm-forming genes did not show a correlation with specific agr groups in the current study. Additionally, the frequencies of MSCRAMM genes were different from other studies [30, 41]. Differences in primers and sources of S. aureus between studies, and in the predominant clones among different countries should be considered.

Two surface components have been implicated in biofilm formation by S. aureus, the product of the icaADBC operon and the Bap [42]. Our data were in agreement with previous studies, which have demonstrated that biofilm genes icaA/icaC/icaD were present in all isolates, whereas bap was always missing [43, 44]. The relatively low detection rate of bap indicates that the ica-dependent mechanism might be primarily responsible for adhesion and biofilm production in S. aureus strains [45]. Notably, we found the icaC gene was not detectable in all ST25 strains, the lack of icaC gene could be interpreted as indication for an aberrant or absent icaC sequence. To our knowledge, which has not been reported yet and needs further confirmation by testing more ST25 strains or by genome sequencing.

We found the agr group IV carried the highest number of virulence factors, followed by the agr group III. The main reason was that the eta, etb, bbp, ebpS, and tst genes were more likely to belong to these two

groups. Our finding was different from a previous study [46], probably due to the different sources of the available strains in these two studies.

Previously, Wright et al. [34] confirmed that there are four well-documented clinical situations where agr group may be highly relevant: the association of agr III with menstrual toxic shock syndrome and with PVL-induced necrotizing pneumonitis, the association of agr IV with exfoliatin production and SSSS, and a predilection for reduced vancomycin sensitivity in agr I and II. Our results also demonstrated that there was a possible relationship between agr groups and particular diseases. As Jarraud et al. [10] stated, in most cases, the association between the agr types and certain diseases (mainly toxin-mediated diseases) reflected the link between the disease types, the pattern of toxin genes, and the genetic background of the strains, and this preferential association might make the activation of virulence factors more efficient.

This study had some limitations. First, the limitation of sample size, as the agr classification was not uniform, most of them were in group I. A relatively small sample of agr group II, III, IV and some toxin-mediated diseases (e.g. SSSS) impacted our ability to see the true correlation between the virulence genes or clonal groups and some of the clinical characteristics and diagnoses. Furthermore, the diagnoses were reviewed and collected from patient's medical records and were made by pediatricians, inconsistencies between diagnoses and actual diseases might exist. Additionally, although all isolates were collected from a single medical center in this study, there were more than 60% of inpatients come from all over the country. We consider that our data are likely to be representative of children from the whole China.

Conclusions

In summary, the correlation we observed suggests a clear link between agr typing and genetic background. Furthermore, we observed a number of virulence factors and certain staphylococcal syndromes, such as tst, eta, etb, lukS/F-PV, bbp, and ebpS genes as well as SSSS and cellulitis, to a lesser extent, were preferentially associated with one or more specific agr groups. These findings could provide novel insights into further understanding the molecular characteristics and pathogenesis of the S. aureus agr system in Chinese children.

Abbreviations

agr: accessory gene regulator; CA: community-associated; CC: clonal complex; CLSI: Clinical and Laboratory Standards Institute; CARSS: China Antimicrobial Resistance Surveillance System; ET: exfoliative toxin; goeBURST: global optimal eBURST; HA: healthcare-associated; IBSC: Institutional Biosafety Committee; MLST: multilocus sequence typing; MRSA: methicillin-resistant *S. aureus*; MSSA: methicillin-susceptible *S. aureus*; MSCRAMMs: microbial surface components recognizing adhesive matrix molecules; PBP2a: penicillin-binding protein 2a; PCR: polymerase chain reaction; PICU: pediatric intensive care unit; PVL: Panton-Valentine leukocidin; QS: quorum-sensing; SSSS: staphylococcal scalded skin syndrome; *S. aureus*: *staphylococcus aureus*; SCC*mec*: staphylococcal cassette chromosome *mec*;

spa: staphylococcal protein A; SSTI: skin and soft tissue infection; ST: sequence type; TSS: toxic shock syndrome; TSST: toxic shock syndrome toxin; VRSA: vancomycin-resistant *S. aureus*.

Declarations

Ethics approval and consent to participate

The bacterial isolates used in this study were isolated from the biological specimens obtained during the management of patients, with no threat to the subjects' rights and health. The applications for the exemption of written informed content and ethical review were approved by the Ethics Committee of Beijing Children's Hospital Affiliated to Capital Medical University according to national regulations (No. 2016–93, 23/06/2016). Verbal consent was obtained from the parents or legal guardians of the patients. This study also obtained clearance from the Institutional Biosafety Committee (IBSC) ([2017] No.43).

Consent for publication

Not applicable.

Availability of data and materials

The raw data will be provided upon request by Suyun Qian (Correspondence author), Email: syqian1211@163.com.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by the Beijing Natural Science Foundation (No.7172075) and the National Natural Science Foundation of China (No. 81571948). Except for the financial support needed to conduct this study, the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Suyun Qian is the funding recipient, designed the study and revised the manuscript.

Authors' contributions

YX collected the medical records, performed *agr*, MLST, SCC*mec* and *spa* typing, detected the virulence genes, analyzed the data, and drafted the manuscript. SQ designed the study and revised the article. KY statistically analyzed the data and revised the article. FD, WS and XY revised the article. CS performed MLST and *spa* typing. JZ, XL, ZL, and XY collected and identified the clinical strains of *S. aureus*. All authors had read and approved the final manuscript.

Acknowledgements

We thank the research group of Xuzhuang Shen, who worked in Beijing Pediatric Research Institute in China, for supplying the *S. aureus* standard strains used in this study.

Authors' information

[1]Pediatric Intensive Care Unit, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, No.56 Nan-Li-Shi Road, Beijing 100045, China. [2]Key Laboratory of Major Diseases in Children, Ministry of Education, National Key Discipline of Pediatrics (Capital Medical University), Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing 100045, China. [3]Bacteriology Laboratory, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing 100045, China.

References

1. Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998;339(8):520-532.
2. Liu Y, Xu Z, Yang Z, Sun J, Ma L. Characterization of community-associated Staphylococcus aureus from skin and soft-tissue infections: a multicenter study in China. Emerg Microbes Infect. 2016;5(12):e127.
3. Hu F, Zhu D, Wang F, Wang M. Current Status and Trends of Antibacterial Resistance in China. Clin Infect Dis. 2018;67:S128-S134.
4. China Antimicrobial Resistance Surveillance S. [Surveillance of bacterial resistance in children and newborns across China from 2014 to 2017]. Zhonghua Yi Xue Za Zhi. 2018;98(40):3279-3287.
5. David MZ, Boyle-Vavra S, Zychowski DL, Daum RS. Methicillin-Susceptible Staphylococcus aureus as a Predominantly Healthcare-Associated Pathogen: A Possible Reversal of Roles? PLoS One. 2011;6(4):e18217.
6. Ikonomidis A, Vasdeki A, Kristo I, Maniatis AN, Tsakris A, Malizos KN, Pournaras S. Association of biofilm formation and methicillin-resistance with accessory gene regulator (agr) loci in Greek Staphylococcus aureus clones. Microb Pathog. 2009;47(6):341-344.
7. Bronner S, Monteil H, Prevost G. Regulation of virulence determinants in Staphylococcus aureus: complexity and applications. FEMS Microbiol Rev. 2004;28(2):183-200.
8. Martinez-Garcia S, Ortiz-Garcia CI, Cruz-Aguilar M, Zenteno JC, Murrieta-Coxca JM, Perez-Tapia SM, Rodriguez-Martinez S, Cancino-Diaz ME, Cancino-Diaz JC. Competition/antagonism associations of biofilm formation among Staphylococcus epidermidis Agr groups I, II, and III. J Microbiol. 2019;57(2):143-153.
9. Holtfreter S, Grumann D, Schmudde M, Nguyen HTT, Eichler P, Strommenger B, Kopron K, Kolata J, Giedrys-Kalemba S, Steinmetz I et al. Clonal distribution of superantigen genes in clinical Staphylococcus aureus isolates. J Clin Microbiol. 2007;45(8):2669-2680.
10. Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Forey F, Nesme X, Etienne J, Vandenesch F. Relationships between Staphylococcus aureus genetic background, virulence factors, agr groups

(Alleles), and human disease. Infect Immun. 2002;70(2):631-641.

11. Bal AM, Coombs GW, Holden MTG, Lindsay JA, Nimmo GR, Tattevin P, Skov RL. Genomic insights into the emergence and spread of international clones of healthcare-, community- and livestock-associated meticillin-resistant Staphylococcus aureus: Blurring of the traditional definitions. J Glob Antimicrob Re. 2016;6:95-101.
12. Kateete DP, Kimani CN, Katabazi FA, Okeng A, Okee MS, Nanteza A, Joloba ML, Najjuka FC. Identification of Staphylococcus aureus: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Ann Clin Microbiol Antimicrob. 2010;9:23.
13. Petersson AC, Olsson-Liljequist B, Miorner H, Haeggman S. Evaluating the usefulness of spa typing, in comparison with pulsed-field gel electrophoresis, for epidemiological typing of methicillin-resistant Staphylococcus aureus in a low-prevalence region in Sweden 2000-2004. Clin Microbiol Infect. 2010;16(5):456-462.
14. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, twenty-ninth informational supplement; M100-S29. Wayne: Clin Lab Stand Institute; 2019.
15. Li SP, Sun J, Zhang JZ, Li XM, Tao XX, Wang LJ, Sun MJ, Liu YC, Li J, Qiao YH, et al. Comparative analysis of the virulence characteristics of epidemic methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from Chinese children: ST59 MRSA highly expresses core gene-encoded toxin. Apmis. 2014;122(2):101-114.
16. Gilot P, Lina G, Cochard T, Poutrel B. Analysis of the genetic variability of genes encoding the RNA III-activating components Agr and TRAP in a population of Staphylococcus aureus strains isolated from cows with mastitis. J Clin Microbiol. 2002;40(11):4060-4067.
17. Enright MC, Day NPJ, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol. 2000;38(3):1008-1015.
18. Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JA, Kreiswirth BN. spa Typing method for discriminating among Staphylococcus aureus isolates: Implications for use of a single marker to detect genetic micro- and macrovariation. J Clin Microbiol. 2004;42(2):792-799.
19. Milheirico C, Oliveira DC, de Lencastre H. Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome mec type IV in methicillin-resistant Staphylococcus aureus: 'SCCmec IV multiplex'. J Antimicrob Chemother. 2007;60(1):42-48.
20. Ote I, Taminiau B, Duprez JN, Dizier I, Mainil JG. Genotypic characterization by polymerase chain reaction of Staphylococcus aureus isolates associated with bovine mastitis. Vet Microbiol. 2011;153(3-4):285-292.
21. Pereyra EAL, Picech F, Renna MS, Baravalle C, Andreotti CS, Russi R, Calvinho LF, Diez C, Dallard BE. Detection of Staphylococcus aureus adhesion and biofilm-producing genes and their expression during internalization in bovine mammary epithelial cells. Vet Microbiol. 2016;183:69-77.
22. Tristan A, Ying L, Bes M, Etienne J, Vandenesch F, Lina G. Use of multiplex PCR to identify Staphylococcus aureus adhesins involved in human hematogenous infections. J Clin Microbiol.

2003;41(9):4465-4467.

23. Suryadevara M, Clark AE, Wolk DM, Carman A, Rosenbaum PF, Shaw J. Molecular Characterization of Invasive Staphylococcus aureus Infection in Central New York Children: Importance of Two Clonal Groups and Inconsistent Presence of Selected Virulence Determinants. J Pediatric Infect Dis Soc. 2013;2(1):30-39.
24. Yang Y, Hu Z, Shang W, Hu Q, Zhu J, Yang J, Peng H, Zhang X, Liu H, Cong Y, et al. Molecular and Phenotypic Characterization Revealed High Prevalence of Multidrug-Resistant Methicillin-Susceptible Staphylococcus aureus in Chongqing, Southwestern China. Microb Drug Resist. 2017;23(2):241-246.
25. Wu DJ, Li XM, Yang YH, Zheng YJ, Wang CQ, Deng L, Liu L, Li CC, Shang YX, Zhao CA, et al. Superantigen gene profiles and presence of exfoliative toxin genes in community-acquired meticillin-resistant Staphylococcus aureus isolated from Chinese children. J Med Microbiol. 2011;60(1):35-45.
26. Monecke S, Slickers P, Ehricht R. Assignment of Staphylococcus aureus isolates to clonal complexes based on microarray analysis and pattern recognition. FEMS Immunol Med Microbiol. 2008;53(2):237-251.
27. Robinson DA, Monk AB, Cooper JE, Feil EJ, Enright MC. Evolutionary genetics of the accessory gene regulator (agr) locus in Staphylococcus aureus. J Bacteriol. 2005;187(24):8312-8321.
28. Gu FF, Chen Y, Dong DP, Song Z, Guo XK, Ni YX, Han LZ. Molecular Epidemiology of Staphylococcus aureus among Patients with Skin and Soft Tissue Infections in Two Chinese Hospitals. Chin Med J (Engl). 2016;129(19):2319-2324.
29. van Leeuwen W, van Nieuwenhuizen W, Gijzen C, Verbrugh H, van Belkum A. Population studies of methicillin-resistant and -sensitive Staphylococcus aureus strains reveal a lack of variability in the agrD gene, encoding a staphylococcal autoinducer peptide. J Bacteriol. 2000;182(20):5721-5729.
30. Azmi K, Qrei W, Abdeen Z. Screening of genes encoding adhesion factors and biofilm production in methicillin resistant strains of Staphylococcus aureus isolated from Palestinian patients. Bmc Genomics. 2019;20(1):578.
31. Tahmasebi H, Dehbashi S, Arabestani MR. Association between the accessory gene regulator (agr) locus and the presence of superantigen genes in clinical isolates of methicillin-resistant Staphylococcus aureus. BMC Res Notes. 2019;12(1):130.
32. Chong YP, Kim ES, Park SJ, Park KH, Kim T, Kim MN, Kim SH, Lee SO, Choi SH, Woo JH, et al. Accessory gene regulator (agr) dysfunction in Staphylococcus aureus bloodstream isolates from South Korean patients. Antimicrob Agents Chemother. 2013;57(3):1509-1512.
33. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant Staphylococcus aureus (MRSA). Proc Natl Acad Sci U S A. 2002;99(11):7687-7692.
34. Wright JS, 3rd, Traber KE, Corrigan R, Benson SA, Musser JM, Novick RP. The agr radiation: an early event in the evolution of staphylococci. J Bacteriol. 2005;187(16):5585-5594.
35. Wang X, Shen Y, Huang W, Zhou Y. Characterisation of community-acquired Staphylococcus aureus causing skin and soft tissue infections in a children's hospital in Shanghai, China. Epidemiol Infect.

2019;147:e323.
36. Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2003;47(12):3926-3934.
37. Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M, Vandenesch F, Piemont Y, Brousse N, Floret D, et al. Association between Staphylococcus aureus strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet. 2002;359(9308):753-759.
38. Otto M. Community-associated MRSA: what makes them special? Int J Med Microbiol. 2013;303(6-7):324-330.
39. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME, et al. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis. 2003;9(8):978-984.
40. Montanaro L, Speziale P, Campoccia D, Pirini V, Ravaioli S, Cangini I, Visai L, Arciola CR. Polymorphisms of agr locus correspond to distinct genetic patterns of virulence in Staphylococcus aureus clinical isolates from orthopedic implant infections. J Biomed Mater Res A. 2010;94(3):825-832.
41. Serray B, Oufrid S, Hannaoui I, Bourjilate F, Soraa N, Mliji M, Sobh M, Hammoumi A, Timinouni M, El Azhari M. Genes encoding adhesion factors and biofilm formation in methicillin-resistant Staphylococcus aureus in Morocco. J Infect Dev Ctries. 2016;10(8):863-869.
42. Cucarella C, Tormo MA, Knecht E, Amorena B, Lasa I, Foster TJ, Penades JR. Expression of the biofilm-associated protein interferes with host protein receptors of Staphylococcus aureus and alters the infective process. Infect Immun. 2002;70(6):3180-3186.
43. Mottola C, Semedo-Lemsaddek T, Mendes JJ, Melo-Cristino J, Tavares L, Cavaco-Silva P, Oliveira M. Molecular typing, virulence traits and antimicrobial resistance of diabetic foot staphylococci. J Biomed Sci. 2016;23(1):33.
44. Monecke S, Ludicke C, Slickers P, Ehricht R. Molecular epidemiology of Staphylococcus aureus in asymptomatic carriers. Eur J Clin Microbiol Infect Dis. 2009, 28(9):1159-1165.
45. Vautor E, Magnone V, Rios G, Le Brigand K, Bergonier D, Lina G, Meugnier H, Barbry P, Thiery R, Pepin M. Genetic differences among Staphylococcus aureus isolates from dairy ruminant species: A single-dye DNA microarray approach. Vet Microbiol. 2009;133(1-2):105-114.
46. Zhang Y, Xu D, Shi L, Cai R, Li C, Yan H. Association Between agr Type, Virulence Factors, Biofilm Formation and Antibiotic Resistance of Staphylococcus aureus Isolates From Pork Production. Front Microbiol. 2018;9:1876.

Tables

Table 1 Patient demographics and characteristics of *S. aureus* isolates.

Characteristics	*S. aureus* isolates (n=191)
Age (years), median (range)	12.0 (2.0-72.0)
Gender (male), n (%)	102 (53.4)
Infection Characteristics	
CA, n (%)	79 (41.4)
MRSA, n (%)	120 (62.8)
Clinical sources	
Respiratory tract	94 (49.2)
Skin and soft tissue	62 (33.5)
Sterile sites	28 (13.6)
Other sources	7 (3.7)
Clinical characteristics*	
Hospital stay length (days), median (range)	11.0 (7.0-18.0)
PICU transfer, n (%)[&]	24 (12.6)
PICU stay length (days), median (range)	18.5 (6.5-28.0)
Intravenous antibiotics, n (%)	119 (100.0)
vancomycin, n (%)	24 (20.2)
linezolid, n (%)	29 (24.4)
Accessory gene regulator, *agr*, n (%)	
agr group I	162 (84.8)
agr group II	16 (8.4)
agr group III	9 (4.7)
agr group IV	4 (2.1)

*Only the clinical characteristics of 119 inpatients were available. [&]The PICU transfer rate of *agr* group III was significantly higher than that of other *agr* groups ($p = 0.009$). There were no statistical differences betweeen other variables among different *agr* groups ($p > 0.05$).

Table 2 Disease types distribution according to different *agr* groups.

Diease type*	Total (n=233)	*agr* group (n, %)				*P*-value[#]
		I (n=199)	II (n=18)	III (n=12)	IV (n=4)	
Respiratory tract infection						
Pneumonia	53 (22.7)	45 (22.6)	3 (16.7)	4 (33.3)	1 (25.0)	0.399
Necrotzing pneumonia	2 (0.9)	1 (0.5)	0	1 (8.3)	0	0.145
Neonatal pneumonia	9 (3.9)	9 (4.5)	0	0	0	0.250
Upper respiratory tract infection	8 (3.4)	7 (3.5)	1 (5.6)	0	0	0.493
Others	5 (2.1)	5 (2.5)	0	0	0	0.459
Subtotal	66 (28.3)	57 (28.6)	4 (22.2)	4 (33.3)	1 (25.0)	0.539
SSTIs						
Wound infection	26 (11.2)	22 (11.1)	4 (22.2)	0	0	0.352
Cellulitis	4 (1.7)	2 (1.0)	0	2 (16.7)	0	**0.046**
Neonatal omphalitis	6 (2.6)	6 (3.0)	0	0	0	0.394
Others	2 (0.9)	1 (0.5)	1 (5.6)	0	0	0.276
Subtotal	38 (16.3)	31 (15.6)	5 (27.8)	2 (16.7)	0	0.289
Invasive diseases						
Septicemia	22 (9.4)	19 (9.5)	2 (11.1)	1 (8.3)	0	0.463
Suppurative invasive infection	17 (7.3)	13 (6.5)	1 (5.6)	3 (25.0)	0	0.174
Bone and joint infection	9 (3.9)	9 (4.5)	0	0	0	0.250
Medical device-related infection	4 (1.7)	2 (1.0)	2 (11.1)	0	0	0.277
Subtotal	52 (22.3)	43 (21.6)	5 (27.8)	4 (33.3)	0	0.455
Exfoliative toxin-mediated diseases						
SSSS	1 (0.4)	0	0	0	1 (25.0)	**0.021**
Toxic epidermal necrolysis	2 (0.9)	2 (1.0)	0	0	0	0.731
Neonatal bullous impetigo	2 (0.9)	2 (1.0)	0	0	0	0.731
Subtotal	5 (2.1)	4 (2.0)	0	0	1 (25.0)	0.180
Asymptomatic carriers	46 (19.7)	40 (20.1)	3 (16.7)	1 (8.3)	2 (50.0)	0.500
Outpatients	26 (11.2)	24 (12.1)	1 (5.6)	1 (8.3)	0	0.239

*Clinical Diagnoses were not mutually exclusive. [#]Comparison between the distribution of each diagnosis and the overall total diagnoses according to *agr* groups. Note: significant *P*-values were highlighted in bold.

Table 3 Virulence genes distribution according to different *agr* groups.

	Total (n, %)	*agr* group (n, %)				*P*-value*
		I (n=162)	II (n=16)	III (n=9)	IV (n=4)	
Toxin genes						
hla	191 (100.0)	162 (100.0)	16 (100.0)	9 (100.0)	4 (100.0)	1.000
hlb	191 (100.0)	162 (100.0)	16 (100.0)	9 (100.0)	4 (100.0)	1.000
hld	191 (100.0)	162 (100.0)	16 (100.0)	9 (100.0)	4 (100.0)	1.000
eta	7 (3.7)	4 (2.5)	0	0	3 (75.0)	**0.003**
etb	2 (1.0)	0	0	0	2 (50.0)	**0.001**
tst	15 (7.9)	8 (4.9)	2 (12.5)	5 (55.6)	0	**0.006**
lukS/F-PV	60 (31.4)	59 (36.4)	0	1 (11.1)	0	**0.004**
MSCRAMM genes						
bbp	6 (3.1)	1 (0.6)	0	2 (22.2)	3 (75.0)	**< 0.001**
ebpS	38 (19.9)	12 (7.4)	13 (81.3)	9 (100.0)	4 (100.0)	**< 0.001**
cna	22 (11.5)	19 (11.7)	2 (12.5)	0	1 (25.0)	0.566
eno	191 (100.0)	162 (100.0)	16 (100.0)	9 (100.0)	4 (100.0)	1.000
clfA	191 (100.0)	162 (100.0)	16 (100.0)	9 (100.0)	4 (100.0)	1.000
clfB	191 (100.0)	162 (100.0)	16 (100.0)	9 (100.0)	4 (100.0)	1.000
fib	143 (74.9)	117 (72.2)	15 (93.8)	7 (77.8)	4 (100.0)	0.292
fnbB	32 (16.8)	30 (18.5)	2 (12.5)	0	0	0.068
Biofilm-forming genes						
icaA	191 (100.0)	162 (100.0)	16 (100.0)	9 (100.0)	4 (100.0)	1.000
icaC	179 (93.7)	150 (92.6)	16 (100.0)	9 (100.0)	4 (100.0)	0.486
icaD	191 (100.0)	162 (100.0)	16 (100.0)	9 (100.0)	4 (100.0)	1.000
bap	1 (0.5)	1 (0.6)	0	0	0	0.849

*Comparison between the distribution of each gene and the total isolates according to *agr* groups. Note: significant *P*-values were highlighted in bold.

Additional File Legends

Additional file 1 Molecular characteristics of 191 *S. aureus* stratified by *agr* types.

[a]including ST45[#]-t116-SCC*mec*IV (n=1); ST188[&]-t189-NT (n=1); ST5413-t324-SCC*mec*V (n=1); ST5413-t2431-SCC*mec*V (n=1); ST4845-t437-SCC*mec*IV (n=1). [b]including ST6*-t2467 (n=1); ST7-t796 (n=1); ST944-t364 (n=1); ST1419-t085 (n=1); ST5413-t148 (n=1); ST5435-t1485 (n=1); ST1492-t078 (n=1). (*CC5 strains mostly belonged to agr group II had one isolate which belonged to agr group I; [&]CC1 strains belonged to agr I, II and III groups; [#]CC45 strains belonged to both agr I and IV groups). NT, non-typable by the method used.

Additional file 2 Virulent genes distribution according to different clinical sources.

*Comparison between the distribution of each gene and the total isolates according to different clinical sources. N/A: not applicable.

Figures

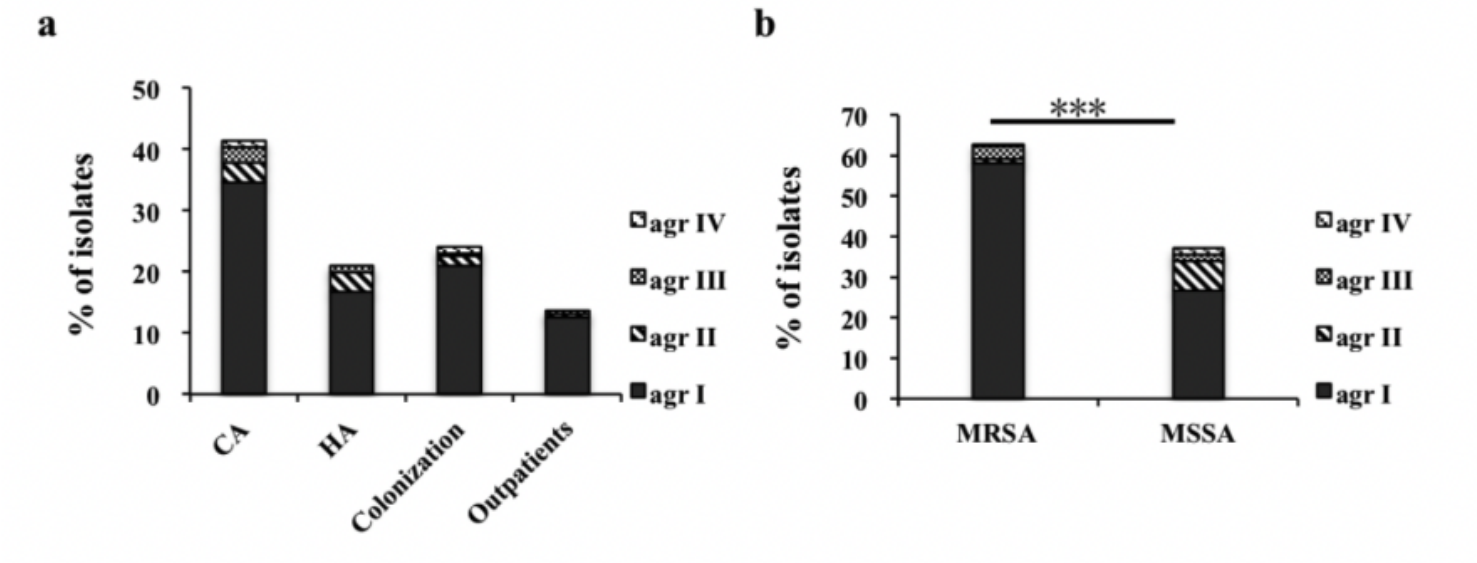

Figure 1

The distribution of strains from different sources and of MRSA and MSSA isolates among different agr groups. a There was no difference between S. aureus isolated from different clinical sources among different agr groups. b The number of MRSA isolates belonged to agr group I was significantly higher than that of MSSA, whereas the number of MRSA isolates belonged to agr group II was significantly lower than that of MSSA isolates (***$p < 0.001$).

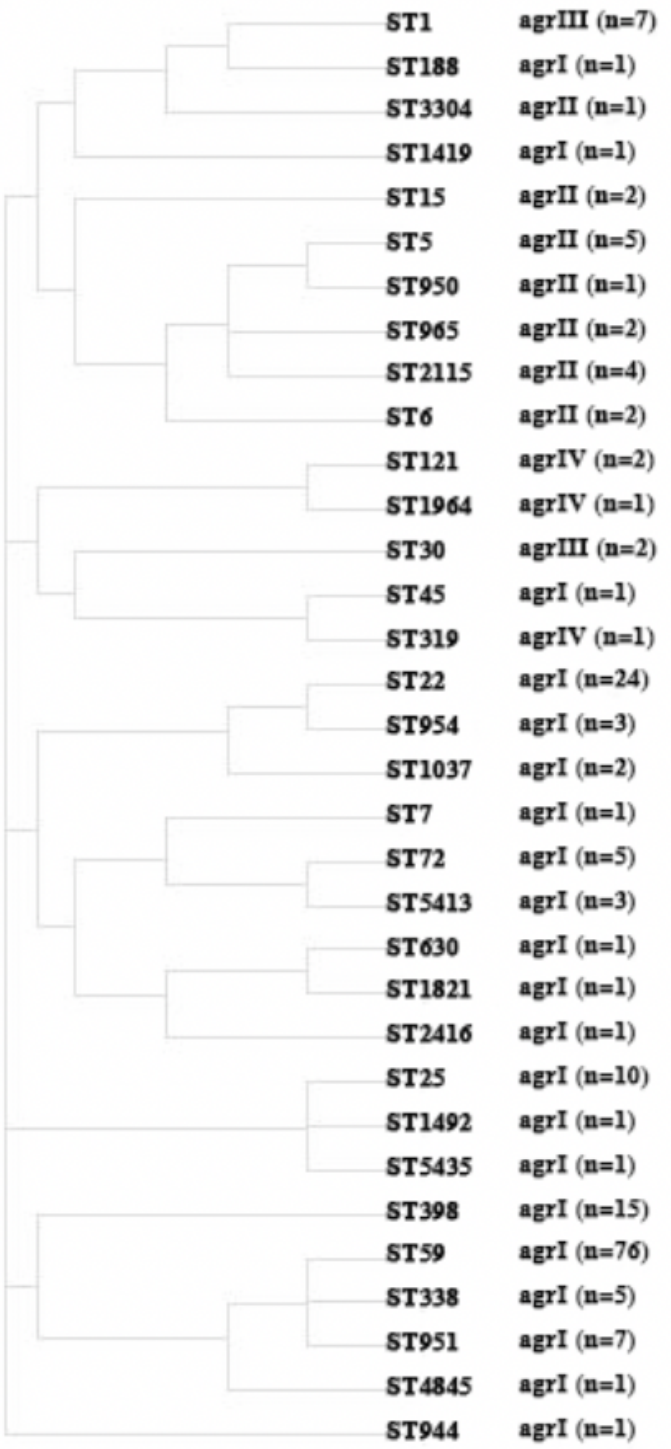

Figure 2

The evolution patterns of the 191 S. aureus strains (calculated by goeBURST hierarchical clustering analysis) and the STs distribution among different agr groups.

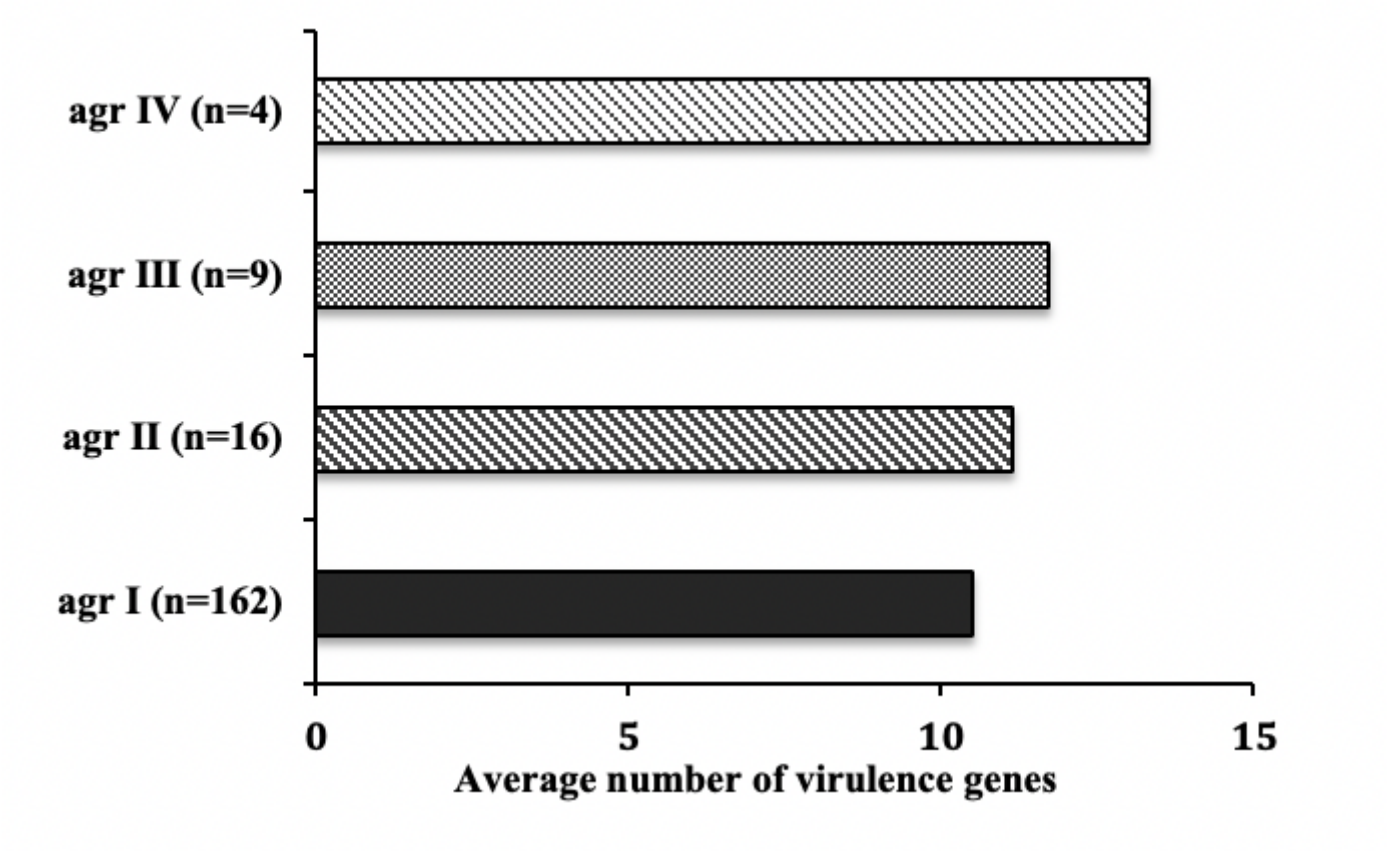

Figure 3

The virulence gene content of S. aureus isolates based on agr genotyping. The number in parentheses on the Y-axis represents the total number of isolates in the corresponding agr group.

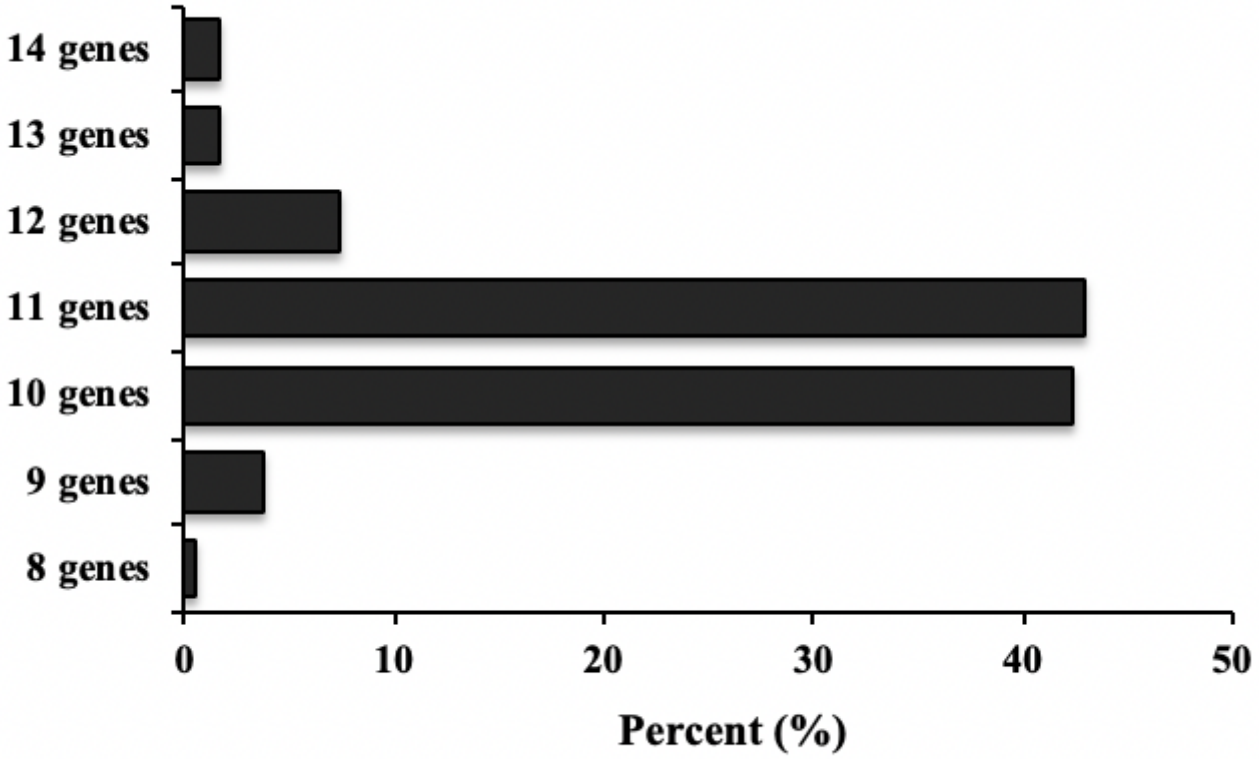

Figure 4

The percentage of S. aureus isolates with different coexisting studied virulence genes. The number on the Y-axis represents the total number of toxin genes harbored by each isolate.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- additionalfile2.doc
- Additionalfile1.doc