

# Prevalence, risk factors, phenotypic and molecular characteristics for methicillin-resistant *Staphylococcus aureus* carriage in community-based drug users in Guangzhou, China

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## Research

**Keywords:** *S. aureus*, MRSA, risk factor, antimicrobial susceptibility, drug user, molecular characteristics

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# Abstract

**Background:** *Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *Staphylococcus aureus* (MRSA) remained the predominant cause of infections in drug users. The cross-sectional study aimed to elucidate the prevalence, risk factors, phenotypic and molecular characteristics of *S. aureus* and MRSA carriage among community-based drug users.

**Methods:** Eligible drug users were asked to complete questionnaires and collect nasal swabs during May and December 2017 in Guangzhou, China. Swabs were processed for identification of *S. aureus* and MRSA. Antimicrobial susceptibility test and polymerase chain reaction assays were used to detect phenotypic and molecular characteristics for identified isolates. Univariate and multivariate logistic regression analyses were used to assess risk factors for *S. aureus* and MRSA carriage.

**Results:** Overall, the prevalence of *S. aureus* and MRSA carriage in 353 drug users were 15.01% and 6.79%, respectively. Cohabitation was a risk factor for *S. aureus* (adjusted OR=8.80, 95% CI: 1.89-40.99) and MRSA (adjusted OR=14.30, 95% CI: 2.67-76.46) carriage. The proportions of multidrug resistance were respectively 72.41% and 89.47% for *S. aureus* and MRSA isolates and were simultaneously resistant to penicillin, erythromycin and clindamycin. The results of clonal complexes and sequence types for *S. aureus* and MRSA isolates were diverse. The proportions of virulence genes were high for MRSA isolates.

**Conclusion:** The prevalence of *S. aureus* nasal carriage was lower while the prevalence of MRSA nasal carriage was moderate. Phenotypic and molecular characteristics of MRSA isolates revealed serious antibiotic resistance, indicating the cross-circulation of MRSA isolates, and imply high opportunity of virulence-related diseases. Decolonization might be considered for drug users with MRSA carriage, especially for those with risk factors.

## Background

*Staphylococcus aureus* (*S. aureus*) continues to be a major pathogen in both hospital- and community-associated infections [1]. Worldwide, the increasing resistance of *S. aureus* and methicillin-resistant *S. aureus* (MRSA) to various antibiotics complicates treatment of infections [2]. It has been reported that nasal carriers of *S. aureus* and MRSA have an increased risk of acquiring an infection with this pathogen [3].

Based on the latest World Drug Report, an estimated 271 million people aged 15–64 had used drugs in the previous year, while 35 million people are estimated to be suffering from drug use disorders in 2017 [4]. Obviously, illicit Drug use is a global public health problem. In recent studies, the prevalence of *S. aureus* and MRSA carriage among drug users is higher compared to the general population [5,6]. The phenotypic-molecular characteristics of *S. aureus* and MRSA carriage in drug users were little reported. Most of these studies were conducted in developed countries, including the United States of America, Canada, and European countries.

According to what we mentioned above, it is necessary to investigate the epidemiology of *S. aureus* and MRSA carriage among drug users, in China. Therefore, in this study, we aimed to elucidate the prevalence, risk factors, phenotypic and molecular characteristics for *S. aureus* and MRSA nasal carriage in community-based drug users in Guangzhou, China.

## Methods

### Ethics statement

The study was approved by the Ethics Committee of Guangdong Pharmaceutical University, and it was performed in accordance with the approved guidelines. Written informed consent were obtained from all participants.

### Study design and participants

A cross-sectional study of *S. aureus* and MRSA nasal carriage among drug users was conducted between May and December 2017 in three communities, Guangzhou, China. Participants had drug use in the previous 12 months were voluntarily recruited in the study. Those participants were with psychiatric illness or acute diseases were excluded. A face-to-face questionnaire was used to collect relevant information, including demographics (age, sex), socio-related characteristics (employed status, living conditions, income level, history of homelessness, and history of incarceration), behavior (history of sex and the number of sexual partner), health-related characteristics (human immunodeficiency virus (HIV) status, hepatitis, antibiotic use, skin infection, hospitalization, and history of needle exchange), and period and route of drug use.

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

After completing the questionnaire, trained personnel collected swabs from both anterior nares of participants. The swabs were soaked in enrichment broth at 4 °C during transportation, and incubated for 24 hours for further experiments. The swabs were then transferred to mannitol salt agar for 24-48 hours incubation. Samples were identified as *S. aureus* isolates when they had specific colony morphology and were positive for gram staining, catalase reaction, hemolysis test, DNase test, coagulase tests, and *16S rRNA* and *nuc* genes. Those *S. aureus* isolates that were resistant to cefoxitin and positive for *mecA* gene were identified as MRSA isolates. More details were described previously [7].

### Phenotypic characterization

The antimicrobial susceptibility of all *S. aureus* isolates was determined by the disk diffusion method. The following antibiotics were tested: clindamycin, erythromycin, penicillin, linezolid, gentamicin, teicoplanin, moxifloxacin, trimethoprim-sulfamethoxazole, rifampin, chloramphenicol, and tetracycline. The reference *S. aureus* strain ATCC 25923 and ATCC 29213 were respectively used for quality and positive control. We classified the isolates as susceptible or resistant to each antibiotic. Those isolates resistant to  $\geq 3$  classes of antibiotics were identified as multidrug resistant (MDR) [8]. All *S. aureus* isolates were also tested for the carriage of tetracycline-resistant genes [*tet*(M), *tet*(K)] and erythromycin-resistant genes [*erm*(A), *erm*(C)]. More details were described previously [7].

## Molecular characterization

All *S. aureus* isolates were further tested to confirm the presence of toxic gene including Panton-Valentine leukocidin genes (*pvl*), Toxic shock syndrome toxin-1 gene (*tst*), Exfoliative toxin A gene (*eta*), Exfoliative toxin B gene (*etb*) and Staphylococcal enterotoxins (SEs) (*sea-see*, *seg-ser*, *seu*) genes. Multilocus sequence typing (MLST) was performed to confirm clonal complexes (CCs) and sequence types (STs). Additionally, all MRSA isolates were tested for Staphylococcal cassette chromosome *mec* (SCC*mec*) typing. More details were described previously [7].

## Statistical analysis

The data were entered using Epidata 3.1 (EpiData Association, Odense Denmark) and exported to Stata 14.2 (College Station, Texas, USA) software for further analysis. We assessed the associations between non-*S. aureus* and *S. aureus* /MRSA carriers by the following methods. Univariate analyses were used by Pearson's chi-squared test or Fisher's exact test when appropriate. Multivariate logistic regression models were then used to assess the adjusted odds ratios (aORs) and 95% confidence intervals (CIs). Potential confounding covariates were adjusted in the models. A two-sided *P*-value of  $\leq 0.05$  was defined as statistical significance.

# Results

## Prevalence of *S. aureus* and MRSA carriage

A total of 353 drug users were eligible for inclusion in the study. The prevalence of *S. aureus* nasal carriage in drug users was 15.01% (53/353). Twenty-nine drug users carried with MDR *S. aureus* and the prevalence was 8.22%. The prevalence of MRSA carriage was 6.79% (24/353). Seventeen drug users were with MDR MRSA carriage and the prevalence was 4.82%.

## Risk factors of *S. aureus* and MRSA carriage

Table 1 showed univariate analyses of *S. aureus* and MRSA carriage among drug users. Drug users with current cohabitation (Fisher's exact test,  $P=0.011$ ) and had history of heroin snorting in previous 3 months were more likely to be with *S. aureus* carriage ( $\chi^2=4.266$ ,  $P=0.039$ ). For MRSA carriage, current cohabitation status (Fisher's exact test,  $P=0.006$ ), income level ( $\chi^2=3.867$ ,  $P=0.49$ ), and history of hospitalization in previous 1 year (Fisher's exact test,  $P=0.047$ ) were significant factors.

After adjusting for confounding covariables, cohabitation was still a risk factor for *S. aureus* carriage (aOR=8.80, 95% CI: 1.89-40.99) and MRSA carriage (aOR=14.30, 95% CI: 2.67-76.46) in drug users (Figure 1). Drug users who were cohabiting with others were more likely to be with nasal carriage of *S. aureus* and MRSA.

## Phenotypic characteristics

The antibiotic susceptibility testing results revealed that most of *S. aureus* isolates were susceptible to linezolid, rifampin and gentamicin, but resistant to penicillin (92.45%), erythromycin (49.06%), clindamycin (45.28%) and tetracycline (32.08%) (Table 2). MRSA isolates were with higher proportions of antibiotic resistance than *S. aureus* isolates. Notably, 72.41% of MDR *S. aureus* and 89.47% of MDR MRSA isolates were simultaneously resistant to penicillin, erythromycin and clindamycin (Figure 2).

In terms of macrolide-resistant genes, five (9.43%) *S. aureus* isolates were positive to the *erm(C)* and one (1.89%) was positive to the *erm(A)* gene. Only one *S. aureus* isolate was simultaneously positive to both the *erm(C)* and *erm(A)* genes. For tetracycline-resistant genes, four (7.55%) *S. aureus* isolates were positive to the *tet(K)* and no isolate was positive to *tet(M)* gene. Additionally, only one *S. aureus* isolate was simultaneously positive to *erm(C)*, *erm(A)* and *tet(K)* genes. These gene-positive *S. aureus* isolates were all MRSA isolates.

## Molecular characteristics

Overall, eight CCs and 18 STs were detected from 53 *S. aureus* isolates (Figure 2). Three of the most predominant CCs were CC5 (34), CC59 (6), and CC7 (4). Three of the most predominant STs were ST188 (11), ST5 (6), and ST59 (6). Seven CCs and 13 STs were detected from 24 MRSA isolates (Figure 2). Two of the most predominant CCs were CC5 (13) and CC59 (6). Two of the most predominant STs were ST188 (6) and ST59 (6).

In terms of virulence genes (Table 2), 5.66% of *S. aureus* isolates and 12.50% of MRSA isolates were positive to *pvl* genes. Two MRSA isolates were positive to the *tst* gene. Notably, only one MRSA isolate was positive to the *eta* gene and one to the *etb* gene. For the SEs genes, 26 (49.06%) *S. aureus* isolates

were positive to the *seg*, 18 (34.96%) to the *sei*, 17 (32.08%) to the *sad*, 16 (30.19%) to the *sem*, 13 (24.53%) to the *seo*, 12 (22.64%) to the *sen*, 12 (22.64%) to the *seu*, 11 (20.75%) to the *sek*, 6 (11.32%) to the *sej*, 5 (9.43%) to the *seq*, 5 (9.43%) to the *ser*, 4 (7.55%) to the *sel*, 4 (7.55%) to the *sep*, 3 (3.45%) to the *sec*, 3 (3.45%) to the *seh*, and 1 (1.89%) to the *seb* gene. All *S. aureus* isolates were negative to the *sea* and *see* genes. The proportions of positive SEs genes were mostly higher in MRSA than *S. aureus* isolates.

A total of four SCC*mec* types were detected from the 24 MRSA isolates, in which 12 isolates were type IVd, five were type IVa, one was type V, one was type II, and five were non-typeable (Figure 2).

## Discussion

To our knowledge, this is a relatively comprehensive study which contributes to the prevalence, risk factors, and phenotypic-molecular characteristics for *S. aureus* and MRSA nasal carriage among community-based drug users in China. The prevalence of *S. aureus* carriage in the study (15.01%) is lower than previously reported estimates ranged from 19.79% to 45.05% [5,9–12]. Participants of those previous studies were injection drug users. In the study, however, only 64.02% of participants had history of using injection drugs in previous 3 months. Additionally, we found that a majority of long-term drug users who took drugs by snorting had few vibrissae. This might also be a potential factor leading to a low prevalence of *S. aureus* carriage. Further studies need to explore in the future. The prevalence of MRSA nasal carriage (6.79%) in the study is similar to observed studies in other countries [5,13–15]. Notably, the proportion of MRSA in *S. aureus* isolates were higher than observed studies [5,15].

In the present study, we found that cohabitation was a risk factor for *S. aureus* carriage and MRSA carriage in drug users, which is different from other study [5]. One of the possible reasons might be that most drug users cohabitated with other drug users. This could provide more opportunities for sharing drugs [12]. HIV infection has been reported to be a risk factor for *S. aureus* carriage [16], however, we did not find any significance. This could be caused by the limited number of drug users with HIV infection. Therefore, further studies need to identify the risk factors for *S. aureus* carriage and MRSA carriage in drug users.

The proportions of antibiotic resistance on *S. aureus* and MRSA isolates are consistent to limited available studies [9,17,18]. However, the proportion of MDR MRSA (70.83%) is high. The most predominant MDR pattern could partially demonstrate the high use of antibiotics in community-based drug users and provide evidence that healthcare workers need to be more careful with selection of antibiotics for drug users. The high proportions of erythromycin and tetracycline resistance and low proportions of erythromycin- and tetracycline-resistance genes could demonstrate that resistant genes are always attributed to the presence of antibiotic resistance.

The proportions of virulence genes were higher in MRSA than in *S. aureus* isolates, suggesting the higher risks of MRSA isolates in causing virulence-related diseases, including Staphylococcal scalded skin syndrome, toxic shock syndrome, Staphylococcal food poisoning, etc. [19–21]. The proportions of

virulence genes for MRSA isolates were higher than observed studies [7,22–24]. The results implied that drug users with MRSA carriage harbouring virulence associated genes, might have higher risks for relevant disease and should be paid greater attention to.

The results of CCs and STs for *S. aureus* and MRSA isolates could demonstrate the multiple transmissions among human beings, livestock and environment, which are similar to previous studies [7,22]. Although we included community-based drug users in the study, according to the results of SCC*mec* types we could know the source of MRSA isolates were from both communities and healthcare settings, which is similar to observed studies [5,10]. MRSA isolates could circulate between communities and healthcare settings and this might be a potential risk for other populations. Therefore, relevant decolonization methods could be used for drug users with MRSA carriage, which would help prevent further MRSA circulation.

Our study contributes to the prevalence, risk factors, phenotypic and molecular characteristics for *S. aureus* and MRSA carriage among drug users in China. Despite of the strengths, there are several limitations. Firstly, it was a cross-sectional study, thus, we could not determine the persistence of *S. aureus* and MRSA carriage. Secondly, we only obtained cultures from the anterior nares, which may lead to an underestimation of the prevalence of *S. aureus* and MRSA carriage. Finally, the generality of this study is limited owing to the small number of drug users.

## Conclusion

In summary, the prevalence of *S. aureus* nasal carriage was lower while the prevalence of MRSA nasal carriage was moderate among community-based drug users, China. Cohabitation is a risk factor for *S. aureus* and MRSA carriage. Phenotypic-molecular characteristics of MRSA isolates reveal serious antibiotic resistance, indicate the cross-circulation of MRSA isolates between communities and healthcare settings, and imply high opportunity of virulence-related diseases. Decolonization might be considered for drug users with MRSA carriage, especially for those with risk factors.

## Abbreviations

*S. aureus*: *Staphylococcus aureus*; MRSA: methicillin-resistant *S. aureus*; HIV: human immunodeficiency virus; MDR: multidrug resistance; SE: Staphylococcal enterotoxin; MLST: multilocus sequence typing; CC: clonal complex; ST: sequence type; SCC*mec*: Staphylococcal cassette chromosome *mec*; aOR: adjusted odds ratio; CI: confidence interval; P: penicillin; E: erythromycin; DA: clindamycin; TE: tetracycline; C: chloramphenicol; SXT: trimethoprim-sulfamethoxazole; TEC: teicoplanin; MXF: moxifloxacin; CN: gentamicin; RD: rifampin; LZD: linezolid.

## Declarations

## Acknowledgements

Not applicable.

### **Authors' contributions**

WY and JL performed the experiments, participated in data analysis and contributed to manuscript writing. JZ collected information, performed the experiments, and analyzed the results. ZY and ZH designed the study and critically reviewed the manuscript. All authors revised the manuscript and approved the final form.

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### **Availability of data and materials**

The data supporting the conclusions of this manuscript will be made available by the corresponding authors to any qualified researcher.

### **Ethics approval and consent to participate**

The study was approved by the Ethics Committee of Guangdong Pharmaceutical University, and it was performed in accordance with the approved guidelines. Written informed consent were obtained from all participants.

### **Consent for publication**

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.



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## Tables

**Table 1** Univariate analysis of risk factor for *S. aureus* and MRSA carriage among drug users in Guangzhou, China, 2017.

Characteristics	Non- <i>S. aureus</i> carriage (N=300)	<i>S. aureus</i> carriage (N=53)		MRSA carriage (N=24)	
		<i>n</i> (%)	<i>P</i> - value*	<i>n</i> (%)	<i>P</i> - value†
Demographics-level					
Sex (Male)	262 (87.33)	47 (88.68)	0.784	21 (87.50)	1.000
age (>50)	169 (56.33)	26 (49.06)	0.326	16 (66.67)	0.325
Social-level					
Current employed (Yes)	80 (26.67)	14 (26.42)	0.970	6 (25.00)	0.859
Current cohabitataion (Yes)	3 (1.00)	4 (7.55)	0.011	3 (12.50)	0.006
Low income (Yes)	61 (20.33)	17 (32.08)	0.058	9 (37.50)	0.049
History of homelessness in previous 6 months (Yes)	9 (3.00)	3 (5.66)	0.400	0 (0.00)	1.000
History of incarceration (Yes)	240 (80.00)	44 (83.02)	0.609	22 (91.67)	0.277
Behavior-level					
History of vaginal sex in previous 1 month (Yes)	97 (33.68)	13 (26.53)	0.324	7 (31.82)	0.858
Number of sexual partners in previous 1 year (>1)	28 (9.33)	2 (3.77)	0.283	0 (0.00)	0.246
Health-level					
Current HIV positive (Yes)	22 (7.33)	2 (3.77)	0.553	1 (4.17)	1.000
Current hepatitis (Yes)	114 (38.00)	24 (45.28)	0.316	12 (50.00)	0.246
Current antibiotics use (Yes)	83 (27.67)	16 (30.19)	0.706	10 (41.67)	0.145
History of hospitalization in previous 1 year (Yes)	32 (10.67)	9 (16.98)	0.186	6 (25.00)	0.047
History of skin infection in previous 6 months (Yes)	130 (43.33)	25 (47.17)	0.604	15 (62.50)	0.069
History of needle exchange in previous 1 year (Yes)	26 (8.67)	4 (7.55)	1.000	0 (0.00)	0.238
Drug use-level					
Period of drug use (>10 years)	18 (6.00)	0 (0.00)	0.087	0 (0.00)	0.380
History of heroin snorting in previous 3 months (Yes)	188 (62.67)	41 (77.36)	0.039	18 (75.00)	0.227
History of intravenous heroin in previous 3 months (Yes)	113 (37.67)	14 (26.42)	0.116	6 (25.00)	0.215
History of using injection drugs in previous 3 months (Yes)	187 (62.33)	39 (73.58)	0.116	18 (75.00)	0.215

*S. aureus*, *Staphylococcus aureus*; MRSA, Methicillin-resistant *S. aureus*; N, Number of total participants; HIV, Human immunodeficiency virus.

\* Comparison of characteristic in *S. aureus* carriage versus non-*S. aureus* carriage;

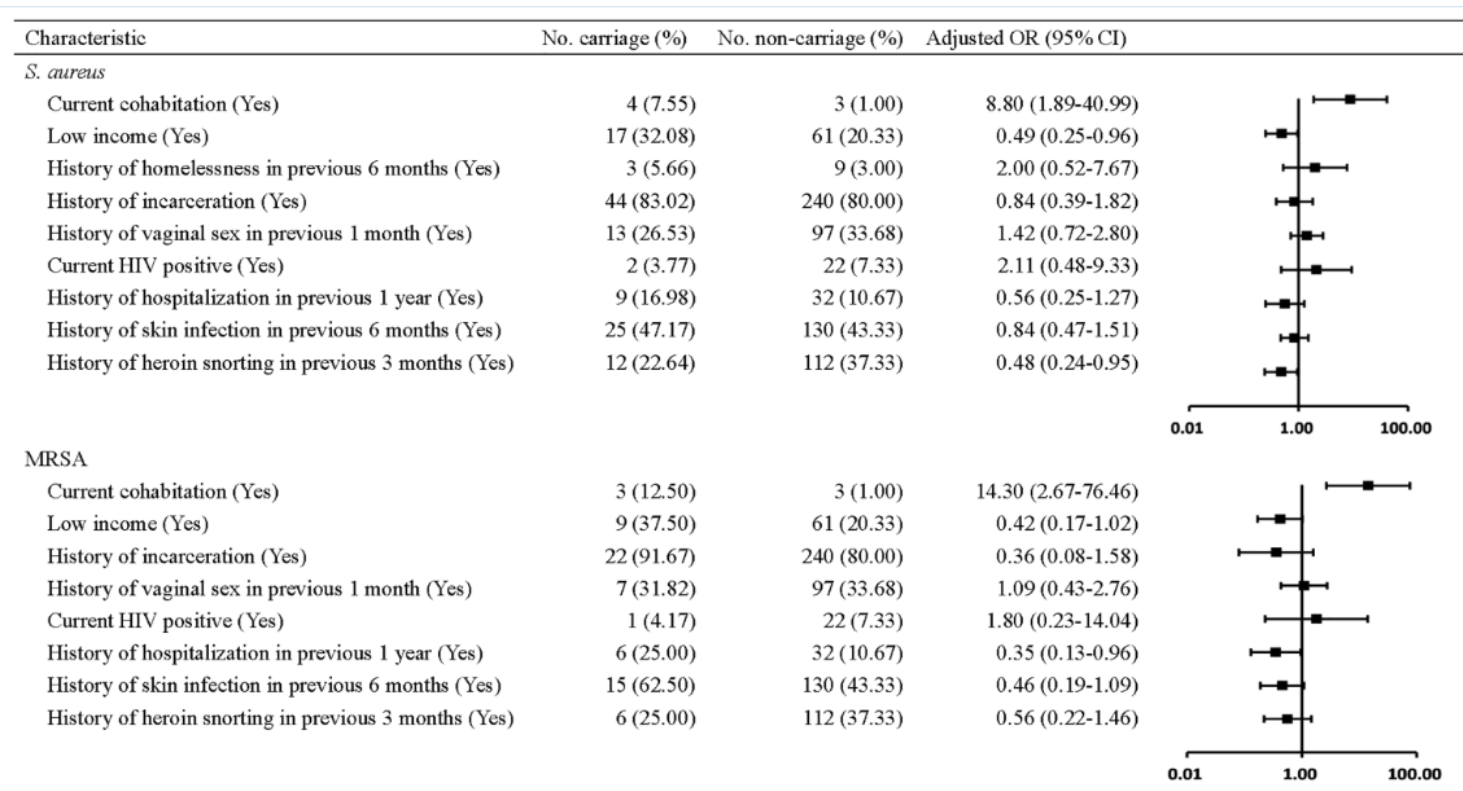
‡ Comparison of characteristic in MRSA carriage versus non-*S. aureus* carriage.

**Table 2** Phenotypic and molecular characteristics of *S. aureus* isolates among drug users in Guangzhou, China, 2017.

Characteristics	<i>S. aureus</i> (N=53)	MRSA(N=24)
Resistant phenotype (resistant)		
Clindamycin	24 (45.28)	15 (62.50)
Erythromycin	26 (49.06)	15 (62.50)
Penicillin	49 (92.45)	23 (95.83)
Linezolid	1 (1.89)	1 (4.17)
Gentamicin	4 (7.55)	3 (12.50)
Teicoplanin	10 (18.87)	8 (33.33)
Trimethoprim-sulfamethoxazole	7 (13.21)	4 (16.67)
Moxifloxacin	5 (9.43)	4 (16.67)
Rifampin	2 (3.77)	2 (8.33)
Chloramphenicol	11 (20.75)	7 (29.17)
Tetracycline	17 (32.08)	9 (37.50)
Resistant genotype (positive)		
<i>erm</i> (A)	1 (1.89)	1 (4.17)
<i>erm</i> (C)	5 (9.43)	5 (20.83)
<i>tet</i> (K)	4 (7.55)	4 (16.67)
<i>tet</i> (M)	0 (0.00)	0 (0.00)
Virulence genes (positive)		
<i>pvl</i>	3 (5.66)	3 (12.50)
<i>tst</i>	2 (3.77)	2 (8.33)
<i>eta</i>	1 (1.89)	1 (4.17)
<i>etb</i>	1 (1.89)	1 (4.17)
<i>sea</i>	0 (0.00)	0 (0.00)
<i>seb</i>	1 (1.89)	0 (0.00)
<i>sec</i>	3 (5.66)	1 (4.17)
<i>sed</i>	17 (32.08)	11 (45.83)
<i>see</i>	0 (0.00)	0 (0.00)
<i>seg</i>	26 (49.06)	13 (54.17)
<i>seh</i>	3 (3.45)	3 (12.50)
<i>sei</i>	18 (33.96)	9 (37.50)
<i>sej</i>	6 (11.32)	3 (12.50)
<i>sek</i>	11 (20.75)	8 (33.33)
<i>sel</i>	4 (7.55)	2 (8.33)
<i>sem</i>	16 (30.19)	6 (25.00)
<i>sen</i>	12 (22.64)	4 (16.67)
<i>seo</i>	13 (24.53)	4 (16.67)
<i>sep</i>	4 (7.55)	1 (4.17)
<i>seq</i>	5 (9.43)	2 (8.33)
<i>ser</i>	5 (9.43)	2 (8.33)
<i>seu</i>	12 (22.64)	7 (29.17)

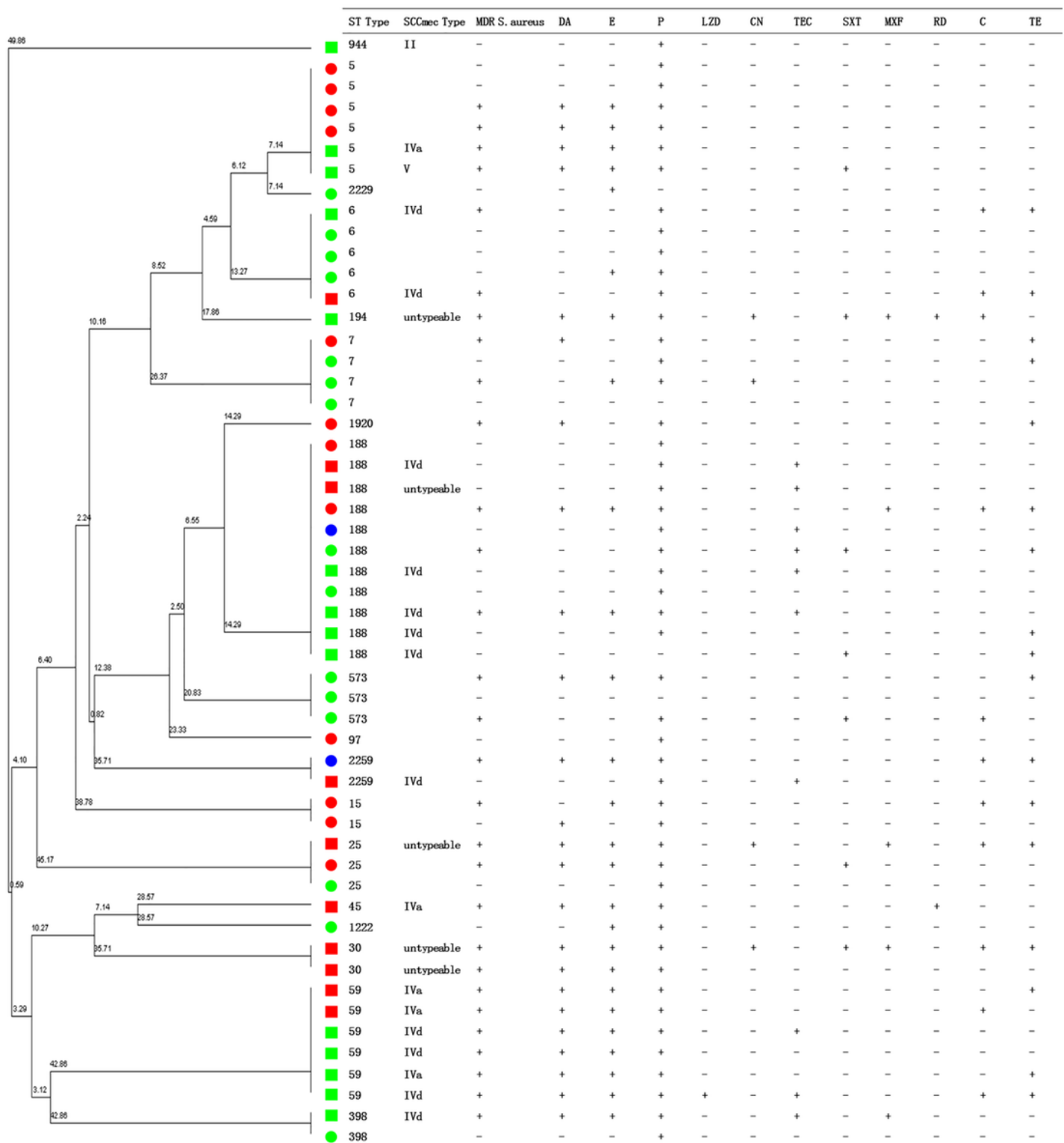
*S. aureus*, *Staphylococcus aureus*; MRSA, Methicillin-resistant *S. aureus*; MDR, Multidrug resistant.

## Figures



**Figure 1**

Multivariate analysis of risk factors for *S. aureus* and MRSA carriage among community-based drug users in Guangzhou, China, 2017. *S. aureus*, *Staphylococcus aureus*; MRSA, Methicillin-resistant *S. aureus*; No., Number of; OR, Odds ratio; CI, Confidence interval; HIV, Human immunodeficiency virus.



**Figure 2**

Clonal dendrogram and detailed information of *S. aureus* isolates for community-based drug users in Guangzhou, China, 2017. Same color represented isolates were from the same community; squares represented MRSA isolates and circles represented non-MRSA isolates. *S. aureus*, Staphylococcus aureus; MRSA, Methicillin-resistant *S. aureus*; ST, Sequence type; SCCmec, Staphylococcal cassette chromosome mec; MDR, Multidrug resistance; P, Penicillin; E, Erythromycin; DA, Clindamycin; TE, Tetracycline; C,

Chloramphenicol; SXT, Trimethoprim-sulfamethoxazole; TEC, Teicoplanin; MXF, Moxifloxacin; CN, Gentamicin; RD, Rifampin; LZD, Linezolid.