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RESEARCH ARTICLE

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Characteristics of *Staphylococcus aureus* small colony variants isolated from wound specimen of a tertiary care hospital in China

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Abstract

Background: Small colony variants (SCVs) of *Staphylococcus aureus* (*S. aureus*) frequently lead to chronic and recurrent infections, but they are always ignored and there are few researches on their clinical isolates. We intended to investigate the prevalence and characteristics of *S. aureus* SCVs.

Methods: None-duplicated *S. aureus* strains isolated from wound samples were collected from January 2018 to December 2020. The characteristics (i.e. colony morphology, growth rate, coagulase, biofilm formation, and pathogenic characteristics), antimicrobial susceptibilities, and resistance mechanisms of SCVs were also investigated. The genetic background of SCVs was analyzed through staphylococcal protein A (SPA) typing, sequence typing, and pulse field gel electrophoresis (PFGE).

Results: Three SCVs were screened from 278 *S. aureus* strains (1.1%). They formed pinpoint white colonies on blood agar plates with weak hemolysis. The reproduction speed in liquid medium was very slow for SCVs strains. The coagulase weakened or disappeared, and the ability to form biofilm varied greatly. Only slight inflammation was triggered when wound infected. The SPA typing was t2592, t233, and t023, and the sequence typing was ST88, ST239, and ST965, respectively. The PFGE revealed three SCVs were singletons.

Conclusions: The rate of SCVs in wound sample is low in our hospital, and the formation is associated with the usage of antimicrobial. SCVs grow slowly, and their colony morphology and biochemical characteristics are significantly different from classic *S. aureus*. SCVs may cause chronic infection and weak inflammation. SCVs form in resistant or susceptible strains, and there is no clonal epidemic in this hospital.

KEYWORDS
genetic background, growth characteristics, pathogenic characteristics, small colony variants, *Staphylococcus aureus*

1 | INTRODUCTION

Small colony variants (SCVs) are subpopulation of bacterium which exhibit slow growth rate, abnormal colony morphology, and different pathogenic characteristics, compared with wild-type strains. There are many kinds of bacteria that can form SCVs, such as *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa*, *and Enterococcus*.[1] Among them, *S. aureus* is the most common pathogen causing skin and soft tissue infections and may lead to severe sepsis or even septic shock.[2]

Different from wild-type *S. aureus*, SCVs may exhibit auxotrophy for thymidine, menadione, or hemin, so the nucleic acid synthesis and oxidation of the respiratory chain are blocked.[1,3,4] As a result, their reproduction and metabolism rates get slower significantly and the resistance to aminoglycosides, sulfamethoxazole-trimethoprim (SXT), and antibiotics acting against cell wall increases.[5,6] Meanwhile, both methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) can form SCVs, and the MRSA-SCVs lead to the worse condition.[7] These characteristics of SCVs have brought great challenges to anti-infection treatment. However, the treatment of antimicrobial contributes to the formation of SCVs. The application of aminoglycosides has been reported to relate to menadione- and hemin-auxotrophic SCVs, and SXT treatment is associated with thymidine-auxotrophic SCVs.[8,9] Fatty acid and CO_2-dependent SCVs also have been reported while the mechanisms are still unambiguous.[10,11] Moreover, the virulence of SCVs is weakened so they can survive intracellularly without inducing an immune response. Therefore, SCVs often cause persistent or recurrent infections[12,13] and are closely related to chronic infection, including osteomyelitis, endocarditis, wound infection, and pulmonary cystic fibrosis infection.[14–16]

As a result, the identification of SCVs is important for effective treatment. However, SCVs are often ignored in clinical microbiology laboratory since the slow growth rate and abnormal biochemical reaction.[17] In addition, some SCVs could revert to wild type after being cultured on solid medium for several generations or even several hours.[1] Meanwhile, there is no laboratory standard to define SCVs.

Due to the high instability of SCVs, most current studies are concerned with laboratory-induced mutants rather than naturally derived SCVs.[4,18] There are few researches on SCVs among *S. aureus* clinical isolates, especially the prevalence and characteristics of SCVs isolated from wound samples are relatively lacking. Therefore, in our study, we intended to investigate the prevalence, characteristics, antimicrobial susceptibility, resistance mechanisms, and genetic background of SCVs among *S. aureus* from wound samples.

2 | MATERIALS AND METHODS

2.1 | Bacteria source and growth characteristics

Non-duplicated *S. aureus* strains isolated from wound samples were collected from Xiangya Hospital, Central South University, China from January 2018 to December 2020. All strains were identified by Microflex™ MALDI-TOF MS system (Bruker Daltonik, Bremen, Germany). The strains were cultured on the blood agar plate (Beiruite Biotechnology, Zhengzhou, China) for 24 to 72 h. According to the colony morphology and growth rate, the strains were identified as SCVs or wild phenotype.

The growth curve for SCVs in Luria-Bertani (LB) broth was recorded by measuring the optical density (OD) at 600 nm at different time points.

2.2 | Clinical manifestations and laboratory data of patients

The patients' clinical information was collected from the medical record system, including the medication history, clinical manifestations, and laboratory test data, such as white blood cells (WBC), neutrophils, C-reactive protein (CRP), and procalcitonin (PCT).

2.3 | Coagulase test

The plasma was diluted with sterile normal saline (1:4, v/v); then, 0.5 ml dilution was added to the tube. A single colony was grinded to the plasma evenly and incubated at 37°C for 16 h. The result was identified as positive if the contents were completely solidified. *S. aureus* ATCC25923 and *S. epidermidis* ATCC57625 were used as positive and negative control, respectively.

2.4 | Ability for biofilm formation

According to previous literature,[19] the crystal violet staining method was used to detect the biofilm-forming ability of SCVs. *S. aureus* ATCC29213 and *S. epidermidis* ATCC35984 were used as controls, and *S. epidermidis* ATCC35984 was strong biofilm-forming strain. Briefly, fresh colony was inoculated in trypticase soy broth (TSB) supplemented with 0.25% glucose (TSBG) at 200 rpm 37°C for 16–18 h. The culture density was adjusted to 0.5 McFarland and diluted 1:100 (v/v) in TSBG; then, 200 μl of each diluted culture was distributed in five wells of 96-well plate and incubated for 48 h at 37°C. Negative control was included. After that, the supernatant was discarded and the wells were washed with sterile phosphate-buffered saline for three times. Then, the wells were stained with 200 μl of 0.1% (w/v) crystal violet for 20 min and rinsed with running water. The dye attached to the wells was dissolved with 200 μl of 95% (v/v) ethanol, and the OD570nm was measured.

2.5 | Antimicrobial susceptibility testing

The antimicrobial susceptibility was accomplished by VITEK-2 Compact system (bioMérieux, Marcy L'Etoile, France). In addition, the susceptibility of SCVs to some antibiotics (ie, penicillin, cefoxitin, ciprofloxacin, tigecycline, etc.) was measured by disk diffusion

method following the Clinical and Laboratory Standards Institute (CLSI) recommendations.[20] As SCVs grew slowly on the Müeller-Hinton broth (Oxoid, unipath, UK) plate, the results were recorded at 24, 48, and 72 h. Moreover, the minimum inhibitory concentration (MIC) to vancomycin and oxacillin was also detected by broth microdilution method according to the CLSI guidelines.[20] *S. aureus* ATCC25923 and ATCC29213 severed as quality control for disk diffusion and broth microdilution method, respectively.

2.6 | Resistance mechanisms of penicillin and oxacillin

The genome of SCVs was extracted by DNA genome extraction kit (TianGen Biotech Co., Ltd, Beijing, China). The *MecA*, *MecC*, and *MecA subtype f2* were detected by polymerase chain reaction (PCR) according to previous reports.[21,22] Nitrocefin test and PCR were used to detect β-lactamase and gene *blaZ*, respectively.[23]

2.7 | Bacteria homology analysis

Pulse field gel electrophoresis (PFGE) was utilized to analyze the genomic relationship of SCVs according to previous study.[24] In short, the genomic DNA was treated with SmaI restriction enzyme for 12 h and separated with 1% agarose gel under the condition of 12°C and 6.0 V/cm, and the pulses were alternated with time gradient of 0.5-70 s at an angle of 120° for 21 h. Strains were categorized into the same PFGE group if they possessed ≥80% genetic similarity. *Salmonella enterica* H9812 was used as the size marker.

Additionally, multilocus sequence typing (MLST) was carried to analyze ST type of SCVs. Seven house-keeping genes of *S. aureus* (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) were amplified and sequenced. The ST type and their homology were analyzed according to protocol of Pasteur website (http://bigsdb.web.pasteur.fr).

As described in standard documents, the staphylococcal protein A (SPA) gene was amplified and sequenced. The sequence of *spa* was compared with the SPA database (http://spatyper.fortinbras.us/) to determine the SPA type of SCVs.

2.8 | Statistical analysis

Wilcoxon-Mann-Whitney test was used for qualitative variables, and $p < 0.05$ was regarded as statistically significant (SPSS 22.0, SPSS Inc., Chicago, IL, USA). The images were edited by GraphPad PRISM v 5.0.3.477.

2.9 | Ethics Statement

This study did not exert any influence on the patients. In accordance with Ethics Committee of Central South University (Changsha, Hunan Province, China), written informed patient consent was not required for any part of the study.

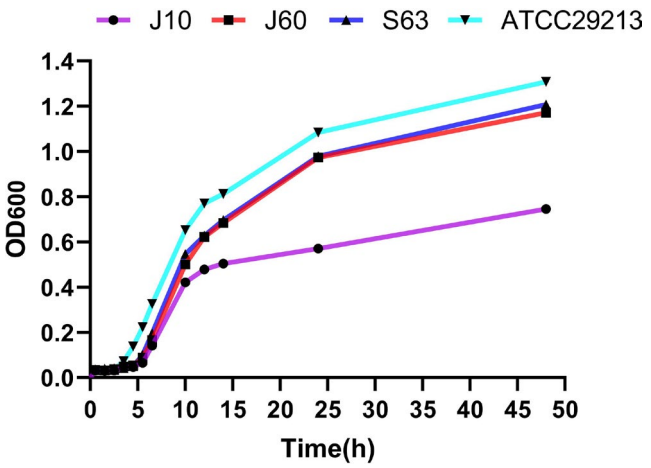

FIGURE 1 Growth curves of three SCVs and control ATCC29213

3 | RESULTS

3.1 | Growth characteristics

Three SCVs were screened from 278 non-duplicated *S. aureus* strains (1.1%), named J10, J60, and S63, respectively. After cultured on blood plate for 24 h, the three SCVs formed pinpoint round white colonies with complete bitty hemolytic ring (Figures S1 and S2). The colonies of J10 adhered to the plate extremely tightly. At 48 h, the colonies developed at least five times bigger and the hemolysis also became obvious. Meanwhile, the colonies turned pale yellow, and the colonies of J10 still adhered tightly. After 72 h, the colony size did not change significantly from the previous day, while the colonies became flat and yellow, and the hemolytic ring was bigger and more obvious.

In LB broth, three SCVs grew in suspension. Compared with *S. aureus* ATCC29213, J10, J60, and S63 grew more slowly and the plateaus of the growth curves were lower. In particular, J10 had the slowest growth rate, and the OD600 of the plateau phase was close to half of the OD600 value for the ATCC29213 (Figure 1).

3.2 | Pathogenic characteristics

The patient of J10 had pain in the right back and occasionally fever for more than one month. During the period, the patient took amoxicillin and the symptoms improved. After admission, he was diagnosed with multiple stones in the right kidney, hydronephrosis, and urinary tract infection. SCVs of *S. aureus* were isolated from renal fistula wound and urine specimens. After being treated with cefoxitin, the patient's wound and urinary tract infection improved. The patient of J60 was diagnosed with diabetic foot and

was amputated two months ago. The diabetic foot relapsed and the toe ulcerated for two weeks. The usage of antimicrobial during this period was unknown. On admission, the wound was red and swollen with peculiar smell and yellow-white discharge from which this SCV strain was isolated. The wound infection improved significantly after treatment with piperacillin-tazobactam and vancomycin. The patient of S63 was diagnosed with skin squamous cell carcinoma seven years ago and started chemotherapy for one year. After first chemotherapy, the patient developed small patches of erythema and pustules on multiple places and had high fever. There was no improvement after changing the chemotherapy protocols. The symptoms improved significantly after treatment with moxifloxacin and tripterygium wilfordii. When stopping the antimicrobial and starting chemotherapy, the skin erythema and pustules recurred. This SCV strain was isolated from the pustules after several times of treatment.

The WBC, neutrophils, CRP, PCT, and other infection indicators of the three patients before and after the infection showed no significant changes or only slightly increased (Table S1).

3.3 | Coagulase and biofilm formation

As shown in Figure S3, both J10 and S63 were negative for coagulase, and J60 was weakly positive. Moreover, the results of biofilm staining showed that J10 was strong biofilm-forming and stronger than *S. aureus* ATCC29213 and even *S. epidermidis* ATCC35984 ($p < 0.05$). S63 and J60 were weak biofilm-forming and non-biofilm-forming, respectively. Same as *S. epidermidis* ATCC35984, J10 formed biofilm both on the bottom and sidewall (Figure 2).

3.4 | Antibiotic susceptibility profiles

According to VITEK-2, J10 and J60 were resistant to penicillin and oxacillin. As for cefoxitin, J60 was resistant and J10 was susceptible. The two strains were identified as MRSA. However, S63 was susceptible to penicillin, oxacillin, and cefoxitin, and it was identified as MSSA. The results of antibiotic susceptibility profiles were listed in Table 1.

The results of disk diffusion and broth microdilution method were displayed in Table 2. J60 was still resistant to penicillin and cefoxitin. S63 was also still susceptible to penicillin and cefoxitin. Due to the slow growth rate, J10 did not show an obvious inhibition zone within 24 h, and it was hard to judge the results. At 48 h, the results showed that J10 was resistant to penicillin but susceptible to cefoxitin. As for vancomycin, all the SCVs were susceptible and the MIC was 1μg/ml of J60 and S63, and ≤0.25 μg/ml of J10. Moreover, J10 and J60 were resistant to oxacillin and the MIC was 16 μg/ml. S63 was susceptible to oxacillin, and the MIC was 0.25 μg/ml. There was no difference after 48 and 72 h.

3.5 | Penicillin and oxacillin resistance mechanism

J60 harbored with *MecA* and *MecA subtype f2* and was positive with β-lactamase and *blaZ*, but it was negative with *MecC* (Figures 3 and 4). J10 and S63 were negative with *MecA*, *MecC*, and *MecA subtype f2*. However, J10 was positive with β-lactamase and *blaZ*, and S63 was negative (Figures 3 and 4).

3.6 | Genomic background of SCVs

The SPA types of J10, J60, and S63 were t2592, t233, and t023, respectively. The MLST of J10, J60, and S63 was ST88, ST239, and ST965, respectively. Through homology analysis, the three SCVs were singletons. The results of PFGE were displayed in Figure 5, and the three SCVs were singletons.

4 | DISCUSSION

In this study, we screened three SCVs from 278 non-repetitive *S. aureus* strains (1.1%), indicating a relatively lower isolating rate than other studies. For instance, Ansari S et al. screened 10 SCVs from 66 *S. aureus* strains and Cervantes-García E et al. screened 3 SCVs from 47 *S. aureus* strains.[25,26] Unequal incidence may be related to the different treatment strategies of *S. aureus* in countries. There is no widely accepted standard to identify SCVs. In our study, the colony characteristics including colony size, hemolysis, and pigment production were different from classic *S. aureus*, which is similar to previous studies.[6,27] In addition, the colony characteristics were also different among three SCVs which would make the development of SCVs identification standard more difficult. The difference in growth rate between SCVs and classic strains can be clearly observed from the growth curves. Therefore, to identifying SCVs, detecting auxotrophy types and growth curves are useful but complex for routine diagnostic laboratories. PCR and sequencing for molecular targets which have been proven for *S. aureus* identification (eg, *nuc*, *clfA*, *eap*, *coa*, and *sodM*) also have good performance for identifying SCVs.[1] What's more, MALDI-TOF MS has great potential in the identification of *S. aureus* SCVs. It has been used to identify an SCV phenotype of *Enterococcus faecium*.[28] However, mass spectrometry and genetic analysis need further verification. Therefore, combining the colony characteristics, slow growth rate, and antibiotic usage records of patient is still necessary for SCVs diagnosis.

The long-term usage of antibiotics plays an important role in the formation of SCVs. In this study, the patients of J10 and S63 had taken antibiotic for a long time but the exact medication records of J60 were unavailable owing to the lack of integral clinical data records in other hospitals or at home. The patient of J10 had been taking amoxicillin for a long time due to gastric ulcer and continued after developing symptoms of urinary tract infection. Although the use of amoxicillin has not been reported to be related to the formation of SCVs, bacteria

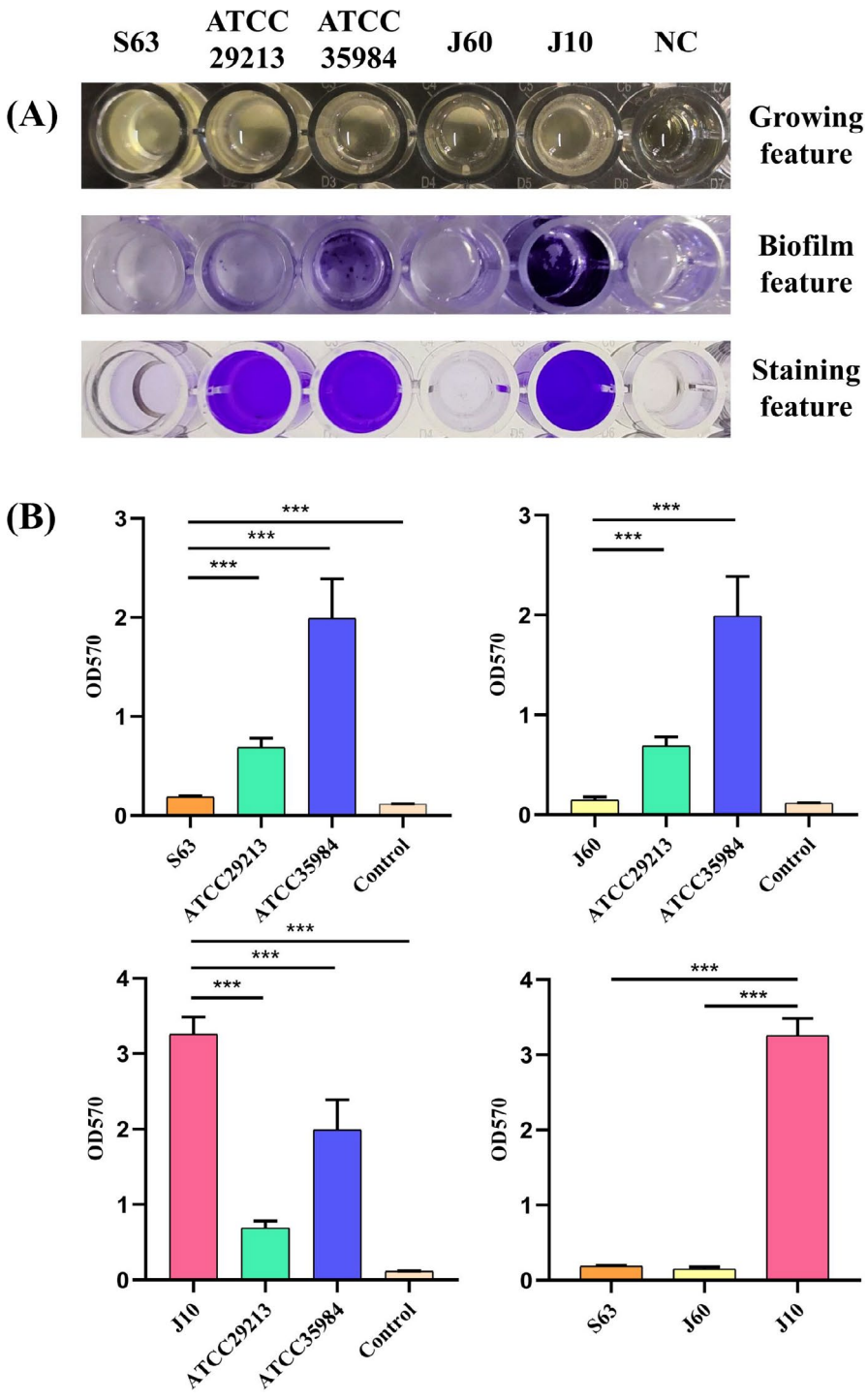

FIGURE 2 Results of SCVs biofilm formation and staining. After being incubated for 48 h, (A) the growing status, the biofilm characteristics after crystal violet staining and the results after dissolving the dye; (B) statistical analysis results of biofilm staining. ***$p < 0.05$

often undergo genetic mutations in the process of interaction with the immune system, especially after anti-infection treatment.[29,30] The long-term application of antibiotics which act on the bacterial wall-like amoxicillin may be related to the formation of SCVs. The skin pustules of patient of S63 may be closely related to SCVs chronic infection according to long-term usage of moxifloxacin and recurrent infection. It has been proved that treatment with low concentration of moxifloxacin could stimulate the formation of SCVs.[31]

At the same time, no significant changes of infection-related biomarkers were observed including WBC, PCT, CRP, and neutrophils, indicating that SCVs infection may not induce serious inflammation. The reduced inflammation is associated with the intracellular feature and weakened virulence of SCVs. SCVs present in cells can evade the host's immune system and effect of antibiotics, thereby causing persistent and recurrent infections.[13,32] Previous study showed that the increase in pro-inflammatory cytokines such *IL1B*, *IL6*, and *IL12* caused by SCVs cell infection is significantly lower than those of wild-type strains.[33] In the study of chronic *S. aureus* infection models, long-term environmental adaptation leads to the down-regulation of virulence genes such as toxin-encoding genes,

TABLE 1 Antimicrobial susceptibility results of three SCVs according to VITEK-2 Compact system

	J10		J60		S63	
Antimicrobial	MIC(μg/mL)	Susceptibility	MIC(μg/mL)	Susceptibility	MIC(μg/mL)	Susceptibility
PEN	≥0.5	R	≥0.5	R	≤0.03	S
OXA	≥4	R	≥4	R	≤0.25	S
FOX[a]		Ne		Po		Ne
CIP	≥8	R	≥8	R	≥8	R
LVX	4	R	≥8	R	≥8	R
MFX	2	R	≥8	R	4	R
CLI[b]	≤0.25	S	≤0.25	R	≤0.25	R
ERY	1	I	≥8	R	≥8	R
GEN	≤0.5	S	≥16	R	≥16	R
RIF	≤0.5	S	≥32	R	≤0.5	S
SXT	≤0.5	S	≤0.5	S	≤0.5	S
TCY	≤1	S	≥16	R	≥16	R
TGC	≤0.12	S	0.25	S	0.5	S
LNZ	2	S	2	S	2	S
QDA	≤0.25	S	≤0.25	S	≤0.25	S
VAN	≤0.5	S	≤0.5	S	1	S

Abbreviations: CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; PEN, penicillin G; OXA, oxacillin; FOX, cefoxitin screen; I, intermediate; LNZ, linezolid; LVX, levofloxacin; MFX, moxifloxacin; Ne, negative; QDA, quinupristin/dalfopristin; Po, positive; R, resistance; RIF, rifampicin; S, susceptibility; SXT, sulfamethoxazole/trimethoprim; TCY, tetracycline; TGC, tigecycline; VAN, vancomycin.

[a]According to VITEK-2 Compact system, the result of cefoxitin was expressed as positive or negative

[b]According to CLSI, when erythromycin is resistant and clindamycin is susceptible or intermediary, the result of clindamycin is reported as high-level mupirocin resistance if the disk diffusion is positive.

TABLE 2 Antimicrobial susceptibility results of three SCVs by disk diffusion or broth microdilution method

SCVs	PEN	OXA[a]	FOX	CIP	LVX	MFX	CLI	ERY	GEN	RIF	SXT	TCY	TGC	LNZ	QDA	VAN[a]
J10	R	R	S	R	R	R	S	I	S	N	S	N	S	S	N	S
J60	R	R	R	R	R	R	R	R	R	N	S	N	S	S	N	S
S63	S	S	S	R	R	R	R	R	R	N	S	N	S	S	N	S

Abbreviations: CLI, clindamycin; CIP, ciprofloxacin; ERY, erythromycin; FOX, cefoxitin; GEN, gentamicin; I, intermediate; LNZ, linezolid; LVX, levofloxacin; MFX, moxifloxacin; N, no result; OXA, oxacillin; PEN, penicillin G; QDA, quinupristin/dalfopristin; R, resistance; RIF, rifampicin; S, susceptibility; SXT, sulfamethoxazole/trimethoprim; TCY, tetracycline; TGC, tigecycline; VAN, vancomycin.

[a]broth microdilution method.

increased expression of adhesion and biofilm formation genes, slow growth rate, and formation of SCVs.[34] Therefore, the formation of SCVs may be the result of the interaction between *S. aureus* and the host immune system and antimicrobial. Since the insufficient SCVs and the lack of parent wild-type strains, it is unable to confirm the pathogenic characteristics of SCVs. The analysis of more samples and animal experiments may be helpful to reveal the pathogenic characteristics and mechanisms of SCVs.

The incubation time should be extended for antimicrobial susceptibility test when SCV is suspected. The results might not be determined within the standard time of routine experimental procedures due to the slow growth rate which could bring difficulties to detect the growth by visual inspection or measuring the optical density. In the present study, the results of disk diffusion method of J10 cannot be determined at 24 h for unobvious inhibition zone. Moreover, the results of VITEK-2 and disk diffusion method showed that J10 was MRSA, but it had no PBP2a genes. The positive phenotype and gene results of β-lactamase confirmed that J10 might be borderline oxacillin resistance, which is different from MRSA and often associated with hyperproduction of β-lactamases or sometimes PBP2a genes' point mutations.[35] However, similar to SCVs, borderline oxacillin resistance is not valued in clinical laboratories either. These results indicate that SCVs can be formed in MRSA, MSSA, and borderline oxacillin-resistant *S. aureus*. Since the original wild-type strains cannot be obtained, the difference in antimicrobial susceptibility between wild types and SCVs is unintelligible.

The results of *in vitro* susceptibility experiments may not be suitable to guide clinical anti-SCVs infection treatment. The formation

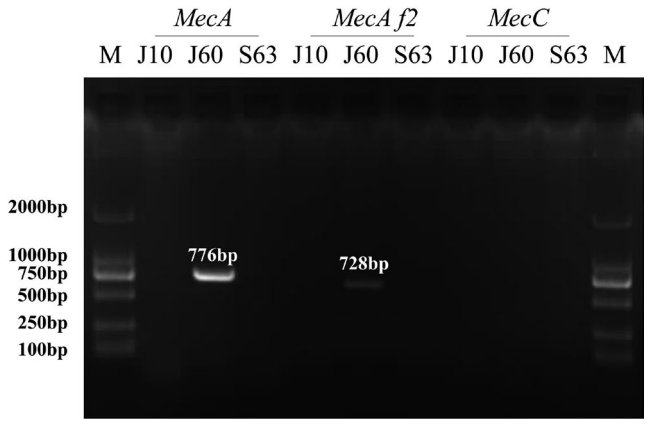

FIGURE 3 Agarose gel electrophoresis results of resistance genes

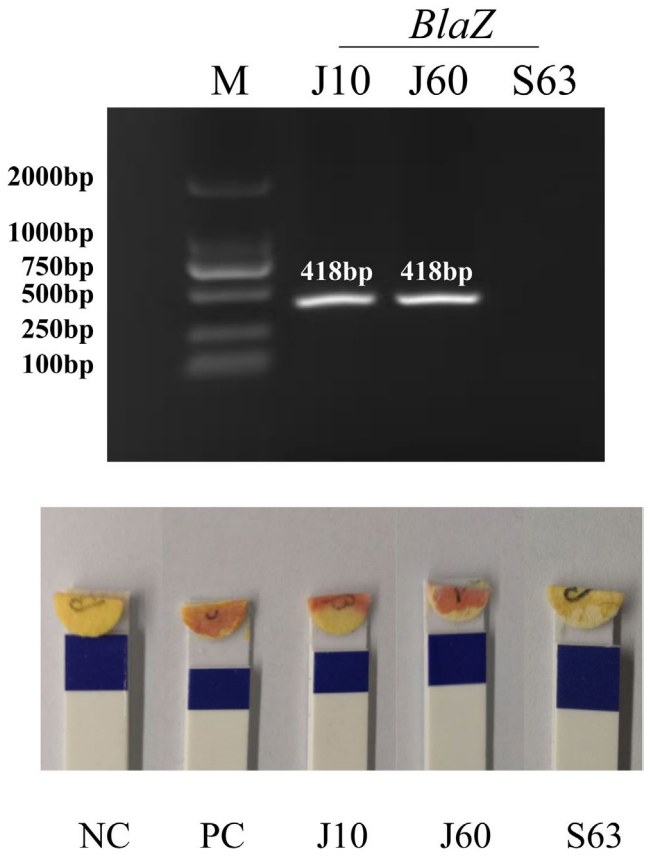

FIGURE 4 Nitrocefin test and *blaZ* gene agarose gel electrophoresis results. After scraping the colonies with nitrocefin disk, the disk turned red within 15 min meant the β-lactamase was positive. NC negative control; PC positive control

of SCVs will reduce the antimicrobial susceptibility such as aminoglycosides, SXT, and vancomycin because of the slow growth rate, reduced basal metabolism, weakened membrane potential, and thymidine dependence.[36,37] In addition, since the intracellular feature of SCVs, the antimicrobial activity *in vivo* is weakened partially and even invalid.[31] Moreover, the formation of biofilm can protect the strains from the effect of antimicrobial and even lead to failure of

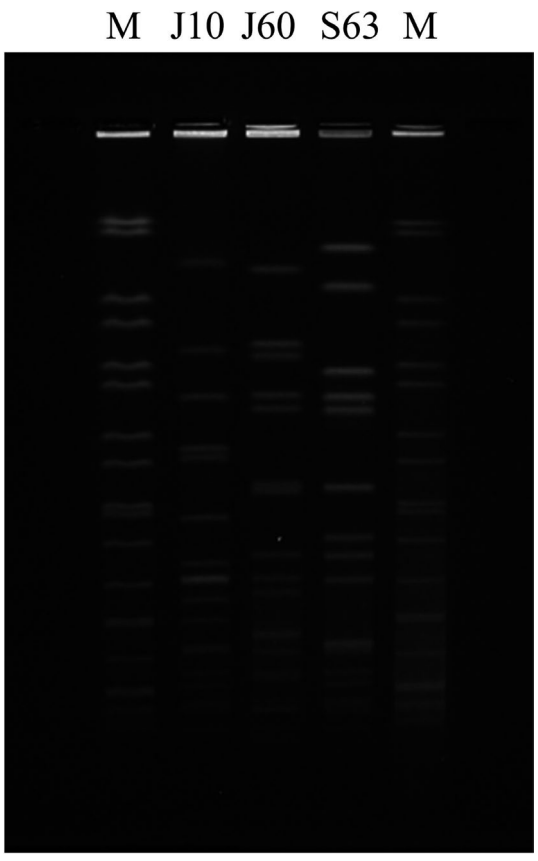

FIGURE 5 The PFGE results of three SCVs

treatment of device-related infections, such as pacemakers, prosthetic joints, and artificial valves.[36,38–40] As a result, standard antimicrobial regimens are not sufficient to clear the SCVs infection.[41] According to the antimicrobial susceptibility results and treatment course of patients, for MSSA-SCVs, oxacillin still has good antimicrobial effects. Moreover, for infections caused by MRSA-SCVs, vancomycin is one of the significant options. However, vancomycin has a weaker killing effect on SCVs, and low-concentration vancomycin contributes to the formation and growth of SCVs.[37,42] Therefore, the combination of vancomycin and MRSA-susceptible antibiotics is worthy of consideration.

Through MLST and PFGE analysis, no homology between the three SCVs was identified in our study, which indicates there is no clonal propagation among SCVs. Meanwhile, different ST types, PFGE types, and SPA types of *S. aureus* may form SCVs. That means the practical significance of epidemiological monitoring of SCVs is limited, and more standardized management and use of antibiotics play an important role in controlling the formation of SCVs.

In conclusion, wound infections caused by SCVs are rare. However, chronic infections caused by SCVs are still worthy of attention. SCVs can appear in antimicrobial-resistant and non-resistant strains. Therefore, it is very essential to establish appropriate identification and treatment standards for SCVs. The formation of SCVs may be related to long-term use of antimicrobial and chronic inflammation. However, the underline mechanism and pathogenic

characteristics of SCVs still need further studies. At present, the poor homology between SCVs indicates that the formation of SCVs may not be limited to some special ST or PFGE types.

There are some limitations to this study. First, the wild-type strains cannot be obtained, so it is impossible to compare the biological characteristics and the difference in antimicrobial resistance between SCVs and their parent strains. Next, the number of SCV strains is small, and the sample size needs to be expanded for further research.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Changhang Min, Haichen Wang, and Mingxiang Zou designed the study; Changhang Min, Jun Li, Yongmei Hu, and Qingya Dou collected and analyzed clinical and laboratory data; Changhang Min, Fengjun Xia, and Mengli Tang carried out experiments; Changhang Min, Haichen Wang, and Fengjun Xia analyzed experimental results; Changhang Min and Haichen Wang wrote the manuscript; Changhang Min, Haichen Wang, and Mingxiang Zou made some critical revisions of the manuscript.

DATA AVAILABILITY STATEMENT

The data and strains that support this study are obtained from Xiangya Hospital, but restrictions apply to the availability of these data and strains which were used under license for the current study, and so are not publicly available. However, data are available from the authors with reasonable request and the permission of Xiangya Hospital.

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REFERENCES

1. Kahl BC, Becker K, Löffler B. Clinical significance and pathogenesis of staphylococcal small colony variants in persistent infections. *Clin Microbiol Rev*. 2016;29(2):401-427.
2. Jung N, Rieg S. Essentials in the management of S. aureus bloodstream infection. *Infection*. 2018;46(4):441-442.
3. Seggewiss J, Becker K, Kotte O, et al. Reporter metabolite analysis of transcriptional profiles of a Staphylococcus aureus strain with normal phenotype and its isogenic hemB mutant displaying the small-colony-variant phenotype. *J Bacteriol*. 2006;188(22):7765-7777.
4. Kohler C, von Eiff C, Peters G, Proctor RA, Hecker M, Engelmann S. Physiological characterization of a heme-deficient mutant of Staphylococcus aureus by a proteomic approach. *J Bacteriol*. 2003;185(23):6928-6937.
5. Johns BE, Purdy KJ, Tucker NP, Maddocks SE. Phenotypic and genotypic characteristics of small colony variants and their role in chronic infection. *Microbiol Insights*. 2015;8:15-23.
6. Proctor RA, von Eiff C, Kahl BC, et al. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. *Nat Rev Microbiol*. 2006;4(4):295-305.
7. Mirani ZA, Urooj S, Khan MN, Khan AB, Shaikh IA, Siddiqui A. An effective weapon against biofilm consortia and small colony variants of MRSA. *Iran J Basic Med Sci*. 2020;23(11):1494-1498.
8. Besier S, Zander J, Siegel E, et al. Thymidine-dependent Staphylococcus aureus small-colony variants: human pathogens that are relevant not only in cases of cystic fibrosis lung disease. *J Clin Microbiol*. 2008;46(11):3829-3832.
9. Chatterjee I, Kriegeskorte A, Fischer A, et al. In vivo mutations of thymidylate synthase (encoded by thyA) are responsible for thymidine dependency in clinical small-colony variants of Staphylococcus aureus. *J Bacteriol*. 2008;190(3):834-842.
10. Bazaid AS, Forbes S, Humphreys GJ, Ledder RG, O'Cualain R, McBain AJ. Fatty acid supplementation reverses the small colony variant phenotype in triclosan-adapted staphylococcus aureus: genetic, proteomic and phenotypic analyses. *Sci Rep*. 2018;8(1):3876.
11. Bhattacharyya S, Jaiswal NK, Kumar M, Ansari MA, Sarfraz A. Breast abscess in a case of duct atresia caused by CO2-auxotrophic small colony variants of Staphylococcus aureus: case report and review of literature. *J Nat Sci Biol Med*. 2015;6(2):475-476.
12. Tuchscherr L, Löffler B, Proctor RA. Persistence of Staphylococcus aureus: multiple metabolic pathways impact the expression of virulence factors in small-colony variants (SCVs). *Front Microbiol*. 2020;11:1028.
13. Tuchscherr L, Heitmann V, Hussain M, et al. Staphylococcus aureus small-colony variants are adapted phenotypes for intracellular persistence. *J Infect Dis*. 2010;202(7):1031-1040.
14. Wolter DJ, Onchiri FM, Emerson J, et al. Prevalence and clinical associations of Staphylococcus aureus small-colony variant respiratory infection in children with cystic fibrosis (SCVSA): a multicentre, observational study. *Lancet Respir Med*. 2019;7(12):1027-1038.
15. Kahl B, Herrmann M, Everding AS, et al. Persistent infection with small colony variant strains of Staphylococcus aureus in patients with cystic fibrosis. *J Infect Dis*. 1998;177(4):1023-1029.
16. Loss G, Simões PM, Valour F, et al. Staphylococcus aureus small colony variants (SCVs): news from a chronic prosthetic joint infection. *Front Cell Infect Microbiol*. 2019;9:363.
17. Tuchscherr L, Medina E, Hussain M, et al. Staphylococcus aureus phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. *EMBO Mol Med*. 2011;3(3):129-141.
18. Seaman PF, Ochs D, Day MJ. Small-colony variants: a novel mechanism for triclosan resistance in methicillin-resistant Staphylococcus aureus. *J Antimicrob Chemother*. 2007;59(1):43-50.
19. Hashem YA, Amin HM, Essam TM, Yassin AS, Aziz RK. Biofilm formation in enterococci: genotype-phenotype correlations and inhibition by vancomycin. *Sci Rep*. 2017;7(1):5733.
20. Huse HK, Miller SA, Chandrasekaran S, et al. Evaluation of oxacillin and cefoxitin disk diffusion and MIC breakpoints established by the clinical and laboratory standards institute for detection of mecA-mediated oxacillin resistance in Staphylococcus schleiferi. *J Clin Microbiol*. 2018;56(2):10.
21. Mohajeri PIB, Rezaei M, Farahani A. Frequency distribution of hospital-acquired mrsa nasal carriage among hospitalized patients in West of Iran. *Jundishapur J Microbiol*. 2013;6(6):e9076.
22. Giacinti G, Carfora V, Caprioli A, et al. Prevalence and characterization of methicillin-resistant Staphylococcus aureus carrying mecA or mecC and methicillin-susceptible Staphylococcus

aureus in dairy sheep farms in central Italy. *J Dairy Sci*. 2017;100(10):7857-7863.
23. Ferreira AM, Martins KB, Silva VR, Mondelli AL, Cunha ML. Correlation of phenotypic tests with the presence of the blaZ gene for detection of beta-lactamase. *Braz J Microbiol*. 2017;48(1):159-166.
24. Poormohammadi S, Farahani A, Mohajeri P. Genomic diversity and antimicrobial susceptibility profiling of nasal carriage Staphylococcus aureus isolated from pediatric ward in Western Iran. *Saudi J Biol Sci*. 2019;26(1):1-7.
25. Ansari S, Nepal HP, Gautam R, Shrestha S, Chhetri MR, Chapagain ML. Staphylococcus aureus: methicillin resistance and small colony variants from pyogenic infections of skin, soft tissue and bone. *J Nepal Health Res Counc*. 2015;13(30):126-132.
26. Cervantes-García E, García-Gonzalez R, Reyes-Torres A, Resendiz-Albor AA, Salazar-Schettino PM. Staphylococcus aureus small colony variants in diabetic foot infections. *Diabet Foot Ankle*. 2015;6:26431.
27. Kahl BC, Belling G, Reichelt R, Herrmann M, Proctor RA, Peters G. Thymidine-dependent small-colony variants of Staphylococcus aureus exhibit gross morphological and ultrastructural changes consistent with impaired cell separation. *J Clin Microbiol*. 2003;41(1):410-413.
28. Gröbner S, Beck J, Schaller M, Autenrieth IB, Schulte B. Characterization of an Enterococcus faecium small-colony variant isolated from blood culture. *Int J Med Microbiol*. 2012;302(1):40-44.
29. Foster PL. Stress responses and genetic variation in bacteria. *Mutat Res*. 2005;569(1–2):3-11.
30. Durão P, Balbontín R, Gordo I. Evolutionary mechanisms shaping the maintenance of antibiotic resistance. *Trends Microbiol*. 2018;26(8):677-691.
31. Tuchscherr L, Kreis CA, Hoerr V, et al. Staphylococcus aureus develops increased resistance to antibiotics by forming dynamic small colony variants during chronic osteomyelitis. *J Antimicrob Chemother*. 2016;71(2):438-448.
32. Kalinka J, Hachmeister M, Geraci J, et al. Staphylococcus aureus isolates from chronic osteomyelitis are characterized by high host cell invasion and intracellular adaptation, but still induce inflammation. *Int J Med Microbiol*. 2014;304(8):1038-1049.
33. Ou JJ, Drilling AJ, Cooksley C, et al. Reduced innate immune response to a staphylococcus aureus small colony variant compared to its wild-type parent strain. *Front Cell Infect Microbiol*. 2016;6:187.
34. Pouget C, Gustave CA, Ngba-Essebe C, et al. Adaptation of staphylococcus aureus in a medium mimicking a diabetic foot environment. *Toxins (Basel)*. 2021;13(3).
35. Hryniewicz MM, Garbacz K. Borderline oxacillin-resistant Staphylococcus aureus (BORSA) - a more common problem than expected? *J Med Microbiol*. 2017;66(10):1367-1373.
36. Maduka-Ezeh A, Seville MT, Kusne S, et al. Thymidine auxotrophic Staphylococcus aureus small-colony variant endocarditis and left ventricular assist device infection. *J Clin Microbiol*. 2012;50(3):1102-1105.
37. Lenhard JR, von Eiff C, Hong IS, et al. Evolution of Staphylococcus aureus under vancomycin selective pressure: the role of the small-colony variant phenotype. *Antimicrob Agents Chemother*. 2015;59(2):1347-1351.
38. Sendi P, Rohrbach M, Graber P, Frei R, Ochsner PE, Zimmerli W. Staphylococcus aureus small colony variants in prosthetic joint infection. *Clin Infect Dis*. 2006;43(8):961-967.
39. Seifert H, Oltmanns D, Becker K, Wisplinghoff H, von Eiff C. Staphylococcus lugdunensis pacemaker-related infection. *Emerg Infect Dis*. 2005;11(8):1283-1286.
40. Roy R, Tiwari M, Donelli G, Tiwari V. Strategies for combating bacterial biofilms: a focus on anti-biofilm agents and their mechanisms of action. *Virulence*. 2018;9(1):522-554.
41. Bui LM, Conlon BP, Kidd SP. Antibiotic tolerance and the alternative lifestyles of Staphylococcus aureus. *Essays Biochem*. 2017;61(1):71-79.
42. Garcia LG, Lemaire S, Kahl BC, et al. Pharmacodynamic evaluation of the activity of antibiotics against hemin- and menadione-dependent small-colony variants of Staphylococcus aureus in models of extracellular (broth) and intracellular (THP-1 monocytes) infections. *Antimicrob Agents Chemother*. 2012;56(7):3700-3711.

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