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ORIGINAL RESEARCH

Characterization of *Staphylococcus aureus* Small-Colony Variants Isolated from Lower Respiratory Tract Specimens

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Purpose: Epidemiological data on small colony variants (SCVs) of *Staphylococcus aureus* (*S. aureus*) in China are lacking. This study aimed to investigate the prevalence and characteristics of *S. aureus* SCVs in patients with *Pseudomonas aeruginosa* (*P. aeruginosa*) pneumonia.

Methods: From October 2024 to September 2025, *S. aureus* SCVs were collected from lower respiratory tract specimens at two tertiary hospitals in Fuzhou and identified using MALDI-TOF MS. Antibiotic susceptibility testing was performed using a VITEK® 2 Compact System. Genetic diversity and virulence were analyzed using multilocus sequence typing (MLST), staphylococcal protein A (spa) typing, and toxin gene profiling. Biofilm formation was assessed using a microtiter plate assay, and patient characteristics were analyzed using the Hospital Information System.

Results: Thirty-eight *S. aureus* SCVs (2.1%) were isolated from 1,832 lower respiratory tract specimens collected from patients with *P. aeruginosa* pneumonia. Compared to normal phenotype strains, SCVs exhibited smaller colonies and reduced hemolysis. Among resistant strains, 20 were methicillin-resistant *S. aureus* SCVs (MRSA-SCVs), with ST1-t128 (25.0%) being the most prevalent. ST764-t1084 MRSA-SCVs were resistant to penicillin, oxacillin, gentamicin, ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, clindamycin, and tetracycline. Eighteen strains were methicillin-susceptible *S. aureus* SCVs (MSSA-SCVs), predominantly ST72-t3735 (16.7%). Virulence analysis showed adhesion-related gene carriage rates of 47.4–100.0% and immune evasion gene carriage rates of 52.6–73.7%. In addition, most *S. aureus* SCVs showed strong biofilm production.

Conclusion: This study identified a 2.1% prevalence of *S. aureus* SCVs (often undetected) in *P. aeruginosa* pneumonia patients. More than half of patients were methicillin-resistant (MRSA), with strong biofilm-forming capacity and a potential association with prolonged hospitalization. Vigilance is warranted against potential outbreaks of the predominant MRSA-SCV clone ST1-t128, as well as the severe drug resistance observed in ST764-t1084 MRSA-SCVs.

Keywords: *Staphylococcus aureus*, small colony variants, MLST, antibiotic susceptibility, biofilm

Introduction

Small colony variants (SCVs) are a subpopulation of nutrient-deficient bacteria that exhibit a unique phenotype as an adaptive response to environmental stress. They are characterized by small colony size, slow growth, reduced metabolic activity, and intracellular parasitism.[1] The first SCV was reported in *Salmonella typhi* in 1910 and, since then, SCVs have been identified in various bacterial species, including *Staphylococcus aureus, Vibrio cholerae*, and *Escherichia coli*.[2] Among these, *S. aureus* is a major pathogen responsible for hospital-acquired pneumonia (HAP) and hemorrhagic necrotizing pneumonia, and it can lead to severe conditions such as sepsis and septic shock.[3]

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The formation of SCVs is primarily attributed to specific nutritional auxotrophies affecting the respiratory chain, including deficiencies in thymidine, menadione, or heme.[4] These metabolic impairments lead to obstructed oxidative phosphorylation in the electron transport chain, resulting in significantly reduced bacterial replication and metabolic rates that ultimately manifest as the SCV phenotype. Unlike wild-type *S. aureus*, small colony variants of *S. aureus* demonstrate enhanced resistance to antibiotics such as aminoglycosides and trimethoprim-sulfamethoxazole. Both methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) can develop SCVs. Notably, MRSA-SCVs are particularly challenging to treat, warranting heightened clinical vigilance among physicians and clinical microbiologists.[5] Inappropriate antimicrobial therapy may facilitate SCV development.[6] Prolonged use of sulfonamides such as trimethoprim-sulfamethoxazole is associated with thymidine-auxotrophic SCVs, as this antibiotic combination inhibits the synthesis of tetrahydrofolate, an essential cofactor for thymidylate synthase.[7] Similarly, aminoglycoside usage correlates with the emergence of menadione- or heme-auxotrophic SCVs, although the precise underlying mechanisms remain incompletely understood. Furthermore, SCVs exhibit attenuated virulence and an enhanced biofilm-forming capacity. By downregulating host chemokines, including CCL5 and CXCL8, SCVs impair the rapid recruitment of neutrophils and other immune cells to infection sites, thereby facilitating persistent host colonization.[8] Consequently, SCVs are frequently implicated in chronic and recurrent infections, such as periprosthetic joint infections, pulmonary cystic fibrosis-related infections, and refractory wound infections.[8–10]

The rapid and accurate identification of SCVs is crucial for effective clinical treatment. However, owing to their slow growth, SCVs are frequently overlooked by clinical microbiologists in routine practice. Although certain chromogenic media can facilitate convenient screening of *S. aureus* SCVs, most clinical laboratories in China and other countries do not routinely test for these variants.[11,12] Furthermore, clinically isolated *S. aureus* SCVs often exhibit phenotypic instability and tend to revert to the wild-type phenotype.

Owing to the phenotypic instability of SCVs, researchers often employ stable site-directed mutant strains (hemB or menD mutants) to simulate SCV phenotypes.[13] Consequently, studies on clinically derived SCVs remain limited. Existing evidence has established a significant association between *S. aureus* SCV formation and *P. aeruginosa* coinfection.[14] However, epidemiological data on *S. aureus* SCVs in China are notably scarce. To address this knowledge gap, our study aimed to investigate the prevalence, antimicrobial susceptibility patterns, and genetic characteristics of *S. aureus* SCVs isolated from lower respiratory tract specimens of patients with *P. aeruginosa* pneumonia.

Materials and Methods

Sample Collection and Identification of S. aureus SCVs

From October 2024 to September 2025, 1,832 lower respiratory tract specimens (including sputum and bronchoalveolar lavage fluid) were collected from patients with *P. aeruginosa* pneumonia at two major tertiary hospitals in Fuzhou, Fujian Province. All specimens suspected of harboring *S. aureus* SCVs were processed by isolating single colonies through quadrant streaking, followed by confirmatory identification using MALDI-TOF MS (Bruker Daltonik, Germany), according to the manufacturer's protocol. Confirmed *S. aureus* isolates were cultured on blood agar plates for 24–48 hours. Colonies exhibiting characteristic SCV phenotypes, including approximately 10% reduction in size compared to normal morphology, grayish-white coloration, and diminished hemolytic activity, were identified as *S. aureus* SCVs.[15] All verified *S. aureus* SCVs were subsequently lyophilized and preserved at −80°C for future research purposes.

DNA Extraction of S. aureus SCVs

DNA was extracted from *S. aureus* SCVs using previously described methods.[16] Briefly, preserved SCVs were streaked onto blood agar plates and incubated overnight at 35°C. Subsequently, the bacterial colonies were suspended in 300 μL sterile distilled water, heated at 95°C for 10 min, and centrifuged at 12,000 × g for 5 min to remove cellular debris. The supernatant was stored at 4°C and used as the DNA template for amplification. DNA concentration and purity were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

Molecular Typing of S. aureus SCVs

Multilocus sequence typing (MLST) analysis was performed as described previously.[17] Seven *S. aureus* house-keeping genes (*arcC, aroE, glpF, gmk, pta, tpi*, and *yqiL*) of *S. aureus* were amplified and sequenced. The sequencing data were compared with existing *S. aureus* sequences in the MLST database (https://pubmlst.org/) to determine the allelic profiles and sequence types (STs). Spa typing was performed based on polymorphisms in the X-region of the staphylococcal protein A (spa) gene.[18] The X-region of each isolate was amplified by PCR, and the PCR products were subjected to Sanger sequencing. The obtained spa sequences were compared with the database (https://spa.ridom.de/) to determine the spa types of *S. aureus* SCVs.

Antimicrobial Susceptibility Testing of S. aureus SCVs

Antimicrobial susceptibility testing was performed using the Vitek® 2 Compact system (bioMérieux, France). The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[19] The study evaluated susceptibility to 13 antibiotics: penicillin, oxacillin, gentamicin, rifampin, ciprofloxacin, levofloxacin, moxifloxacin, clindamycin, erythromycin, linezolid, vancomycin, tetracycline, and tigecycline. *Staphylococcus aureus* ATCC 25923 was used as a quality control strain. Methicillin-resistant *S. aureus* (MRSA) strains were confirmed based on phenotypic resistance patterns and detection of the *mecA* gene.

PCR Detection of Antimicrobial Resistance and Virulence Genes

The detection of 15 virulence genes (*sea, icaA, icaC, icaD, clfA, clfB, hla, hlg, pvl, fnbA, cna, sarA, sdrD*, and *sdrE, ebpS*) and the resistance gene *mecA* in *S. aureus* SCVs was performed by PCR according to the methods described by a previous study.[20,21] The PCR amplification protocol consisted of: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 53°C for 30 sec, and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. The PCR products were visualized using 2% agarose gel electrophoresis and subsequently sent to Sangon Biotech (Shanghai, China) for sequencing. The sequencing results were analyzed using SnapGene 7.1.2 software.

Detection of Biofilm Formation

Crystal violet staining measured biofilm formation.[16] Briefly, fresh *S. aureus* SCVs were inoculated into LB broth, cultured at 37°C, and shaken (220 rpm) for 18 h. The bacterial suspension was adjusted to a McFarland standard of 0.5, and diluted at a ratio of 1:100 in fresh LB broth. Aliquots (200 μL) of the diluted culture were dispensed into eight wells of a 96-well microtiter plate and incubated statically at 37°C for 48 h. Sterile LB broth served as the negative control. After incubation, the plates were washed thrice with phosphate-buffered saline PBS (pH 7.0) and air-dried at room temperature. The biofilms were then fixed with methanol for 20 min, after which the methanol was discarded. The fixed biofilms were stained with 1% crystal violet solution for 15 min, followed by washing with PBS until the solution became colorless. After drying, the bound crystal violet was solubilized in 200μL of absolute ethanol and the solution was transferred to a new microtiter plate. Optical density (OD) was measured at 570 nm. Mean OD values were calculated for all tested strains and negative controls. The optical density cutoff (ODc) was defined as 3 standard deviations (SDs) above the mean optical density (OD) of the negative control. Classification: non-biofilm producers: $OD \leq ODc$; weak-biofilm producers: $ODc < OD \leq 2ODc$; moderate-biofilm producers: $2ODc < OD \leq 4ODc$; strong-biofilm producers: $OD > 4ODc$.

Clinical Data Collection

Clinical data of hospitalized and outpatient patients with *P. aeruginosa* pneumonia were collected from the Hospital Information System (HIS), including patient age, sex, department, treatment course, and underlying diseases. The data were organized using Microsoft Excel.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 26.0. Categorical variables were analyzed using either the chi-square test or Fisher's exact test, while continuous variables were evaluated using either the Student's *t*-test or Mann–Whitney *U*-test. Statistical significance was set at a *p*-value of <0.05.

Results

Prevalence of S. aureus SCV Isolates

Thirty-eight *S. aureus* SCVs were isolated from 1,832 sputum and bronchoalveolar lavage fluid specimens collected from patients with *P. aeruginosa* pneumonia, yielding a detection rate of 2.1%. After 24 h of incubation on blood agar plates, *S. aureus* SCVs formed pinpoint-sized round white colonies. As shown in Figure 1, when cultured on blood agar plates at 37°C for 24 h, *S. aureus* SCVs demonstrated distinct morphological differences from normal *S. aureus* ATCC 29213.

Molecular Characteristics of S. aureus SCV Isolates

MLST typing of 38 *S. aureus* SCVs revealed 15 distinct sequence types (STs) (Figure 2). ST1 (15.8%, 6/38) and ST398 (15.8%, 6/38) were the predominant clones, followed by ST965 (13.2%, 5/38), ST72 (10.5%, 4/38), ST764 (10.5%, 4/38), and ST7 (7.9%, 3/38). The remaining nine ST types collectively accounted for 26.3% of isolates. Among the methicillin-resistant *S. aureus* SCVs (MRSA-SCVs), ST1 (25.0%, 5/20) and ST965 (25.0%, 5/20) were the most prevalent, followed by ST764 (20.0%, 4/20).

Spa typing revealed 23 distinct types, predominantly t128 (13.2%, 5/38). Notably, 25.0% (5/20) of MRSA-SCVs carried the t128 spa type (Table 1). The most prevalent *S. aureus* SCV in lower respiratory tract samples was ST1-t128 (13.2%, 5/38), followed by ST965-t062 (7.9%, 3/38), ST72-t3735 (7.9%, 3/38) and ST764-t1084 (7.9%, 3/38). Genotypic distribution analysis showed that ST1-t128 was the dominant MRSA-SCVs genotype, whereas ST72-t3735 was predominant among methicillin-susceptible *S. aureus* SCV (MSSA-SCVs) isolates. These findings suggest a potential association between specific molecular types of *S. aureus* SCV and methicillin resistance.

Figure 1 Colony morphology of *S. aureus* strains ATCC 29213 and *S. aureus* SCV isolates.
Notes: The clinical isolate of *S. aureus* SCVs was grown on the upper section of a blood agar plate, whereas the normal morphology *S. aureus* ATCC 29213 strain was cultured on the lower section.

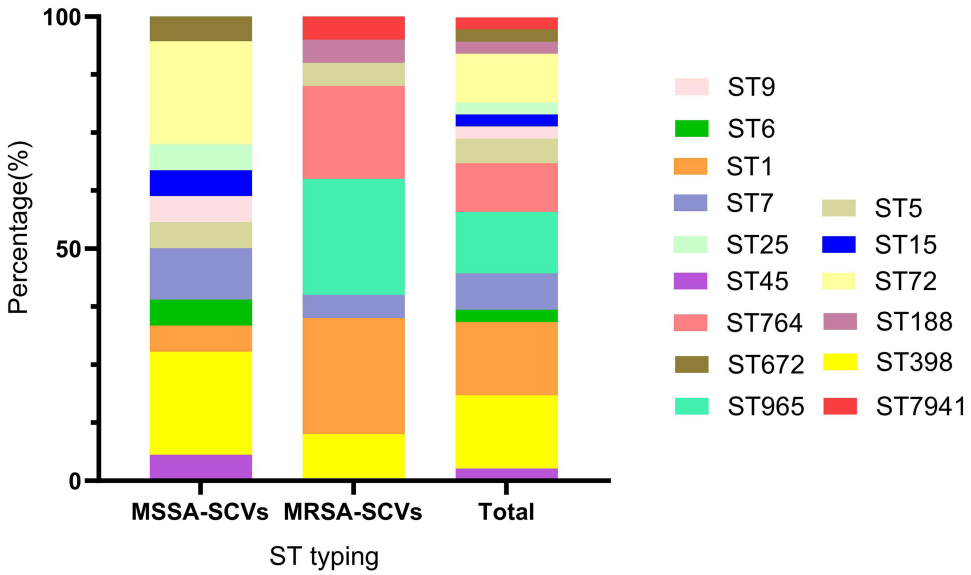

Figure 2 Distribution of sequence types (STs) of all isolates of *S. aureus* SCVs, MRSA-SCVs and MSSA-SCV isolates.

Antibiotic Susceptibility of S. aureus SCV Isolates

The VITEK® 2 Compact results revealed that 86.8% of the isolated *S. aureus* SCVs were resistant to at least one antibiotic. However, all *S. aureus* SCVs remained susceptible to linezolid, vancomycin, and tigecycline. The isolated *S. aureus* SCVs showed high resistance rates to the β-lactam antibiotics, penicillin (81.6%) and oxacillin (52.6%). Lower

Table 1 Molecular Characteristics of *S. aureus* SCV Isolates

MLST	spa (no.)	PVL+	MRSA-SCVs	MSSA-SCVs
ST1	t128(5)	5	5	0
	t127(1)	1	0	1
ST5	t548(2)	2	1	1
ST6	t1476(1)	0	0	1
ST7	t091(2)	1	0	2
	t1943(1)	1	1	0
ST9	t899(1)	1	0	1
ST15	t1492(1)	1	0	1
ST25	t078(1)	0	0	1
ST45	t116(1)	0	0	1
ST72	t3735(3)	1	0	3
	t148(1)	0	0	1
ST188	t189(1)	1	1	0
ST398	t034(2)	2	0	2
	t3625(2)	1	0	2
	t571(1)	1	1	0
	t16437(1)	0	1	0
ST672	t535(1)	0	0	1
ST764	t1084(3)	3	3	0
	t034(1)	1	1	0
ST965	t062(3)	2	3	0
	t571(1)	1	1	0
	t575(1)	1	1	0
ST7941	t1381(1)	1	1	0

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Table 2 Susceptibility of *S. aureus* SCVs to Antimicrobials

Antibiotic	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I(%)	R (%)	*P*-value*
	Total			MRSA-SCVs			MSSA-SCVs			
Penicillin	18.4	0	81.6	0	0	100	38.9	0	61.1	0.003
Oxacillin	47.4	0	52.6	0	0	100	100	0	0	0.000
Gentamicin	65.8	7.9	26.3	50	15	35	83.3	0	16.7	0.278
Rifampin	94.8	2.6	2.6	100	0	0	88.8	5.6	5.6	0.474
Ciprofloxacin	55.3	7.9	36.8	40	5	55	72.2	11.1	16.7	0.020
Levofloxacin	60.6	2.6	36.8	45	0	55	77.7	5.6	16.7	0.020
Moxifloxacin	63.2	0	36.8	45	0	55	83.3	0	16.7	0.020
Clindamycin	57.9	0	42.1	40	0	60	77.8	0	22.2	0.025
Erythromycin	57.9	0	42.1	40	0	60	77.8	0	22.2	0.025
Linezolid	100	0	0	100	0	0	100	0	0	N
Vancomycin	100	0	0	100	0	0	100	0	0	N
Tetracycline	65.8	0	34.2	65	0	35	66.7	0	33.3	1.000
Tigecycline	100	0	0	100	0	0	100	0	0	N

Notes: *Comparison of antimicrobial resistance rates between MRSA-SCVs and MSSA-SCV isolates.
Abbreviations: S, susceptible; I, intermediate; R, resistant; N, no result.

resistance rates were observed for other antibiotics, including gentamicin (26.3%), tetracycline (34.2%), rifampin (2.6%), ciprofloxacin (36.8%), levofloxacin (36.8%), and moxifloxacin (36.8%). Methicillin resistance was observed in 52.6% (20/38) of *S. aureus* SCVs, with the remaining (18/38) being MSSA-SCVs. Compared to MSSA-SCV strains, MRSA-SCV strains demonstrated significantly higher resistance rates to clindamycin, erythromycin,ciprofloxacin, levofloxacin, moxifloxacin and oxacillin ($P<0.05$) (Table 2). Additionally, it should be noted that all ST764-t1084 MRSA-SCVs were resistant to penicillin, oxacillin, gentamicin, ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, clindamycin, and tetracycline. ST764 *S. aureus* SCVs showed high resistance to multiple antibiotics, whereas ST72 and ST9 remained susceptible to all tested antibiotics (Figure 3).

Detection of Virulence and Resistance Genes

The distribution of virulence genes among the isolated *S. aureus* SCVs is summarized in Table 3 and Figure 3, demonstrating that most *S. aureus* SCVs co-harbored multiple virulence determinants. The analysis revealed universal positivity (100%) for *icaD, hla, hlg, and fnbA*, whereas other virulence genes, including *icaA, icaC, clfB, cna* and *pvl*,

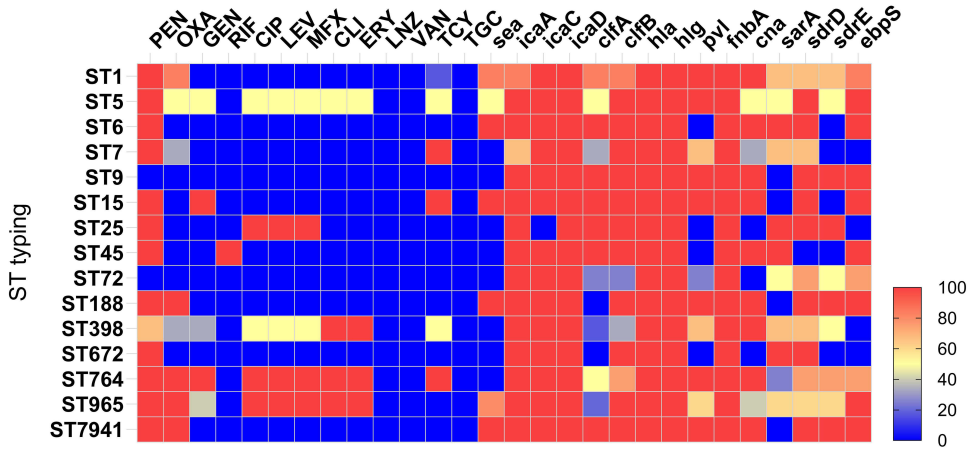

Figure 3 Heatmap of antimicrobial resistance rates and virulence gene carrier rates in different ST *S. aureus* SCV isolates.
Abbreviations: PEN, penicillin; OXA, oxacillin; GEN, gentamicin; RIF, rifampin; CIP, ciprofloxacin; LEV, levofloxacin; MFX, moxifloxacin; CLI, clindamycin; ERY, erythromycin; LNZ, linezolid; VAN, vancomycin; TCY, tetracycline; TGC, tigecycline.

Table 3 Carrying Rates of Virulence Genes in *S. aureus* SCV Isolates

Virulence Gene	Total n (%)	MRSA-SCVs n (%)	MSSA-SCVs n (%)	*P*-value*
Sea	14 (36.8)	11 (55)	3 (16.7)	0.020
icaA	36 (94.7)	19 (95)	17 (94.4)	1.000
icaC	37 (97.4)	20 (100)	17 (94.4)	0.474
icaD	38 (100)	20 (100)	18 (100)	N
clfA	18 (47.4)	10 (50)	8 (44.4)	0.757
clfB	29 (76.3)	18 (90)	11 (61.1)	0.058
hla	38 (100)	20 (100)	18 (100)	N
hlg	38 (100)	20 (100)	18 (100)	N
pvl	27 (71.1)	18 (90)	9 (50)	0.011
fnbA	38 (100)	20 (100)	18 (100)	N
cna	27 (71.1)	18 (90)	9 (50)	0.011
sarA	21 (55.3)	10 (50)	11 (61.1)	0.532
sdrD	28 (73.7)	13 (65)	15 (83.3)	0.278
sdrE	20 (52.6)	14 (70)	6 (33.3)	0.050
ebpS	24 (63.2)	15 (75)	9 (50)	0.179

Notes: *Comparison of virulence gene carriage rates between MRSA-SCVs and MSSA-SCV isolates.
Abbreviation: N, no result.

showed positivity rates exceeding 70%. In contrast, the enterotoxin gene *sea* and immune evasion-associated gene *sdrE* exhibited lower positivity rates of 36.8% and 52.6%, respectively. Notably, MRSA-SCVs displayed significantly higher positivity rates for *cna, sea*, and *pvl* than their MSSA-SCV counterparts ($P<0.05$), with no other statistically significant differences observed. Furthermore, strain typing analysis revealed distinct genotype-specific patterns: *pvl* was predominantly associated with the ST1, ST5, and ST764 lineages, whereas *sarA* and *sdrD* genes were primarily detected in the ST6, ST25, and ST672 strains.

Biofilm Formation Capacity of S. aureus SCV Isolates

All *S. aureus* SCVs obtained in this study exhibited biofilm production, as observed by crystal violet staining. Among these, 25 isolates (65.8%) exhibited strong biofilm-forming capacity, 10 isolates (26.3%) showed moderate biofilm formation, and the remaining isolates (7.9%) displayed weak biofilm formation (Table 4). No significant difference in biofilm-forming capacity was observed between MRSA-SCVs and MSSA-SCVs.

Clinical Characteristics

Clinical data from *P. aeruginosa* pneumonia were extracted using the HIS. *S. aureus* SCV carriers showed significant differences in epidemiological and clinical features compared with non-carriers (Table 5). Patients with *P. aeruginosa* pneumonia coinfected with *S. aureus* SCVs experienced significantly longer hospital stays and antimicrobial therapy duration than those without SCVs ($P = 0.010$ and 0.002, respectively). *S. aureus* SCV carriers also required fluoroquinolone or vancomycin treatment more frequently ($P = 0.022$). However, no significant differences were observed between the two groups in terms of sex ($P = 0.156$), age ($P = 0.071$), PCT level ($P = 0.673$), or prevalence of underlying conditions, such as diabetes ($P = 0.082$) and hypertension ($P = 0.100$).

Table 4 Biofilm Formation of *S. aureus* SCV Isolates

Biofilm-Forming Capacity	Total n (%)	MRSA-SCVs n (%)	MSSA-SCVs n (%)	*P*-value*
None/Weak	3 (7.9)	1 (5)	2 (11.1)	0.595
Moderate	10 (26.3)	5 (25)	5 (27.8)	1.000
Strong	25 (65.8)	14 (70)	11 (61.1)	0.734

Notes: *Comparison of different biofilm formation rates between MRSA-SCVs and MSSA-SCV isolates.

Table 5 Epidemiological and Clinical Characteristics of Study Patients

Variable	Patients with SCVs (n=38)	Patients Without SCVs (n=38)	*P*-value
Age, years	71(61, 79)	63(53, 74)	0.071
Female, no. (%)	11(28.9%)	18(47.4%)	0.156
PCT (ng/mL)	0.09(0.05, 0.23)	0.07(0.04, 0.20)	0.673
Hb (g/L)	116.0(102.8, 132.5)	115(89.3, 129.8)	0.855
CRP (nmol/L)	8.12(5.38, 17.04)	17.78(8.47, 35.99)	0.012
Blood WBC (*10^9/L)	7.96(6.51, 9.27)	6.90(5.87, 10.00)	0.638
Blood PMN (%)	71.55(65.30, 75.73)	72.15(64.53, 82.60)	0.497
LY%	17.40(12.13, 24.13)	17.15(7.63, 24.50)	0.670
Hospitalization days	27(16, 100)	18(12, 28)	0.010
Antimicrobial exposure days	18(12, 42)	13(7, 18)	0.002
No. (%) with hypertension	19(50.0%)	11(28.9%)	0.100
No. (%) with diabetes	11(28.9%)	4(10.5%)	0.082
No. (%) with cancer	3(7.9%)	3(7.9%)	1.000
No. (%) who smoke	4(10.5%)	5(13.2%)	1.000
No. (%) with COPD	7(18.4%)	4(10.5%)	0.516
No. (%) with heart disease	6(15.8%)	1(2.6%)	0.108
No. (%) using FQ/VAN	16(42.1%)	6(15.8%)	0.022

Notes: Data are presented as median (interquartile range) or n (%).
Abbreviations: PCT, procalcitonin; Hb, hemoglobin; CRP, C-reactive protein; WBC, white blood cell; PMN, polymorphonuclear neutrophil; LY, lymphocyte; COPD, chronic obstructive pulmonary disease; FQ, fluoroquinolone; VAN, vancomycin.

Discussion

In this study, we isolated 38 *S. aureus* SCVs from 1,832 lower respiratory tract specimens obtained from patients with *P. aeruginosa* pneumonia, yielding a detection rate of 2.1%. Similarly, Min[10] screened three *S. aureus* SCVs from 278 *S. aureus* isolates obtained from wound specimens, with a detection rate of 1.1%. Zheng[22] identified three stable *S. aureus* SCVs from 41 rifampin-resistant *S. aureus* isolates, with a detection rate of 7.3%. The higher detection rate among rifampin-resistant *S. aureus* may be attributed to mutations in the *rpoB* gene, which reduce the transcription of tricarboxylic acid (TCA) cycle and oxidative phosphorylation-related genes, forcing the bacteria to rely on fermentation pathways and thereby adopting SCV characteristics. Cervantes-García[23] detected four *S. aureus* SCVs from 47 pus samples of diabetic foot ulcer patients infected with *S. aureus*, with a detection rate of 8.5%. Further investigation revealed that all four patients carrying *S. aureus* SCVs had received multiple antibiotic treatments, including trimethoprim-sulfamethoxazole, for one month or longer. We propose that the variation in detection rates of *S. aureus* SCVs across different studies may be linked to its survival environment. When *S. aureus* is exposed to adverse conditions, such as prolonged antibiotic exposure, the small-colony variant phenotype may provide a survival advantage for it. Additionally, we investigated the carriage rate of *S. aureus* SCVs in patients with *P. aeruginosa* pneumonia in Southeast China, which distinguishes our study from previous research in terms of population and geographic location. The carriage rate of S. aureus SCVs may vary across different populations and regions.

Among the *S. aureus* SCVs isolated, ST1-t128 (13.2%, 5/38) were the predominant clones. The ST1 lineage, originally prevalent in North America (the United States and Canada) before spreading to Europe and other regions, had not been previously reported as circulating in China.[24,25] Fujian Province's status as a southeastern coastal region with extensive exchanges with North America may explain ST1 emergence in this area. Notably, ST1 represents one of the major community-associated MRSA (CA-MRSA) clones.[25] Unlike multidrug-resistant hospital-associated MRSA (HA-MRSA) strains such as ST5 and ST239, ST1 typically exhibits a narrower resistance profile. Our study confirmed this pattern, with ST1 *S. aureus* SCVs showing resistance only to penicillin and oxacillin, while remaining susceptible to the other tested antibiotics.

Globally, the ST5 lineage is one of the most widely disseminated HA-MRSA lineages. ST764 is a single-locus variant of the ST5 HA-MRSA lineage, exhibiting characteristics of community-associated MRSA, and was first identified in Japan in 2006.[26] The ST764 clone has become increasingly prevalent in China, Japan, and other Asian regions, which

may be associated with its strong biofilm formation and cell adhesion capabilities.[27] Our study found that ST764-t1084 is resistant to all tested antibiotics, including penicillin, oxacillin, gentamicin, ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, clindamycin, and tetracycline. These results highlight the necessity for increased surveillance of ST764-t1084 *S. aureus* SCVs in the clinical setting.

PVL, encoded by the *lukF-PV* and *lukS-PV* genes, forms pores in target cell membranes leading to membrane damage and subsequent leukocyte necrosis.[28] The ST1 strain of *S. aureus* frequently carries *PVL* and represents a highly virulent form of CA-MRSA. *PVL*-mediated destruction of white blood cells and cytokine storms by ST1 *S. aureus* are key factors in necrotizing pneumonia.[29] In our study, 71.1% of *S. aureus* SCVs carried *PVL* genes, with 100% carriage among ST1 isolates. Previous research has shown that *PVL*-positive MRSA strains can evade host immunity and induce keratinocyte apoptosis, thereby promoting local inflammatory spread.[30] However, some studies found no correlation between *PVL* production levels and clinical infection severity, indicating the need for further investigation into *PVL*'s role in disease progression.[31] The intercellular adhesion gene cluster (*icaADBC*) collectively encodes enzymes for the polysaccharide intercellular adhesin (PIA) synthesis essential for biofilm formation, while *icaR* encodes a repressor protein regulating *icaADBC* expression.[32] In this study, the positivity rates of *icaA, icaC, and icaD* genes in *S. aureus* SCVs all reached 90.0% or higher. However, in normal-phenotype *S. aureus* infections, the positivity rates of *icaA* and *icaD* genes were only 77.6%.[33] This suggests that, compared to normal-phenotype *S. aureus*, the high expression of the intercellular adhesion gene cluster *icaADBC* may contribute to the strong biofilm-forming capacity of *S. aureus* SCVs. The *cna* gene encodes a collagen-binding protein that mediates bacterial adhesion. Among our SCVs, the *cna* positivity rate was 71.1%, with a significantly higher prevalence in MRSA-SCVs than in MSSA-SCVs ($P = 0.011$). The hemolysins encoded by *hla* and *hlg* genes can penetrate eukaryotic membranes and lyse target cells. In our SCVs, both *hla* and *hlg* genes showed 100.0% positivity rates, that is, markedly higher than the 83.5% and 69.5% rates observed in normal-phenotype *S. aureus*.[20] Additionally, the immune evasion-related gene *sdrD* demonstrated a positivity rate of 73.7%, exceeding the 63.5% rate reported for normal-phenotype strains in previous studies.[20] The elevated expression of these virulence factors in *S. aureus* SCVs likely plays a crucial role in their pathogenic processes, including adhesion, invasion, and host immune evasion.

Finally, *S. aureus* SCVs displayed enhanced biofilm-forming capabilities. Our data showed that 92.1% of *S. aureus* SCVs exhibited moderate to strong biofilm production, with 65.8% of isolates demonstrating particularly robust biofilm formation. This pronounced biofilm-forming capacity may contribute to chronic and persistent infections associated with SCVs. Through analysis of clinical data from the Hospital Information System, we observed that *P. aeruginosa* pneumonia patients co-infected with *S. aureus* SCVs experienced significantly prolonged hospitalization and required extended courses of antimicrobial therapy. These findings suggest that *S. aureus* SCVs may play a critical role in sustaining persistent pulmonary infections in *P. aeruginosa* pneumonia patients.

In China and other countries, routine screening through specialized methods such as chromogenic media for *S. aureus* SCVs may be beneficial but substantially increase patient treatment costs. Extended culture duration may represent a cost-effective alternative for detecting *S. aureus* SCVs. In future studies, we will employ whole-genome sequencing to elucidate the formation mechanisms of *S. aureus* SCVs in patients with *P. aeruginosa* pneumonia, which will contribute to improving the diagnosis and treatment of these patients, particularly those with refractory infections.

Conclusion

In Fujian, China, *S. aureus* SCVs were detected in 2.1% of lower respiratory tract specimens from *P. aeruginosa* pneumonia cases, representing missed coinfection in clinical practice. Among these *S. aureus* SCVs, the ST1-t128 type was predominant and most strains carried the *PVL* gene. The ST1 *S. aureus* SCVs showed susceptibility to all tested antibiotics except penicillin and oxacillin. All ST764-t1084 MRSA-SCVs exhibited pan-resistance to tetracycline, β-Lactam antibiotics (penicillin and oxacillin), fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin), and macrolide-lincosamides (erythromycin/clindamycin), highlighting the critical need for clinical vigilance. Notably, the strong biofilm-forming ability of *S. aureus* SCVs may be associated with prolonged treatment duration and extended hospitalization.

There were some limitations in this study. (1) Although we detected previously overlooked *S. aureus* SCVs in patients with *P. aeruginosa* pneumonia at two tertiary hospitals in Fujian Province, our findings are intended primarily as a reference for clinicians and microbiologists in other regions managing refractory *P. aeruginosa* pneumonia. (2) Future studies utilizing whole-genome sequencing and animal models to elucidate the mechanisms underlying the formation and persistent colonization of *S. aureus* SCVs in the lower respiratory tract of these patients will be crucial for alleviating disease burden.

Data Sharing Statement

Data will be available upon reasonable request from the corresponding author (Bin Yang, Email: yangbin2864@163.com).

Ethics Approval and Consent to Participate

This study exclusively involved bacterial isolates and did not include any human or animal subjects. It utilized only anonymized residual clinical samples from routine hospital laboratory tests and was approved by the Ethics Committee of the First Affiliated Hospital of Fujian Medical University and Fuzhou Pulmonary Hospital of Fujian Province. As this was a retrospective study, the requirement for informed consent was waived by the ethics committee.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Zhou S, Rao Y, Li J, Huang Q, Rao X. Staphylococcus aureus small-colony variants: formation, infection, and treatment. *Microbiol Res*. 2022;260:127040. doi:10.1016/j.micres.2022.127040
2. 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. doi:10.1038/nrmicro1384
3. Jung N, Rieg S. Essentials in the management of S. aureus bloodstream infection. *Infection*. 2018;46(4):441–442. doi:10.1007/s15010-018-1130-8
4. Mapar M, Rydzak T, Hommes JW, Surewaard BGJ, Lewis IA. Diverse molecular mechanisms underpinning staphylococcus aureus small colony variants. *Trends Microbiol*. 2025;33(2):223–232. doi:10.1016/j.tim.2024.09.007
5. Liu S, Chen HB, Chen J, et al. Transcriptome and proteome of methicillin-resistant staphylococcus aureus small-colony variants reveal changed metabolism and increased immune evasion. *Microbiol Spectr*. 2023;11(2):e01898–22. doi:10.1128/spectrum.01898-22
6. Chen J, Zhou H, Huang J, Zhang R, Rao X. Virulence alterations in staphylococcus aureus upon treatment with the sub-inhibitory concentrations of antibiotics. *J Adv Res*. 2021;31:165–175. doi:10.1016/j.jare.2021.01.008
7. Kriegeskorte A, Lorè NI, Bragonzi A, et al. Thymidine-dependent staphylococcus aureus small-colony variants are induced by trimethoprim-sulfamethoxazole (SXT) and have increased fitness during SXT challenge. *Antimicrob Agents Chemother*. 2015;59(12):7265–7272. doi:10.1128/aac.00742-15
8. Cai Y, Huang C, Chen X, et al. The role of *Staphylococcus aureus* small colony variants in intraosseous invasion and colonization in periprosthetic joint infection. *Bone Joint Res*. 2022;11(12):843–853. doi:10.1302/2046-3758.1112.BJR-2021-0590.R1
9. De Souza DC, Cogo LL, Dalla-Costa LM, et al. Emergence of thymidine-dependent staphylococcus aureus small-colony variants in cystic fibrosis patients in Southern Brazil. *Microbiol Spectr*. 2021;9(1):11.e00614–21. doi:10.1128/spectrum.00614-21
10. Min C, Wang H, Xia F, et al. Characteristics of staphylococcus aureus small colony variants isolated from wound specimen of a tertiary care hospital in China. *J Clin Lab Anal*. 2022;36(1):e24121. doi:10.1002/jcla.24121
11. Watanabe Y, Oikawa N, Hariu M, Seki M. Evaluation of agar culture plates to efficiently identify small colony variants of methicillin-resistant staphylococcus aureus. *Infect Drug Resist*. 2019;12:1743–1748. doi:10.2147/IDR.S207057
12. Kipp F, Kahl BC, Becker K, et al. Evaluation of two chromogenic agar media for recovery and identification of *Staphylococcus aureus* small-colony variants. *J Clin Microbiol*. 2005;43(4):1956–1959. doi:10.1128/jcm.43.4.1956-1959.2005
13. Wong Fok Lung T, Monk IR, Acker KP, et al. Staphylococcus aureus small colony variants impair host immunity by activating host cell glycolysis and inducing necroptosis. *Nature Microbiology*. 2019;5(1):141–153. doi:10.1038/s41564-019-0597-0
14. Hoffman LR, Déziel E, D'Argenio DA, et al. Selection for *Staphylococcus aureus* small-colony variants due to growth in the presence of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci*. 2006;103(52):19890–19895. doi:10.1073/pnas.0606756104
15. Becker K. Detection, identification and diagnostic characterization of the staphylococcal small colony-variant (SCV) phenotype. *Antibiotics*. 2023;12(9):1446. doi:10.3390/antibiotics12091446

16. Luo C, Chen Y, Hu X, et al. Genetic and functional analysis of the *pks* gene in clinical *Klebsiella pneumoniae* isolates. *Microbiol Spectr*. 2023;11(4):e00174–23. doi:10.1128/spectrum.00174-23
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. doi:10.1128/JCM.38.3.1008-1015.2000
18. Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, 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. doi:10.1128/JCM.42.2.792-799.2004
19. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 30th. In: *CLSI Supplement M100*. PA: CLSI; 2020.
20. Titouche Y, Akkou M, Campaña-Burguet A, et al. Phenotypic and genotypic characterization of staphylococcus aureus isolated from nasal samples of healthy dairy goats in Algeria. *Pathogens*. 2024;13(5):408. doi:10.3390/pathogens13050408
21. Gu J, Xiong M, Zhang J, Li Y. Prevalence and characterization of community-associated Staphylococcus aureus isolates from human mastitis in Beijing, China. *Int J Med Microbiol*. 2024;315:151623. doi:10.1016/j.ijmm.2024.151623
22. Zheng X, Fang R, Wang C, et al. Resistance profiles and biological characteristics of rifampicin-resistant staphylococcus aureus small-colony variants. *Infect Drug Resist*. 2021;14:1527–1536. doi:10.2147/IDR.S301863
23. Cervantes-García E, García-Gonzalez R, Reyes-Torres A, Resendiz-Albor AA, Salazar-Schettino PM. Staphylococcus aureus small colony variants in diabetic foot infections. *Diabetic Foot & Ankle*. 2015;6:26431. doi:10.3402/dfa.v6.26431
24. Golding GR, Levett PN, McDonald RR, et al. High rates of *Staphylococcus aureus* USA400 infection, Northern Canada. *Emerg Infect Dis*. 2011;17(4):722–725. doi:10.3201/eid1704.100482
25. David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev*. 2010;23(3):616–687. doi:10.1128/CMR.00081-09
26. Zaraket H, Otsuka T, Saito K, et al. Molecular characterization of methicillin-resistant staphylococcus aureus in hospitals in Niigata, Japan: divergence and transmission. *Microbiol Immunol*. 2007;51(2):171–176. doi:10.1111/j.1348-0421.2007.tb03898.x
27. Xiao Y, Han W, Wang B, et al. Phylogenetic analysis and virulence characteristics of methicillin-resistant Staphylococcus aureus ST764-SCC mec II: an emerging hypervirulent clone ST764-t1084 in China. *Emerging Microbes Infect*. 2023;12(1):2165969. doi:10.1080/22221751.2023.2165969
28. Balakirski G, Hischebeth G, Altengarten J, et al. Recurrent mucocutaneous infections caused by PVL-positive *Staphylococcus aureus* strains: a challenge in clinical practice. *JDDG J Dtsch Dermatol Ges*. 2020;18(4):315–322. doi:10.1111/ddg.14058
29. Diep BA, Chan L, Tattevin P, et al. Polymorphonuclear leukocytes mediate *Staphylococcus aureus* Panton-Valentine leukocidin-induced lung inflammation and injury. *Proc Natl Acad Sci*. 2010;107(12):5587–5592. doi:10.1073/pnas.0912403107
30. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev*. 2015;28(3):603–661. doi:10.1128/CMR.00134-14
31. McGuire E, Neill C, Collin SM, et al. Is panton–valentine leucocidin (PVL) toxin associated with poor clinical outcomes in patients with community-acquired staphylococcus aureus bacteraemia? *J Med Microbiol*. 2023;72(4):001683. doi:10.1099/jmm.0.001683
32. Fluckiger U, Ulrich M, Steinhuber A, et al. Biofilm formation, *icaADBC* transcription, and polysaccharide intercellular adhesin synthesis by staphylococci in a device-related infection model. *Infect Immun*. 2005;73(3):1811–1819. doi:10.1128/IAI.73.3.1811-1819.2005
33. Omidi M, Firoozeh F, Saffari M, Sedaghat H, Zibaei M, Khaledi A. Ability of biofilm production and molecular analysis of spa and ica genes among clinical isolates of methicillin-resistant Staphylococcus aureus. *BMC Res Notes*. 2020;13(1):19. doi:10.1186/s13104-020-4885-9