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ORIGINAL RESEARCH

ST7 Becomes One of the Most Common *Staphylococcus aureus* Clones After the COVID-19 Epidemic in the City of Wuhan, China

Jihong Gu[1,*], Shucheng Shen[1,*], Mengyuan Xiong[1,2], Jin Zhao[1,2], Hongpan Tian[1], Xiao Xiao[1,3], Yirong Li[1–3]

[1]Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan, People's Republic of China; [2]Wuhan Research Center for Infectious Diseases and Tumors of the Chinese Academy of Medical Sciences, Wuhan, People's Republic of China; [3]Hubei Engineering Center for Infectious Disease Prevention, Control and Treatment, Wuhan, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yirong Li, Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan, 430061, People's Republic of China, Tel +8618602718052, Email liyirong838@163.com

Background: *Staphylococcus aureus* (*S. aureus*) was able to rapidly evolve and adapt under the pressure of antibiotics, host immune and environmental change. After Corona Virus Disease 2019 (COVID-19) epidemic in Wuhan, China, a large number of disinfectants were used, which might result in rapid evolution of *S. aureus*.

Methods: A total of 619 *S. aureus* isolates were collected from Zhongnan Hospital, Wuhan University from 2018 to 2021, including group BEFORE (309 strains collected before COVID-19 pandemic) and group AFTER (310 strains collected after COVID-19 pandemic), for comparing the changes of molecular epidemiology. The molecular characteristics of isolates were analyzed by Multi-locus sequence typing (MLST), spa, chromosomal cassette *mec* (SCCmec) typing, virulence genes were screened by the PCR, antibiotic susceptibility test was carried out by the VITEK system.

Results: Thirty-six sequence types (STs) belonging to 14 clone complexes (CCs) were identified. ST5 was the most prevalent clone in both groups, and ST7, ranking the sixth in group BEFORE, became the second dominant clone in group AFTER (6.5% vs 10.0%), whereas ST239 decreased from the seventh to the fourteenth (5.8% vs 1.9%). ST7 in group AFTER had a higher positive rate of virulence genes, including *hlb, fnbB, seb, lukDE, sdrE* and the proportion of ST7-t091 MRSA strains increased from 19.1% to 50% compared with group BEFORE. Though no significant difference of MRSA proportion was found between two groups, SCCmec type-III in group AFTER decreased *(p<0.01)*. Though the rate of multidrug-resistance (MDR) decreased, the virulence genes *hlb, hlg, fnbB, seb* and *pvl* carrying rates were significantly elevated in MRSA strains of group AFTER.

Conclusion: After COVID-19 pandemic, ST7 becomes one of the predominant *S. aureus* clones in Wuhan and the carrying rate of SCCmec and virulence genes is on the rise. Therefore, it is essential to strengthen the surveillance of ST7 *S. aureus* clone.

Keywords: *Staphylococcus aureus*, molecular characterization, antimicrobial resistance, virulence gene, disinfectant

Introduction

Staphylococcus aureus (*S. aureus*), a major human opportunistic pathogen, can cause a variety of infectious diseases including skin and soft-tissue infections, bacteremia, ventilator-associated pneumonia and toxic shock syndrome.[1,2] A rapid and proper administration of antibiotics is the most important means to inhibit *S. aureus*' growth and prevent disease progression. Under selective pressure of antibiotics, *S. aureus* can rapidly evolve and adapt with the help of gene mutation or horizontal gene transfer (HGT), leading to the emergence of multidrug-resistance (MDR).[3,4] The most notorious MDR *S. aureus* was Methicillin-resistant *Staphylococcus aureus* (MRSA), which was first found in the United Kingdom in 1961, two years after the administration of Methicillin. To date, it is still a MDR bacteria puzzling global doctors. MRSA in most cases accounts for at least 25% to 50% of *S. aureus* infections in hospital settings and mortalities of 33% was reported for MRSA.[5,6] MRSA infections are responsible for longer hospital stays, increased hospital costs, and poorer outcomes compared to methicillin-sensitive *S. aureus*

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(MSSA) infections.[7] Moreover, MRSA clones have been reported to be associated with a certain of molecular type.[8–10] ST239-t030, ST5-t2460 and ST59-t437 isolates were most found to be MRSA, whereas ST188-t189 and ST7-t091 isolates were predominant MSSA clones,[4,11–13] which suggests that molecular characteristics surveillance contributes to the empirical use of antibiotics in patients with *S. aureus* infection.

In addition to the selective pressure of antibiotics, host immune system, especially innate immunity system, can encircle and intercept, and finally kill *S. aureus*, which prevents the progress of infection. Cells involved in innate immunity include neutrophils, monocytes, macrophages, and NK cells, which have specific receptors on their surface, so-called pattern recognition receptors (PRRs), for recognizing bacteria following by the clear of bacteria.[14,15] *S. aureus* consists of a variety of virulent proteins, which promotes the adhesion and invasion to host cells, and the evasion of innate immune responses. Fibronectin-binding protein A (*fnbA*), fibronectin-binding protein B (*fnbB*), clumping factor A (*clfA*) and clumping factor B (*clfB*) push *S. aureus* to bind to host cells at the initial stage of infection.[16] Cytolytic toxins including hemolysin and Panton-Valentine Leukocidin (*pvl*) can lyse host cells, causing the release and spread of *S. aureus*, and even tissue damage.[17] More than 70% of *S. aureus* isolates are exotoxin-producing strains, such as staphylococcal enterotoxins A, B, and C (*sea, seb, sec*), which can act as superantigens to penetrate the epidermal barrier and exacerbate the inflammatory process.[14] Recent studies showed imbalanced cell-mediated immune response was found in severe and critical Coronavirus disease 2019 (COVID-19) patients,[18,19] but it was not clear whether the immune imbalance influences on the virulence of *S. aureus*.

Stress from the environment can also cause *S. aureus* to adapt and evolve rapidly. Recent studies showed that widely used benzalkonium chloride disinfectants promote antibiotic resistance due to co-selection.[20] Studies have shown that diluted or residual disinfectants in the environment can increase bacterial resistance through phenotypic adaptation, genetic mutation and HGT.[21] One study reported that high concentration and frequent application of disinfection increase the detection of MRSA infections in psychiatric hospitals during the COVID-19 pandemic.[22] And one study indicated that the molecular characteristics of the epidemic strains in Kunming had changed significantly since the COVID-19 epidemic.[23] These studies have a common limitation; the sample size is small and type II errors can easily occur. Patients with COVID-19 were firstly identified in Wuhan, and as of March 2020, 2000 tons of disinfectants have been used in this region,[24,25] therefore, we collected a total of 619 *S. aureus* isolates before and after the extensive use of disinfectants in a tertiary hospital in Wuhan, for comparing the changes of molecular typing, antimicrobial resistance and virulence factors.

The molecular characteristics of these isolates were analysed by Multi-locus sequence typing (MLST), spa, SCCmec typing, whereas virulence genes were screened by the PCR assay, and the antibiotic susceptibility tests were carried out by the VITEK system.

Materials and Methods

Bacterial Isolates

A total of 619 non-repetitive *S. aureus* isolates were collected in Zhongnan Hospital, Wuhan University, during the period from 2018 to 2021, including group BEFORE (309 strains collected before COVID-19 pandemic) and group AFTER (310 strains collected after COVID-19 pandemic). These strains were isolated from sputum, alveolar lavage fluid, pus, wound secretions, blood, urine, catheter tips and other clinical specimens. Repeated strains of the same patient were excluded and only the first isolated strains were included. All isolates were initially identified by colony morphology, gram staining, coagulase tests at the clinical microbiology laboratory, and then MALDI-TOF/MS (VITEK MS, bioMérieux) was used to further confirm. All isolates were stored in Tryptic Soy Broth (TSB) with glycerol at −80°C for later study.

S. aureus DNA Extraction

The stored *S. aureus* isolates were streaked on blood agar and incubated at 37°C overnight, and a single colony was transferred into 5mL TSB medium and incubated at 37°C overnight at 220 rpm. *S. aureus* pellets were then suspended in 200 μL of buffer containing 10 μg of lysostaphin (Coolaber, China) at a concentration of 1mg/mL. After incubation at 37°C for 30 minutes, DNA was extracted using a rapid DNA extraction kit (Aidlab, China) according to the

manufacturer's protocol. DNA quantity and purity were determined using the NanoDrop 2000 (Thermo Fisher Scientific, USA). All of the extracted DNAs were stored at −20° for future experiments.

Molecular Typing

Molecular characteristics of all *S. aureus* isolates were analyzed by Staphylococcal protein A (spa) typing and Multi-locus sequence typing (MLST). The reaction conditions and primers required for PCR were similar to previous study.[26] PCR products were then subject to sanger sequencing (Tsingke Biotechnology, China). The resulting sequences were compared with the existing sequences available on the MLST website (http://www.pubmlst.net) for *S. aureus*, which designated sequence type (ST) for *S. aureus*. The clone complex (CC) was analyzed through the eBURST. The spa typing was based on variations in the polymorphic X region of the staphylococcal protein A (*spa*) gene. The X region of each isolate was amplified using PCR and sequenced with DNA sequencing (Tsingke Biotechnology, China). The sequence data of the amplified products were submitted to the website (http://www.spaserver.ridom.de) to obtain the spa type for each isolate.[27] Primers used for MLST and spa typing were shown in the Supplementary Table S1. SCCmec is a mobile genetic element, which is the site of insertion and integration of antibiotic resistance genes. *S. aureus* usually develops resistance to methicillin by acquiring SCCmec elements. A multiplex PCR assay was used to analyze MRSA SCCmec I–V types. Primers and reaction conditions required for PCR, and interpretation of results were as described in the literature.[28]

Screening for Virulence Factors

All isolates were screened by a conventional PCR assay for the following 18 staphylococcal virulence factors: hemolysin genes (*hla, hlb, hld, hlg*), adhesion genes (*clfA, clfB, fnbA, fnbB*), enterotoxin gene (*sea, seb, sec*), exfoliative gene (*eta, etb*), Panton-Valentine Leukocidin (*pvl), lukED, sdrC, sdrD, sdrE*. The annealing temperature and elongation time were determined according to the primer and product length. Some of the virulence gene primers were from the references.[4] Primers used for PCR were shown in the Supplementary Table S1.[4,16] All PCR products were analyzed by 1.5% agarose gel electrophoresis.

Antimicrobial Susceptibility Testing

The antibiotic susceptibility testing of all isolates in this study was performed on a VITEK system (BioMérieux, Marcy l′ Etoile, France) according to manufacturer's instructions. Results were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.[29] The antibiotics tested in the present study were as follows: levofloxacin (LEV), gentamicin (GEN), ciprofloxacin (CIP), vancomycin (VAN), erythromycin (ERY), clindamycin (CLI), linezolid (LZD), tigecycline (TGC), moxifloxacin (MXF), oxacillin (OXA), quinupristin-dalfopristin (QUD), tetracycline (TET), rifampicin (RIF), penicillin (PEN). The MRSA isolates were further identified using PCR to confirm the presence of the *mecA*. ATCC 25923 were used as a quality control strain.

Statistical Analysis

All statistical analyses were performed using the IBM SPSS Statistics 25.0. GraphPad Prism 9.0 software was used to plot the experimental data. The virulence gene profiles and antimicrobial susceptibilities were analyzed using two tailed chi-square test or Fisher's exact test. $p<0.05$ was considered to be statistically significant.

Results

Clinical Characteristics of *S. aureus* Isolates

A total of 619 non-repetitive *S. aureus* isolates were collected from inpatients during the period from 2018 to 2021. Of them, 267 (43.1%) were found to be MRSA and there was no significant difference of MRAS proportion between group BEFORE and group AFTER (42.4% vs 43.9%, $p>0.05$) (Table 1). There was also no significant difference of patient's gender, age and specimen source between two groups (Table 1). These isolates were commonly recovered from the male patients rather than the female (62.7% vs 37.3%, $p<0.05$), most of whom were aged over 20 years old (95.3%, 590/619). In addition, *S. aureus* were mostly isolated from skin and soft tissue specimens (47.0%, 291/619), followed by respiratory system specimens (26.0%, 161/619), blood (14.7%, 91/619) and other specimen sources (12.3%, 76/619).

Table 1 Specimen Information from Inpatients Infected with *S. aureus*

No.	Total n=619	BEFORE n=309	AFTER n=310	*P*-value
Gender				
Male	388 (62.7%)	196 (63.4%)	192 (61.9%)	>0.05
Female	231 (37.3%)	113 (36.6%)	118 (38.1%)	>0.05
Age				
≤20	29 (4.7%)	18 (5.8%)	11 (3.5%)	>0.05
21–64	390 (63.0%)	196 (63.4%)	194 (62.6%)	>0.05
≥65	200 (32.3%)	95 (30.7%)	105 (33.9%)	>0.05
Specimen sources				
Respiratory system	161 (26.0%)	86 (27.8%)	75 (24.2%)	>0.05
Skin/soft tissue	291 (47.0%)	153 (49.5%)	138 (44.5%)	>0.05
Blood	91 (14.7%)	40 (12.9%)	51 (16.5%)	>0.05
Others	76 (12.3%)	30 (9.7%)	46 (14.8%)	>0.05
MRSA	267 (43.1%)	131 (42.4%)	136 (43.9%)	>0.05

Molecular Characteristics of *S. aureus* Isolates

Among the 619 *S. aureus* isolates, 36 STs were identified. ST types identified in both groups included ST1, ST188, ST2315, ST5, ST6, ST306, ST7, ST8, ST72, ST239, ST630, ST15, ST1281, ST22, ST25, ST30, ST59, ST88, ST121, ST398. ST9, ST573, ST764, ST845, ST217, ST954, ST4121, ST1163. ST45 and ST5615 were detected only in group BEFORE, whereas ST6159, ST2112, ST5459 ST308, ST7241 and ST946 were found only in group AFTER. The most predominant ST type was ST5 in two groups, followed by ST188, ST22, ST59 and ST398 in group BEFORE, and ST7, ST22, ST188 and ST398 in group AFTER (Figure 1A and Table 2). ST7, ranking the sixth in group BEFORE, was elevated to the second in group AFTER (6.5% vs 10.0%, $p>0.05$), while ST239 decreased from the seventh to the fourteenth (5.8% vs 1.9%, $p<0.05$). Fourteen CC types were identified according to eBURST, namely CC1, CC5, CC7, CC8, CC15, CC20, CC22, CC25, CC30, CC45, CC59, CC88, CC121, CC398, and the most prevalent CC was CC5 (26.2%, 162/619). When spa typing and MSLT were combined to analyze, the most prevalent clone was ST5-t2460 (11.0%, 34/309), followed by ST188-t189 (9.1%, 28/309), ST22-t309 (8.1%, 25/309), ST59-t437 (4.9%, 15/309), ST239-t030 (4.9%, 15/309), ST1-t127 (3.9%, 12/309) and ST7-t091 (3.6%, 11/309) in group BEFORE. In group AFTER, the

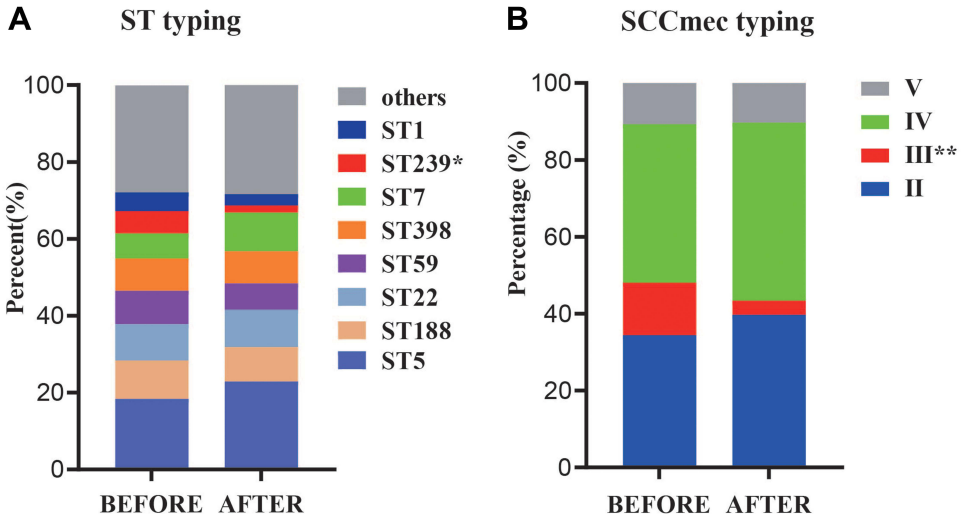

Figure 1 Distribution in Sequence Types (STs) of Staphylococcus aureus isolates between group BEFORE and group AFTER (**A**). SCCmec typing of MRSA isolates between group BEFORE and group AFTER (**B**). *$p < 0.05$, **$p < 0.01$.

Table 2 Molecular Characteristics of *S. aureus* Isolates

CC	ST	spa[a]	BEFORE			AFTER		
			spa (n, %)	MRSA (No.)	SCCmec Typing (No.)	spa (n, %)	MRSA (No.)	SCCmec Typing (No.)
CC1	ST1	t127	12, 3.9	3	IV (2), II (1)	6, 1.9	2	II (1), IV (1)
	ST188	t189	28, 9.1	2	IV (2)	24, 7.7	3	IV (3)
CC5	ST5	t002	3, 1.0	1	II (1)	0, 0.0		
		t2460	34, 11.0	33	II (32), IV (1)	50, 16.1	44	II (41), IV (3)
		t311	4, 1.3	4	II (4)	0, 0.0		
		t548	8, 2.6	1	V (1)	5, 1.6	1	IV (1)
	ST6	t701	9, 2.9			8, 2.6		
		t304	0, 0.0			5, 1.6	1	IV (1)
	ST764	t002	3, 1.0	3	II (3)	0, 0.0		
CC7	ST7	t091	11, 3.6	2	II (1), IV (1)	20, 6.5	10	II (7), IV (3)
		t289	0, 0.0			4, 1.3	1	II (1)
		t796	5, 1.6			0, 0.0		
CC8	ST239	t030	15, 4.9	14	III (13), IV (1)	3, 1.0	3	III (2), IV (1)
		t037	0, 0.0			3, 1.0	3	III (3)
	ST72	t148	0, 0.0			5, 1.6	1	V (1)
	ST630	t377	5, 1.6			5, 1.6	1	V (1)
CC15	ST15	t084	6, 1.9			3, 1.0		
CC20	ST1281	t164	7, 2.3			7, 2.3		
CC22	ST22	t309	25, 8.1	21	IV (20), V (1)	27, 8.7	14	IV (13), II (1)
CC25	ST25	t078	0, 0.0			4, 1.3	2	IV (2)
		t258	0, 0.0			4, 1.3	2	IV (2)
CC45	ST45	t116	3, 1.0	3	IV (3)	0, 0.0		
CC59	ST59	t172	5, 1.6	5	IV (5)	0, 0.0		
		t437	15, 4.9	6	IV (6)	13, 4.2	11	IV (11)
CC88	ST88	t1376	4, 1.3	2	III (1), IV (1)	0, 0.0		
CC398	ST398	t034	9, 2.9	3	V (3)	12, 3.9	3	IV (1), V (2)
		t571	6, 1.9			4, 1.3		

Note: [a]Only *S. aureus* with spa frequency ≥3 was displayed.

most prevalent clone was also ST5-t2460 (16.1%, 50/310), followed by ST22-t309 (8.7%, 27/310), ST188-t189 (7.7%, 24/310), ST7-t091 (6.5%, 20/310), ST59-t437 (4.2%, 13/310), ST398-t034 (3.9%, 12/310) (Table 2). The proportion of ST7-t091 and ST239-t030 changed obviously, especially ST239-t030 has fallen out of the top ten. There was a strong association between some certain spa types and STs, ST188 was associated with t189 (88.1%, 52/59), ST22 was primarily associated mainly with t309 (88.1%, 52/59), and ST1281 was mainly associated with t164 (82.4%, 14/17) (Table 2). There were four SCCmec types, including type II (37.1%, 99/267), III (8.6%, 23/267), IV (43.8%, 117/267), V (10.5%, 28/267). Type III decreased significantly in group AFTER (13.7% vs 3.7%, $p< 0.01$) (Figure 1B). Certain association was observed among specific SCCmec, ST and spa types in MRSA isolates. Most ST5-t2460 were MRSA-II isolates, and ST239-t030 were MRSA-III isolates, whereas most ST188-t189, ST6-t701 and ST1281-t164 were MSSA isolates. In addition, most ST7-t091 in group BEFORE were MSSA (81.9%, 9/11), while half of ST7-t091 in group AFTER were MRSA isolates (50%, 10/20), and there was no significant difference of MRSA proportion in ST7-t091clone between two groups ($p > 0.05$). ST22-t309 was considered as the main MSSA isolate type in previous reports,[4,30,31] but MRSA accounted for 67.3% (35/52) of the isolates in this study (Table 2).

Antimicrobial Resistance

The results of antibiotic susceptibility testing showed in Figure 2. All of isolates were susceptible to VAN, TGC and QUD, whereas the majority of these strains were resistant to PEN (93.4%, 578/619). The resistance rates to remaining antibiotics ranged from 0.2% to 61.5%. There was no significant difference in the resistance rate to tested antibiotics except RIF

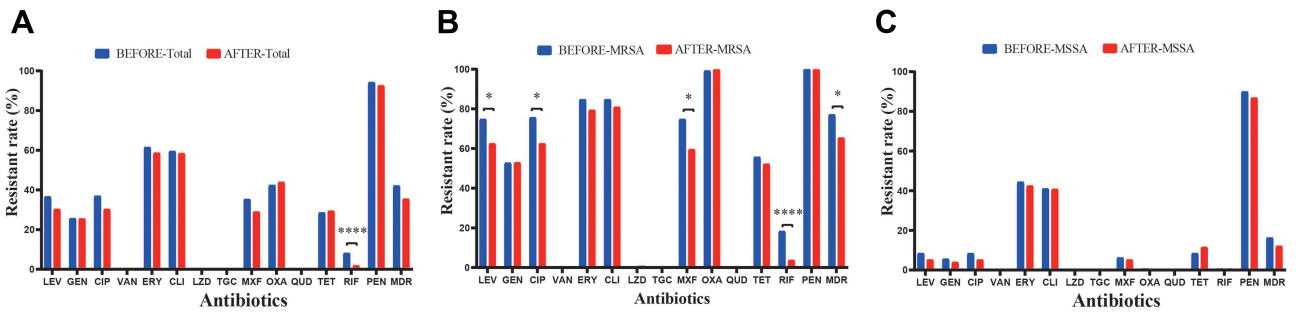

Figure 2 The antimicrobial resistance among BEFORE *S. aureus* isolates were compared with those AFTER *S. aureus* isolates (**A**). The antimicrobial resistance among BEFORE MRSA isolates were compared with those AFTER MRSA isolates (**B**). The antimicrobial resistance among BEFORE MSSA isolates were compared with those AFTER MSSA isolates (**C**). *$p < 0.05$, ****$p < 0.0001$.
Abbreviation: MSSA, Methicillin-sensitive*Staphylococcus aureus*.

between two groups (Figure 2A). The antimicrobial resistance rate to LEV, CIP, MXF and RIF decreased significantly in MRSA strains of group AFTER as well as the prevalence of MDR (77.1%, 65.4%, $p< 0.05$) (Figure 2B). In addition, MRSA isolates had higher resistance rates to most antibiotics and MDR rate than MSSA isolates (Figure 2B and C). Three representative MRSA clones including ST5-II/t2460, ST22-IV/t309 and ST239-III/t030 were selected for further analyzing antimicrobial resistance profiles (ARPs) to 6 non-beta-lactam antibiotics. ARPs included antimicrobial agents which more than 70% MRSA isolates were resistant.[32] It was found that antibiotics GEN was included in the APRs of ST5-II/t2460 clone of group AFTER rather group BEFORE (Table 3).

Virulence Gene Profiles

All isolates contained five detected virulence genes at least, and 97.6% (604/619) isolates have seven or more tested virulence genes. The frequencies of virulence genes among *S. aureus*, MRSA and MSSA isolates in the two groups were shown in Supplementary Table S2. ST22-t309 had a *pvl* carrying rate of 96.2%, while ST7-t091 had a *pvl* carrying rate of only 25.8%. The carrying rates of some hemolysin genes and adhesins were also different among different types of *S. aureus* (Table 4). In addition, the virulence genes *hlb, fnbB, seb, lukDE, sdrE* were found to be significantly higher in ST7 clone of group AFTER (Table 5).

Discussion

S. aureus can invade different organs and systems, causing a wide range of infections, from mild skin and soft tissue infections to life-threatening diseases including infective endocarditis, bacteremia and sepsis.[33] Although the antimicrobial resistance rate of MRSA strains has declined in some countries and regions in recent years, MRSA is still an important pathogen of community and health-care-related infections.[34,35] Studies have shown that diluted or residual disinfectants in the environment can increase bacterial resistance and resistance through phenotypic adaptation, genetic mutation, and horizontal gene transfer (HGT).[21] One study reported that high concentration and frequent application of

Table 3 Antimicrobial Resistance Profiles (APRs) of Some Major MRSA Clones

MRSA (NO.)	GEN	CIP	ERY	CLI	TET	RIF	ARPs
BEFORE-ST5-II/t2460(32)	46.9%	96.9%	93.8%	93.8%	93.8%	6.3%	CIP, ERY, CLI, TET
AFTER-ST5-II/t2460(41)	82.9%	97.6%	100.0%	100.0%	97.6%	2.4%	GEN, CIP, ERY, CLI, TET
BEFORE-ST22-IV/t309 (20)	100.0%	100.0%	100.0%	100.0%	5.0%	0.0%	GEN, CIP, ERY, CLI
AFTER-ST22-IV/t309(13)	84.6%	84.6%	84.6%	84.6%	0.0%	0.0%	GEN, CIP, ERY, CLI
BEFORE-ST239-III/t030 (13)	84.6%	100.0%	69.2%	69.2%	76.9%	100.0%	GEN, CIP, TET, RIF
AFTER- ST239-III/t030(2)	100.0%	100.0%	0.0%	0.0%	100.0%	100.0%	GEN, CIP, TET, RIF

Abbreviations: GEN, gentamicin; CIP, ciprofloxacin; ERY, erythromycin; CLI, clindamycin; TET, tetracycline; RIF, rifampicin.

Table 4 Carrying Rates of Virulence Genes in Main ST-Spa Isolates

Virulence Genes	ST5-t2460 n=84	ST188-t189 n=52	ST7-t091 n=31	ST22-t309 n=52	ST398-t034 n=21
hla	98.8%	100.0%	100.0%	84.6%	57.1%
hlb	98.8%	76.9%	80.6%	96.2%	76.2%
hld	97.6%	90.4%	90.3%	86.5%	90.5%
hlg	84.5%	92.3%	74.2%	100.0%	100.0%
clfA	100.0%	100.0%	100.0%	98.1%	100.0%
clfB	46.4%	80.8%	96.8%	98.1%	95.2%
fnbA	14.3%	3.8%	12.9%	0.0%	0.0%
fnbB	33.3%	38.5%	38.7%	19.2%	66.7%
sea	3.6%	5.8%	0.0%	19.2%	0.0%
seb	38.1%	51.9%	41.9%	71.2%	66.7%
sec	25.0%	13.5%	0.0%	53.8%	0.0%
eta	6.0%	3.8%	3.2%	3.8%	0.0%
etb	7.1%	11.5%	12.9%	1.9%	14.3%
pvl	35.7%	32.7%	25.8%	96.2%	57.1%
lukED	98.8%	100.0%	71.0%	26.9%	28.6%
sdrC	100.0%	11.5%	51.6%	11.5%	4.8%
sdrD	96.4%	15.4%	100.0%	98.1%	4.8%
sdrE	97.6%	92.3%	80.6%	100.0%	90.5%

Table 5 The Frequencies of Virulence Genes Between ST7 and ST239

Virulence Genes	BEFORE-ST7 n=20	AFTER-ST7 n=31	*P*-value[a]	BEFORE-ST239 n=18	AFTER-ST239 n=6	*P*-value[b]
hla	95.0%	100.0%	-	100.0%	100.0%	-
hlb	40.0%	96.8%	<0.001	44.4%	100.0%	0.024
hld	100.0%	87.1%	0.145	100.0%	83.3%	0.250
hlg	75.0%	80.6%	0.897	61.1%	66.7%	1.000
clfA	100.0%	100.0%	-	100.0%	100.0%	-
clfB	100.0%	93.5%	0.514	100.0%	100.0%	-
fnbA	0.0%	12.9%	0.145	77.8%	16.7%	0.015
fnbB	10.0%	48.4%	0.005	5.6%	66.7%	0.006
sea	5.0%	0.0%	-	27.8%	66.7%	0.150
seb	10.0%	54.8%	0.001	11.1%	16.7%	1.000
sec	5.0%	3.2%	1.000	0.0%	0.0%	-
eta	5.0%	0.0%	-	0.0%	0.0%	-
etb	10.0%	9.7%	1.000	0.0%	50.0%	0.010
pvl	20.0%	41.9%	0.105	27.8%	16.7%	1.000
lukED	40.0%	83.9%	0.001	94.4%	100.0%	1.000
sdrC	85.0%	35.5%	0.001	94.4%	100.0%	1.000
sdrD	100.0%	96.8%	1.000	94.4%	100.0%	1.000
sdrE	50.0%	93.5%	0.001	94.4%	83.3%	0.446

Notes: [a]The positive rates of virulence genes among BEFORE ST7 isolates were compared with those among AFTER ST7 isolates. [b]The positive rates of virulence genes among BEFORE ST239 isolates were compared with those among AFTER ST239 isolates.

disinfection increase the detection of MRSA infections in psychiatric hospitals during the COVID-19 pandemic.[22] However, in the present study, it was shown that there was no significant difference of the resistance rate to each antibiotic between group BEFORE and group AFTER as well as MRSA proportion, which was not consistent to the previous study and need to be studied intensively. The antimicrobial resistance rate to LEV, CIP, MXF and RIF decreased significantly in MRSA strains of group AFTER as well as the prevalence of MDR and GEN was included in the APRs of

ST5-II/t2460 clone of group AFTER. These changes in antibiotic sensitivity and resistance information can guide clinical selection of antibiotics in the area.

Molecular typing showed that ST5-t2460 was the most common *S aureus* clone, followed by ST188-t189, ST22-t309, ST59-t437 and ST239-t030 before COVID-19 pandemic in Wuhan. After COVID-19 pandemic, the most frequent type was still ST5-t2460, following by ST22-t309, ST188-t189, ST7-t091 and ST59-t437. An obvious change was that ST239-t030 was not only replace by ST7-t091 in the top five *S aureus* clones, but also fall out of the top ten after COVID-19 pandemic. In our previous studies during the period from 2017 to 2018, ST239-t030 was found to rank second to ST5-t2460,[4,36] which indicating that ST239-t030 clone decreased rapidly in the last four years, and to date, was not one of the most prevalent clones in *S. aureus* isolates in Wuhan. Supporting our result, ST239-t030 was found to significantly decreased from 20.3% to 1% during the period from 2008 to 2017.[35] As the predominant healthcare-associated MRSA (HA-MRSA) clones, ST239 infections are transmitted mainly through hand contact,[35,37] therefore, it is reasonable to speculate that due to the use of large quantities of disinfectants and greater attention to hand disinfection and personal protection during the COVID-19 pandemic, ST239-t030 clones may be less adaptable and competitive when faced with highly selective pressures in health-care settings, which probably lead to the reduction of ST239-t030. An article reported that the dominant molecular type was ST22-t309 in 2019 and 2021, while in 2020, it was ST59-t437 in Kunming.[23] These changes suggest that the possible impact of disinfectant use during the COVID-19 pandemic on the evolution of *S. aureus*.

It is another important to point that ST7 isolates show an increasing trend and becomes the second dominant strain after COVID-19 pandemic. One previous study reported ST7 as the predominant clone in *S. aureus* skin and soft tissue infections isolates from Wenzhou, China, and this clone was rarely found in Chinese isolates before.[38] In our previous study in 2018, although ST7 was found to be one of the most dominant clone, which was found to rank second to ST5, ST239, ST59, and ST188, and associated with MSSA.[36] In the present study, three main subclones were identified and ST7-t091 was the predominant. The MRSA proportion of ST7-t091 increased from 19.1% (before COVID-19 pandemic) to 50% (after COVID-19 pandemic). Moreover, ST7 clone of group AFTER had a higher positive rate of a variety of virulence genes, including *hlb, fnbB, seb, lukDE, sdrE*. These data indicating ST7 isolates have a strong ability to acquire SCCmec element and virulence genes under the pressure of disinfectant agent, which in part interpreted why ST7 became a common *S. aureus* clone after pandemic. A recent study revealed that ST7 was the main reported genotype of *S. aureus* in wholesale and retail pork in Wuhan (detection rate 57.5%) and the results reveal new potential threats to food safety and public health from wholesale and retail pork.[39] In addition, ST22 clone, a community-associated *S. aureus*, was found to be the main MSSA isolate type in Wuhan in our previous reports,[4] but 67.3% ST22 clones presented with resistance to methicillin in this study, which reminds us to alert that ST22 has the potential to be one of the most common MRSA strains in the next few years.

It is well known that *S. aureus* is notorious for its ability to produce a series of virulence factors.[40] Previous studies have shown that *pvl* is closely related to the prognosis and mortality of infected patients. Among patients with *S. aureus* pneumonia, the survival rate at 48 hours after admission was 63% for *pvl*-positive patients and 94% for *pvl*-negative patients.[41,42] Specific molecular typing was associated with virulence genes. For example, ST22-t309 had a *pvl* carrying rate of 96.2%, while ST7-t091 had a *pvl* carrying rate of only 25.8%. The carrying rates of some hemolysin genes and adhesins were also different among different types of *S. aureus*. Furthermore, the rate of *pvl* in this study, was significantly higher than the previous detection rate in this area (48.6% vs 18.9%, $p<0.001$),[4] and moreover, *S. aureus* isolates collected after pandemic had higher positive rate of the *pvl* than those before pandemic. Therefore, we should attach great importance to the relationship between the change of molecular prevalence of *S. aureus* and the virulence gene carrying rate. The limitation should be acknowledged was that our results were from a single center, which may affect the representativeness of the samples to this study. These changes and limitation remind that more isolates for longer period should be collected and analysed to show how *S. aureus* has evolved and adapted rapidly to this stressful environment.

Conclusion

In conclusion, before and after the epidemic, the molecular typing, antimicrobial resistance and virulence of *S. aureus* have changed. ST5-t2460 is still the most common clone in the region and ST22-t309 had a *pvl* carrying rate of 96.2%. ST7 becomes the second dominant clone after COVID-19 pandemic with an increasing trend of both carrying SCCmec and more

virulent genes in Wuhan. CC/ST/spa typing was closely related to antimicrobial resistance and virulence genes of *S. aureus*. So, more isolates for longer period should be collected to detect changes in the prevalence of this important pathogen.

Data Sharing Statement

Data sets used and analyzed in the current study are included in this published article.

Ethics Approval and Consent to Participate

This study did not involve patient data and only used anonymous clinical residue samples from routine laboratory tests in hospital and was approved by the Zhongnan hospital of Wuhan university.

Consent for Publication

All authors approved the final manuscript and gave consent for publication.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for the contents of the article.

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Disclosure

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