



Prevalence, antibiotic resistance and molecular characterization of *Staphylococcus aureus* in ready-to-eat fruits and vegetables in Shanghai, China

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ABSTRACT

Staphylococcus aureus (*S. aureus*) is one of the foodborne pathogens. This study aimed to investigate the prevalence of *S. aureus* in ready-to-eat (RTE) fruits and vegetables in Shanghai, China. We evaluated antibiotic resistance patterns and genetic diversity of isolates through whole genome sequencing. Our findings demonstrated that out of 143 market samples, 47 (32.87%) tested positive for *S. aureus*, with the prevalence rates ranging from 10% to 57.14% among 12 types of RTE fruits and vegetables. Most isolates were resistant to trimethoprim-sulphamethoxazole, oxacillin, and ampicillin. We identified a total of 15 antibiotic resistance genes associated with resistance to 6 antibiotics, such as fosfomicin, fluoroquinolone, and β -lactam. Adhesion genes and enterotoxin genes, including *icaA*, *icaB*, *icaC*, *set*, *seg*, and *sec*, were also identified. Seven multi-locus sequence types (MLST) were detected, two of which were novel (ST7208 and ST7986). Notably, ST705-t529 (34.04%) and ST6-t701 (27.79%) represented the predominant types of *S. aureus*. Furthermore, three of the isolates were confirmed to be methicillin-resistant *S. aureus* by *mecA* genes. Taken together, our results highlight the high prevalence of *S. aureus* in RTE fruits and vegetables, posing a potential threat to food safety, particularly due to its high level of antibiotic resistance.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a significant foodborne pathogen responsible for global gastroenteritis cases. Under favorable conditions, approximately 50%–75% of *S. aureus* strains can produce extracellular heat-stable Staphylococcal enterotoxins (SEs) (Hennekinne et al., 2011). In 2014, the European Union documented 393 SE-related foodborne outbreaks, whereas Staphylococcal foodborne intoxication (SFI) outbreaks ranked third for foodborne diseases in China (EFSA, 2015; Li et al., 2021). Given its wide prevalence in the food chain, comprehensive surveillance and assessment of *S. aureus* are imperative.

In the agricultural sector, pathogenic bacteria are commonly transported via agricultural irrigation water and soil, which can contaminate ready-to-eat (RTE) fruits and vegetables that are rich in various nutrients, such as sugar, organic acids, and vitamins (Gurtler et al., 2018). Moreover, RTE fruits and vegetables can be cross-contaminated during consumption due to unclean transport vehicles, storage equipment, or

substandard manual operations involving cleaning water sources (Lynch et al., 2009; Doren et al., 2022). Certain fruits and vegetables, including leafy greens (such as lettuce and coriander; Shi et al., 2018), tomatoes (Kwon et al., 2017), and grapes (Botondi et al., 2015), are particularly susceptible to pathogen contamination. Accounting for 72,855 infections and 173 fatalities between 1980 and 2016, the distribution of RTE fruits and vegetables linked to microbial disease outbreaks revealed an overall rising trend in the number of these incidents (Bernardino et al., 2019). Foodborne diseases caused by microbial contamination are one of the primary factors that influence the quality and safety of RTE fruits and vegetables.

Although *S. aureus* has been reported previously in retail foods in China, limited studies were available on RTE fruits and vegetables. Rong et al. (2018) reported the detection of 6 strains of *S. aureus* in 150 vegetable samples from 15 provincial capitals in China. Shi et al. (2018) found 24 samples of *S. aureus* out of 419 commercially available vegetable samples from 39 cities in China. In Shanghai, the positive rate of

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S. aureus in 102 vegetable samples was 11.76% (Song et al., 2015). Similarly, in a study involving 75 samples of loose-leaf and knotty lettuce from Zhejiang province, the positive rate for *S. aureus* was 4% (Xiao et al., 2020). RTE fruits and vegetables are popular among residents in Shanghai, China. Nowadays, it is noteworthy to mention that the risk of foodborne transmission associated with *S. aureus* in RTE fruits and vegetables is increasing.

Antibiotic resistance poses a significant global public health challenge owing to the persistent dissemination of resistant strains in the environment. Numerous studies have been conducted worldwide to describe the rates of antibiotic resistance exhibited by *S. aureus* in RTE fruits and vegetables. In Korea, all *S. aureus* isolates obtained from leafy vegetables demonstrated resistance to multiple tested antibiotics, with 35.85% of the isolates exhibiting multidrug resistance (MDR) (Hong et al., 2015). Similarly, in China, the prevalence of antibiotic-resistant *S. aureus* contamination in RTE fruits and vegetables was observed to be 2.2%, with 21 isolates identified as MDR *S. aureus* (Lin et al., 2019). Of the 14 strains of *S. aureus* isolated from the various fruits, 21.4% exhibited resistance to at least one antibiotic (Feng et al., 2014). Overall, further researches are needed to determine the underlying causes of *S. aureus*'s high-level antibiotic resistance in RTE fruits and vegetables.

The molecular characterization of *S. aureus* is essential to trace the origin and spread of infection and contamination, as well as to gain a deeper epidemiological understanding of foods and environmental issues. Both multi-locus sequence typing (MLST) and Staphylococcal protein A (*spa*) typing have proved effective in characterizing and identifying genetic relationships among *S. aureus* isolates (Aggarwal et al., 2019; Dai et al., 2019; Wang et al., 2018; Bletz et al., 2015). In recent years, whole genome sequencing (WGS) technology based on high-throughput sequencing has emerged as a promising approach for bacterial typing, including *S. aureus*, due to its ability to provide more comprehensive genomic information (Angen et al., 2017; Kyung-Hwa et al., 2017). Nevertheless, publicly available data regarding *S. aureus* characterization have primarily focused on clinical settings, animal foods, and dairy products, with limited information available for RTE fruits and vegetables.

Overall, this study aimed to provide valuable qualitative data concerning the prevalence of *S. aureus* in RTE fruits and vegetables in Shanghai, China, as well as their antibiotic resistance profiles, virulence genes, MLST typing, and *spa* typing. This information can help us determine the risks associated with *S. aureus* contamination in RTE fruits and vegetables.

2. Materials and methods

2.1. Sample collection

A total of 143 samples of RTE fruits and vegetables were sold at two farmers' markets, two supermarkets, and two e-commerces in Shanghai, China, between June and September 2021. Of this total, 66 samples were fruits, and 77 were vegetables. The fruits category comprised fresh-cut honeydew melon ($n = 13$), fresh-cut watermelon ($n = 13$), cherry tomato ($n = 10$), fresh-cut pear ($n = 10$), fresh-cut peach ($n = 10$), and grape ($n = 10$). The vegetable category comprised lettuce ($n = 37$), chicory ($n = 15$), purple cabbage ($n = 6$), cucumber ($n = 7$), coriander ($n = 5$), and carrot ($n = 7$). All the aforementioned samples were collected in sterile homogeneous bags and transported immediately to the laboratory for subsequent microbiological analysis. These 12 fruits and vegetables were representative in terms of consumption and nutrient quality scores and met the needs of the study.

2.2. Isolation and identification of *S. aureus*

All samples underwent qualitative analysis for the presence of *S. aureus* using an enrichment method as described by the National Food Safety Standard of China (GB4789.10-2016, 2016). Briefly, sterile

sample bags were opened in a clean bench and 25 g portions of each sample were weighed, diluted to a ratio of 1:10 with 7.5 % NaCl solution, sealed up and mixed in a homogenizer (Interscience, Cantal, France). The samples were then divided into two groups and plated onto Baird Parker Agar (Hope Bio-Technology Co., Ltd., Qingdao, China) and Columbia Blood Agar (Hope Bio-Technology Co., Ltd., Qingdao, China), respectively, and incubated at 37 °C for 48 h. Putative colonies were transferred to Brain-Heart Infusion Agar (Hope Bio-Technology Co., Ltd., Qingdao, China) and cultured at 37 °C for 24 h for further confirmation and identification.

0.3 mL of Brain-Heart Infusion Broth (Hope Bio-Technology Co., Ltd., Qingdao, China), which contains presumptive *S. aureus* strains, was combined with 0.5 mL of Freeze-Dried Rabbit Plasma (Hope Bio-Technology Co., Ltd., Qingdao, China) to determine coagulation activity. This mixture was subsequently cultured at 37 °C for 6 h. Results were monitored every 30 min and considered positive if the coagulation was greater than half of the original volume. *Staphylococcus epidermidis* ATCC 12228 and *S. aureus* ATCC 29213 were utilized as reference strains.

2.3. Antibiotic susceptibility testing

All *S. aureus* isolates were used for antibiotic susceptibility testing by broth dilution method provided by the Clinical and Laboratory Standards Institute (CLSI, 2021). The following 17 antibiotics were selected in this study: chloramphenicol (CHL) (2–16 µg/mL), gentamicin (GEN) (2–16 µg/mL), erythromycin (ERY) (0.25–4 µg/mL), clindamycin (CLI) (0.5–2 µg/mL), oxacillin (OXA⁺) (0.25–4 µg/mL), ampicillin (AMP) (0.12–8 µg/mL), linezolid (LZD) (1–8 µg/mL), penicillin (PEN) (0.06–8 µg/mL), rifampin (RIF) (0.5–4 µg/mL), vancomycin (VAN) (0.25–32 µg/mL), sulfamethoxazole-trimethoprim (SXT) (0.5/9.5–4/76 µg/mL), levofloxacin (LEVO) (0.25–4 µg/mL), quinupristin-dalfopristin (SYN) (0.5–4 µg/mL), tigecycline (TGC) (0.03–0.5 µg/mL), tetracycline (TET) (2–16 µg/mL), moxifloxacin (MXF) (0.25–4 µg/mL), and ciprofloxacin (CIP) (1–2 µg/mL).

MDR was defined as resistance to at least one agent in three or more antibiotic categories, while extensively drug-resistant (XDR) was defined as susceptible to two antibiotic categories at most (Magiorakos et al., 2012). Both MDR and MAR indices were used to characterize the different patterns of resistance found in healthcare-associated and foodborne antibiotic-resistant bacteria. The MAR index is expressed as:

$$MAR = a/b \quad (1)$$

where a is the number of antibiotics to which the isolate displayed resistance, and b is the number of antibiotics to which the isolate was exposed. *S. aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were utilized for quality control strains.

2.4. Whole genome sequencing

All isolates recovered in this study were subjected to WGS. Genomic DNA was extracted and purified using the Sangon DNA Kit (Sangon., Shanghai, China), and purified genomic DNA was quantified by Nano-Drop 2000 (Thermo Fisher Scientific., Wilmington, USA). High-quality DNA (OD_{260/280} = 1.8–2.0, >1 µg) was used for further research. Illumina sequencing libraries were prepared from sheared DNA fragments (400–500 bp) using the NEXTflex™ Rapid DNA-Seq Kit (PerkinElmer, Massachusetts, USA). The libraries were then sequenced using Illumina paired-end sequencing. Raw reads were filtered, assembled, and submitted to the National Library of Medicine (NCBI).

2.5. Antimicrobial resistance and virulence determinants

Antimicrobial resistance genes (ARGs) were analyzed using Res-Finder 4.1, available on the Centre for Genomic Epidemiology (CGE) website (<http://www.genomicepidemiology.org/>), while virulence

factors were estimated using VFAnalyzer available in the Virulence Factor Database (VFDB) (<http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi>).

2.6. MLST, *spa*, and *SCCmec* typing

STs and clonal complexes typing (CCs), *spa* typing, and *SCCmec* typing were analyzed using MLST 2.0, *spa*Typer 1.0, and *SCCmec*Finder 1.2, respectively, all available on the CGE website. Newly identified MLST allele sequences were submitted to PubMLST (<https://pubmlst.org>) for definitions. Untypable data results were recorded if they did not match any *spa* repeat or *spa* types in the database.

2.7. Statistical analysis and data availability

The Chi-square test and Bonferroni correction were performed to compare the difference in the positive rate among isolates from various products or sampling locations. Data analysis was performed using SPSS 24.0 (SPSS, Inc., Chicago, USA), and $p < 0.05$ were considered statistically significant.

Table 1

Prevalence of *S. aureus* in different RTE fruits and vegetables.

Types of products	Sampling location	No. of <i>S. aureus</i> isolate/No. of samples	No. (%) of positive samples for <i>S. aureus</i> ^a	Strain number of <i>S. aureus</i> isolates
Cucumber	Farmers' market	4/7	4 (57.14)	SA22 SA23 SA24 SA25
Cherry tomato	Local supermarket e-commerce	1/4 4/6	5 (50.00)	SA10 SA34 SA39 SA40 SA46
Fresh cut watermelon	Local supermarket e-commerce	1/2	6 (46.15)	SA06 SA15 SA16 SA33 SA37
Carrot	Farmers' market	5/11 3/7	3 (42.86)	SA38 SA28 SA29 SA30
Chicory	Local supermarket e-commerce	3/6	6 (40.0)	SA08 SA09 SA17 SA31 SA36 SA45
Fresh cut peach	Local supermarket e-commerce	3/9 1/6	4 (40.00)	SA11 SA12 SA35 SA42
Coriander	Farmers' market	3/4 2/5	2 (40.00)	SA26 SA27
Fresh cut honeydew melon	Local supermarket e-commerce	1/2	4 (30.77)	SA05 SA13 SA14 SA32
Lettuce	Farmers' market Local supermarket e-commerce	3/11 7/24	8 (21.62)	SA01 SA02 SA03 SA04 SA19 SA20 SA21
Purple cabbage	e-commerce	0/5 1/8		SA44
Grape	e-commerce	1/6 1/10	1 (16.67) 1 (10.00)	SA18 SA41
Fresh cut pear	Local supermarket e-commerce	1/10 0/2	3 (30.00)	SA07 SA43
Total		3/8 143	47 (32.87)	SA47

^a $p > 0.05$: non-significance among the different types of fruits and vegetables.

The raw sequence reads were deposited to the Short Read Archive (SRA) database of the NCBI under the BioProject accession number: PRJNA972510 and PRJNA972517.

3. Results

3.1. The prevalence of *S. aureus* in RTE fruits and vegetables

The selected farmers' markets in this research were prominent local hubs for agricultural product wholesale, while the supermarkets represent renowned chain-operated establishments. Meanwhile, e-commerce outlets were the favored platform among consumers. In total, 66 retail fruits and 77 vegetables were examined, and 47 (32.87%) samples were positive for *S. aureus* (Table 1). Overall, excluding grapes (10.00%), the prevalence of *S. aureus* in fruits was relatively high (30.00%–50.00%), particularly in fresh-cut fruits (30.00%–46.15%). Among vegetables, cucumbers had the highest contamination rate of *S. aureus* (57.14%), followed by carrots (42.86%). The contamination rates of leafy green vegetables, including chicory, coriander, and lettuce range from 21.62%

to 40.00%, while purple cabbage has the lowest contamination rate (16.67%).

Analysis of different sampling matrices and locations revealed the prevalence of *S. aureus* in RTE fruits and vegetables was 34.8% and 31.2%. For samples from farmers' markets, local supermarkets, and e-commerces, the positive rates were 37.2%, 27.6%, and 32.4%, respectively. However, no significant differences were observed among the groups ($p > 0.05$) (Tables 1 and 2). Differences in sample size and distribution of varieties across each stage might lead to non-significant differences.

3.2. High-level multidrug resistance occurred in *S. aureus* isolates from RTE fruits and vegetables

The antibiotic susceptibility results of 47 *S. aureus* isolates were analyzed (Fig. 1). The majority of isolates showed high resistance rates to antibiotics, including SXT (97.87%), OXA⁺ (95.74%), AMP (95.74%), PEN (95.74%), SYN (93.62%), RIF (89.36%), GEN (85.11%), CLI (85.11%), LEVO (34.04%), LZD (31.91%), CIP (27.66%), ERY (25.53%), VAN (23.40%), MXF (23.40%), and others (<20%). Overall, all the isolates were resistant to at least one antibiotic. MDR and XDR patterns were observed, indicating severe antibiotic resistance in RTE fruits and vegetables. Notably, lettuce and purple cabbage strains exhibited lower resistance overall, while isolates from coriander demonstrated the highest resistance to multiple antibiotics.

Antibiotic resistance phenotypes and classification of *S. aureus* isolates were illustrated in Table 3. It was worth noting that all isolates were resistant to more than three tested antibiotics, with 65.96% of them classified as MDR. Unfortunately, 34.04% of the isolates were XDR, and none of them were LDR. In this regard, the average MAR index for all *S. aureus* isolates was 0.777, all of them showed MAR indices >0.5. The strains were found to be associated with the overuse or misuse of antibiotics in highly selective pressure environments, as mentioned by Krumperman (1983). Out of the tested isolates, 9 (19.15%) isolates were resistant to 16 antibiotics, resulting in a high MAR index of 0.941. Similarly, 7 (14.89%) isolates were resistant to 16 antibiotics with a MAR index of 0.824, 17 (36.17%) were resistant to 13 antibiotics with a MAR index of 0.765, 12 (25.53%) were resistant to 10 antibiotics with a MAR index of 0.588, and 2 (4.26%) were resistant to 9 antibiotics with a MAR index of 0.529. The most common spectrum of resistance was GEN-RIF-SXT-SYN-OXA⁺-AMP-PEN-LEVO-CLI-CIP-MXF-ERY-LZD.

In addition, macrolide resistance gene *mdf(A)*, aminoglycoside resistance gene *aph(3')-Ia*, fosfomycin resistance gene *fosA*, lincosamide resistance gene *lnu(A)*, *lsa(A)* and *sal(A)*, fluoroquinolone resistance gene (*oqxA*, *oqxB* and *qnrS1*), as well as β -lactam resistance genes (*mecA*, *bla*_{TEM-116}, *bla*_{ACT-15}, *blaZ*, *bla*_{LAP-2} and *bla*_{OXY-1-3}) were detected. Analysis of ARGs based on the food categories revealed that all ARGs were identified in *S. aureus* isolates from lettuce (Fig. 2).

A combination of antibiotic resistance phenotypes and genotypes showed that OXA⁺, AMP, PEN, SYN and GEN resistance were the predominant antibiotics resisted by *S. aureus* isolates (Table 3). Consequently, the corresponding resistant genes, i.e. *mecA*, *qnrS1*, *aph(3')-Ia*, *bla* (*bla*_{TEM-116}, *bla*_{ACT-15}, *blaZ*, *bla*_{LAP-2} and *bla*_{OXY-1-3}), and *oqx* (*oqxA* and *oqxB*), were also measured. This highlights the informative nature of

antibiotic-resistance genes in evaluating microbial resistance. However, some isolates with resistance genes did not exhibit phenotypic resistance to certain antibiotics, and no relevant positive resistance genes were detected for CHL, LZD, RIF, VAN, SXT, TGC, and TET.

3.3. Thirty-eight virulence gene determinants were distributed with varying frequencies in *S. aureus* isolates

Known virulence genes were identified in all isolates by comparing nucleotides with the VFDB, a total of 38 toxin genes were found in the 47 *S. aureus* isolates (Fig. 3). Regardless of the source, all isolates carried at least three toxin genes. To classify the isolates based on the virulence genes, they were grouped into 10 virulence profiles. The most common profile observed was profile 1 (34.04%). This was followed by profiles 2 (29.79%), 3 (10.64%), 4 (8.51%), and profiles 5 and 6, each occurring with a frequency of 4.26%.

All isolates carried the virulence genes encoding for host cell adhesion (*icaA*, *icaB*, *icaC*). However, only two classical enterotoxin genes were identified in this study, *sea* (29.79%) and *sec* (44.68%). Non-classical enterotoxin genes *set* (89.36%) had the highest positive rate among the enterotoxin genes. Almost all isolates (89.36%) harbored multiple genes (*efb*, *sspB*, *geh*, *lip*, *spIC*, *hly/hla*, *hlyB*, *set*, *hlgB*, and *hlgC*), but none had the *pvl* genes that belong to the two-component toxin family.

3.4. MLST, *spa* typing and SCCmec typing of *S. aureus*

As shown in Fig. 3, a total of seven sequence types (STs) were identified among these 47 *S. aureus* isolates, namely ST705, ST6, ST243, ST7208, ST72, ST188, and ST7986. Among them, ST705 (n = 16) and ST6 (n = 14) were the predominant types. In addition, two previously unreported STs, namely ST7208 and ST7986, were detected in *S. aureus* isolates. Four clonal complexes (CC5, CC8, CC1, and CC30) were identified, with CC5 being the most prevalent and diverse, comprising ST6 and ST7208. Interestingly, CC5 was widely distributed in multiple food categories excluding coriander and grape. Furthermore, the clonal lineages of *S. aureus* isolates were scrutinized based on the specific categories. ST705 was the predominant genotype among isolates from various food categories, whereas ST7986 was exclusively identified in *S. aureus* isolates from lettuce.

Furthermore, *spa* typing showed results similar to those of MLST, with 47 *S. aureus* isolates classified into 5 *spa* types. The most prevalent *spa* type was t529 (40.43%), predominantly associated with ST705. Based on MLST, all 14 isolates belonging to t701 shared an identical sequence type (ST6), highlighting the superior discriminating power of *spa* typing. Interestingly, certain isolates exhibited identical *spa* types but different STs (t529-ST705 and t529-ST7986). Furthermore, when considering both the STs and *spa* types, ST705-t529 (16/47, 34.04%) and ST6-t701 (14/47, 27.79%) were the predominant types of *S. aureus* isolates, demonstrating a high level of concordance between STs and *spa* types.

Three isolates were confirmed to be methicillin-resistant *Staphylococcus aureus* (MRSA) as they tested positive for *mecA*. However, none of the isolates were *mecC*-negative. The *mecA*-positive isolates, isolated

Table 2
The prevalence of *Staphylococcus aureus* under different classifications.

Classifications	Total	No. (%) of positive samples	No. (%) of negative samples	chi-square test		
				χ^2	p	
Sampling sources	Fruits	66	23 (34.8)	43 (65.2)	0.218	>0.05
	Vegetables	77	24 (31.2)	53 (68.8)		
Sampling locations	Farmers' market	43	16 (37.2)	27 (62.8)	0.741	>0.05
	Local supermarket	29	8 (27.6)	21 (72.4)		
	e-commerce	71	23 (32.4)	48 (67.6)		

$p < 0.05$: statistical significance.

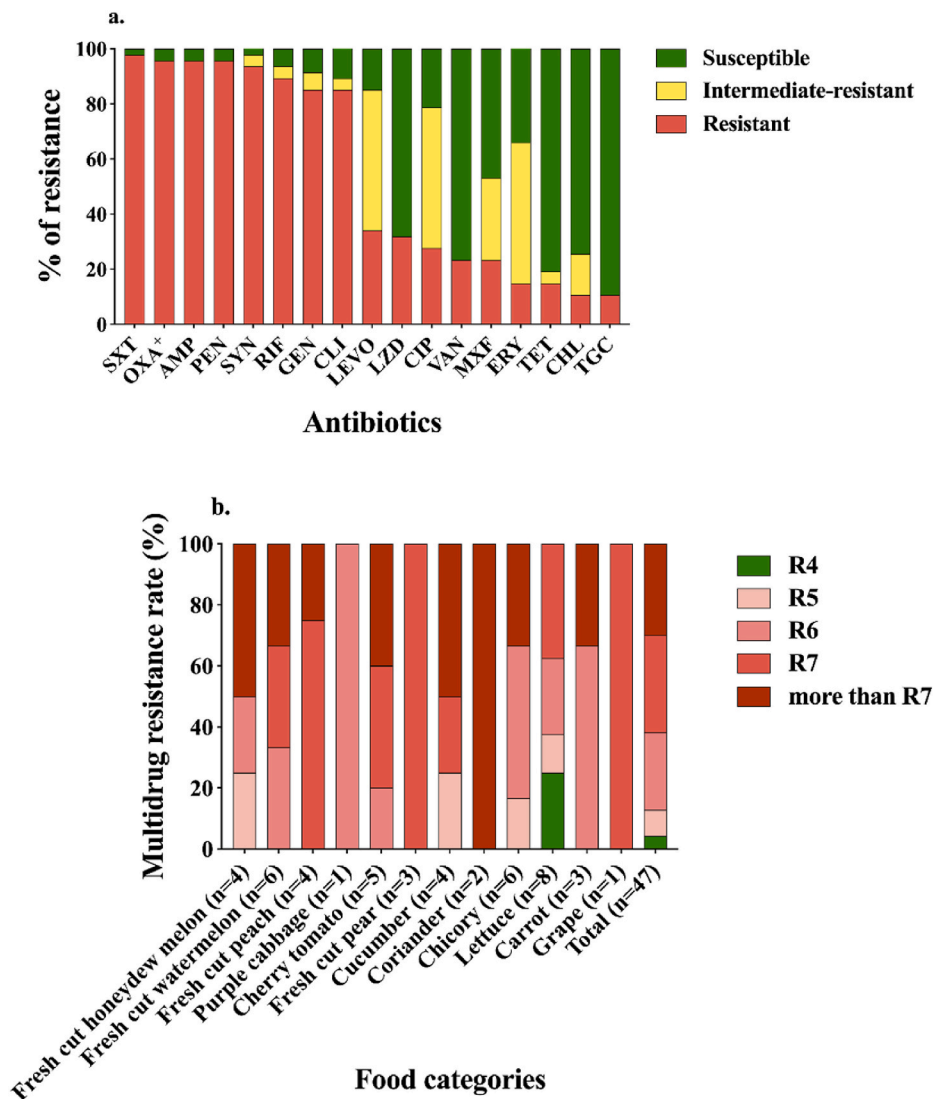


Fig. 1. Antibiotic resistance profiles of *S. aureus* strains isolated from fruits and vegetables. a. Antibiotic resistance phenotypes of 47 *S. aureus* to 17 kinds of antibiotics isolates from different RTE fruits and vegetables. SXT = trimethoprim-sulfamethoxazole; OXA⁺ = oxacillin; AMP = ampicillin; PEN = penicillin; SYN = quinupristin-dalfopristin; RIF = rifampicin; GEN = gentamycin; CLI = clindamycin; LEVO = levofloxacin; LZD = linezolid; CIP = ciprofloxacin; ERY = erythromycin; VAN = vancomycin; MXF = moxifloxacin; TET = tetracycline; CHL = chloramphenicol; TGC = tigecycline. b. The multi-drug resistance rate of 47 *Staphylococcus aureus* isolates from different fruits and vegetables. R4: resistant to 4 kinds of antibiotics; R5: resistant to 5 kinds of antibiotics; R6: resistant to 6 kinds of antibiotics; R7: resistant to 7 kinds of antibiotics; more than R7: at least resistant to 7 kinds of antibiotics.

from lettuce, pear, and cherry tomato samples, respectively, were all classified as SCCmecIV, and shared the same sequence type, ST7208 (Fig. 3).

4. Discussion

RTE fruits and vegetables are composed of raw ingredients from multiple sources and are processed by frequent hand contact, indicating significant potential for contamination by *S. aureus* from both human and environmental sources. Previous studies have highlighted the frequent isolation of *S. aureus* in salad vegetables, fruits, and sprouts (Viswanathan and Kaur, 2001; Seo et al., 2010). However, limited research is available regarding the prevalence of *S. aureus* in RTE fruit and vegetable products in China. In this study, the fresh-cut fruits were categorized as semi-processed products, undergoing procedures such as peeling, cutting, and packaging. Conversely, the remaining samples were non-prepackaged products. We discovered that 32.87% of RTE fruit and vegetable samples in Shanghai were contaminated with *S. aureus*. The prevalence observed in this study was lower than that

found in street-vended fruits products in Africa (61%–76%), aquatic products in 32 provincial capitals of China (37.2%), and raw milk in China (33.5%) (Kalsoom et al., 2011; Rong et al., 2017; Ou et al., 2018). However, it was higher than the rates reported for RTE fruits and vegetables in Sichuan province of China (20.5%), Saudi Arabia (11.8%) and Korea (1.8% and 1.4%) (Wang et al., 2019; Hassan et al., 2011; Lin et al., 2019; Tango et al., 2018).

A potential limitation of our study is the collection of only eight batches of RTE fruits and vegetables samples over four months. This research focused on sampling during the summer when microbial growth and reproduction are active, enabling a representative evaluation and inference of *S. aureus* contamination in fruits and vegetables year-round in Shanghai. However, this approach may not fully account for the influence of different seasons and produce types on the contamination rate, potentially limiting result accuracy. To enhance the credibility and generalizability of these findings, we planned to prolong the sampling duration, increase the number of sampling batches, and enlarge the number of samples per batch in future research.

The relatively low *S. aureus* contamination rate observed on grape

Table 3

Antibiotic resistance phenotypes of *S. aureus* strains isolated from RTE fruits and vegetables and classification of isolates according to their antibiotic resistance.

Antibiotic resistance phenotype	No. and (%) of isolates	MAR index ^a	Classification of isolates ^b	
			Type of resistance	No. and (%) of isolate
LZD-SXT-OXA ⁺ -AMP-CLI-PEN-MXF-CIP-SYN	2 (4.26)	0.529	Multi-drug resistant	31 (65.96)
GEN-RIF-SXT-SYN-OXA ⁺ -AMP-PEN-CLI-TGC-CIP	12 (25.53)	0.588		
GEN-RIF-SXT-SYN-OXA ⁺ -AMP-PEN-LEVO-CLI-CIP-MXF-ERY-LZD	15 (31.91)	0.765		
GEN-RIF-SXT-SYN-ERY-OXA ⁺ -AMP-PEN-LEVO-CLI-CIP-VAN-MXF	2 (4.26)	0.765		
GEN-RIF-SXT-OXA ⁺ -AMP-PEN-CLI-SYN-TGC-LZD-LEVO-CIP-MXF-TET	4 (8.51)	0.824	Extensive-drug resistant	16 (34.04)
CHL-GEN-LZD-RIF-SXT-SYN-TET-ERY-OXA ⁺ -AMP-PEN-VAN-MXF-CLI	2 (4.26)	0.824		
CHL-GEN-LZD-RIF-SXT-SYN-TET-ERY-OXA ⁺ -AMP-PEN-VAN-MXF-CLI-LEVO	1 (2.13)	0.824		
LZD-RIF-SXT-SYN-ERY-OXA ⁺ -AMP-PEN-VAN-TGC-CLI-TET-GEN-CHL-MXF-CIP	5 (10.64)	0.941		
GEN-LZD-RIF-SXT-SYN-TET-OXA ⁺ -ERY-AMP-PEN-VAN-MXF-LEVO-CLI-TGC-CHL	4 (8.51)	0.941		

^a The average multiple antibiotic resistance (MAR) index is 0.777.

^b According to Magiorakos et al. (2012); SXT = trimethoprim-sulfamethoxazole; OXA⁺ = oxacillin; AMP = ampicillin; PEN = penicillin; SYN = quinupristin-dalfopristin; RIF = rifampicin; GEN = gentamycin; CLI = clindamycin; LEVO = levofloxacin; LZD = linezolid; CIP = ciprofloxacin; ERY = erythromycin; VAN = vancomycin; MXF = moxifloxacin; TET = tetracycline; CHL = chloramphenicol; TGC = tigecycline.

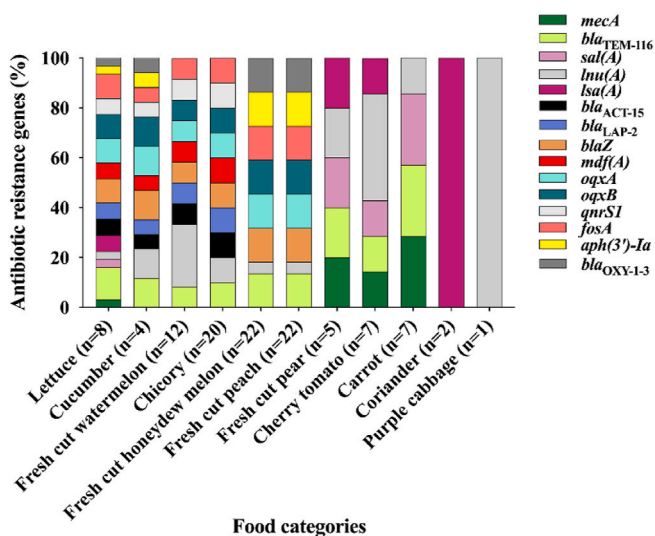


Fig. 2. Genetic diversity of *S. aureus* isolates from different RTE fruits and vegetables based on WGS. Percent prevalence of each antibiotic resistance genes (ARGs) is expressed as the number of isolates within the same ARG over the total number of *S. aureus* strains isolated from the same food category.

and purple cabbage was attributed to the natural wax coating present on their surfaces. Grapes, being more acidic and contain polyphenolic substances, exhibit properties that can inhibit bacterial growth. Furthermore, the thick, compact, spherical shape of purple cabbage leaves minimizes bacterial accumulation. In contrast, cherry tomatoes and fresh-cut fruits are prone to bacterial contamination due to their smooth, unwaxed surfaces and thin skins, which can be easily damaged. Similarly, bacteria from cutting tools, cutting boards, or handlers could be transferred to the surface of fresh-cut fruits during cutting procedures. Fresh-cut fruits were usually not consumed immediately, the nutrient-rich juice on their surface, combined with inadequate refrigeration or prolonged storage, created an environment conducive to bacterial growth. Cucumbers and carrots, commonly associated with a higher prevalence of *S. aureus*, often grow in soil and water-rich environments. Their rough surface structures made them prone to cross-contamination. On the other hand, leafy vegetables grow in open-air environments with larger leaves that easily accumulate bacteria on their surfaces.

From the results available, the contamination rates for *S. aureus* in lettuce, cherry tomatoes, chicory, and cucumber were 21.62%, 50.00%, 40.00%, and 57.14%, respectively, which were also higher than those reported in previous studies (15.48%, 6.36%, 2.30%, and 1.56%) (Shi et al., 2018). In recent years, the consumption of raw or minimally processed fruits and vegetables has been on the rise, leading to the development of various disinfection technologies (Ramos et al., 2013). These technologies include physical methods such as UV-light, ionizing radiation, and high-intensity ultrasound, chemical disinfection methods like chlorine dioxide, ozone, and organic acid, as well as hurdle technology, which effectively reduces microbial loads while minimizing sensory and nutritional impacts (Deng et al., 2020). It is also advisable to extend the washing duration or use appropriate fruit and vegetable cleaners before consumption to mitigate the risk of pathogenic bacterial infections.

S. aureus is known for its exceptional ability to acquire antibiotic resistance from other organisms, which is further aggravated by the extensive use and misuse of antibiotics in humans, farms, or agriculture (Phillips et al., 2004; Ma et al., 2017). Our resistance phenotypes findings were consistent with other relevant vegetable research conducted in China (Shi et al., 2018; Hong et al., 2015), but higher than previous reports among different food sources from other countries (Shahraz et al., 2012; Papadopoulos et al., 2018). Among them, 95.74% of isolates were resistant to ampicillin and penicillin, consistent with investigations by Hong et al. (2015) and Shi et al. (2018) on retail vegetables (96.3% and 93.3%) in Korea and China, respectively. Since the first identification of vancomycin-resistant *S. aureus* (VRSA) strain in Michigan, USA in 2002, it has been successively isolated in laboratories worldwide. (Cong et al., 2020; Chang et al., 2003; Pan et al., 2018; Ahsanul et al., 2021). The emergence of VRSA in RTE fruits and vegetables is of concern since vancomycin is the preferred antibiotic for treating MRSA infections. This study suggested a potential association with animal or clinical usage, as identified by the presence of fluoroquinolone, aminoglycoside, and β -lactams resistance in *S. aureus*. Post-harvest cross-contamination of fruits and vegetables and horizontal genes transfer may contribute to this situation (Usui et al., 2019; Iseppi et al., 2018; Skořková et al., 2013; Yang et al., 2019; Kabir et al., 2015).

We have observed variations in antibiotic resistance profiles of *S. aureus* isolated from coriander and purple cabbage. This difference might be attributed to their potential exposure to different types or concentrations of antibiotics during their growth. Thus, selective pressure or genomic differences from different plants could contribute to antibiotic resistance in coriander and purple cabbage. Lastly, the small sample sizes and potential biases should be considered. *S. aureus* isolated from grapes, without carrying any known resistant genes, exhibited multidrug resistance, possibly due to natural resistance mechanisms or genetic mutations. In addition to coriander and grape, the *lnu(A)* and *bla*_{TEM-116} were detected in all other fruits and vegetables, which could

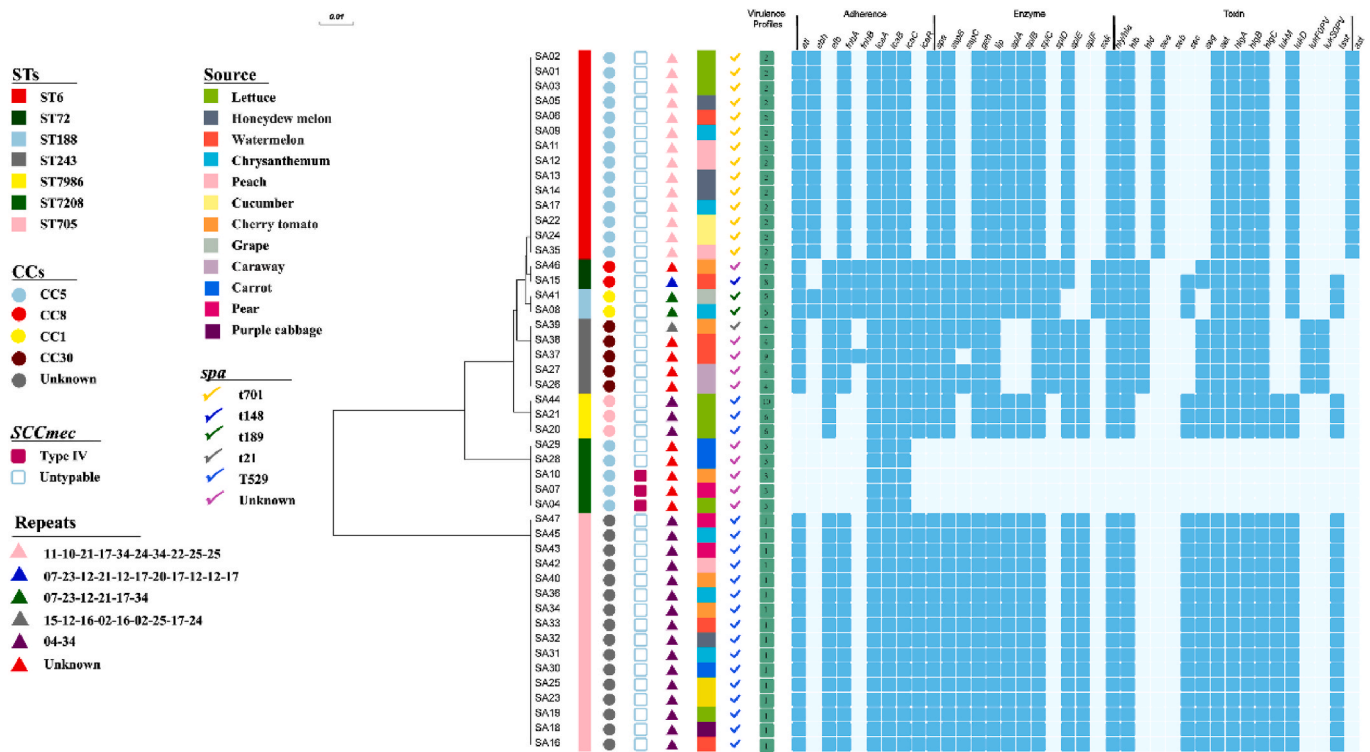


Fig. 3. Dendrogram displaying the MLST of *S. aureus* isolates, and heatmap analysis of *spa* typing and virulence genes harbored in the *S. aureus* isolates.

explain the higher resistance rate of *S. aureus* to β -lactam antibiotics in antimicrobial susceptibility tests. Interestingly, *S. aureus* isolated from purple cabbage solely carried *lnu(A)*, consistent with its lower resistant phenotype.

Based on the identified resistance genes, the majority of tested isolates contained at least one determinant conferring resistance. The most frequently identified gene, similar to Piekarska et al. (2022), was *bla*_{TEM-116}, conferring resistance to β -lactams. Fig. 2 also depicted the common presence of genes conferring resistance to fosfomycin (*fosA*), lincosamide (*lnu(A)*), and fluoroquinolone (*oqxA* and *oqxB*) were also commonly found in the tested strains. These results, both phenotypic and genotypic, showed a high level of consistency. Notably, the VRSA measured in this study did not harbor the *vanA* gene, possibly as a result of cross-resistance.

MRSA, a potential cause of hospital-acquired infections, is associated with the continuous acquisition of resistance genes by *S. aureus* strains carrying mobile elements like *SCCmec*, which promoted the spread of MDR. Five MRSA isolates were identified from lettuce, fresh-cut pear, cherry tomato, and carrot, exhibiting phenotypic resistance to OXA⁺-AMP-PEN, and belonging to *SCCmec IV*. While cross-contamination from environments is a possible contributing factor, further studies are required to elucidate the transmission routes of MRSA with retail foods in order to provide the tools for preventing the spread of MRSA.

Both the experimental and WGS analyses confirmed strong antibiotic resistance in the *S. aureus* strains. Although there were inconsistencies between phenotype and genotype in a few isolates, these discrepancies may be attributed to other undetected resistance genes, the influence of other genetic factors, or environmental conditions. Nevertheless, the phenotypic and genetic profiling of antibiotic-resistant *S. aureus* isolates provides valuable insights into antibiotic usage in our society at large.

In this study, the *eta*, *etb*, and *etd* genes, encoding exfoliative toxins, were not detected in *S. aureus* isolates. This suggests a low frequency of these toxin genes in RTE fruits and vegetables, consistent with previous research (Song et al., 2015). However, 22 isolates tested positive for the *tst* gene, which has been associated with important poisoning symptoms in clinical *S. aureus* isolates (Schlievert et al., 2010). Furthermore, seven

genes encoding pathogenic SEs were detected. The gene *set* (89.36%) was frequently detected, with *seg* (55.32%) and *sec* (46.81%) following closely behind, whereas *sea* (29.79%) had a lower frequency. As we all know, about 95% of Staphylococcal food poisoning (SFP) outbreaks were caused by strains producing the classical SEs (SEA-SEE), and the *egc* cluster (*seg* and *set*) was widely distributed in clinical isolates (Mashouf et al., 2015; Jarraud et al., 2001; Johler et al., 2015). Nonetheless, most SEs genes were carried and transmitted by mobile genetic elements, and their transmission in *S. aureus* isolates could alter their pathogenicity, contributing to the evolution of this significant pathogen. Therefore, consumers should be aware of the pathogenicity of typical clinical strains carrying non-classical enterotoxin genes as well.

In this study, 47 *S. aureus* isolates were analyzed using two typing methods, revealing the presence of 7 STs, including 2 new types and 5 *spa* types. Some major STs, such as ST705-t529 and ST6-t701, corresponded to previous reports (Song et al., 2015; Shi et al., 2018; Liu et al., 2020). Interestingly, the ST705 strains displayed identical CC typing and *spa* typing, as well as virulence profiles of *hlgA-hlgB-hlgC-lukD*, consistent with other studies (Hata et al., 2010; Asai et al., 2012). However, this study also discovered that these ST705 strains, which were prominent bovine *S. aureus* lineages in Japan, the UK, and the Netherlands, and the most recently established pandemic bovine lineage, were found in our samples, indicating possible introduction through food sources (Ikawaty et al., 2009). Furthermore, the ST6-t701 clone (71.4%) was the most predominant strain with multiple virulence genes in seven SFP outbreaks in Xi'an, northwest China, between 2006 and 2013. The most frequently detected virulence genes in these outbreaks were *lukD*, *hly*, and *hld* (Li et al., 2015).

All three ST7986 *S. aureus* isolates were obtained from lettuce samples. These isolates belonged to the t529 lineage and harbored the resistant gene *Isa(A)*. ST7986 was distinguished from the predominant ST705 genotype in this research by a single allele. Furthermore, five strains of the ST7208-type were identified as MRSA, belonging to the clonal complexes CC5. MRSA CC5 was prevailing in clinical isolates associated with bacteremia in China and showed strong invasion ability on cells (He et al., 2013; Zautner et al., 2010). It suggested foods as a

potential environmental source of *S. aureus* isolates that have an important clinical relevance. Future research should investigate the evolutionary relationships of *S. aureus* from agricultural, husbandry, and hospital origins to prevent its spread through the food chain.

Overall, there was diversity in virulence profiles among different sources and STs. For instance, ST188, a double locus variant (DLV) of ST1, showed strong virulence-related adhesion and colonization ability, and WGS analysis demonstrated it might evolve from livestock. ST188 is the major lineage causing multiple host species infections in Shanghai, China (Wang et al., 2018). Furthermore, both international transmission of ST72 isolates and interregional transmission within China were observed, with t148 being the most common *spa* typing (Zhou et al., 2021). Although ST243 has fewer reports, it may colonize patient-to-contaminated healthcare worker transmission in hospital settings (Ferreira et al., 2013). The presence of antibiotic-resistant, enterotoxigenic *S. aureus* strains acquired from livestock and hospitals in RTE fruits and vegetables is a public health concern.

5. Conclusions

In summary, this study investigated the prevalence of *S. aureus* isolated from RTE fruits and vegetables in Shanghai, China. The relatively high positive rate and antibiotic resistance of *S. aureus* were observed, which also showed a high degree of genetic relatedness. Hence, it is important to strengthen hygiene supervision in the production bases of fruits and vegetables and enhance the establishment of guidelines and standards for cultivation and harvesting practices. Furthermore, it is suggested that, after harvesting, strict control measures be implemented for storage temperatures to inhibit the growth of *S. aureus*, thereby ensuring the safety of fruits and vegetables and reducing the risk of *S. aureus* contamination. In the future, it is advisable to conduct risk assessments of high-risk foodborne pathogens in fruits and vegetables and perform a comprehensive microbial risk assessment from farm to fork specifically for *S. aureus* along the entire supply chain. This will help identify key control points for risk management.

CRedit authorship contribution statement

Kai Jia: Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. **Xiaojie Qin:** Conceptualization, Formal analysis, Supervision, Resources, Writing – review & editing. **Xiangfeng Bu:** Methodology, Investigation. **Huajian Zhu:** Formal analysis, Investigation. **Yangtai Liu:** Methodology, Writing – review & editing. **Xiang Wang:** Methodology, Writing – review & editing. **Qingli Dong:** Validation, Resources, Project administration, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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