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Phenotypic and genotypic characterization of PVL-positive *Staphylococcus aureus* isolated from retail foods in China

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ABSTRACT

Staphylococcus aureus encodes numerous toxins that are known or strongly suspected to cause specific diseases or symptoms. Panton-Valentine leukocidin (PVL) is one of these important toxins that is associated with high mortality rates. In our previous study, 1581 *S. aureus* strains were isolated from 4300 samples of retail foods obtained from most of the provincial capitals in China from 2011 to 2016. This study aimed to investigate the prevalence of PVL-positive *S. aureus* isolates from retail foods in China and characterize these isolates by antibiotic resistance testing, *spa* typing, multilocus sequence typing (MLST) and enterotoxin gene analyses. In total, seventy-two isolates (72/1581, 4.6%) possessed *pvl* genes, including 24.1% MRSA isolates (26/108) and 3.1% MSSA isolates (46/1473), covering different types of food. The strains were divided into seventeen sequence types (STs) and twenty-seven *spa* types, and 43.1% (31/72) of the PVL-positive *S. aureus* isolates belonged to CC59-t437. These isolates contained at least one of the following enterotoxin genes: *sei* (97.2%), *sem* (86.1%), *seq* (80.6%), *seg* (68.1%), *sek* (68.1%), *seb* (62.5%), *sel* (52.8%), *sej* (50.0%), *seh* (48.6%), *sep* (45.8%), *sea* (38.9%), *ser* (37.5%), *sen* (27.8%), *sec* (16.7%), *see* (16.7%), *sed* (6.9%), *seo* (6.9%) and *seu* (6.7%). A total of 87.5% of the *S. aureus* isolates (63/72) harboured the classic SE genes (*sea*, *seb*, *sec*, *sed*, and *see*), whereas all the *S. aureus* isolates harboured the genes of the *egc* cluster (*seg*, *sei*, *sem*, *sen*, *seo*, and *seu*). In antimicrobial susceptibility tests, 98.6% of the isolates (71/72) exhibited resistance to at least one antibiotic, including 47 multi-drug-resistant isolates. Resistance to penicillin (94.4%), erythromycin (83.4%), clindamycin (63.9%), kanamycin (61.1%), telithromycin (58.3%), streptomycin (51.4%), tetracycline (47.2%), chloramphenicol (27.8%), fusidic acid (27.8%) and other antibiotics (< 20%) was observed. All the PVL-positive MRSA isolates belonged to CC59-t437, which is the predominant type of community-associated (CA)-MRSA in China. The presence of these isolates in food represents a potential health risk for consumers and warrants further attention.

1. Introduction

Staphylococcus aureus is a bacterial pathogen that can infect humans, animals, plants and contaminate foods that is responsible for a wide variety of diseases. Despite the ubiquitous distribution of *S. aureus* in nature, food remains the most important source of infection, and staphylococcal food poisoning is reported to be the third most prevalent cause of foodborne illness worldwide (Aydin et al., 2011). Every year, approximately 241,000 foodborne illnesses are caused in the United States by *S. aureus* (Scallan et al., 2011). In China, it is estimated that approximately 20–25% of foodborne bacterial outbreaks are caused by *S. aureus*, which ranks just next to *Salmonella* and *Vibrio parahaemolyticus* in the list of important foodborne pathogens (Wang et al., 2013).

As an important pathogen, *S. aureus* encompasses a spectrum of strains with varying virulence and pathogenicity. Differentiation between virulent and non-virulent strains is important for evaluation of the potential implications of the presence of this microorganism for

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food safety and public health. There are many putative virulence markers in *S. aureus*, such as staphylococcal enterotoxins (SEs), exfoliatins, toxic shock syndrome toxin (TSST), haemolysins, and Panton-Valentine leukocidin (PVL) (Grumann et al., 2014). Among these virulence markers, PVL is a bacteriophage-encoded bicomponent leukotoxin that is present in some strains of *S. aureus* and plays a key role in leukocytolysis and tissue necrosis (Shallcross et al., 2013). This toxin belongs to a family of synergohymenotropic toxins that consist of two non-associated components that act synergistically on cell membranes. PVL is encoded by the *lukS-PV* and *lukF-PV* bacteriophage-transmitted genes, the detection of which has been used in epidemiological studies to detect and determine the prevalence of PVL-positive *S. aureus* (Melles et al., 2006). Nowadays, many studies have reported an association between PVL genes and invasive disease (Ellis et al., 2004; Gillet et al., 2002; Department of Health and Human Services, Centers for Disease Control and Prevention, 1999; Kaplan, 2006; Lee et al., 2005). In fact, the risk of death associated with PVL-positive *S. aureus* has been reported to be higher than that associated with non-PVL-producing *S. aureus* (Gillet et al., 2002).

In recent years, antibiotic-resistant *S. aureus* clones have rapidly emerged. Isolation of single- or multiple-drug resistant *S. aureus* strains from food, the environment and clinics has been continuously reported (Gould et al., 2012; Rasigade et al., 2014). Methicillin-resistant *S. aureus* (MRSA) is one of most important types of multi-drug-resistant *S. aureus* and has spread globally in recent years. As we know, MRSA can be resistant to several antibiotics and leading causes of death due to infection by any single infectious agent (Deleo et al., 2010). Distinct epidemiological groups of MRSA have been identified, such as hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA). By contrast, CA-MRSA has been reported to carry the loci for PVL at high frequency (Dufour, 2002). In addition to CA-MRSA, PVL is also carried by some methicillin-susceptible *S. aureus* (MSSA) strains, which exhibit similar disease potentials and epidemiological characteristics to those of MRSA (Rasigade et al., 2010). In some countries, such as the UK, PVL-positive MSSAs are more common than MRSA in community-acquired infections (Holmes et al., 2005).

Globally, the reported incidence of PVL-positive *S. aureus* is variable, and the presence of this pathogen is strongly dependent on strain types/lineages as well as geographical differences. There have been a limited number of studies reported on PVL-positive *S. aureus* isolated from retail food. Previously, to study *S. aureus* present in retail foods in China from July 2011 to June 2016, we collected 4300 retail food samples from supermarkets, fairs and farmers' markets, covering most of the provincial capitals of China, and isolated 1581 *S. aureus* strains from 1063 positive samples from all the sampling sites (Wu et al., 2018a; Wu et al., 2018b). In order to further understand these food related *S. aureus* isolates in China, this study was to identify PVL-positive *S. aureus* isolates from our previous study and to characterize these isolates by phenotyping (antimicrobial susceptibility testing) and genotyping (enterotoxin gene typing, multi-locus sequence typing (MLST) and *spa* typing) methods to understand the genetic background of food-related PVL-positive *S. aureus* in China.

2. Materials and methods

2.1. Bacterial isolates

A total of 1581 *S. aureus* isolates, including 108 MRSA isolates and 1473 MSSA isolates, collected from retail food samples in 39 Chinese cities were analysed, comprising 469 isolates from meat and meat products (bacon/sausage, poultry, pork, mutton and beef), 511 isolates from aquatic products (freshwater fish, shrimp and seafood), 368 isolates from quick-frozen products (frozen dumplings/steamed stuffed buns and frozen meat), 148 isolates from ready-to-eat (RTE) food, 13 isolates from pasteurized milk, 30 isolates from vegetables and 42 isolates from edible mushrooms (Wu et al., 2018a; Wu et al., 2018b). The isolates were obtained between July 2011 and June 2016 according to GB 4789.10-2010 for National Food Safety Standard food microbiological examination of *S. aureus* (Ministry of Health of the PRC, 2010) and the most probable number (MPN) method (Gombas et al., 2003). The isolates were identified by Gram staining, catalase and oxidase tests, and API STAPH test strips (bioMerieux, Marcy-1′Etoile, France).

Table 1
Distribution of PVL-positive *S. aureus* isolates from retail foods in China.

Types of product	No. of isolates	No. (%) of PVL-positive isolates	No. (%) of MRSA
Raw meat	469	20	8
Aquatic products	511	22	11
Ready-to-eat food	148	7	1
Quick-frozen meat[a]	368	17	1
Edible mushrooms	42	5	4
Vegetables	30	1	1
Pasteurized milk	13	0	0
Total	1581	72	26

[a] All quick-frozen meat were stored at −10 °C before being sold.

2.2. PCR method

All the isolates were incubated at 37 °C overnight on BHI (brain heart infusion broth). Genomic DNA was extracted using a Genomic DNA Extraction Kit (Magen Biotech, Guangzhou, China) according to the manufacturer's instructions. The presence of *lukSF-PV* (*pvl*) genes was determined by PCR using primers described previously (Jarraud et al., 2002). All positive PCR products were purified using a PCR Purification Kit (Qiagen, Germany) and sequenced with fluorescent BigDye Terminator on an ABI 3730XL sequencer (Applied Biosystems). PCR-based detection of 18 genes encoding SEs (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, *ser* and *seu*) was performed as previously described (Varshney et al., 2009). The amplified DNA fragments were visualised under a UV transillumination gel imaging system (GE Healthcare, WI, USA) following electrophoresis on a 1.5% agarose gel stained with Gold View, and the images were saved as TIFF files for analysis.

2.3. Antimicrobial susceptibility testing

The Kirby-Bauer disk diffusion method was used to test susceptibility to all the antibiotics, and diameter interpretations were based on the protocol described in the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2015). *Staphylococcus aureus* ATCC25923 was used for quality control. CLSI zone diameter breakpoints were used to interpret the antimicrobial susceptibilities of the analysed strains. All *pvl*-positive isolates were assessed for antimicrobial susceptibility to the 19 antibiotics (Oxoid, UK) used: cefoxitin (FOX, 30 μg), penicillin G (P, 10 U), amikacin (AMK, 30 μg), gentamicin (CN, 10 μg), kanamycin (K, 30 μg), streptomycin (S, 25 μg), chloramphenicol (C, 30 μg), clindamycin (DA, 2 μg), erythromycin (E, 15 μg), telithromycin (TEL, 15 μg), ciprofloxacin (CIP, 5 μg), norfloxacin (NOR, 10 μg), tetracycline (TE, 30 μg), linezolid (LZD, 30 μg), trimethoprim/sulphamethoxazole 1:19 (SXT, 25 μg), rifampicin (RD, 5 μg), quinupristin/dalfopristin (QD, 15 μg), nitrofurantoin (F, 300 μg) and fusidic acid (FD, 10 μg). The isolates determined to have linezolid resistance by disk diffusion were also verified using a microdilution test according to the CLSI method for minimum inhibitory concentration (MIC) determination (CLSI, 2015).

2.4. Molecular typing

All of the PVL-positive *S. aureus* isolates were characterized by MLST and *spa* typing (Enright et al., 2013; Shopsin et al., 1999). The

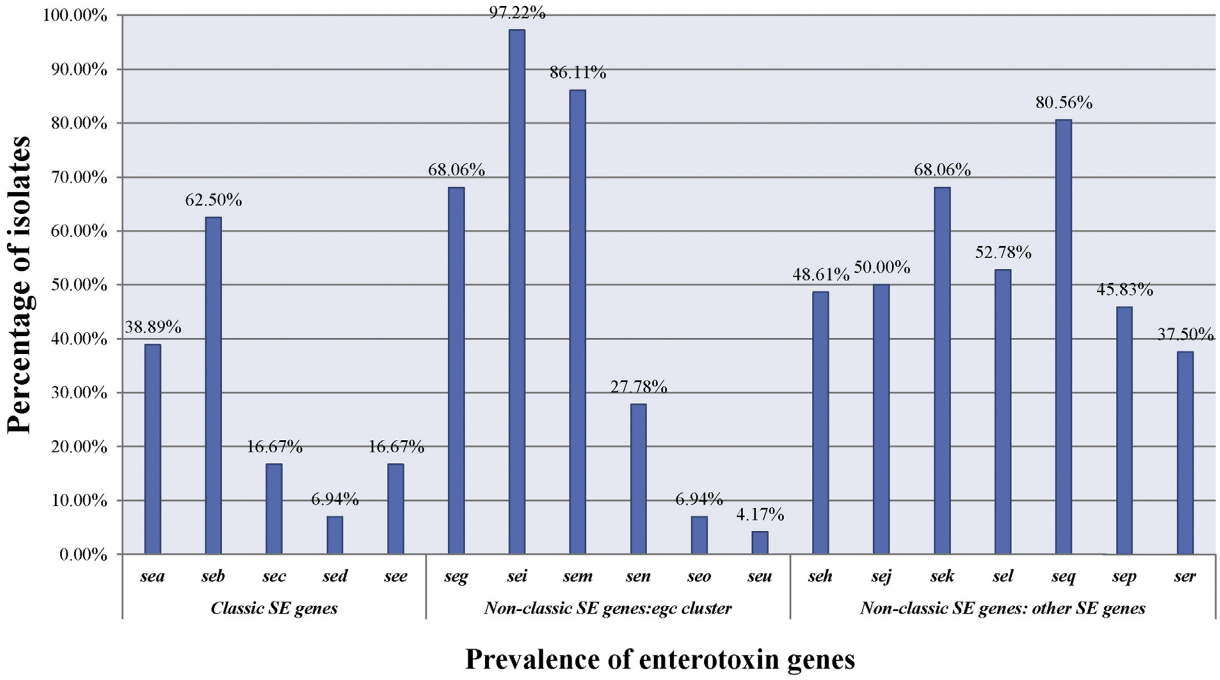


Fig. 1. Distribution of the staphylococcal toxin gene profiles of PVL-positive *S. aureus* isolated from retail food.

Table 2
Results of antimicrobial susceptibility tests of PVL-positive *S. aureus* isolates from China.

Antimicrobial group	Antibiotics[a]	*S. aureus* (n = 72)		
		No. (%) of resistant	No. (%) of intermediate-resistant	No. (%) of susceptible
β-Lactams	Cefoxitin	26 (36.1)	–	46 (63.9)
	Penicillin G	68 (94.4)	–	4 (5.6)
Aminoglycosides	Amikacin	12 (16.7)	28 (38.9)	32 (44.4)
	Gentamicin	8 (11.1)	1 (1.4)	63 (87.5)
	Kanamycin	44 (61.1)	10 (13.9)	18 (25.0)
	Streptomycin	37 (51.4)	28 (38.9)	7 (9.7)
Phenicols	Chloramphenicol	20 (27.8)	19 (26.4)	33 (45.8)
Lincosamides	Clindamycin	46 (63.9)	10 (13.9)	16 (22.2)
Macrolides	Erythromycin	60 (83.4)	5 (6.9)	7 (9.7)
	Telithromycin	42 (58.3)	13 (18.1)	17 (23.6)
Fluoroquinolones	Ciprofloxacin	4 (5.5)	19 (26.4)	49 (68.1)
	Norfloxacin	7 (9.7)	5 (6.9)	60 (83.4)
Tetracyclines	Tetracycline	34 (47.2)	3 (4.2)	35 (48.6)
Oxazolidinones	Linezolid	2 (2.8)	–	70 (97.2)
Ansamycins	Rifampicin	3 (4.2)	1 (1.4)	68 (94.4)
Sulphonamides	Trimethoprim/sulphamethoxazole 1:19	1 (1.4)	1 (1.4)	70 (97.2)
Streptogramin combination	Quinupristin/dalfopristin	0 (0.0)	9 (12.5)	63 (87.5)
Nitrofurantoins	Nitrofurantoin	0 (0.0)	9 (12.5)	63 (87.5)
Isoprenoid	Fusidic acid	20 (27.8)	–	52 (72.2)
Antimicrobial	1–3 antimicrobial	24		
	4–10 Antimicrobial	47		
	11–13 Antimicrobial	0		

[a] Breakpoints from the CLSI (2015).

PCR products were purified by using a PCR Purification Kit (Qiagen, Germany) and sequenced in each direction with fluorescent BigDye Terminator on an ABI 3730XL sequencer (Applied Biosystems). STs were determined using the *S. aureus* MLST database (https://pubmlst.org/saureus/). Clonal complex (CC) analysis was performed in eBURST v.3 as previously described (Feil et al., 2004). The *spa* types were randomly assigned using the SpaServer website (http://spaserver2.ridom.de). Sequence Type Analysis and Recombinational Tests software (S.T.A.R.T. ver. 2; http://pubmlst.org/software/analysis/start2) was used to analyse the MLST data.

3. Results

Overall, of the 1581 *S. aureus* isolates from retail foods in China, 72 (4.6%) isolates tested positive for the *pvl* gene, including 20 isolates (20/469, 4.3%) from raw meat, 22 isolates (22/511, 4.3%) from aquatic products, 17 isolates (17/368, 4.6%) from quick-frozen food, 7 isolates (7/148, 4.7%) from RTE food, 5 isolates (5/42, 11.9%) from edible mushrooms and one isolate (1/30, 3.3%) from vegetables, whereas all the isolates from pasteurized milk lacked the *pvl* gene (Table 1). Twenty-six (24.1%) of the PVL-positive *S. aureus* isolates were MRSA and 46 (3.1%) of them were PVL-positive MSSA.

Among the 72 food-related PVL-positive isolates analysed, each

Table 3
The STs and *spa* types of the PVL-positive *S. aureus* isolated from retail food in China.

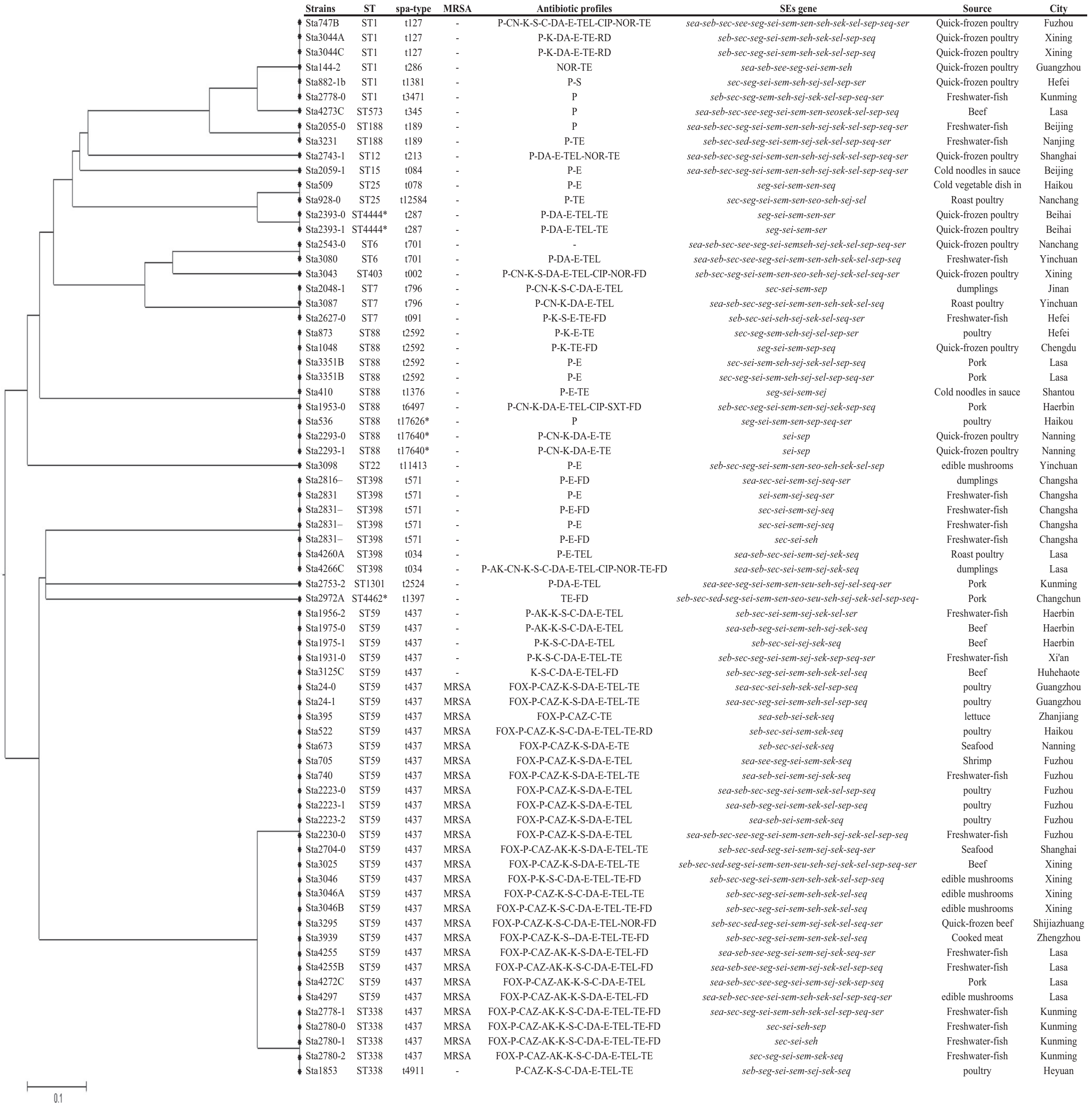
Method	Criterion	No. (%) of positive isolates						
		Total (n = 72)	Raw meat (n = 20)	Edible mushrooms (n = 5)	Vegetables (n = 1)	Ready-to-eat food (n = 7)	Aquatic products (n = 22)	Quick-frozen meat[a] (n = 17)
MLST	ST59	27 (37.50)	11 (15.28)	4 (5.56)	1 (1.39)	1 (1.39)	9 (12.50)	1 (1.39)
	ST88	9 (12.50)	5 (6.94)	–	–	1 (1.39)	–	3 (4.17)
	ST398	7 (9.72)	–	–	–	1 (1.39)	4 (5.56)	2 (2.78)
	ST1	6 (8.33)	–	–	–	–	1 (1.39)	5 (6.94)
	ST338	5 (6.94)	1 (1.39)	–	–	–	4 (5.56)	–
	ST7	3 (4.17)	–	–	–	1 (1.39)	1 (1.39)	1 (1.39)
	ST6	2 (2.78)	–	–	–	–	1 (1.39)	1 (1.39)
	ST25	2 (2.78)	–	–	–	2 (2.78)	–	–
	ST188	2 (2.78)	–	–	–	–	2 (2.78)	–
	ST4444[a]	2 (2.78)	–	–	–	–	–	2 (2.78)
	ST12	1 (1.39)	–	–	–	–	–	1 (1.39)
	ST15	1 (1.39)	–	–	–	1 (1.39)	–	–
	ST22	1 (1.39)	–	1 (1.39)	–	–	–	–
	ST403	1 (1.39)	–	–	–	–	–	1 (1.39)
	ST573	1 (1.39)	1 (1.39)	–	–	–	–	–
	ST1301	1 (1.39)	1 (1.39)	–	–	–	–	–
	ST4462[a]	1 (1.39)	1 (1.39)	–	–	–	–	–
spa	t437	31 (43.06)	11 (15.28)	4 (5.56)	1 (1.39)	1 (1.39)	13 (18.06)	1 (1.39)
	t571	5 (6.94)	–	–	–	–	4 (5.56)	1 (1.39)
	t2592	4 (5.56)	3 (4.17)	–	–	–	–	1 (1.39)
	t127	3 (4.17)	–	–	–	–	–	3 (4.17)
	t034	2 (2.78)	–	–	–	1 (1.39)	–	1 (1.39)
	t17640[b]	2 (2.78)	–	–	–	–	–	2 (2.78)
	t189	2 (2.78)	–	–	–	–	2 (2.78)	–
	t287	2 (2.78)	–	–	–	–	–	2 (2.78)
	t701	2 (2.78)	–	–	–	–	1 (1.39)	1 (1.39)
	t796	2 (2.78)	–	–	–	1 (1.39)	–	1 (1.39)
	t002	1 (1.39)	–	–	–	–	–	1 (1.39)
	t078	1 (1.39)	–	–	–	1 (1.39)	–	–
	t084	1 (1.39)	–	–	–	1 (1.39)	–	–
	t091	1 (1.39)	–	–	–	–	1 (1.39)	–
	t11413	1 (1.39)	–	1 (1.39)	–	–	–	–
	t12584	1 (1.39)	–	–	–	1 (1.39)	–	–
	t1376	1 (1.39)	–	–	–	1 (1.39)	–	–
	t1381	1 (1.39)	–	–	–	–	–	1 (1.39)
	t1397	1 (1.39)	1 (1.39)	–	–	–	–	–
	t17626[b]	1 (1.39)	1 (1.39)	–	–	–	–	–
	t213	1 (1.39)	–	–	–	–	–	1 (1.39)
	t2524	1 (1.39)	1 (1.39)	–	–	–	–	–
	t286	1 (1.39)	–	–	–	–	–	1 (1.39)
	t345	1 (1.39)	1 (1.39)	–	–	–	–	–
	t3471	1 (1.39)	–	–	–	–	1 (1.39)	–
	t4911	1 (1.39)	1 (1.39)	–	–	–	–	–
	t6497	1 (1.39)	1 (1.39)	–	–	–	–	–

[a] Novel ST types.
[b] Novel *spa* types.

isolate harboured at least one of the SEs gene, including 29 isolates carrying > 10 SEs gene. As shown in Fig. 1, the *sei* gene was the most frequently detected gene, whereas *seu* gene was the lowest detected gene among the 18 investigated SE genes. In this study, 87.5% of the *S. aureus* isolates (63/72) harboured one or more genes for the classic SE genes (*sea*, *seb*, *sec*, *sed*, and *see*), whereas all of the *S. aureus* isolates harboured one or more genes for the *egc* cluster (*seg*, *sei*, *sem*, *sen*, *seo*, and *seu*). The classic SE genes accounted for 22.6% (142/627) of the detected genes, whereas the *egc* cluster accounted for 33.3% (209/627). There was no significant difference between MRSA and MSSA in terms of most of the enterotoxin genes detected.

The food related PVL-positive isolates were all susceptible to linezolid, but high level of resistance was observed for penicillin (94.4%) and erythromycin (83.4%; Table 2). Resistance to clindamycin, kanamycin, telithromycin, streptomycin, and tetracycline were also common in this study. Most of the isolates (71/72, 98.6%) exhibited resistance to at least one antibiotic. Multi-drug resistance (resistant to > 3 antimicrobial groups) was observed in 47 (65.3%) PVL-positive *S. aureus* isolates. In this study, the proportion of PVL-positive MRSA was higher than PVL-positive MSSA. Most food-related PVL-positive MRSA isolates (84.6%, 22/26) were resistant to > 10 antibiotics, including kanamycin, streptomycin, clindamycin, erythromycin and telithromycin. Interestingly, a comparison of the MRSA and MSSA isolates revealed that resistance to gentamicin, ciprofloxacin and trimethoprim/sulphamethoxazole 1:19 was detected in only MSSA.

All PVL-positive *S. aureus* isolates were subjected to *spa* typing and MLST. Among these isolates, a total of seventeen STs and twenty-seven *spa* types were identified, including two novel STs (ST4444 and ST4462) and two novel *spa* types (t17626 and t17640). The most commonly observed *spa* type was t437 (43.1%), followed by t571 (6.9%), t2592 (5.6%), t127 (4.2%), t034 (2.8%), t189 (2.8%), t287 (2.8%), t701 (2.8%), t796 (2.8%), t17640 (2.8%) and other *spa* types (1.4%) (Table 3). The predominant MLST types were ST59 (37.5%), ST88 (12.5%), ST398 (9.7%), ST1 (8.3%), and ST338 (6.9%) (Table 3). Based on eBURST analysis, three CCs were identified, including CC59 (ST59, ST338), CC1 (ST1, ST573), and CC25 (ST25, ST4444). Compared with MLST, *spa* typing exhibited superior discriminatory power. Some isolates had identical STs but differing *spa* types (ST1-t127, ST1-



Strains	ST	spa-type	MRSA	Antibiotic profiles	SEs gene	Source	City
Sta747B	ST1	t127	-	P-CN-K-S-C-DA-E-TEL-CIP-NOR-TE	*sea-seb-sec-see-seg-sei-sem-sen-seh-sek-sel-sep-seq-ser*	Quick-frozen poultry	Fuzhou
Sta3044A	ST1	t127	-	P-K-DA-E-TE-RD	*seb-sec-seg-sei-sem-seh-sek-sel-sep-seq*	Quick-frozen poultry	Xining
Sta3044C	ST1	t127	-	P-K-DA-E-TE-RD	*seb-sec-seg-sei-sem-seh-sek-sel-sep-seq*	Quick-frozen poultry	Xining
Sta144-2	ST1	t286	-	NOR-TE	*sea-seb-see-seg-sei-sem-seh*	Quick-frozen poultry	Guangzhou
Sta882-1b	ST1	t1381	-	P-S	*sec-seg-sei-sem-seh-sej-sel-sep-ser*	Quick-frozen poultry	Hefei
Sta2778-0	ST1	t3471	-	P	*seb-sec-seg-sem-seh-sej-sek-sel-sep-seq-ser*	Freshwater-fish	Kunming
Sta4273C	ST573	t345	-	P	*sea-seb-sec-see-seg-sei-sem-sen-seosek-sel-sep-seq*	Beef	Lasa
Sta2055-0	ST188	t189	-	P	*sea-seb-sec-seg-sei-sem-sen-seh-sej-sek-sel-sep-seq-ser*	Freshwater-fish	Beijing
Sta3231	ST188	t189	-	P-TE	*seb-sec-sed-seg-sei-sem-sej-sek-sel-sep-seq-ser*	Freshwater-fish	Nanjing
Sta2743-1	ST12	t213	-	P-DA-E-TEL-NOR-TE	*sea-seb-sec-seg-sei-sem-sen-seh-sej-sek-sel-sep-seq-ser*	Quick-frozen poultry	Shanghai
Sta2059-1	ST15	t084	-	P-E	*sea-seb-sec-seg-sei-sem-sen-seh-sej-sek-sel-sep-seq-ser*	Cold noodles in sauce	Beijing
Sta509	ST25	t078	-	P-E	*seg-sei-sem-sen-seq*	Cold vegetable dish in	Haikou
Sta928-0	ST25	t12584	-	P-TE	*sec-seg-sei-sem-sen-seo-seh-sej-sel*	Roast poultry	Nanchang
Sta2393-0	ST4444*	t287	-	P-DA-E-TEL-TE	*seg-sei-sem-sen-ser*	Quick-frozen poultry	Beihai
Sta2393-1	ST4444*	t287	-	P-DA-E-TEL-TE	*seg-sei-sem-ser*	Quick-frozen poultry	Beihai
Sta2543-0	ST6	t701	-	-	*sea-seb-sec-see-seg-sei-semseh-sej-sek-sel-sep-seq-ser*	Quick-frozen poultry	Nanchang
Sta3080	ST6	t701	-	P-DA-E-TEL	*sea-seb-sec-see-seg-sei-sem-sen-seh-sek-sel-sep-seq*	Freshwater-fish	Yinchuan
Sta3043	ST403	t002	-	P-CN-K-S-DA-E-TEL-CIP-NOR-FD	*seb-sec-seg-sei-sem-sen-seo-seh-sej-sek-sel-seq-ser*	Quick-frozen poultry	Xining
Sta2048-1	ST7	t796	-	P-CN-K-S-C-DA-E-TEL	*sec-sei-sem-sep*	dumplings	Jinan
Sta3087	ST7	t796	-	P-CN-K-DA-E-TEL	*sea-seb-sec-seg-sei-sem-sen-seh-sek-sel-seq*	Roast poultry	Yinchuan
Sta2627-0	ST7	t091	-	P-K-S-E-TE-FD	*seb-sec-sei-seh-sej-sek-sel-seq-ser*	Freshwater-fish	Hefei
Sta873	ST88	t2592	-	P-K-E-TE	*sec-seg-sem-seh-sej-sel-sep-ser*	poultry	Hefei
Sta1048	ST88	t2592	-	P-K-TE-FD	*seg-sei-sem-sep-seq*	Quick-frozen poultry	Chengdu
Sta3351B	ST88	t2592	-	P-E	*sec-sei-sem-seh-sej-sek-sel-sep-seq*	Pork	Lasa
Sta3351B	ST88	t2592	-	P-E	*sec-seg-sei-sem-seh-sej-sel-sep-seq-ser*	Pork	Lasa
Sta410	ST88	t1376	-	P-E-TE	*seg-sei-sem-sej*	Cold noodles in sauce	Shantou
Sta1953-0	ST88	t6497	-	P-CN-K-DA-E-TEL-CIP-SXT-FD	*seb-sec-seg-sei-sem-sen-sej-sek-sep-seq*	Pork	Haerbin
Sta536	ST88	t17626*	-	P	*seg-sei-sem-sen-sep-seq-ser*	poultry	Haikou
Sta2293-0	ST88	t17640*	-	P-CN-K-DA-E-TE	*sei-sep*	Quick-frozen poultry	Nanning
Sta2293-1	ST88	t17640*	-	P-CN-K-DA-E-TE	*sei-sep*	Quick-frozen poultry	Nanning
Sta3098	ST22	t11413	-	P-E	*seb-sec-seg-sei-sem-sen-seo-seh-sek-sel-sep*	edible mushrooms	Yinchuan
Sta2816–	ST398	t571	-	P-E-FD	*sea-sec-sei-sem-sej-seq-ser*	dumplings	Changsha
Sta2831	ST398	t571	-	P-E	*sei-sem-sej-seq-ser*	Freshwater-fish	Changsha
Sta2831–	ST398	t571	-	P-E-FD	*sec-sei-sem-sej-seq*	Freshwater-fish	Changsha
Sta2831–	ST398	t571	-	P-E	*sec-sei-sem-sej-seq*	Freshwater-fish	Changsha
Sta2831–	ST398	t571	-	P-E-FD	*sec-sei-seh*	Freshwater-fish	Changsha
Sta4260A	ST398	t034	-	P-E-TEL	*sea-seb-sec-sei-sem-sej-sek-seq*	Roast poultry	Lasa
Sta4266C	ST398	t034	-	P-AK-CN-K-S-C-DA-E-TEL-CIP-NOR-TE-FD	*sea-seb-sec-sei-sem-sej-sek-seq*	dumplings	Lasa
Sta2753-2	ST1301	t2524	-	P-DA-E-TEL	*sea-see-seg-sei-sem-sen-seu-seh-sej-sel-seq-ser*	Pork	Kunming
Sta2972A	ST4462*	t1397	-	TE-FD	*seb-sec-sed-seg-sei-sem-sen-seo-seu-seh-sej-sek-sel-sep-seq-*	Pork	Changchun
Sta1956-2	ST59	t437	-	P-AK-K-S-C-DA-E-TEL	*seb-sec-sei-sem-sej-sek-sel-ser*	Freshwater-fish	Haerbin
Sta1975-0	ST59	t437	-	P-AK-K-S-C-DA-E-TEL	*sea-seb-seg-sei-sem-seh-sej-sek-seq*	Beef	Haerbin
Sta1975-1	ST59	t437	-	P-K-S-C-DA-E-TEL	*seb-sec-sei-sej-sek-seq*	Beef	Haerbin
Sta1931-0	ST59	t437	-	P-K-S-C-DA-E-TEL-TE	*seb-sec-seg-sei-sem-sej-sek-sep-seq-ser*	Freshwater-fish	Xi'an
Sta3125C	ST59	t437	-	K-S-C-DA-E-TEL-FD	*seb-sec-seg-sei-sem-seh-sek-sel-seq*	Beef	Huhehaote
Sta24-0	ST59	t437	MRSA	FOX-P-CAZ-K-S-DA-E-TEL-TE	*sea-sec-sei-seh-sek-sel-sep-seq*	poultry	Guangzhou
Sta24-1	ST59	t437	MRSA	FOX-P-CAZ-K-S-DA-E-TEL-TE	*sea-sec-seg-sei-sem-seh-sel-sep-seq*	poultry	Guangzhou
Sta395	ST59	t437	MRSA	FOX-P-CAZ-C-TE	*sea-seb-sei-sek-seq*	lettuce	Zhanjiang
Sta522	ST59	t437	MRSA	FOX-P-CAZ-K-S-C-DA-E-TEL-TE-RD	*seb-sec-sei-sem-sek-seq*	poultry	Haikou
Sta673	ST59	t437	MRSA	FOX-P-CAZ-K-S-DA-E-TE	*seb-sec-sei-sek-seq*	Seafood	Nanning
Sta705	ST59	t437	MRSA	FOX-P-CAZ-K-S-DA-E-TEL	*sea-see-seg-sei-sem-sek-seq*	Shrimp	Fuzhou
Sta740	ST59	t437	MRSA	FOX-P-CAZ-K-S-DA-E-TEL-TE	*sea-seb-sei-sem-sej-sek-seq*	Freshwater-fish	Fuzhou
Sta2223-0	ST59	t437	MRSA	FOX-P-CAZ-K-S-DA-E-TEL	*sea-seb-sec-seg-sei-sem-sek-sel-sep-seq*	poultry	Fuzhou
Sta2223-1	ST59	t437	MRSA	FOX-P-CAZ-K-S-DA-E-TEL	*sea-seb-seg-sei-sem-sek-sel-sep-seq*	poultry	Fuzhou
Sta2223-2	ST59	t437	MRSA	FOX-P-CAZ-K-S-DA-E-TEL	*sea-seb-sei-sem-sek-seq*	poultry	Fuzhou
Sta2230-0	ST59	t437	MRSA	FOX-P-CAZ-K-S-DA-E-TEL	*sea-seb-sec-see-seg-sei-sem-sen-seh-sej-sek-sel-sep-seq*	Freshwater-fish	Fuzhou
Sta2704-0	ST59	t437	MRSA	FOX-P-CAZ-AK-K-S-DA-E-TEL-TE	*seb-sec-sed-seg-sei-sem-sej-sek-seq-ser*	Seafood	Shanghai
Sta3025	ST59	t437	MRSA	FOX-P-CAZ-K-S-DA-E-TEL-TE	*seb-sec-sed-seg-sei-sem-sen-seu-seh-sej-sek-sel-sep-seq-ser*	Beef	Xining
Sta3046	ST59	t437	MRSA	FOX-P-K-S-C-DA-E-TEL-TE-FD	*seb-sec-seg-sei-sem-sen-seh-sek-sel-sep-seq*	edible mushrooms	Xining
Sta3046A	ST59	t437	MRSA	FOX-P-CAZ-K-S-C-DA-E-TEL-TE	*seb-sec-seg-sei-sem-seh-sek-sel-seq*	edible mushrooms	Xining
Sta3046B	ST59	t437	MRSA	FOX-P-CAZ-K-S-C-DA-E-TEL-TE-FD	*seb-sec-seg-sei-sem-seh-sek-sel-seq*	edible mushrooms	Xining
Sta3295	ST59	t437	MRSA	FOX-P-CAZ-K-S-C-DA-E-TEL-NOR-FD	*seb-sec-sed-seg-sei-sem-sej-sek-sel-seq-ser*	Quick-frozen beef	Shijiazhuang
Sta3939	ST59	t437	MRSA	FOX-P-CAZ-K-S--DA-E-TEL-TE-FD	*seb-sec-seg-sei-sem-sen-sek-sel-seq*	Cooked meat	Zhengzhou
Sta4255	ST59	t437	MRSA	FOX-P-CAZ-AK-K-S-DA-E-TEL-FD	*sea-seb-see-seg-sei-sem-sej-sek-seq-ser*	Freshwater-fish	Lasa
Sta4255B	ST59	t437	MRSA	FOX-P-CAZ-AK-K-S-C-DA-E-TEL-FD	*sea-seb-see-seg-sei-sem-sej-sek-sel-sep-seq*	Freshwater-fish	Lasa
Sta4272C	ST59	t437	MRSA	FOX-P-CAZ-AK-K-S-C-DA-E-TEL	*sea-seb-sec-see-seg-sei-sem-sej-sek-sep-seq*	Pork	Lasa
Sta4297	ST59	t437	MRSA	FOX-P-CAZ-AK-K-S-DA-E-TEL-FD	*sea-seb-sec-see-sei-sem-seh-sek-sel-sep-seq-ser*	edible mushrooms	Lasa
Sta2778-1	ST338	t437	MRSA	FOX-P-CAZ-AK-K-S-C-DA-E-TEL-TE-FD	*sea-sec-seg-sei-sem-seh-sek-sel-sep-seq-ser*	Freshwater-fish	Kunming
Sta2780-0	ST338	t437	MRSA	FOX-P-CAZ-AK-K-S-C-DA-E-TEL-TE-FD	*sec-sei-seh-sep*	Freshwater-fish	Kunming
Sta2780-1	ST338	t437	MRSA	FOX-P-CAZ-AK-K-S-C-DA-E-TEL-TE-FD	*sec-sei-seh*	Freshwater-fish	Kunming
Sta2780-2	ST338	t437	MRSA	FOX-P-CAZ-AK-K-S-C-DA-E-TEL-TE	*sec-seg-sei-sem-sek-seq*	Freshwater-fish	Kunming
Sta1853	ST338	t4911	-	P-CAZ-K-S-C-DA-E-TEL-TE	*seb-seg-sei-sem-sej-sek-seq*	poultry	Heyuan

Fig. 2. The UPGMA (unweighted pair group method with arithmetic mean) tree of the 7 multi-locus sequence typing loci of PVL-positive *S. aureus* isolates. * represents novel types. This tree was generated using the S.T.A.R.T. (version 2).

t286, ST1-t1381 and ST1-t3471). A phylogenetic tree based on the 7 concatenated MLST sequences (Fig. 2) shows the relationships among the PVL-positive *S. aureus* strains. However, most STs, such as ST1-t127, ST188-t189, ST88-t2592, ST398-t571 and ST59-t437, correlated well with *spa* types. Notably, all the PVL-positive MRSA isolates belonged to CC59-t437, which grouped into one cluster and exhibited multi-drug resistance.

4. Discussion

Recent years, PVL-positive *S. aureus* has received a significant amount of attention which can cause highly necrotizing pneumonia, necrotizing dermatitis, and other primary diseases in humans (Shallcross et al., 2013). Approximately, the mortality of PVL-positive *S. aureus* infection were ranges from 40% to 60% (Lina et al., 1999). PVL-positive *S. aureus* frequently detected in clinical (Asiimwe et al., 2017; Brown et al., 2011; Hewagama et al., 2016; Krziwanek et al., 2007; Monecke et al., 2014; Vourli et al., 2009; Yamamoto, 2004; Yu et al., 2010). For food-related *S. aureus*, PVL-positive *S. aureus* was either not detected or exhibited very low prevalence rates (Argudín et al., 2012; Feßler et al., 2011; Gharsa et al., 2012; Hanson et al., 2011; Papadopoulos et al., 2018). In this study, 4.6% of the food-related *S. aureus* isolates were PVL positive. It is the first systematical investigation of PVL-positive *S. aureus* isolated from retail foods in China which showed lower than the results of studies on clinical *S. aureus* but higher than those of most food-related studies. However, the prevalence of PVL-positive *S. aureus* is associated with sample type and geographic location, which might impact the prevalence of these organisms.

Antimicrobial resistance was noted in food related PVL-positive *S. aureus* isolates in this study. 98.6% of the isolates exhibited resistance to at least one antibiotic and 65.3% of the isolates were multi-drug resistant. The frequency of resistance to some antimicrobial agents, including penicillin, erythromycin, and tetracycline was higher than that reported in previous report by Yu et al. (2010) from hospitalized patients in China. The resistant profiles of mis consistent with those of many previous studies conducted worldwide (Otter et al., 2010; Takizawa et al., 2005; Xiao et al., 2013; Yu et al., 2010). Thus, the scenario regarding the presence of PVL-positive *S. aureus* in retail food in China is not favourable and warrants public attention.

In this study, the presence of SE genes was determined in PVL-positive *S. aureus* isolates. The SE genes produced by enterotoxigenic *S. aureus* are associated with most types of staphylococcal food poisoning. These toxins are heat stable, stable to proteolysis or exhibit emetic activity. Generally, 95% of staphylococcal food poisoning was found to be caused by classical SE genes (Kokan and Bergdoll, 1987). In the present study, 87.5% of the isolates (63/72) harboured the classic SE genes. These proportions are higher than those of other studies, indicating the high potential of these isolates to cause staphylococcal food poisoning. Currently, *lukS-PV* and *lukF-PV* are located on several temperate *Siphoviridae* phages (Li et al., 2012). In addition, some SE genes (*sea*, *sek*, *sep* and *seq*) are harboured by some *S. aureus Siphoviridae* phages, which may facilitate the transmission of PVL-carrying phages among the *S. aureus* population. In fact, most SE genes are carried and disseminated by different mobile genetic elements (e.g., on plasmids, prophages, *S. aureus* pathogenicity islands (SaPIs), and the genomic island *v*Sa or adjacent to staphylococcal cassette chromosome (SCC) elements), and the spread of these genes among *S. aureus* isolates can modify the ability of the isolates to cause disease and contribute to the evolution of this important pathogen (Argudín et al., 2010).

Of the various molecular typing methods, MLST and *spa* typing showed the highly clonal population structure identified for *S. aureus* in many previous studies (Basanisi et al., 2017; Fetsch et al., 2014; Kanika et al., 2011; Wu et al., 2018b). In this study, these PVL-positive *S. aureus* strains belonged to 17 STs and 27 *spa* types. Except for the novel types, most of these types, such as ST1, ST188, ST15, ST398 and ST59, have been linked to human infections around the world (Silva-Carvalho et al., 2009; Wang et al., 2018). In this study, CC59-t437 was the predominant type of the PVL-positive MRSA isolates, which is consistent with the results of previous studies that isolated *S. aureus* from hospitalized patients in China (Chen et al., 2009; Fan et al., 2009; Hu et al., 2014; Li et al., 2012; Xiao et al., 2013; Yu et al., 2010; Zhao et al., 2016). Currently, PVL is the most consistently observed transferable toxin locus among CA-MRSA strains and is considered a stable marker of CA-MRSA strains worldwide (Lo and Wang, 2011). In 2013, Chuang and Huang (2013) reviewed the molecular epidemiology of CA-MRSA in Asia and noted that CC59-SCC*mec*IV-t437 was the major lineage of CA-MRSA in China. Our result implied that food-related PVL-positive MRSA may be an important source of CA-MRSA in China. In addition, ST88 and ST398 were the most common clones among PVL-positive MSSA in this study. In recent years, the isolation of ST398 strains has been increasingly reported from farms and individuals around the world (Argudín et al., 2011; Huber et al., 2010; Smith and Pearson, 2011). Interestingly, > 60% of the infections of livestock and individuals that occurred via live animal contact have been linked to MRSA ST398, whereas human infections with MSSA ST398 were apparently more frequent than those caused by MRSA ST398 (Deiters et al., 2015; Grundmann et al., 2010). This finding is also consistent with previous studies conducted in China (Fan et al., 2009; Zhao et al., 2016). The positive detection of the *pvl* gene in this study may explain why MSSA ST398 has been frequently detected in humans. In addition, MSSA ST88 appears to always carry the *pvl* gene, as reported in previous studies in China (Zhao et al., 2016). According to previous studies, ST88 is not the predominant *S. aureus* lineage observed in hospitals, animals or food in China (Chao et al., 2015; He et al., 2013; Song et al., 2015; Xiuli et al., 2016; Yang et al., 2018; Ye et al., 2016). Thus, further research should be conducted to determine why PVL-positive MSSA ST88 became the dominant ST in China.

5. Conclusion

In conclusion, this study shows the existence of PVL-positive *S. aureus* in retail food and the presence of different *S. aureus* strains harbouring important resistance and virulence determinants. The molecular epidemiological study conducted herein showed the relationships between these food-related PVL-positive *S. aureus* strains and hospital-acquired isolates. Notably, 24.1% of the MRSA isolates were PVL positive and belonged to the predominant type of CA-MRSA (CC59-t437) in China. Our study is the first systematic and full-scale investigation of the prevalence of PVL-positive *S. aureus* isolated from retail foods in China, revealing the genetic background of food-related PVL-positive *S. aureus* isolates in China. However, the presence of these isolates in food represents a potential health risk for consumers and warrants further attention.

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References

Argudín, M.Á., Mendoza, M.C., Rodicio, M.R., 2010. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins 2, 1751–1773.

Argudín, M.A., Tenhagen, B.A., Fetsch, A., Sachsenröder, J., Käsbohrer, A., Schroeter, A., Hammerl, J.A., Hertwig, S., Helmuth, R., Bräunig, J., 2011. Virulence and resistance determinants of German *Staphylococcus aureus* ST398 isolates from nonhuman sources. Appl. Environ. Microb. 77, 3052–3060.

Argudín, M.A., Mendoza, M.C., Gonzálezhevia, M.A., Bances, M., Guerra, B., Rodicio, M.R., 2012. Genotypes, exotoxin gene content, and antimicrobial resistance of *Staphylococcus aureus* strains recovered from foods and food handlers. Appl. Environ. Microb. 78, 2930–2935.

Asiimwe, B.B., Baldan, R., Trovato, A., Cirillo, D.M., 2017. Molecular epidemiology of Panton-Valentine Leukocidin-positive community-acquired methicillin resistant *Staphylococcus aureus* isolates in pastoral communities of rural south western Uganda. BMC Infect. Dis. 17, 24.

Aydin, A., Sudagidan, M., Muratoglu, K., 2011. Prevalence of staphylococcal enterotoxins, toxin genes and genetic-relatedness of foodborne *Staphylococcus aureus* strains isolated in the Marmara Region of Turkey. Int. J. Food Microbiol. 148, 99–106.

Basanisi, M.G., Bella, G.L., Nobili, G., Franconieri, I., Salandra, G.L., 2017. Genotyping of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and dairy products in South Italy. Food Microbiol. 62, 141–146.

Brown, M.L., O'Hara, F.P., Close, N.M., Mera, R.M., Miller, L.A., Suaya, J.A., Amrine-Madsen, H., 2011. Prevalence and sequence variation of panton-valentine leukocidin in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains in the United States. J. Clin. Microbiol. 50, 86–90.

Chao, G., Bao, G., Cao, Y., Yan, W., Wang, Y., Zhang, X., Zhou, L, Wu, Y., 2015. Prevalence and diversity of enterotoxin genes with genetic background of Staphylococcus aureus isolates from different origins in China. Int. J. Food Microbiol. 211, 142–147.

Chen, F.J., Hiramatsu, K., Huang, I.W., Wang, C.H., Lauderdale, T.L., 2009. Panton–Valentine leukocidin (PVL)-positive methicillin-susceptible and resistant *Staphylococcus aureus* in Taiwan: identification of oxacillin-susceptible mecA-positive methicillin-resistant *S. aureus*. Diagn. Microbiol. Infec. Dis. 65, 351–357.

Chuang, Y.Y., Huang, Y.C., 2013. Molecular epidemiology of community-associated meticillin-resistant *Staphylococcus aureus* in Asia. Lancet Infect. Dis. 13, 698–708.

CLSI, 2015. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth InformationalSupplement. Approved Standard-M02-A12. Wayne,. PA: The Clinical andLaboratory Standards Institute.

Deiters, C., Günnewig, V., Friedrich, A.W., Mellmann, A., Köck, R., 2015. Are cases of methicillin-resistant *Staphylococcus aureus* clonal complex (CC) 398 among humans still livestock-associated? Int. J. Med. Microbiol. 305, 110–113.

Deleo, F.R., Otto, M., Kreiswirth, B.N., Chambers, H.F., 2010. Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet 375, 767–768.

Department of Health and Human Services, Centers for Disease Control and Prevention, 1999. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*- Minnesota and North Dakota, 1997–1999. Mmwr Morbid. Mortal. W. 48, 707–710.

Dufour, P., 2002. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton-Valentine leukocidin. Clin. Infect. Dis. 35, 819–824.

Ellis, M.W., Hospenthal, D.R., Dooley, D.P., Gray, P.J., Murray, C.K., 2004. Natural history of community-acquired methicillin-resistant *Staphylococcus aureus* colonization and infection in soldiers. Clin. Infect. Dis. 39, 971–979.

Enright, M.C., Day, N.P.J., Davies, C.E., Peacock, S.J., Spratt, B.G., 2013positive S. aureus isolates were characterized. Multilocus Sequence Typing for Characterization of Methicillin-Resistant and Methicillin-Susceptible Clones of Staphylococcus aureus. J.Clin. Microbl. 51, 306–310.

Fan, J., Shu, M., Zhang, G., Zhou, W., Jiang, Y., Zhu, Y., Chen, G., Peacock, S.J., Wan, C., Pan, W., 2009. Biogeography and virulence of *Staphylococcus aureus*. PLoS One 4, e6216.

Feil, E.J., Li, B.C., Aanensen, D.M., Hanage, W.P., Spratt, B.G., 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J. Bacteriol. 186, 1518–1530.

Fetsch, A., Contzen, M., Hartelt, K., Kleiser, A., Maassen, S., Rau, J., Kraushaar, B., Layer, F., Strommenger, B., 2014. *Staphylococcus aureus* food-poisoning outbreak associated with the consumption of ice-cream. Int. J. Food Microbiol. 187, 1–6.

Gharsa, H., Slama, K.B., Lozano, C., Gómez-Sanz, E., Klibi, N., Sallem, R.B., Gómez, P., Zarazaga, M., Boudabous, A., Torres, C., 2012. Prevalence, antibiotic resistance, virulence traits and genetic lineages of *Staphylococcus aureus* in healthy sheep in Tunisia. Vet. Microbiol. 156, 367–373.

Gillet, Y., Issartel, B., Vanhems, P., Fournet, J.C., Lina, G., Bes, M., Vandenesch, F., Piémont, Y., Brousse, N., Floret, D., 2002. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet 359, 753–759.

Gombas, D.E., Chen, Y., Clavero, R.S., Scott, V.N., 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. J. Food Protect. 66, 559–569.

Gould, I.M., David, M.Z., Esposito, S., Garau, J., Lina, G., Mazzei, T., Peters, G., 2012. New insights into meticillin-resistant *Staphylococcus aureus* (MRSA) pathogenesis, treatment and resistance. Int. J. Antimicrob. Ag. 39, 96–104.

Grumann, D., Nübel, U., Bröker, B.M., 2014. *Staphylococcus aureus* toxins—their functions and genetics. Infect. Genet. Evo. 21, 583–592.

Grundmann, H., Aanensen, D.M., Wijngaard, C.C.V.D., Spratt, B.G., Harmsen, D., Friedrich, A.W., 2010. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. PLoS Med. 7, e1000215.

Hanson, B.M., Dressler, A.E., Harper, A.L., Scheibel, R.P., Wardyn, S.E., Roberts, L.K., Kroeger, J.S., Smith, T.C., 2011. Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. J. Infect. Public Heal. 4, 169–174.

He, W., Chen, H., Zhao, C., Zhang, F., Li, H., Wang, Q., Wang, X., Wang, H., 2013. Population structure and characterisation of *Staphylococcus aureus* from bacteraemia at multiple hospitals in China: association between antimicrobial resistance, toxin genes and genotypes. Int. J. Antimicrob. Ag. 42, 211–219.

Hewagama, S., Spelman, T., Woolley, M., Mcleod, J., Gordon, D., Einsiedel, L., 2016. The epidemiology of *Staphylococcus aureus* and Panton-Valentine leucocidin (pvl) in Central Australia, 2006–2010. BMC Infect. Dis. 16, 382.

Holmes, A., Ganner, M., Mcguane, S., Pitt, T.L., Cookson, B.D., Kearns, A.M., 2005. *Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. J. Clin. Microbiol. 43, 2384–2390.

Hu, Q., Cheng, H., Yuan, W., Zeng, F., Shang, W., Tang, D., Xue, W., Fu, J., Zhou, R., Zhu, J., 2014. Panton-Valentine leukocidin (PVL)-positive health care-associated methicillin-resistant *Staphylococcus aureus* isolates are associated with skin and soft tissue infections and colonized mainly by infective PVL-encoding bacteriophages. J. Clin. Microbiol. 53, 67–72.

Huber, H., Koller, S., Giezendanner, N., Stephan, R., Zweifel, C., 2010. Prevalence and characteristics of meticillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009. Euro Surveill. 15 (pii = 19542).

Jarraud, S., Mougel, C., Thioulouse, J., Lina, G., Meugnier, H., Forey, F., Nesme, X., Etienne, J., Vandenesch, F., 2002. Relationships between *Staphylococcus aureus* genetic background, virulence factors, agr groups (alleles), and human disease. Infect. Immun. 70, 631–641.

Kanika, B., Wang, X., Susan, D., Marcus, Z., Da, R.L., Zhang, Y., 2011. Methicillin-resistant *Staphylococcus aureus* in retail meat, Detroit, Michigan, USA. Emerg. Infect. Dis. 17, 1135–1137.

Kaplan, S.L., 2006. Infective pyomyositis and myositis in children in the era of community-acquired, methicillin-resistant *Staphylococcus aureus* infection. Clin. Infect. Dis. 43, 953–960.

Kokan, N.P., Bergdoll, M.S., 1987. Detection of low-enterotoxin-producing *Staphylococcus aureus* strains. Appl. Environ. Microb. 53, 2675–2676.

Krziwanek, K., Luger, C., Sammer, B., Stumvoll, S., Stammler, M., Metz-Gercek, S., Mittermayer, H., 2007. PVL-positive MRSA in Austria. Eur. J. Clin. Microbiol. Infect. Dis. 26, 931–935.

Lee, N.E., Taylor, M.M., Bancroft, E., Ruane, P.J., Morgan, M., Mccoy, L., Simon, P.A., 2005. Risk factors for community-associated methicillin-resistant *Staphylococcus aureus* skin infections among HIV-positive men who have sex with men. Clin. Infect. Dis. 40, 1529–1534.

Li, X., Sun, J., Wu, D., Wang, L., Yang, Y., Wang, C., Liu, L., Zhao, C., Du, P., Yu, S., 2012. Panton–Valentine leukocidin gene sequence variation and phage in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* from children in mainland China. Microbiol. Immunol. 56, 155–162.

Lina, G., Piemont, Y., Godail-Gamot, F., Bes, M., Peter, M.O., Gauduchon, V., Vandenesch, F., Etienne, J., 1999. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin. Infect. Dis. 29 (5), 1128–1132.

Lo, W.T., Wang, C.C., 2011. Panton-Valentine leukocidin in the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. Pediatr. Neonatol. 52, 59–65.

Melles, D.C., Leeuwen, W.B.V., Boelens, H.A.M., Peeters, J.K., Verbrugh, H.A., Belkum, A.V., 2006. Panton-Valentine leukocidin genes in *Staphylococcus aureus*. Emerg. Infect. Dis. 12, 1174–1175.

Monecke, S., Stieber, B., Ruppelt, A., Aamot, H.V., Ehricht, R., 2014. Characterization of PVL-positive MRSA from Norway. APMIS 122, 580–584.

Otter, J.A., Kearns, A.M., French, G.L., Ellington, M.J., 2010. Panton–Valentine leukocidin-encoding bacteriophage and gene sequence variation in community-associated methicillin-resistant *Staphylococcus aureus*. Clin. Microbiol. Infect. 16, 68–73.

Papadopoulos, P., Papadopoulos, T., Angelidis, A.S., Boukouvala, E., Zdragas, A., Papa, A., Hadjichristodoulou, C., Sergelidis, D., 2018. Prevalence of *Staphylococcus aureus* and of methicillin-resistant *S. aureus* (MRSA) along the production chain of dairy products in north-western Greece. Food Microbiol. 69, 43–50.

Rasigade, J.P., Laurent, F., Lina, G., Meugnier, H., Bes, M., Vandenesch, F., Etienne, J., Tristan, A., 2010. Global distribution and evolution of Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus*, 1981–2007. J. Infect. Dis. 201, 1589–1597.

Rasigade, J.P., Dumitrescu, O., Lina, G., 2014. New epidemiology of *Staphylococcus aureus* infections. Clin. Microbiol. Infect. 20, 587–588.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.-A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States—major pathogens. Emerg. Infect. Dis. 17, 7–15.

Shallcross, L.J., Ellen, F., Johnson, A.M., Hayward, A.C., 2013. The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis. Lancet Infect. Dis. 13, 43–54.

Shopsin, B., Gomez, M., Montgomery, S.O., Smith, D.H., Waddington, M., Dodge, D.E., Bost, D.A., Riehman, M., Naidich, S., Kreiswirth, B.N., 1999. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J. Clin. Microbiol. 37, 3556–3563.

Silva-Carvalho, M.C., Bonelli, R.R., Souza, R.R., Moreira, S., Santos, L.C.G.D., Conceição, M.D.S., Mello Junior, S.J.D., Carballido, J.M., Rito, P.N., Vieira, V.V., 2009. Emergence of multiresistant variants of the community-acquired methicillin-resistant *Staphylococcus aureus* lineage ST1-SCCmecIV in 2 hospitals in Rio de Janeiro, Brazil. Diagn. Micr. Infec. Dis. 65, 300–305.

Smith, T.C., Pearson, N., 2011. The emergence of *Staphylococcus aureus* ST398. Vector-Borne Zoonot 11, 327–339.

Song, M., Bai, Y., Xu, J., Carter, M.Q., Shi, C., Shi, X., 2015. Genetic diversity and virulence potential of *Staphylococcus aureus* isolates from raw and processed food commodities in Shanghai. Int. J. Food Microbiol. 195, 1–8.

Takizawa, Y., Taneike, I., Nakagawa, S., Oishi, T., Nitahara, Y., Iwakura, N., Ozaki, K., Takano, M., Nakayama, T., Yamamoto, T., 2005. A Panton-Valentine leucocidin (PVL)-positive community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) strain, another such strain carrying a multiple-drug resistance plasmid, and other more-typical PVL-negative MRSA strains found in Japan. J. Clin. Microbiol. 43, 3356–3363.

Varshney, A.K., Mediavilla, J.R., Robiou, N., Guh, A., Wang, X., Gialanella, P., Levi, M.H., Kreiswirth, B.N., Fries, B.C., 2009. Diverse enterotoxin gene profiles among clonal complexes of *Staphylococcus aureus* isolates from the Bronx, New York. Appl. Environ. Microb. 75, 6839–6849.

Vourli, S., Vagiakou, H., Ganteris, G., Orfanidou, M., Polemis, M., Vatopoulos, A., Malamou-Ladas, H., 2009. High rates of community-acquired, Panton-Valentine leukocidin (PVL)- positive methicillin-resistant *S. aureus* (MRSA) infections in adult outpatients in Greece. Euro Surveill 14, 19–22.

Wang, X., Tao, X., Xia, X., Yang, B., Xi, M., Meng, J., Zhang, J., Xu, B., 2013. Staphylococcus aureus and methicillin-resistant *Staphylococcus aureus* in retail raw chicken in China. Food Control 29, 103–106.

Wang, Y., Liu, Q., Liu, Q., Gao, Q., Lu, H., Meng, H., Xie, Y., Huang, Q., Ma, X., Wang, H., 2018. Phylogenetic analysis and virulence determinant of the host-adapted *Staphylococcus aureus* lineage ST188 in China. Emerg. Microbes. Infect. 7, 45.

Wu, S., Huang, J., Wu, Q., Zhang, F., Zhang, J., Lei, T., Chen, M., Ding, Y., Xue, L., 2018a. Prevalence and characterization of *Staphylococcus aureus* isolated from retail vegetables in China. Front. Microbiol. 9, 1263.

Wu, S., Huang, J., Wu, Q., Zhang, J., Zhang, F., Yang, X., Wu, H., Zeng, H., Chen, M., Ding, Y., Wang, J., Lei, T., Zhang, S., Xue, L., 2018b. *Staphylococcus aureus* isolated from retail meat and meat products in China: incidence, antibiotic resistance and genetic diversity. Front. Microbiol. 9, 2767.

Xiao, M., Wang, H., Zhao, Y., Mao, L.L., Brown, M., Yu, Y.S., O'Sullivan, M.V., Kong, F., Xu, Y.C., 2013. National surveillance of methicillin-resistant *Staphylococcus aureus* in China highlights a still-evolving epidemiology with 15 novel emerging multilocus sequence types. J. Clin. Microbiol. 51, 3638–3644.

Xiuli, X., Zhou, S., Bai, L., Jiayun, L., Yueyun, M., Xiaoke, H., 2016. Characteristics of antimicrobial resistance and molecular epidemiology of *Staphylococcus aureus* isolated from patients in intensive care units. Chin. J. Infect. Control 15, 294–298.

Yamamoto, T., 2004. Panton-Valentine leukocidin positive community-acquired MRSA infection in Japan. Euro Surveill. 8 (pii = 2500).

Yang, X., Yu, S., Wu, Q., Zhang, J., Wu, S., Rong, D., 2018. Multilocus sequence typing and virulence-associated gene profile analysis of *Staphylococcus aureus* isolates from retail ready-to-eat food in China. Front. Microbiol. 9, 197.

Ye, X., Wang, X., Fan, Y., Peng, Y., Li, L., Li, S., Huang, J., Yao, Z., Chen, S., 2016. Genotypic and phenotypic markers of livestock-associated methicillin-resistant *Staphylococcus aureus* CC9 in humans. Appl. Environ. Microb. 82, 3892–3899.

Yu, F., Chen, Z., Liu, C., Zhang, X., Lin, X., Chi, S., Zhou, T., Chen, Z., Chen, X., 2010. Prevalence of *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes among isolates from hospitalised patients in China. Clin. Microbiol. Infect. 14, 381–384.

Zhao, H., Hu, F., Shu, J., Xu, X., Zou, Y., Ding, B., He, C., Fang, G., Liu, Q., 2016. Typing of Panton-Valentine leukocidin-encoding phages and lukSF-PV gene sequence variation in *Staphylococcus aureus* from China. Front. Microbiol. 7, 1200.