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Research Note

Characterization of Toxin Genes and Antimicrobial Susceptibility of *Staphylococcus aureus* from Retail Raw Chicken Meat

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ABSTRACT

The aim of this study was to investigate the toxin gene profile and antimicrobial resistance of *Staphylococcus aureus* isolates from raw chicken in the People's Republic of China. In total, 289 *S. aureus* isolates were characterized by antimicrobial susceptibility testing, and genes encoding enterotoxins, exfoliative toxins, Panton-Valentine leukocidin, and toxic shock syndrome toxin were revealed by PCR. Overall, 46.0% of the isolates were positive for one or more toxin genes. A high proportion of toxin genes were *pvl* (26.6%), followed by *sej* (12.5%), *sea* (9.0%), *seh* (8.3%), *seb* (6.9%), *sec* (6.9%), *sed* (4.8%), *sei* (3.1%), and *see* (2.4%). None of the isolates harbored *seg, tsst-1,* or exfoliative toxin genes. In total, 29 toxin gene profiles were obtained, and *pvl* (10.7%) was the most frequent genotype, followed by *sea* (5.9%), *seb* (4.8%), and *sej* (4.2%). Furthermore, 99.7% of the strains were resistant to at least one of the tested antimicrobial agents, and 87.2% of them displayed multidrug resistance. Resistance was most frequently observed to trimethoprim-sulfamethoxazole and erythromycin (86.2% for each), followed by tetracycline (69.9%), amoxicillin–clavulanic acid (45.0%), and ampicillin (42.6%). None of the strains were resistant to vancomycin. This study indicates that *S. aureus* isolates from raw chicken harbored multiple toxin genes and exhibited multiple antimicrobial resistance, which represents a potential health hazard for consumers.

Key words: Antimicrobial resistance; Raw chicken meat; *Staphylococcus aureus*; Toxin genes

Staphylococcus aureus is an important foodborne pathogen that can cause food poisoning and infection in humans and animals. Its pathogenicity is caused by extracellular toxins such as staphylococcal enterotoxins (SEs), exfoliative toxins, toxic shock syndrome toxin 1 (TSST-1), and Panton-Valentine leukocidin *(9)*. The SE group is made up of extracellular proteins with molecular masses between 27,000 and 30,000 Da, and they are the main cause of food poisoning *(2, 18)*. To date, 23 kinds of SEs have been found, including the classical SEs (SEA through SEE) and the new types of SEs (SEG through SEX) *(5)*. Among strains associated with staphylococcal food poisoning, SEA was the most common SE, followed by SED, SEC, SEB, and SEE *(5)*.

In recent years, the emergence and spread of multidrug-resistant *S. aureus* strains has gained more and more attention. It is reported that many food poisoning outbreaks are caused by the drug-resistant *S. aureus* strains mainly exhibiting penicillin, ampicillin, erythromycin, and tetracycline resistance *(12, 19, 36),* and an alarming increase in resistance of *S. aureus* from foods, especially raw meat, has been reported worldwide *(2, 6, 24, 28)*. Isolates from different foods have shown that resistance to penicillin, ampicillin, streptomycin, erythromycin, and tetracycline are common. Especially, methicillin-resistant *S. aureus* has become a major public health concern *(7, 25, 28)*. Because food may act as a vector for the transfer of antimicrobial resistant bacteria to humans, it forms an indirect risk to public health *(5)*. Therefore, the monitoring of drug resistance of foodborne and pathogenic *S. aureus* is of great significance.

Previously, we reported the prevalence of *S. aureus* in raw chicken from retail markets in the People's Republic of China. However, no comprehensive study has been performed on the characterization of *S. aureus* isolated from raw chicken in China. Therefore, this study aimed to investigate the toxin genes and antimicrobial resistance of *S. aureus* strains isolated from raw chicken meat in four provinces and one major city of China.

MATERIALS AND METHODS

Bacterial strains. In total, 289 *S. aureus* strains were characterized in this study; they were recovered from 720 retail chicken samples. Isolation and identification of *S. aureus* strains were performed as described previously *(33)*. The number of isolates obtained from chicken meat in four provinces and one city

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TABLE 1. *Oligonucleotide primers for amplification of various toxin genes*

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	Product size (bp)
pvl	ATCATTAGGTAAAATGTCTGGACATGATCCA	GCATCAAGTGTATTGGATAGCAAAAGC	433
sea	GGTTATCAATGTGCGGGTGG	CGGCACTTTTTTCTCTTCGG	102
seb	GTATGGTGGTGTAACTGAGC	CCAAATAGTGACGAGTTAGG	164
sec	AGATGAAGTAGTTGATGTGTATGG	CACACTTTTAGAATCAACCG	451
sed	CCAATAATAGGAGAAAATAAAAG	ATTGGTATTTTTTTTCGTTC	278
see	AGGTTTTTTCACAGGTCATCC	CTTTTTTTTCTTCGGTCAATC	209
seg	TGCTATCGACACACTACAACC	CCAGATTCAAATGCAGAACC	704
seh	CGAAAGCAGAAGATTTACACG	GACCTTTACTTATTTCGCTGTC	495
sei	GACAACAAAACTGTCGAAACTG	CCATATTCTTTGCCTTTACCAG	630
sej	CATCAGAACTGTTGTTCCGCTAG	CTGAATTTTACCATCAAAGGTAC	142
tst	ACCCCTGTTCCCTTATCATC	TTTTCAGTATTTGTAACGCC	326
eta	ATATCAACGTGAGGGCTCTAGTAC	ATGCAGTCAGCTTCTTACTGCTA	1155
etb	CACACATTACGGATAATGCAAG	TCAACCGAATAGAGTGAACTTATCT	604

in China was as follows: Shaanxi province ($n = 75$); Guangdong province ($n = 55$); Guangxi province ($n = 24$); Fujian province ($n = 73$), and Shanghai city ($n = 62$). All isolates were stored at −80°C in tryptic soy broth plus 20% (v/v) glycerol until used.

PCR detection of toxin genes. PCR amplification was performed for nine *S. aureus* enterotoxin genes (*sea, seb, sec, sed, see, seg, seh, sei,* and *sej*) *(26), tsst-1* gene *(26),* exfoliative toxin genes (*eta* and *etb*) *(23),* and Panton-Valentine leukocidin gene (*pvl*) *(31)*. The primers for the PCRs are listed in Table 1. The PCR products were resolved by 1.5% (w/v) agarose gel electrophoresis in 0.5× Tris-borate-EDTA buffer.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed using the agar dilution method on Mueller-Hinton agar (Beijing Land Bridge Technology Ltd., Beijing, China) plates *(8)*. Twelve kinds of antimicrobial drugs were tested: trimethoprim-sulfamethoxazole, cefoperazone, cefoxitin, erythromycin, gentamicin, ampicillin, chloramphenicol, ciprofloxacin, tetracycline, amoxicillin–clavulanic acid, amikacin, and vancomycin. *Escherichia coli* ATCC 25922 and *S. aureus* ATCC 29213 were included as quality control strains in each run.

Statistical analysis. Chi-square tests were performed with Minitab statistical software for Windows (Minitab Inc., State College, PA). Statistical significance was set at P values of <0.05.

RESULTS

Prevalence of toxin genes. Of the 289 *S. aureus* strains, 133 (46.0%) of 289 contained one or more toxin genes, and nine toxin genes (*pvl, sea, seb, sec, sed, see, seh, sei,* and *sej*) were observed in these isolates. The *seg, tsst-1,* and exfoliative toxin genes were not detected (Table 2). The most predominant toxin genes were *pvl* (77 of 289, 26.6%), *sej* (36 of 289, 12.5%), *sea* (26 of 289, 9.0%), and *seh* (24 of 289, 8.3%), followed by *seb* (20 of 289, 6.9%), *sec* (20 of 289, 6.9%), *sed* (14 of 289, 4.8%), *sei* (9 of 289, 3.1%), and *see* (7 of 289, 2.4%). For most of the toxin genes in the 289 *S. aureus* isolates from raw chicken, no statistically significant difference ($P > 0.05$) was found in the detection rate among the locations.

Table 3 lists the toxin gene profiles of the *S. aureus* isolates from raw chicken. Twenty-nine toxin gene profiles were observed. Among all genotypes, the most frequent toxin genotype was *pvl* (31 of 289, 10.7%), followed by *sea* (17 of 289, 5.9%), *seb* (14 of 289, 4.8%), and *sej* (12 of 289, 4.2%). *pvl-seh* and *pvl-sei* were detected in 2.4% (7 of 289) and 2.1% (6 of 289) of all the isolates, respectively. *pvl-sej, pvl-sec-seh, pvl-sec-sed-sej,* and *pvl-sea-see-seh* were detected in 1.4% (4 of 289, each) of the isolates.

TABLE 2. *Toxin genes in 289* S. aureus *isolates from raw chicken from four provinces and one city of China*[a]

Gene	Shannxi ($n = 75$)	Guangdong ($n = 55$)	Shanghai ($n = 62$)	Fujian ($n = 73$)	Guangxi ($n = 24$)	Total ($n = 289$)
sea	3 (4.0)	6 (10.9)	9 (14.5)	6 (8.2)	2 (8.3)	26 (9.0)
seb	3 (4.0)	8 (14.5)	2 (3.2)	6 (8.2)	1 (4.2)	20 (6.9)
sec	4 (5.3)	0 (0)	8 (12.9)	3 (4.1)	5 (20.8)	20 (6.9)
sed	2 (2.7)	0 (0)	4 (6.4)	5 (6.8)	3 (12.5)	14 (4.8)
see	2 (2.7)	0 (0)	4 (6.4)	0 (0)	1 (4.2)	7 (2.4)
seg	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
seh	13 (17.3) A	0 (0) B	8 (12.9) AB	1 (1.4) A	2 (8.3) AB	24 (8.3)
sei	0 (0)	0 (0)	3 (4.8)	1 (1.4)	5 (20.8)	9 (3.1)
sej	24 (32.0) A	0 (0) B	4 (6.4) B	6 (8.2) B	2 (8.3) B	36 (12.5)
pvl	25 (33.3) A	0 (0) C	24 (38.7) AB	13 (17.8) B	15 (62.5) A	77 (26.6)
eta	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
etb	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
tsst-1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

[a] Values in the same row followed by different letters are significantly different ($P < 0.05$). Values in parentheses are percentages.

TABLE 3. *Toxin gene profiles in 289* S. aureus *isolates from raw chicken from four provinces and one city of China*[a]

Profile of toxin gene	Shaanxi ($n = 75$)	Guangdong ($n = 55$)	Shanghai ($n = 62$)	Fujian ($n = 73$)	Guangxi ($n = 24$)	Total ($n = 289$)
pvl	9 (12.0)	0 (0)	8 (12.9)	8 (11.0)	6 (25.0)	31 (10.7)
sea	0 (0)	6 (10.9)	4 (6.5)	6 (8.2)	1 (4.2)	17 (5.9)
seb	1 (1.3)	8 (14.5)	0 (0)	5 (6.8)	0 (0)	14 (4.8)
sec	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.2)	1 (0.3)
sed	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.3)
sej	11 (14.7)	0 (0)	0 (0)	1 (1.4)	0 (0)	12 (4.2)
pvl-seb	0 (0)	0 (0)	2 (3.2)	1 (1.4)	0 (0)	3 (1.0)
pvl-sec	0 (0)	0 (0)	1 (1.6)	0 (0)	0 (0)	1 (0.3)
pvl-sed	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.2)	1 (0.3)
pvl-seh	3 (4.0)	0 (0)	3 (4.8)	1 (1.4)	0 (0)	7 (2.4)
pvl-sei	0 (0)	0 (0)	2 (3.2)	1 (1.4)	3 (12.5)	6 (2.1)
pvl-sej	4 (5.3)	0 (0)	0 (0)	0 (0)	0 (0)	4 (1.4)
seb-sej	2 (2.7)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.7)
sec-sej	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.3)
sec-seh	1 (1.3)	0 (0)	1 (1.6)	0 (0)	0 (0)	2 (0.7)
sed-sej	0 (0)	0 (0)	0 (0)	2 (2.7)	0 (0)	2 (0.7)
seh-sej	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.3)
pvl-seh-sej	3 (4.0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (1.0)
pvl-sea-see	0 (0)	0 (0)	2 (3.2)	0 (0)	0 (0)	2 (0.7)
pvl-sec-seh	2 (2.7)	0 (0)	1 (1.6)	0 (0)	1 (4.2)	4 (1.4)
pvl-seb-sei	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.2)	1 (0.3)
pvl-sed-sej	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.3)
sec-sed-sej	0 (0)	0 (0)	2 (3.2)	1 (1.4)	0 (0)	3 (1.0)
pvl-sec-sed-sej	0 (0)	0 (0)	1 (1.6)	2 (2.7)	1 (4.2)	4 (1.4)
pvl-sea-sec-seh	0 (0)	0 (0)	1 (1.6)	0 (0)	0 (0)	1 (0.3)
pvl-sea-seh-sej	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.3)
pvl-sea-see-seh	2 (2.7)	0 (0)	2 (3.2)	0 (0)	0 (0)	4 (1.4)
pvl-sec-sed-sei-sej	0 (0)	0 (0)	1 (1.6)	0 (0)	1 (4.2)	2 (0.7)
pvl-sea-sec-see-seh	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.2)	1 (0.3)
SEs	34 (45.3)	14 (25.4)	23 (37.1)	20 (27.4)	11 (45.8)	102 (35.3)
Total of toxin gene	43 (57.3)	14 (25.4)	31 (50.0)	28 (38.4)	17 (70.8)	133 (46.0)

[a] Values in parentheses are percentages.

Antimicrobial susceptibility testing. Of the 289 *S. aureus* isolates, resistance was most common to trimethoprim-sulfamethoxazole and erythromycin (249 of 289, each 86.2%), followed by tetracycline (202 of 289, 69.9%). The percentages for amoxicillin–clavulanic acid and ampicillin were 45.0% (130 of 289) and 42.6% (123 of 289), respectively. Some isolates were resistant to amikacin (75 of 289, 26.0%), ciprofloxacin (62 of 289, 21.5%), chloramphenicol (41 of 289, 14.2%), gentamicin (36 of 289, 12.5%), cefoxitin (26 of 289, 9.0%), and cefoperazone (10 of 289, 3.5%). All isolates were sensitive to vancomycin (Table 4). Overall, 288 (99.7%) of strains exhibited

TABLE 4. *Antimicrobial resistance in 289* S. aureus *isolates from raw chicken from four provinces and one city of China*[a]

Antimicrobial agent[b]	Shannxi ($n = 75$)	Guangdong ($n = 55$)	Shanghai ($n = 62$)	Fujian ($n = 73$)	Guangxi ($n = 24$)	Total ($n = 289$)
SXT	69 (92.0) A	51 (92.7) A	61 (98.4) A	62 (84.9) A	6 (25.0) B	249 (86.20)
ERY	71 (94.7) A	54 (98.2) C	48 (77.4) D	56 (76.7) B	20 (83.3) D	249 (86.20)
TET	54 (72.0)	47 (85.5)	39 (62.9)	42 (57.5)	20 (83.3)	202 (69.90)
AMC	33 (44.0)	27 (49.1)	21 (33.9)	42 (57.5)	7 (29.2)	130 (45.00)
AMP	14 (18.7) B	47 (85.5) A	54 (87.1) A	6 (8.2) B	2 (8.3) B	123 (42.60)
AMK	51 (68.0) A	0 (0) B	0 (0) B	2 (2.7) B	22 (91.7) A	75 (26.00)
CIP	35 (46.7) A	2 (3.6) B	11 (17.7) B	3 (4.1) B	11 (45.8) B	62 (21.50)
CHL	21 (28.0) A	6 (10.9) AB	10 (16.1) AB	2 (2.7) B	2 (8.3) B	41 (14.20)
GEN	18 (24.0) A	0 B	5 (8.1) A	7 (9.6) AB	6 (25.0) B	36 (12.50)
FOX	10 (13.3) AB	1 (1.8) B	1 (1.6) B	14 (19.2) A	0 B	26 (9.00)
CEFO	0 (0)	2 (3.6)	5 (8.1)	3 (4.1)	0	10 (3.50)
VAN	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

[a] Values in the same row followed by different letters are significantly different ($P < 0.05$). Values in parentheses are percentages.
[b] SXT, trimethoprim-sulfamethoxazole; ERY, erythromycin; TET, tetracycline; AMC, amoxicillin–clavulanic acid; AMP, ampicillin; AMK, amikacin; CIP, ciprofloxacin; CHL, chloramphenicol; GEN, gentamicin; FOX, cefoxitin; CEFO, cefoperazone; VAN, vancomycin.

TABLE 5. *Multidrug resistance of the 289* S. aureus *isolates from raw chicken*

Province or city	No. (%) of isolates resistant to indicated no. of antimicrobial agents:					
	1	2	3	4	≥5	Total
Shannxi ($n = 75$)	1 (1.3)	5 (6.7)	9 (12.0)	21 (28.0)	39 (52.0)	75 (100)
Guangdong ($n = 55$)	2 (3.6)	2 (3.6)	5 (9.1)	18 (32.7)	28 (50.9)	55 (100)
Shanghai ($n = 62$)	0 (0)	5 (8.1)	18 (29.0)	11 (17.7)	27 (43.5)	61 (98.4)
Fujian ($n = 73$)	5 (6.8)	11 (15.1)	30 (41.1)	16 (21.9)	11 (15.1)	73 (100)
Guangxi ($n = 24$)	1 (4.2)	4 (16.7)	4 (16.7)	4 (16.7)	11 (45.8)	24 (100)
Total ($n = 289$)	9 (3.1)	27 (9.3)	66 (22.8)	70 (24.2)	116 (40.1)	288 (99.7)

resistance to at least one antibiotic (Table 5), and 252 (87.2%) of the strains showed multidrug resistance (MDR) by being resistant to three or more antimicrobial agents.

The percentages of resistance to tetracycline, amoxicillin–clavulanic acid, cefoxitin, and vancomycin between different locations were not significant ($P > 0.05$). However, the percentages of resistant *S. aureus* isolates to other antimicrobial agents differed by the location from which they were isolated. Isolates from Shanghai and Guangdong showed significantly higher rates ($P < 0.05$) of resistance to trimethoprim-sulfamethoxazole and ampicillin than those from Guangxi. Isolates from Guangdong and Shannxi showed significantly higher rates ($P < 0.05$) of resistance to erythromycin than those from Shanghai, Fujian, and Guangxi. Isolates from Shannxi showed significantly higher rates ($P < 0.05$) of resistance to ciprofloxacin, chloramphenicol, and gentamicin than those from Guangxi.

Correlation between antimicrobial resistance and toxin gene carriage. A significant correlation ($P < 0.05$) was found between some antimicrobial susceptibility patterns and toxin genes in the examined isolates (Table 6): trimethoprim-sulfamethoxazole and the *sei* gene; erythromycin and the *sej* or *pvl* gene; amoxicillin–clavulanic acid and *seb, sec, sed, seh, sei,* or *pvl* gene; ampicillin and the *sej* or *pvl* gene; amikacin and *seh, sei, sej,* or *pvl* gene; ciprofloxacin and *sei, sej,* or *pvl* gene; and gentamicin and *sec, sed, sej,* or *pvl* gene.

DISCUSSION

Livestock can carry *S. aureus* in the nasal cavity and on the skin. The food derived from these animals has been considered as a vehicle for transmission of *S. aureus (27)*. Studies on the characteristics of *S. aureus* from raw chicken in China are scarce. The results of the present study showed that 46.0% of the *S. aureus* isolates were positive for one or more toxin genes and that 99.7% exhibited resistance to at least one antibiotic. These isolates may pose a potential threat to human health.

We found 26.6% of isolates carried the *pvl* gene, which was lower than the detection rate (40.7%) reported in another study in China *(32)*. Our rate was higher than those reported in studies in the United States for which the *pvl*-positive rate on raw retail meat products ranged from 0.6% in Iowa to 1.8% in Georgia *(10, 14)*. *tsst*-positive isolates have been isolated from various foods, including meat, poultry, and milk *(1, 6)*. In this study, no isolate harbored *tsst-1,* in agreement with Tsen et al. *(30),* who found that no food isolates contained *tsst-1*. In addition, genes encoding *eta*- and *etb*-linked virulence factors are associated with bullous impetigo. We found that none of the *S. aureus* isolates contained *eta* and *etb* genes, and this finding was in agreement with previous reports *(2, 6)*.

The SEs belong to a family of so-called pyrogenic toxins involved with staphylococcal food poisoning and other types of infections in humans and animals *(2)*. In this study, 35.3% of the isolates carried one or more SE genes, a value that was similar to those of previous studies. For example, there were 30.5% positive isolates from various foods in France *(29)* and 39.2% positive isolates from food samples (cheese, pasta, and sausage) in the Slovak Republic *(11)*. In all *S. aureus* isolates, *sej* (12.5%) was the most commonly detected gene, followed by *sea* (9.0%), *seh* (8.3%), and *seb* and *sec* (each 6.9%). In contrast, in Spain *(4)* the most frequent SE genes of *S. aureus* isolates from foods were *sea* (38.7%), *sed* through *sej* (22.9%), *sec* (16.1%), and *seb* (12.9%), which were higher than in our results. This difference may be caused by the geographic locations and differences in sampling, isolation, and testing methods *(4)*. About 95% of staphylococcal food poisoning outbreaks were caused by strains carrying the classical SEs (SEA through SEE). But the relationship between novel SEs and staphylococcal food poisoning is poorly understood *(5)*. In the present study, *sea* and *seb* were the most commonly detected genes, in accordance with findings in raw chicken by Kitai et al. *(16)* and Il Cho et al. *(13)*. In addition, the *seg* and *sei* genes belong to the same enterotoxin gene cluster (*egc*), and they are usually detected together *(15)*. However, in this study, no *seg* gene was detected, whereas *sei* was present in a few isolates (3.1%). In contrast, only the *seg* gene was detected in isolates from infant foods in a previous study *(32)*. Additional studies are needed to determine whether genes are related to the source of the strains.

We found that 99.7% of *S. aureus* isolates were resistant to at least one antimicrobial agent, a finding that was different from that of other reports *(17, 22, 24)*. In addition, we observed that 86.2% of *S. aureus* isolates were resistant to erythromycin. This rate was higher than the antimicrobial resistance rates of *S. aureus* isolates from other food samples in China and other countries *(6, 25, 28, 32)*. The difference may be explained by the overuse of topical erythromycin often prescribed for *S. aureus* treatment in China *(20)*. Moreover, 87.2% of *S. aureus* isolates showed MDR. Wang et al. *(32)* found that 35.2% of *S. aureus*

TABLE 6. *Antimicrobial resistance in the toxin gene–carrying* S. aureus[a]

Antimicrobial agent[b]	*sea*+ (26)	*sea*− (263)	*P* value	*seb*+ (20)	*seb*− (269)	*P* value	*sec*+ (20)	*sec*− (269)	*P* value	*sed*+ (14)	*sed*− (275)	*P* value	*seh*+ (24)	*seh*− (265)	*P* value	*sei*+ (9)	*sei*− (280)	*P* value	*sej*+ (36)	*sej*− (253)	*P* value	*pvl*+ (77)	*pvl*− (212)	*P* value
SXT	88.5	85.9	0.722	90	85.9	0.606	80	86.7	0.406	78.6	86.5	0.399	91.7	85.7	0.415	33.3	87.8	0.000**[c]	91.7	85.4	0.306	80.5	88.2	0.094
ERY	80.8	86.7	0.404	80	86.7	0.408	95	85.5	0.235	100	85.4	0.124	79.2	86.8	0.300	77.8	86.4	0.459	97.2	84.6	0.040*[d]	77.9	89.2	0.015*
TET	50	71.9	0.020*	65	70.3	0.621	80	69.1	0.307	78.6	71.0	0.488	62.5	70.6	0.409	66.7	70	0.830	69.4	69.9	0.950	63.6	72.2	0.162
AMC	42.3	45.2	0.774	80	42.4	0.001**	10	47.6	0.001**	14.3	46.5	0.018*	25	46.8	0.040*	11.1	46.0	0.038*	36.1	46.2	0.253	19.5	54.2	0.000**
AMP	53.8	41.4	0.222	60	41.3	0.102	45	42.4	0.819	28.6	43.3	0.278	41.7	42.7	0.926	22.2	43.2	0.210	22.2	45.5	0.008**	28.6	47.6	0.004**
AMK	19.2	26.7	0.413	20	26.4	0.529	35	25.3	0.339	28.6	25.8	0.000	54.2	23.4	0.001*	66.7	24.6	0.005**	58.3	21.3	0.000**	46.8	18.4	0.000**
CIP	11.5	22.4	0.197	10	22.3	0.196	10	22.3	0.196	7.1	22.2	0.181	29.2	20.7	0.336	88.9	19.3	0.000**	36.1	19.4	0.022*	35.1	16.5	0.001**
CHL	7.7	14.8	0.320	15	14.1	0.914	10	14.5	0.578	14.3	14.2	0.991	25	13.2	0.113	11.1	14.3	0.788	37.8	12.3	0.012*	16.9	13.2	0.429
GEN	3.8	13.3	0.163	10	12.7	0.730	35	10.8	0.002**	57.1	10.2	0.000**	25	11.3	0.052	33.3	11.8	0.054	44.4	7.9	0.000**	23.4	8.5	0.001**
FOX	0	9.9	0.093	20	8.2	0.075	0	9.7	0.145	0	9.4	0.228	0	9.8	0.108	0	9.3	NA	2.8	9.9	0.163	3.9	10.8	0.068
CEFO	3.8	3.4	NA	0	3.7	NA	0	3.7	NA	0	3.6	NA	3.4	0	NA	3.6	0	NA	0	3.9	0.225	1.3	4.2	0.226

[a] Comparisons of antimicrobial resistance were made between the toxin gene's carriage (+) or lack of carriage (−) in *S. aureus*. NA, not applicable; approximation may be invalid.
[b] SXT, trimethoprim-sulfamethoxazole; ERY, erythromycin; TET, tetracycline; AMC, amoxicillin–clavulanic acid; AMP, ampicillin; AMK, amikacin; CIP, ciprofloxacin; CHL, chloramphenicol; GEN, gentamicin; FOX, cefoxitin; CEFO, cefoperazone.
[c] ** $P < 0.01$.
[d] * $P < 0.05$.

isolated from infant food showed MDR. This difference may be due to the administration of drugs to animals. According to Wu et al. *(35),* more intensive breeding may result in more frequent and larger amounts of antimicrobial agent use, which leads to higher resistance rates in isolates from chickens.

According to the comparison of the detection rates of most toxin genes among samples between the different Chinese locations, this study indicated that the geographic origin of isolates may have little impact on the toxin genes of *S. aureus* isolated from raw chicken meat, whereas isolates recovered from different regions exhibited different resistance levels to most antimicrobial agents. This may suggest that the use of antimicrobial agents in poultry is regional, and there are differences in the amount and range of antimicrobial use in different locations *(35)*.

In this study, there was a strong correlation between toxin genes and antimicrobial resistance of *S. aureus*. Resistance to erythromycin, ampicillin, amikacin, gentamicin, ciprofloxacin, and chloramphenicol in *S. aureus* carrying the *sej* gene was more prevalent than in *S. aureus* without the *sej* gene. In addition, the 14 *sed*-harboring *S. aureus* strains were all resistant to erythromycin. Based on a previous study, plasmids carrying a diverse range of antimicrobial resistance genes may also carry toxin genes *(21)*. The *sed* and *sej* genes were found on the plasmid pIB485, and they were also usually found together *(3)*. Additional studies are needed to discover the relationship between antimicrobial resistance and toxin gene carriage.

In summary, we described the antimicrobial resistance and virulence genes of *S. aureus* isolates from raw chicken meat. The results showed that most of the *S. aureus* isolates exhibited MDR and carried different toxins genes. Although the pathogenicity of the *S. aureus* isolated from food remains unclear, *S. aureus* isolates pose a great concern as they are a potential vehicle for transmission of *S. aureus* from chickens to the human population *(34)*. More research is necessary to determine the survival and growth characteristics of *S. aureus* and its toxin production in raw chicken.

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REFERENCES

1. Adesiyun, A. A., W. Lenz, and K. P. Schaal. 1992. Production of toxic shock syndrome toxin-1 (TSST-1) by *Staphylococcus aureus* strains isolated from humans, animals and foods in Nigeria. *Microbiologica* 15:125–133.
2. Akineden, O., C. Annemuller, A. A. Hassan, C. Lammler, W. Wolter, and M. Zschock. 2001. Toxin genes and other characteristics of *Staphylococcus aureus* isolates from milk of cows with mastitis. *Clin. Diagn. Lab. Immunol.* 8:959–964.
3. Alibayov, B., K. Zdenkova, S. Purkrtova, K. Demnerova, and R. Karpiskova. 2014. Detection of some phenotypic and genotypic characteristics of *Staphylococcus aureus* isolated from food items in the Czech Republic. *Ann. Microbiol.* 64:1587–1596.

4. Argudin, M. A., M. C. Mendoza, M. A. González-Hevia, M. Bances, B. Guerra, and M. R. Rodicio. 2012. Genotypes, exotoxin gene content, and antimicrobial resistance of *Staphylococcus aureus* strains recovered from foods and food handlers. *Appl. Environ. Microbiol.* 78:2930–2935.
5. Argudín, M. Á., M. C. Mendoza, and M. R. Rodicio. 2010. Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins* 2:1751–1753.
6. Aydin, A., M. Sudagidan, and K. Muratoglu. 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.
7. Baumgartner, A., I. Niederhauser, and S. Johler. 2014. Virulence and resistance gene profiles of *Staphylococcus aureus* strains isolated from ready-to-eat foods. *J. Food Prot.* 77:1232–1236.
8. Clinical and Laboratory Standards Institute. 2010. Performance standards for antimicrobial susceptibility testing: fifteenth informational supplement. M100es20. Clinical and Laboratory Standards Institute, Wayne, PA.
9. Dinges, M. M., P. M. Orwin, and P. M. Schlievert. 2000. Exotoxins of *Staphylococcus aureus*. *Clin. Microbiol. Rev.* 13:16–34.
10. Hanson, B. M., A. E. Dressler, A. L. Harper, R. P. Scheibel, S. E. Wardyn, L. K. Roberts, J. S. Kroeger, and T. C. Smith. 2011. Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. *J. Infect. Public Health* 4:169–174.
11. Holeckova, B., E. Holoda, M. Fotta, V. Kalinacova, J. Gondol, and J. Grolmus. 2002. Occurrence of enterotoxigenic *Staphylococcus aureus* in food. *Ann. Agric. Environ. Med.* 9:179–182.
12. Hyeon, J. Y., G. T. Chung, S. H. Bing, K. S. Kwon, H. H. Lee, S. J. Kim, S. E. Jeon, Y. H. Kang, and J. Kim. 2013. A foodborne outbreak of *Staphylococcus aureus* associated with fried chicken in Republic of Korea. *J. Microbiol. Biotechnol.* 23:85–87.
13. Il Cho, J., I. S. Joo, J. H. Choi, K. H. Jung, E. J. Choi, S. H. Lee, and I. G. Hwang. 2012. Prevalence and characterization of foodborne bacteria from meat products in Korea. *Food Sci. Biotechnol.* 21:1257–1261.
14. Jackson, C. R., J. A. Davis, and J. B. Barrett. 2013. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from retail meat and humans in Georgia. *J. Clin. Microbiol.* 51:1199–1207.
15. Jarraud, S., M. A. Peyrat, A. Lim, A. Tristan, M. Bes, C. Mougel, J. Etienne, F. Vandenesch, M. Bonneville, and G. Lina. 2001. *egc,* a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. *J. Immunol.* 166:669–677.
16. Kitai, S., A. Shimizu, J. Kawano, E. Sato, C. Nakano, H. Kitagawa, K. Fujio, K. Matsumura, R. Yasuda, and T. Inamoto. 2005. Prevalence and characterization of *Staphylococcus aureus* and enterotoxigenic *Staphylococcus aureus* in retail raw chicken meat throughout Japan. *J. Vet. Med. Sci.* 67:269–274.
17. Kunz, F., S. Corti, N. Giezendanner, R. Stephan, M. Wittenbrink, and C. Zweifel. 2011. Antimicrobial resistance of *Staphylococcus aureus* and coagulase negative staphylococci isolated from mastitis milk samples from sheep and goats. *Schweiz. Arch. Tierheilkd.* 153:63–69.
18. Le Loir, Y., F. Baron, and M. Gautier. 2003. *Staphylococcus aureus* and food poisoning. *Genet. Mol. Res.* 2:63–76.
19. Li, G. H., S. Z. Wu, W. Luo, Y. L. Su, Y. Luan, and X. Wang. 2015. *Staphylococcus aureus* ST6-t701 isolates from food-poisoning outbreaks (2006-2013) in Xi'an, China. *Foodborne Pathog. Dis.* 12:203–206.
20. Liu, Y., F. Kong, X. Zhang, M. Brown, L. Ma, and Y. Yang. 2009. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from children with impetigo in China from 2003 to 2007 shows community-associated methicillin-resistant *Staphylococcus aureus* to be uncommon and heterogeneous. *Br. J. Dermatol.* 161:1347–1350.
21. McCarthy, A. J., and J. A. Lindsay. 2012. The distribution of plasmids that carry virulence and resistance genes in *Staphylococcus aureus* is lineage associated. *BMC Microbiol.* 12:104.
22. Miranda, J. M., B. I. Vázquez, C. A. Fente, P. Calo-Mata, A. Cepeda, and C. M. Franco. 2008. Comparison of antimicrobial resistance in *Escherichia coli, Staphylococcus aureus,* and *Listeria monocytogenes* strains isolated from organic and conventional poultry meat. *J. Food Prot.* 71:2537–2542.
23. Noguchi, N., H. Nakaminami, S. Nishijima, I. Kurokawa, H. So, and M. Sasatsu. 2006. Antimicrobial agent of susceptibilities and antiseptic resistance gene distribution among methicillin-resistant *Staphylococcus aureus* isolates from patients with impetigo and staphylococcal scalded skin syndrome. *J. Clin. Microbiol.* 44:2119–2125.
24. Normanno, G., G. La Salandra, A. Dambrosio, N. C. Quaglia, M. Corrente, A. Parisi, G. Santagada, A. Firinu, E. Crisetti, and G. V. Celano. 2007. Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. *Int. J. Food Microbiol.* 115:290–296.
25. Ombui, J. N., A. M. Kimotho, and J. G. Nduhiu. 2000. Antimicrobial resistance patterns and plasmid profiles of *Staphylococcus aureus* isolated from milk and meat. *East Afr. Med. J.* 77:463–467.
26. Peles, F., M. Wagner, L. Varga, I. Hein, P. Rieck, K. Gutser, P. Kereszturi, G. Kardos, I. Turcsanyi, B. Beri, and A. Szabo. 2007. Characterization of *Staphylococcus aureus* strains isolated from bovine milk in Hungary. *Int. J. Food Microbiol.* 118:186–193.
27. Pesavento, G., B. Ducci, N. Comodo, and A. Lo Nostro. 2007. Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: a research for methicillin resistant *Staphylococcus aureus* (MRSA). *Food Control* 18:196–200.
28. Pu, S., F. Wang, and B. Ge. 2011. Characterization of toxin genes and antimicrobial susceptibility of *Staphylococcus aureus* isolates from Louisiana retail meats. *Foodborne Pathog. Dis.* 8:299–306.
29. Rosec, J. P., J. P. Guiraud, C. Dalet, and N. Richard. 1997. Enterotoxin production by staphylococci isolated from foods in France. *Int. J. Food Microbiol.* 35:213–221.
30. Tsen, H. Y., G. K. Yu, K. C. Wang, S. J. Wang, M. Y. Chang, and L. Y. Lin. 1998. Comparison of the enterotoxigenic types, toxic shock syndrome toxin I (TSST-1) strains and antibiotic susceptibilities for enterotoxigenic *Staphylococcus aureus* strains isolated from food and clinical samples. *Food Microbiol.* 15:33–41.
31. Wang, Q., L. Yang, F. Luo, and S. Meng. 2009. Antimicrobial resistance and detection of *mecA, qacA/B* and *PVL* genes in *Staphylococcus aureus*. *Chin. J. Clin. Infect. Dis.* 2:4.
32. Wang, X., J. Meng, J. Zhang, T. Zhou, Y. Zhang, B. Yang, M. Xi, and X. Xia. 2012. Characterization of *Staphylococcus aureus* isolated from powdered infant formula milk and infant rice cereal in China. *Int. J. Food Microbiol.* 153:142–147.
33. Wang, X., X. Tao, X. Xia, B. Yang, M. Xi, J. Meng, J. Zhang, and B. Xu. 2013. *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in retail raw chicken in China. *Food Control* 29:103–106.
34. Wieneke, A. A., D. Roberts, and R. J. Gilbert. 1993. Staphylococcal food poisoning in the United Kingdom, 1969–90. *Epidemiol. Infect.* 110:519–531.
35. Wu, Q., M. Xi, X. Lv, Y. Xu, Y. Feng, Q. Li, Q. Yang, and X. Xia. 2014. Presence and antimicrobial susceptibility of *Escherichia coli* recovered from retail chicken in China. *J. Food Prot.* 77:1773–1777.
36. Yan, X. M., B. Wang, X. X. Tao, Q. H. Hu, Z. G. Cui, J. Z. Zhang, Y. M. Lin, Y. H. You, X. L. Shi, and H. Grundmann. 2012. Characterization of *Staphylococcus aureus* strains associated with food poisoning in Shenzhen, China. *Appl. Environ. Microbiol.* 78:6637–6642.