

Antimicrobial Susceptibility and Molecular Typing of Methicillin-Resistant *Staphylococcus aureus* in Retail Foods in Shaanxi, China

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Abstract

The aims of this study were to evaluate the occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) in retail foods in Shaanxi, China and to investigate antimicrobial resistance and molecular characteristics of these strains. A total of 1979 retail food samples were randomly collected during 2008–2012 from supermarkets and farmers markets and screened for *S. aureus*, and then *S. aureus* isolates were further examined to determine whether they were MRSA. MRSA isolates were further characterized by antimicrobial susceptibility test, pulsed-field gel electrophoresis, *spa* typing, multilocus sequence typing, and *SCCmec* typing, and were examined for genes encoding enterotoxins, exfoliative toxins, Panton-Valentine leukocidin (*pvl*), and toxic shock syndrome toxin 1. Among all the samples examined, four (1.4%) raw milk samples, six (2.3%) chicken samples, one (0.6%) pork sample, three (0.6%) ready-to-eat food samples, and three (2.5%) dumpling samples were positive for MRSA. No MRSA isolates were recovered from infant foods. A total of 23 MRSA isolates were recovered from the 17 MRSA-positive samples. Antimicrobial susceptibility tests showed that, among these MRSA isolates, resistance was most frequently observed to penicillin, ampicillin, oxacillin, cefoxitin, and clindamycin (each 100%), followed by erythromycin (95.7%) and clarithromycin (87.0%). The commonly detected toxin genes were *pvl*, *seg*, *seb*, *sed*, followed by *see*, *sec*, and *sei*. Seven *spa* types (t189, t377, t437, t899, t10793, t5762, and a new *spa* type) and three *SCCmec* types (II, IV_b, and V) were identified. More than half (52.2%) of the MRSA isolates belonged to ST9, followed by ST88, ST59, ST188, ST72, and ST630. Our findings indicate that MRSA in food could be from both animal and human origin. Although the prevalence is low, the presence of multidrug resistant and enterotoxigenic MRSA strains in foods poses a potential threat to consumers and emphasizes the need for better control of sources of contamination.

Introduction

METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA) is an important hospital- and community-associated pathogen worldwide (Ho *et al.*, 2008). Methicillin resistance is mediated by the *mecA* gene that encodes penicillin-binding protein 2a, which has a low affinity for essentially all β -lactam antimicrobials (Pinho *et al.*, 2001). In recent years, MRSA strains have been identified in various foods including bovine milk, ice cream, ready-to-eat foods, and meat products (Kwon *et al.*, 2005; de Boer *et al.*, 2009; Pu *et al.*, 2009; Bhargava *et al.*, 2011; Fessler *et al.*, 2011; Lim *et al.*, 2010; Hammad *et al.*, 2012; Vestergaard *et al.*, 2012; Gucukoglu *et al.*, 2013). Foods may serve as an important reservoir and source of community-acquired MRSA (Jones *et al.*, 2002).

Several genotyping methods, such as multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), *SCCmec* typing, and staphylococcal protein A (*spa*) typing, have been applied in typing MRSA strains from various foods (Kwon *et al.*, 2005; de Boer *et al.*, 2009; Pu *et al.*, 2009; Lim *et al.*, 2010; Bhargava *et al.*, 2011; Fessler *et al.*, 2011; Hammad *et al.*, 2012; Vestergaard *et al.*, 2012). It has been demonstrated that the discriminatory power of PFGE is greater than MLST, *spa* typing, and *SCCmec* typing (Rasschaert *et al.*, 2009). Combining these techniques may provide more precision in epidemiological studies (Strommenger *et al.*, 2006).

There was a paucity of data regarding characteristics of MRSA from retail foods in China. Therefore, we carried out this study to determine the prevalence of MRSA strains in retail foods sold in Shaanxi province, China and to

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characterize these strains by antimicrobial susceptibility test and molecular typing.

Materials and Methods

Sample collection

A total of 1979 retail food samples were collected at different times during the period July 2008–December 2012 in 12 cities in Shaanxi province. Whole chickens, pork chops, dumplings, ready-to-eat (RTE) foods (including cooked meat, vegetable salads, boiled peanuts, cold noodles, and dried tofu), powdered infant formula (PIF), and infant rice cereal (IRC) samples were purchased from supermarkets and farmers markets in 12 cities (Xi'an, Yangling, Xianyang, Wugong, Zhouzhi, Liquan, Fufeng, Xingping, Qianxian, Qishan, Meixian, and Baoji). One sample of each type of food was purchased from each store, and stores were visited once each year if possible. Raw milk samples were taken monthly from 10 farms in three cities (Xingping, Xianyang, and Yangling) during July 2008 and July 2009. Solid food samples were placed into a sterile plastic bag and sealed, and a raw milk sample was placed into a 50-mL sterile centrifuge tube. All of the samples were transported on ice to the laboratory at Northwest A&F University (Yangling, Shaanxi, China) and processed within 5 h. For infant foods, they were purchased and transported at ambient temperature to the laboratory and processed within 1 day.

Isolation and identification of *S. aureus* and MRSA

S. aureus was isolated according to the procedures described below. For RTE foods, pork, dumplings, PIF, and IRC, 25 g of each food sample was placed into a sterile glass flask containing 225 mL of buffered peptone water (BPW, Beijing Land Bridge Technology Ltd., Beijing, China). The solution was incubated at 37°C in a water bath with shaking at 150 rpm for 18–24 h. For whole chicken, each chicken sample was placed in a plastic bag (Stomacher 3500; Shanghai Delin Instrument Co., Ltd., Shanghai, China), and manually rinsed in 400 mL of buffered peptone water (BPW; Beijing Land Bridge Technology Ltd.) for 2 min, ensuring that all surfaces had contact with the rinse. The rinse was then incubated at 37°C in a water bath with shaking at 100 rpm for 18–24 h in a plastic bag. After pre-enrichment, a 5-mL aliquot was transferred to 50 mL of trypticase soy broth (TSB, Beijing Land Bridge Technology Ltd.) containing 7.5% NaCl. For raw milk, a 50-mL aliquot of milk sample was enriched in an equal volume of double-strength TSB supplemented with 15% NaCl (Beijing Land Bridge Technology Co. Ltd.). After 18–24 h incubation at 35°C, a loopful (approximately 4 μ L) of the culture was inoculated onto Baird-Parker agar (BPA, Beijing Land Bridge Technology Ltd.) plates with 5% egg yolk and tellurite. Following incubation at 35°C for 24 h, one or two putative *S. aureus* isolates on BPA plates (black colonies surrounded by 2–5-mm clear zones) per sample were transferred to trypticase soy agar (Beijing Land Bridge Technology Ltd.) plates with 0.6% yeast extract for further purification. Colonies were then confirmed as MRSA by polymerase chain reaction (PCR) detection of *nuc* (Brakstad *et al.*, 1992) and *mecA* (Zhang *et al.*, 2004). All MRSA isolates were stored at –80°C in TSB plus 20% (vol/vol) glycerol for further use.

Antimicrobial susceptibility testing

All antimicrobial susceptibility tests were performed using the disk-diffusion method except vancomycin, for which the agar dilution method was used (CLSI, 2012). A panel of 32 antimicrobial agents was used: penicillin (10 μ g, \leq 28 mm), oxacillin (1 μ g, \leq 10 mm), ampicillin (10 μ g, \leq 28 mm), cefoxitin (30 μ g, \leq 21 mm), cefazolin (30 μ g, \leq 14 mm), cefepime (30 μ g, \leq 14 mm), cefoperazone (75 μ g, \leq 15 mm), cefotaxime (30 μ g, \leq 14 mm), ceftazidime (30 μ g, \leq 14 mm), ceftriaxone (30 μ g, \leq 13 mm), cefuroxime (30 μ g, \leq 14 mm), cephalothin (30 μ g, \leq 14 mm), gentamicin (10 μ g, \leq 12 mm), amikacin (30 μ g, \leq 14 mm), kanamycin (30 μ g, \leq 13 mm), spectinomycin (100 μ g, \leq 14 mm), streptomycin (10 μ g, \leq 11 mm), tobramycin (10 μ g, \leq 12 mm), midecamycin (15 μ g, \leq 13 mm), clarithromycin (15 μ g, \leq 13 mm), erythromycin (15 μ g, \leq 13 mm), tetracycline (30 μ g, \leq 14 mm), minocycline (30 μ g, \leq 14 mm), ciprofloxacin (5 μ g, \leq 15 mm), levofloxacin (5 μ g, \leq 15 mm), ofloxacin (5 μ g, \leq 14 mm), norfloxacin (10 μ g, \leq 12 mm), nitrofurantoin (300 μ g, \leq 14 mm), clindamycin (2 μ g, \leq 14 mm), trimethoprim/sulfamethoxazole (1.25 μ g/23.75 μ g, \leq 10 mm), chloromycetin (30 μ g, \leq 12 mm), and vancomycin (minimum inhibitory concentration breakpoint 16 μ g/mL). All antimicrobials discs and vancomycin were purchased from Hangzhou Tianhe Microorganism Reagent Co., Ltd., Hangzhou, China. For all antibiotics except spectinomycin, streptomycin, and midecamycin, the breakpoints used for *Staphylococcus* spp. (CLSI, 2012) were adopted in this study. For spectinomycin, streptomycin, and midecamycin, the breakpoints were taken from the manufacturer's instruction manual. *Escherichia coli* ATCC 25922, *S. aureus* ATCC 25923, and *S. aureus* ATCC 29213 were used as quality-control strains for antimicrobial susceptibility testing.

Molecular characterization of the MRSA isolates

PFGE using *Sma*I was performed to determine genomic DNA fingerprinting patterns of the MRSA isolates, using previously published methods (McDougal *et al.*, 2003). PFGE results were analyzed using BioNumerics software (Applied-Maths, Kortrijk, Belgium), and banding patterns were compared using Dice coefficients with a 1.5% band-position tolerance. MLST analysis was conducted by sequencing fragments of seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) and sequence types (STs) were assigned by comparison with the *S. aureus* MLST database (<http://www.mlst.net/>). *SCCmec* typing was performed by PCR as described previously (Zhang *et al.*, 2005). *Spa* types were defined according to the procedure previously described by Harmsen *et al.* (Harmsen *et al.*, 2003). MRSA isolates were tested by PCR for nine enterotoxin genes (*SEs*) (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, and *sej*) (Peles *et al.*, 2007), toxic shock syndrome toxin 1 (*tst-1*) gene (Peles *et al.*, 2007), exfoliative toxin genes (*eta* and *etb*) (Noguchi *et al.*, 2006), and Panton-Valentine leukocidin gene (*pvl*) (Wang *et al.*, 2009).

Statistical analysis

Proportions of *S. aureus* and MRSA-positive samples in different types of food were compared by chi-square or Fisher's exact tests with SPSS 16.0 statistical software (SPSS

Inc., Chicago, IL) for Windows. The significance level was set at $p=0.05$.

Results

Prevalence of *S. aureus* and MRSA

Table 1 lists the prevalence of *S. aureus* and MRSA strains in the 1979 food samples examined. We recovered 786 *S. aureus* isolates (1–2 isolates per sample) including 763 methicillin-susceptible *S. aureus* isolates (derived from 438 samples) and 23 MRSA isolates (from 17 samples). The overall prevalence of *S. aureus* was 25.1% in RTE foods, 40.9% in chicken, 34.4% in pork, 60.0% in dumplings, 13.2% in PIF, 5.7% in IRC, and 14.6% in raw milk. MRSA was found in four (1.4%) raw milk samples, six (2.3%) chicken samples, one (0.6%) pork sample, three (0.6%) RTE foods, and three (2.5%) dumplings. No MRSA isolates were detected from PIF and IRC. The food type significantly affected *S. aureus* ($p<0.001$) and MRSA ($p=0.0095$) prevalence.

Antimicrobial susceptibility test

All MRSA isolates were resistant to penicillin, ampicillin, oxacillin, cefoxitin, and clindamycin (Fig. 1). Resistance was frequently detected to erythromycin (95.7%), clarithromycin (87.0%), kanamycin, spectinomycin, and midecamycin (73.9% for each), trimethoprim/sulfamethoxazole (69.6%), streptomycin and tetracycline (65.2% for each), cefazolin (60.9%), tobramycin and levofloxacin (56.5%), gentamicin, ciprofloxacin and chloromycetin (52.2% for each), norfloxacin (47.8%), amikacin, cefepime and ceftazidime (39.1% for each), cefuroxime (34.8%), cefotaxime and ceftriaxone (30.4% for each), ofloxacin (21.7%), nitrofurantoin (13.0%), and cefalotin (8.7%). All isolates were susceptible to cefoperazone, vancomycin, and minocycline. All MRSA isolates were resistant to eight or more antimicrobials. One MRSA isolate was resistant to 24 antibiotics. A total of 23 antimicrobial resistance profiles were identified (Fig. 1).

Detection of toxin genes

Of the 23 MRSA strains tested, 21 (91.3%) were positive for one or more toxin genes. Seven toxin genes (*pvl*, *seb*, *sec*, *sed*, *see*, *seg*, and *sei*) were detected in these isolates. The four most frequently detected toxin genes were *pvl* (60.9%), *seg* (56.5%), *seb* (52.2%), and *sed* (43.5%), followed by *see* (8.7%), *sec*, and *sei* (4.3% for each). The *eta*, *etb*, *tsst-I*, *sea*, *seh*, and *sej* genes were not detected in these isolates. A total of 13 toxin gene profiles were identified (Fig. 1).

Molecular typing

Three *SCCmec* types including types II (4, 17.4%), IV_b (9, 39.1%), and V (2, 8.7%) were detected. The remaining eight (34.8%) isolates were nontypeable by *SCCmec* typing (Fig. 1). Six MLST types were identified, including ST9 (12, 52.2%), ST88 (4, 17.4%), ST59 (3, 13.0%), ST188 (2, 8.7%), and ST72 and ST 630 (1, 4.3% each) (Fig. 1). For *spa* typing, seven *spa* types were found, including t899 (12, 52.2%), t437 (3, 13.0%), t189 and t10793 (2, 8.7% each), and t377 and t5762 (1, 4.3% each). The remaining two (8.7%) isolates belonged to a new *spa* type (07-34-34-34-34-33-13) (Fig. 1). PFGE showed that all MRSA isolates were typeable by PFGE using *SmaI* and displayed 15 PFGE patterns. Certain isolates with identical PFGE patterns (such as samples 42 and 48 in P5, samples J43 and sample J105 in P9) were recovered from different samples of the same type of food (Fig. 1).

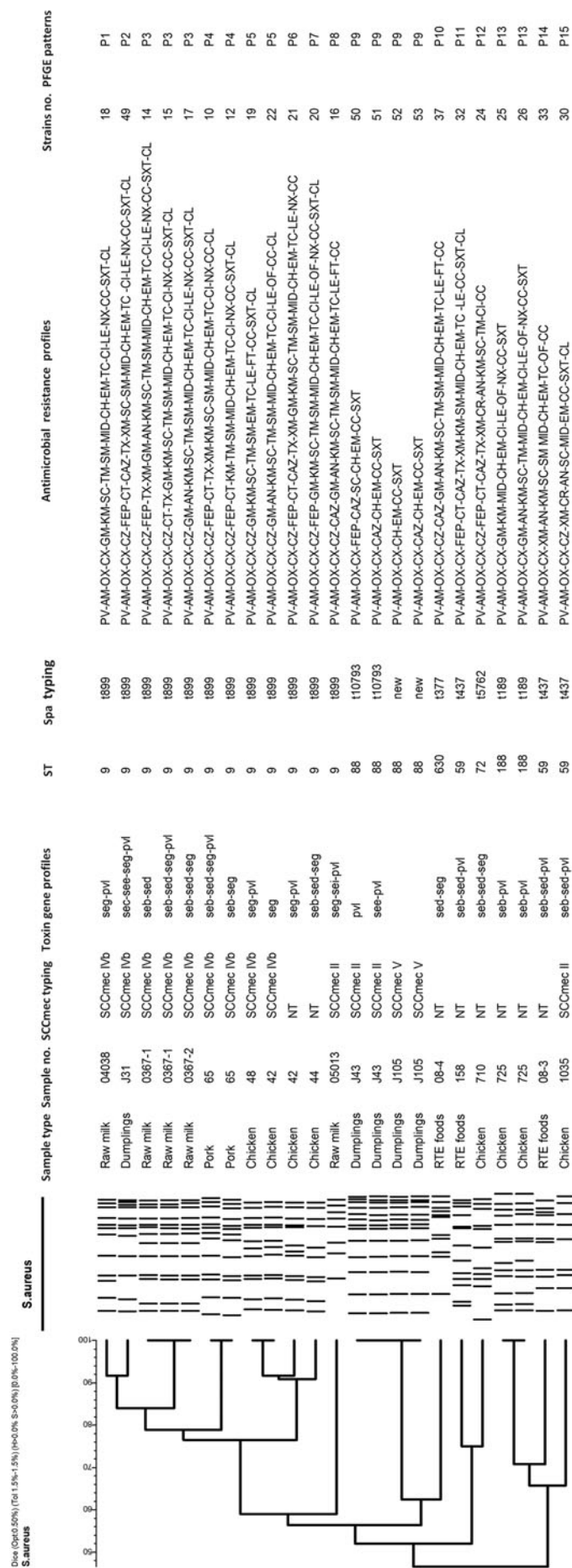
Discussion

To our knowledge, this is the largest and most comprehensive (including various food products) sampling of retail foods in China for MRSA detection. Approximately 0%–2.5% of different types of food samples were contaminated with MRSA, which was similar to the positive rates of 0.3%–3.9% detected in other countries (Lim *et al.*, 2010; Bhargava *et al.*, 2011). In contrast, MRSA was detected at rates ranging from 2.2% to 35.3% in various meat products in another study (de Boer *et al.*, 2009). The difference in isolation methods may contribute to the difference in the positive rates detected. In this study, *S. aureus* was first isolated and up to two isolates were randomly chosen from a few putative isolates for MRSA identification. In addition, nonselective media (TSB) were used in the pre-enrichment step rather than media with antibiotics (such as cefoxitin), which are frequently used for MRSA isolation. These factors may cause an underestimation of the actual positive rate in this study. Although MRSA prevalence in retail foods is relatively low, the risk of its transmission through the food chain, especially by uncooked food, cannot be disregarded. A foodborne outbreak caused by MRSA has been reported previously (Jones *et al.*, 2002).

The high prevalence of resistance of MRSA isolates in different foods to ampicillin, penicillin, trimethoprim/sulfamethoxazole (Fig. 1), which are commonly used for prophylaxis and infection control in food animals as well as for medical use in humans, may be related to the imprudent use of these drugs in food animals and humans in China (Cui *et al.*, 2009; Liu *et al.*, 2009; Chu *et al.*, 2013). All MRSA

TABLE 1. PREVALENCE OF *STAPHYLOCOCCUS AUREUS* AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN DIFFERENT RETAIL FOODS

| Types of food | Total no. of samples | No. (%) <i>S. aureus</i> positive samples | No. of <i>S. aureus</i> recovered | No. (%) of samples positive for MRSA | No. of MRSA recovered |
|-------------------------|----------------------|---|-----------------------------------|--------------------------------------|-----------------------|
| Ready-to-eat foods | 490 | 123 (25.1%) | 208 | 3 (0.6%) | 3 |
| Chicken | 264 | 108 (40.9%) | 198 | 6 (2.3%) | 8 |
| Pork | 160 | 55 (34.4 %) | 94 | 1 (0.6%) | 2 |
| Dumplings | 120 | 72 (60.0%) | 131 | 3 (2.5%) | 5 |
| Powdered infant formula | 228 | 30 (13.2 %) | 49 | 0 (0) | 0 |
| Infant rice cereal | 422 | 24 (5.7 %) | 37 | 0 (0) | 0 |
| Raw milk | 295 | 43 (14.6 %) | 69 | 4 (1.4%) | 5 |
| Total | 1979 | 455 (23.0%) | 786 | 17 (0.9%) | 23 |



isolates showed multidrug resistance. The presence of these multidrug-resistant MRSA strains in food is of concern since it is indicated that multidrug-resistant MRSA strains in foods may be transmitted to humans through the food chain (de Niederhausern *et al.*, 2004). All MRSA isolates were susceptible to vancomycin, which was in agreement with previous studies in which no MRSA isolates recovered from animals and humans demonstrated vancomycin resistance in China (Cui *et al.*, 2009; Liu *et al.*, 2009; Chu *et al.*, 2013).

Many of the MRSA isolates from retail foods were positive for *pvl* and *SE* genes. Similar to this study, the *pvl* gene was detected in MRSA from various foods in the United States and Korea (Kwon *et al.*, 2005; Pu *et al.*, 2009; Hanson *et al.*, 2011). In contrast, none of the MRSA isolates from various foods in Germany and Thailand were *pvl* positive (Rhee and Woo, 2010; Fessler *et al.*, 2011; Vestergaard *et al.*, 2012). Exfoliative toxins genes and *tsst-I*, both of which were rarely reported among MRSA isolates from various foods, were not detected (Rhee and Woo, 2010; Fessler *et al.*, 2011; Vestergaard *et al.*, 2012). Various *SE* genes were detected among food-derived MRSA isolates, which has also been reported in Korea and Germany (Rhee and Woo, 2010; Fessler *et al.*, 2011; Vestergaard *et al.*, 2012). In addition to *see*, *seg*, and *sei*, which were reported in these studies, we detected *seb*, *sec*, and *sed* in MRSA isolates from foods.

MRSA ST398 was first identified in the Netherlands in 2003, and is now recognized as a dominant sequence type among food and food animals in many European countries (de Boer *et al.*, 2009). In contrast, no MRSA ST398 isolates were detected in this study, but methicillin-sensitive *S. aureus* ST398 clones were detected in chicken and RTE foods in our study (data not shown). Many MRSA isolates from animal-derived products (pork, chicken, and raw milk) belonged to MRSA ST9-t899, which was in agreement with previous reports showing that the MRSA ST9-t899 clone is predominant in food animals (Cui *et al.*, 2009; Guardabassi *et al.*, 2009; Wagenaar *et al.*, 2009) and retail pork (Boost *et al.*, 2013) in China. Contamination with MRSA ST9-t899 in foods may be attributable to cross-contamination during slaughtering or food processing. MRSA isolates from hand-made foods (RTE foods and dumplings) overlapped in molecular type (MRSA ST59-t437 and MRSA ST88) with isolates recovered from human in China (Zhang *et al.*, 2009; Geng *et al.*, 2010), and are most likely of human origin. Contamination may originate from poor hygiene of workers during food preparation, since there are plenty of opportunities for workers to have direct contact with the foods. Except for ST9-t899 (Boost *et al.*, 2013) strains that have been detected from food in China, ST72-t5762, ST88-t10793, ST88-t (07-34-34-34-34-33-13), ST59-t437, ST630-t377, and ST188-t189 have never been reported in MRSA from food sources. Eight MRSA isolates were nontypeable by *SCCmec* typing, which is consistent with another study by Zhang *et al.* (2009). Novel types of *SCCmec* have been continuously reported worldwide since *SCCmec* typing was introduced (Zhang *et al.*, 2009). Other studies (Cui *et al.*, 2009; Fessler *et al.*, 2011) have shown that MRSA strains with identical *spa* or MLST types can carry different *SCCmec* elements, which was confirmed in this study.

Certain MRSA isolates (strains 21 and 22 and strains 50–53) with identical PFGE patterns were recovered from the same brand of food products sold in different supermarkets.

For example, strains 21 and 22 were recovered from chickens of the same brand, and strains 50–53 were recovered from dumplings of the same brand. This may reflect a clonal spread of specific strains in the same food plant. In contrast, no PFGE pattern was shared in MRSA strains isolated from different types of food. This may suggest that MRSA isolates from different types of food samples are generally not associated.

In summary, MRSA isolates were detected in retail foods with a low prevalence rate in Shaanxi Province, China. Strain characterization suggests that MRSA isolates from animal-derived foods may originate from food animals, while MRSA from RTE and hand-made foods possibly have a human origin. In addition, these MRSA isolates exhibited multiple drug resistance and carried different toxins genes. Although the real pathogenicity of these MRSA isolates remains unclear, their existence in retail foods still poses a potential health risk to consumers (Jones *et al.*, 2002). Further research to explore how MRSA has made its way into the food chain is warranted to better control its dissemination.

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Disclosure Statement

No competing financial interests exist.

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