**mecA-related structure in methicillin-resistant coagulase-negative staphylococci from street food in Taiwan**

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Antibiotic-resistant patterns, a *mecA* homologue complex, and staphylococcal cassette chromosome *mec* (SCCmec) were analysed in samples of ready-to-eat (RTE) street food in Taiwan. RTE food samples (270) were collected in three densely populated Taiwanese cities between June and November 2014. Among 14 strains being identified as methicillin-resistant coagulase-negative staphylococci (MRCoNS), genetic diversities was determined by PFGE analysis. SCCmec types IV, VIII and TXG-24 were detected in 9, and *mec\(_{ASs}\)* (a *mecA* homologue) detected in 8. The *mec\(_{ASs}\)* gene complex from *S. sciuri* subsp. *sciuri* TXG-24 was found to be closely related to those found in both *S. sciuri* subsp. *sciuri* (ATCC29062) and *S. sciuri* subsp. *rodentium* (ATCC700061). SCCmec\(_{TXG24}\) carries a class A *mec* complex, a *ccrA5B3*-like gene complex, a heavy metal gene complex, and an IS1216 mobile element carrying tet(S). Matching identity to *ccrA5* was 84.5% for *ccrA* in *S. pseudintermedius* KM241. Matching identify to *ccrB3* was 92.1% for *ccrB* in *S. pseudintermedius* AI16. Similar *ccrA* and SCCmec boundary sequences suggest that SCCmec is easily transmitted to coagulase-negative staphylococci (CoNS). Based on MRCoNS strains identified in this research, Taiwanese RTE food products likely carry multiple antibiotic resistance genes that can be transmitted to hospitals and other clinical settings.

Convenient, cheap and popular, ready-to-eat (RTE) food products in Taiwan are found in public markets, train stations, sidewalk stalls, and many other locations. The microbiological quality of RTE foods has gained attention due to the 4,284 cases of food poisoning that were officially reported between 1991 and 2010, with the number of cases increasing yearly. Accordingly, the literature on the microbiological quality of cold RTE foods (e.g., sandwiches, noodles, and rice balls served at 18 °C or lower) and RTE-related ingredients (e.g., staples, meats, vegetables, and seafood) in Taiwan has grown significantly. After analysing 164 RTE food samples served at 18 °C, Fang et al. reported a 42.7% incidence of psychrotrophic *Pseudomonas* spp., 75% coliforms, 7.9% *E. coli*, 49.8% *B. cereus* and 17.9% *S. aureus*. According to a separate study conducted by Wei et al., RTE-related products stored at room temperature had the highest incidence of bacterial contamination, and RTE foods served by street vendors in traditional markets had the highest bacterial counts. However, a review of extant studies indicates that few efforts have been made to determine antibiotic resistance in RTE foods.

The first case of methicillin resistance in *Staphylococcus aureus* (MRSA) was reported in Great Britain in 1961. The resistance mechanism has been linked to an alternative penicillin-binding protein (either PBP2a or PBP2′) encoded by *mecA* and transmitted via the excision and insertion of a SCCmec element\(^6\). SCCmec elements share two important features: a *mec* gene complex carrying a *mecA* homologue, and specific insertion sites with flanking repeat sequences via the *ccr* gene complex. Recently, several research teams have reported the potential of coagulase-negative staphylococci (CoNS) for transmitting antibiotic-resistant genes\(^7–11\). Tulinski et al. found that CoNS strains isolated from pig farms acted as reservoirs for heterogeneous SCCmec elements. Kloos

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et al. have described *S. sciuri* as a reservoir for a methicillin-resistant gene\(^1\), and Ruzauskas et al. have reported the cross-sectional prevalence of methicillin-resistant *S. haemolyticus* in companion animals\(^1\).

It is generally accepted that RTE food products serve as reservoirs for antimicrobial-resistant bacteria, but transmission and resistance mechanisms in Taiwan require further investigation. For this project, we looked at proportions of methicillin-resistant coagulase-negative staphylococci (MRCoNS) found in samples of spring rolls, cold noodles, and fruit platters collected from RTE vendors in the densely inhabited cities of Kaohsiung, Taichung and Taipei, and attempted to determine their antibiotic resistance mechanisms.

### Results

#### MRCoNS characterization

We used *dnaJ* gene sequencing to identify bacterial species in 14 MRCoNS strains (Table 1). The dominant bacteria was *S. sciuri* (6/14, 42.9%), including 3 isolates of *S. sciuri* subsp. *rodentium*, 2 isolates of *S. sciuri* subsp. *sciuri*, and 1 isolate of *S. sciuri* subsp. *carnaticus*, followed by *S. saprophyticus* (4/14, 28.7%), *S. haemolyticus* (2/14, 14.4%), *S. lentus* (1/14, 7%), and *S. pasteuri* (1/14, 7%). The most frequent sources were spring rolls (7/14, 50%), cold noodles (5/14, 35.7%), and fruit platters (2/14, 14.3%). Genetic diversity data as determined by PFGE analysis are shown in Table 1 and Supplementary Fig. S1. Only two *S. saprophyticus* isolates (TPE-21 and TPE-32, isolated from cold noodles and spring rolls, respectively) belong to pulsotype IX (Table 1). According to antimicrobial susceptibility test results, all isolates were resistant to 1–5 antimicrobials, with a list that included oxacillin (14/14, 100%), levofloxacin (2/14, 14%), erythromycin (10/14, 71.4%), tetracycline (9/14, 64.3%), gentamicin (4/14, 28.7%; 1 of the 4 was gentamicin-intermediate), and vancomycin-intermediate (1/14, 7%) (Table 1).

### Genetic analysis of the mecA\(_5\) gene complex and SCCmec\(_{TXG24}\)

Genetic analysis results indicate the presence of two *mecA* homologues (*mecA* and *mecA\(_5\)*) in *S. sciuri* subsp. *sciuri* TXG-24. Genomic structure analysis data for the *mecA\(_5\)* region are shown in Fig. 1. The *mecA\(_5\)* gene complex of TXG-24 is closely related to *S. sciuri* subsp. *sciuri* ATCC29062 (GenBank accession number AB547234.1), with the exception of a downstream 4-gene cobalt ABC transporter homologue. The *mecA\(_5\)* region of *S. sciuri* subsp. *rodentium* ATCC700061 (AB547235.1) was found in all 14 oxacillin-resistant isolates (14/14, 100%) (Table 1). Among the 10 erythromycin-resistant isolates, *S. saprophyticus* KH-2, *S. sciuri* subsp. *sciuri* TXG-24, and *S. sciuri* subsp. *rodentium* TXG-28 carried both *ermA* and *ermC* genes, while *S. haemolyticus* TXG-25, *S. sciuri* subsp. *sciuri* TXG-15, and *S. sciuri* subsp. *rodentium* TPE-18 only carried the *ermC* gene. No *erm* genes were detected in the other 4 erythromycin-resistant isolates. Among the 9 tetracycline-resistant isolates, *S. sciuri* subsp. *sciuri* TXG-15 harboured 3 tetracycline-resistant genes, while *S. haemolyticus* TXG-25 and *S. sciuri* subsp. *rodentium* TXG-28 and TPE-18 contained *tet(K)*. Both *tet(M)* and *tet(K)* were found in *S. saprophyticus* KH-20, and *tet(M)* and *tet(O)* were found in *S. lentus* TXG-26. We also observed *aac(6’)*Ie-*aph(2)”Ia in all gentamicin-resistant isolates (4/4, 100%) (Table 1). Staphylococcal super-antigenic genes encoded staphylococcal enterotoxins (SEs), an SE-related toxin, and toxic shock syndrome toxin-1 (TSS1-1). Among the 14 isolates, 12 (85.7%) carried one or more staphylococcal super-antigenic genes, but they were not detected in *S. haemolyticus* TXG-25 or *S. saprophyticus* TPE-21. Among enterotoxins and enterotoxin-like proteins, the most prevalent genes were sec (3/14, 21.4%), selk (5/14, 35.7%) and seh (5/14, 35.7%). The *sed* and *selp* genes were not detected in any isolates (Table 1).

### Table 1. Antibiotic resistance and pulsotypes identified in CoNS isolated from the 270 RTE food samples.

| Area       | Species and Strain | Isolated Source | Pulsotype | Antibiotic Resistance Phenotype | Antibiotic Resistance Genotype | S. aureus Super Antigenic Toxin Genotype |
|------------|--------------------|----------------|-----------|-------------------------------|-------------------------------|-----------------------------------------|
| Kaohsiung  | *S. saprophyticus* KH-2 | cold noodles  | X         | OXA, ERY                      | mecA, ermA, ermC              | seg, seh, selo                          |
|            | *S. haemolyticus* KH-11 | fruit platter | III       | OXA, ERY                      | mecA                           | sec, seh, selj                          |
|            | *S. saprophyticus* KH-20 | spring roll   | XI        | OXA, TET, ERY                 | mecA, tet(M), tet(K)          | selk, seh                               |
|            | *S. sciuri* subsp. *carnaticus* KH-57 | spring roll | XII       | OXA, TET, ERY                 | mecA                           | sea, selk, seh                          |
| Taichung   | *S. sciuri* subsp. *sciuri* TXG-15 | cold noodles | IV        | OXA, TET, ERY                 | mecA, ermC, tet(M), tet(O), tet(K) | sec, seh, selk, seh                     |
|            | *S. sciuri* subsp. *sciuri* TXG-24 | spring roll   | VI        | OXA, VAN\(^\text{a}\), TET, ERY | mecA, ermA, ermC              | selk, seh                               |
|            | *S. haemolyticus* TXG-25 | cold noodles | XIII      | OXA, TET, GEN, LVX, ERY       | mecA, ermC, tet(K), aac(6’)*Ie-*aph(2)”Ia | ND\(^\text{a}\) |
|            | *S. lentus* TXG-26 | spring roll | I         | OXA, TET, GEN                 | mecA, tet(M), tet(O), aac(6’)*Ie-*aph(2)”Ia | sei, selr |
|            | *S. sciuri* subsp. *rodentium* TXG-28 | spring roll | II        | OXA, TET, GEN, LVX, ERY       | mecA, ermA, ermC, tet(K), aac(6’)*Ie-*aph(2)”Ia | selk, seh |
| Taipei     | *S. pasteuri* TPE-12 | fruit platter | VII       | OXA, GEN\(^\text{a}\), ERY    | mecA, aac(6’)*Ie-*aph(2)”Ia  | see, selm                               |
|            | *S. sciuri* subsp. *rodentium* TPE-18 | cold noodles | VIII      | OXA, TET, ERY                 | mecA, ermC, tet(K)            | sea                                      |
|            | *S. saprophyticus* TPE-21 | cold noodles | IX        | OXA                           | mecA                           | nd\(^\text{a}\)                           |
|            | *S. saprophyticus* TPE-32 | spring roll   | IX        | OXA                           | mecA                           | sell, selq, tetl                         |
|            | *S. sciuri* subsp. *rodentium* TPE-33 | spring roll | V         | OXA, TET                       | mecA                           | sec                                      |

Abbreviations: OXA, oxacillin; ERY, erythromycin; TET, tetracycline; VAN, vancomycin; GEN, gentamicin; LVX, levofloxacin. \(^\text{a}\)Intermediate resistance to antibiotic. \(^\text{b}\)ND, not detected.
shares a high degree of similarity with TXG-24, except for the upstream upgQ of two hypothetical protein genes, the ABC transporter gene, and the amino acid/polyamine/organoacetate (APC) family transporter gene.

The SCCmec element of *S. sciuri* subsp. *sciuri* TXG-24 has a complex genomic structure that contains a class A mec gene complex (IS431-mecA-mecR1-mecI), an IS1216 mobile element carrying tet(S), partial DNA recombinase with methyltransferase, a heavy metal-resistant gene complex, and a ccr gene complex (Fig. 2). The mec gene complex of *SCCmec* _TXG24_ is closely related to *S. sciuri* _carnaticus* GVG2S (HG515014) and *S. pseudintermedius* KM241 (AM904731), except for two hypothetical protein genes and a truncated mecR2 gene. The SCCmec _TXG24_ region containing partial DNA recombinase with methyltransferase is highly similar to the comparative region of Streptococcus suis SC84 (FM252031.1), except for a truncated apt gene. Compared to *S. capitis* CR01 (KF049201), the heavy metal-resistant gene complex is associated with the absence of two cadmium-resistant genes (cadD and cadB). The proximal left boundary of SCCmec consists of the ccr gene complex, the putative helicase gene, and some hypothetical protein genes that are associated with comparative regions in *S. sciuri* subsp. *carnaticus* GVG2S and *S. pseudintermedius* KM241.

**Analysis of insertion sequence element carrying the tet(S) tetracycline-resistant gene.** The tet(S)-carrying IS1216 mobile element was found at the 3′ end of ΔmecR2 (Fig. 2). According to our sequence analysis, orf25-orf26-orf27-tet(S) had a high degree of similarity with both the Lactococcus lactis subsp. lactis pK214 plasmid (GenBank accession number X92946) and Streptococcus dysgalactiae subsp. equisimilis NTUH_1743 (EF682209) (Fig. 3). Comparisons of IS1216 regions revealed exceptionally high degrees of shared identity (99.4% and 99.6%) with the *L. lactis* sp. lactis pK214 plasmid, but much lower degrees of shared identity (69.1% and 76.5%) with *S. dysgalactiae* subsp. *equisimilis* NTUH_1743 due to a truncated gene. The ΔmepA gene was only found downstream of orf25 in *L. lactis* sp. lactis pK214.

**ccr gene phylogenetic trees.** SCCmec is a genetic element that encodes methicillin resistance and that carries a unique site-specific recombinase (the ccr gene) in charge of SCCmec element integration and excision. For the present study, we identified a ccr gene complex in *S. sciuri* subsp. *sciuri* TXG-24. Lengths of ccrA and ccrB were 1350 and 1629 bp, respectively. Phylogenetic trees for the ccrA and ccrB sequences (23 each) are shown in Fig. 4a and b. ccrA matching identity was 84.5% to ccrA5 in *S. pseudintermedius* KM241 (GenBank accession number AM904731). ccrB matching identity was 92.1% to ccrB3 in *S. pseudintermedius* AI16 (LN864705.1).

**SCCmec _TXG24_ boundaries.** To investigate SCCmec _TXG24_ boundaries, we aligned the left and right boundaries of SCCmec types I–VII with the SCCmec element of *S. sciuri* subsp. *carnaticus* GVG2S (Fig. 5). SCCmec _TXG24_ integration occurred at almost the same nucleotide position at the 3′ end of the orfX gene as the SCCmec complex of *S. sciuri* subsp. *carnaticus* GVG2S and *S. pseudintermedius* KM241, with both sharing identical direct repeats (DR) at their left and right boundaries. However, nucleotide positions in the other SCCmec types were different from that of SCCmec _TXG24_ and the inverted repeats (IR) of each SCCmec type were variant.

**SCCmec typing and mecA_α_ detection in 14 MRCoNS strains.** To investigate SCCmec distribution in Taiwan, we analysed 14 strains of MRCoNS from the 270 RTE food samples. Four SCCmec types (IV, V, VIII, and TXG-24) were identified in 9 strains (9/14, 64.3%); the other 5 were non-typeable (Table 2). The dominant form was SCCmec type VIII (3/9, 33.3%), found in *S. sciuri* subsp. *carnaticus* KHH-57 and TPE-33, and in *S. lentus* TXG-26. SCCmec-type IV (2/9, 22.2%) was found in *S. pasteurii* TPE-12 and *S. saprophyticus* TPE-32. SCCmec type V (2/9, 22.2%) was found in *S. haemolyticus* KHH-11 and *S. sciuri* subsp. *rodentium* TXG-28. SCCmec _TXG24_ (2/9, 22.3%) was...
found in *S. sciuri* subsp. *sciuri* TXG-24 and *S. sciuri* subsp. *rodentium* TPE-18. The intrinsic *mecAs* gene was present in 8 of the 14 MRCoNS strains (57.1%).

**Discussion**

In their study of five types of RTE food products in Taiwan, Fang et al. reported 75%, 49.8%, 42.7%, 17.9% and 7.9% contamination rates for coliform, *Bacillus cereus*, *Pseudomonas* spp., *S. aureus* and *E. coli*, respectively, in food products stored at 18 °C\(^2\). *S. aureus* was found in 26.1% of all ham samples, 21.4% of all seafood samples, 15.4% of other meat samples, and 13.6% of all vegetable samples. A separate study conducted in southern Taiwan found a 9.5% incidence of *S. aureus* contamination in RTE food products purchased from warehouse stores, 12.7% from traditional markets, and 19.0% from supermarkets\(^3\). The two research teams reported the presence of different pathogens in RTE food, but did not address antimicrobial susceptibility or resistance pattern tendencies.

For the present study, we isolated 14 MRCoNS strains that were resistant to at least one antibiotic, and identified the dominant sources as spring rolls filled with salad ingredients and stewed ground pork wrapped in thin pastry dough, both prepared by glove-wearing vendors (Table 1). The fillings and pastry cracks are likely bacteria reservoirs\(^13\). The second most common source was cold noodles mixed with some kind of sauce, with bacterial proliferation likely due to the relatively higher pH value of the sauce or improper storage temperature\(^2\). Bacterial

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**Figure 2.** Data from a genomic analysis of SCCmec in *S. sciuri* subsp. *sciuri* TXG-24, compared to data for SCCmec in *S. sciuri* subsp. *carnaticus* GVGS2, *S. pseudintermedius* KM241, *S. capitis* CR01, and a partial sequence in the integrative and conjugative element (ICE) of *Streptococcus suis* SC84. Colours indicate various homologous regions in bacterial isolates.

**Figure 3.** Data from a genetic analysis of the tetracycline-resistant *tet*(S) gene complex inserted in SCCmecTXG24 and compared to the complexes *Lactococcus lactis* subsp. *lactis* plasmid pK214 and *Streptococcus dysgalactiae* subsp. *equisimilis* isolate NTUH_1743. Homologous regions are shaded in gray. Black arrow, transposase. Corresponding regions are shadowed.
contamination of fruit platters (the third most common source) was likely due to the improper cleaning of knives. Regardless of actual cause or transmission route, the data indicate that RTE food contamination is a likely avenue for transmitting antibiotic-resistant genes and food-borne diseases14–16.

Determining genetic relationships in bacterial isolates is an important task for monitoring the spread of bacteria. In one study conducted in Turkey, genetic diversity data for 154 multi-drug-resistant strains of S. aureus found in 1,070 RTE food samples suggested multiple routes for various isolates17. In the present study, only two S. saprophyticus isolates (TPE-21 and TPE-32, both from Taipei city) shared the same pulsotype, indicating genetic diversity in our RTE food samples (Supplementary Fig. S1).

Staphylococcal enterotoxin (SE) contaminated food have been reported in foodborne illness 18. Fijałkowski et al. reported that the prevalence of toxin genes in 75 different staphylococcal isolates from 41 food samples in Poland19. The most prevalent SE genes were sei (27/75, 36%), followed by seln (24/75, 32%) and sed (23/75, 31%). Chiang et al. reported that 109 (74.1%) S. aureus isolates contained one or more SE genes in Taiwan 20. The most detected SE genes were sei (45/147, 30.6%), followed by sea (42/147, 28.6%) and sed (30/147, 20.4%). These studies and our finding revealed that SEs production of staphylococcal isolates may be associated with food poisoning19,20. Our study found that the dominant SE genes were selk (5/14, 35.7%) and seln (5/14, 35.7%), followed by sec (3/14, 21.4%) (Table 1). Further studies are warranted to determine the importance of SEs-producing CoNS in RTE food.

Figure 4. Phylogenetic trees for the cassette chromosome recombinase (ccr) gene. (a) 23 ccrA genes. (b) 23 ccrB genes. Trees were generated using neighbour-joining MEGA7 software. Numbers next to nodes indicate confidence levels, expressed as percentages of occurrence over 2,000 bootstrap samples. Scale bar indicates evolutionary distance.

Figure 5. SCCmec boundaries. Left and right boundaries of SCCmec types I to VII and the SCCmec element of S. sciuri subspp. carniaticus GVGS2 were aligned with SCCmecTXG24. Black arrows indicate direct repeats (DRs). Gray arrows indicate inverted repeats (IRs) of SCCmec elements.
close relationship between the TXG-24 mecA}_{S} gene complex and a comparative region of _S. sciu rium subsp. sciu rium_ ATCC29062 (GenBank accession number AB547234.1) that is not associated with oxacillin resistance (Fig. 1).

The CoNS-acquired mecA gene, which has been the focus of multiple studies, is a likely reservoir for transmitting antibiotic-resistant genes\textsuperscript{7–11}. Of the 14 MRCoNS strains that we tested, the most prevalent was _S. sciu rium subsp. sciu rium_ ATCC29062 (GenBank accession number AB547234.1) that is not associated with oxacillin resistance (Fig. 1).

Methods
Sample collection and microbiological analysis. A total of 270 food samples (90 spring rolls, 90 cold noodle bowls and 90 fruit platters) were collected between June and November of 2014. All samples were randomly procured and transported to our laboratory in their original packaging, either within 1 h at the original temperature (Kaohsiung and Taichung samples) or 2 h refrigerated at 4 \( ^\circ \)C (Taipei samples).

For each sample, 10 g were homogenised using a stomacher sample blender, and enriched in brain-heart infusion broth (BD Biosciences) overnight at 37 \( ^\circ \)C. Single loopfuls of each bacterial suspension were plated on mannitol salt agar. Single colonies were placed on Muller-Hinton agar with 2% NaCl and 4 \( \mu \)g/ml oxacillin. Bacterial identification was performed by _dnaJ_ gene sequencing as previously described\textsuperscript{37}.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed using standard agar dilution methods according to Clinical and Laboratory Standards Institute guidelines\textsuperscript{38}. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic preventing bacterial growth after 16–20 h of incubation at 37 \( ^\circ \)C. The following antimicrobials were tested: erythromycin, gentamicin, levofloxacin, oxacillin, tetracycline and vancomycin.

Pulsed-field gel electrophoresis (PFGE). PFGE typing of _Smal_-digested DNA (New England BioLabs, Ipswich, MA) was performed as previously described\textsuperscript{39}. Electricity (200 volts) was applied for 20 h at 13 \( ^\circ \)C, with

| Strain | _mecA_ | _mecA_ | _mec Complex Class_ | _ccr Gene_ | _SCCaC mec Type_ |
|--------|---------|---------|---------------------|------------|------------------|
| _S. saprophyticus_ KHH-2 | − | + | NT* | _A4B4_ | NT |
| _S. haemolyticus_ KHH-11 | − | + | C2 | _C1_ | V |
| _S. saprophyticus_ KHH-20 | + | + | A | NT | NT |
| _S. sciu rium_ subsp. _carnaticus_ KHH-57 | + | + | A | _A1B1, A4B4_ | VIII |
| _S. sciu rium_ subsp. _sciuri_ TXG-15 | + | + | A | _A5B3_ | TXG-24 |
| _S. sciu rium_ subsp. _sciuri_ TXG-24 | − | + | A | _A1B1, A4B4, A5B3, C1_ | NT |
| _S. haemolyticus_ TXG-25 | − | − | A | _C1_ | NT |
| _S. lentus_ TXG-26 | + | + | A | _A1B1, A4B4_ | VIII |
| _S. sciu rium_ subsp. _rodentium_ TXG-28 | − | + | C2 | _C1_ | V |
| _S. pasteuri_ TPE-12 | − | − | B | _A2B2, A5B3_ | IV |
| _S. pasteuri_ subsp. _rodentium_ TPE-18 | + | + | A | _A5B3_ | TXG-24 |
| _S. pasteuri_ TPE-21 | + | + | NT | NT | NT |
| _S. pasteuri_ TPE-32 | − | + | B | _A2B2, C1_ | IV |
| _S. sciu rium_ subsp. _rodentium_ TPE-33 | + | + | A | _A4B4_ | VIII |

Table 2. **SCCaC mec** types and _mecA_\textsubscript{S} in 14 MRCoNS. *NT, non-typeable.*
pulse durations ranging from 5.3 to 34.9 sec at 6 V/cm. Dice similarity indices were used to construct pulsotype relationship dendrograms using an unweighted pair group method with arithmetic means. Pulsotypes exhibiting 85% similarity were assigned to the same clusters.

**PCR detection of antibiotic-resistant genes and staphylococcal enterotoxin genes.** PCR was used to detect the presence of the following antibiotic-resistant genes: gentamicin (aac(6′)-Ile-aph(3′)Ia), oxacillin (mecA), vancomycin (vanA, vanB), erythromycin (ermA, ermB, ermC), and tetracycline (tet(M), tet(O), tet(K)). Primer sets were selected based on a previous study. The presence of staphylococcal enterotoxin genes, sea, seb, sec, sed, see, seg, seh, sei, sel, selk, sell, selm, seln, selo, sele, selg, selr and tst1, were determined by PCR using primer sets from a previous study.

**Identification of SCC mec and the mecA gene complex.** Genomic DNA from *S. sciuri* subsp. *S. sciuri* TXG-24 was extracted manually. Total DNA was subjected to quality control using agarose gel electrophoresis and quantified by Qubit (Invitrogen, Thermo Fisher Scientific, Waltham, MA). The *S. sciuri* subsp. *S. sciuri* TXG-24 genome was sequenced using massively parallel sequencing Illumina (San Diego, CA). Two DNA libraries were constructed: a paired-end library with a 500 bp insert, and a mate-pair library with a 5 kb insert. Both libraries were sequenced with the HiSeq2500 ultra-high-throughput sequencing system (Illumina, San Diego, CA) (PE125 strategy). Library construction and sequencing was performed at Beijing Novogene Bioinformatics Technology Co., Ltd. An in-house quality control program was used for both paired-end and mate-pair reads. Illumina PCR adapter reads and low quality reads were filtered and assembled with SOAPdenovo to generate scaffolds. All reads were used for subsequent gap closures. SCC mec and mecA gene complex nucleotide sequences from *S. sciuri* subsp. *S. sciuri* TXG-24 were added to GenBank (accession numbers KK774481 and KK774480, respectively).

**Phylogenetic tree analysis.** The ccrA and ccrB genes identified in this work were compared with 22 publicly available *Staphylococcus* spp. sequences: *S. aureus* strains JCSC6943, JCSC6945, COL, NCTC10442, CA05, JH1, JH9, MRSAS252, Mu3, Mu50, MW2, N315, 85/2082, BK20781, CI6082 and HDE288 (GenBank accession numbers AB505628.1, AB505630.1, CP000046, AB033763.2, AB063172.2, CP000736, CP000703, BX571856, AP009324, BA000017, BA000033, BA000018, AB037671.1, FJ670542.1, FJ390057.1, and AF411935.3, respectively); *S. pseudointermedius* strains KM241 and AI16 (AM904731 and LN864705.1); *S. haemolyticus* H9 (EU933405); *S. saprophyticus* subsp. *saprophyticus* TSU33 (AB353724.1); *S. sciuri* MCS24 (AB857080.1); and *S. sciuri* subsp. *carnaticus* GVGS2 (HG515014). Phylogenetic trees were analysed by MEGA7 using the neighbour-joining method; tree topologies were estimated using bootstrap analyses with 2,000 replicates to achieve confidence intervals as indicated on each tree node. Identities shown after each ccr gene were aligned and calculated using DNAMan (Lynnon Biosoft, Quebec).

**SCCmec type determination and mecA gene detection.** SCCmec types were determined by mec and ccrB complexes as described in our previous study. SCCmecTXG24 was determined by the class A mec complex and ccr gene (ccrASB3) (Supplementary Table S1), and mecA was determined by mecA-F and mecA-R (Supplementary Table S1).

**References**

1. Dong, T. T. M. *The knowledge, attitude, and practice of consumers towards food safety issues: A review of Taiwan.* Int J Res Stud Manage 4, 13–22, doi: 10.5861/irsm.2015.976 (2015).
2. Fang, T. J., Wei, Q.-K., Liao, C.-W., Hung, M.-J. & Wang, T.-H. *Microbiological quality of 18 °C ready-to-eat food products sold in Taiwan.* Int J Food Microbiol 80, 241–250, doi: 10.1016/s0168-1605(02)00172-1 (2003).
3. Wei, Q. K., Hwang, S. L. & Chen, T. R. *Microbiological quality of ready-to-eat food products sold in southern Taiwan.* J Food Drug Anal 14, 68–73 (2006).
4. Barber, M. *Methillin-resistant staphylococci.* J Clin Pathol 34, 385–393 (1981).
5. Hurlimann-Dale, R. L., Ryffel, C., Kayser, F. H. & Berger-Bachi, B. *Survey of the methillin resistance-associated genes mecA, mecR1-mecI, and femA-femB in clinical isolates of methillin-resistant Staphylococcus aureus.* Antimicrob Agents Chemother 36, 2617–2621 (1992).
6. Katayama, Y., Io, T. & Hiramatsu, K. *A new class of genetic element, staphylococcus cassette chromosome mec, encodes methillin resistance in Staphylococcus aureus.* Antimicrob Agents Chemother 44, 1549–1555 (2000).
7. Ruzanskas, M. et al. *Prevalence of methillin-resistant Staphylococcus haemolyticus in companion animals: a cross-sectional study.* Ann Clin Microbiol Antimicrob 13, 56–62, doi: 10.1186/s12941-014-0056-y (2014).
8. Barros, E. M., Ceotto, H., Bastos, M. C., Dos Santos, K. R. & Giambiagi-Denmaral, M. *Staphylococcus haemolyticus as an important hospital pathogen and carrier of methillin resistance genes.* J Clin Microbiol 50, 166–168, doi: 10.1128/JCM.05563-11 (2012).
9. Tulinski, P. et al. *Methillin-resistant coagulase-negative staphylococci on pig farms as a reservoir of heterogeneous staphylococcal cassette chromosome mec elements.* Appl Environ Microbiol 78, 299–304, doi: 10.1128/AEM.03594-11 (2012).
10. Soderquist, B. & Berglund, C. *Methillin-resistant Staphylococcus saprophyticus in Sweden carries various types of staphylococcal cassette chromosome mec (SCCmec).* Clin Microbiol Infect 15, 1176–1178, doi: 10.1111/j.1469-0691.2009.02771.x (2009).
11. Kloos, W. E., et al. *Ribotype delineation and description of Staphylococcus sciuiri subspecies and their potential as reservoirs of methillin resistance and staphylococcal enterotoxin genes.* Int J Syst Bacteriol 57, 313–323, doi: 10.1099/00207713-47-4-1279 (1997).
12. Io, T., Katayama, Y. & Hiramatsu, K. *Cloning and nucleotide sequence determination of the entire mec DNA of pre-methillin-resistant Staphylococcus aureus N315.* Antimicrob Agents Chemother 43, 1449–1458 (1999).
13. Sim, B. J., Lucas, P. W., Pereira, B. P. & Oates, C. G. *Mechanical and sensory assessment of the texture of refrigerator-stored spring roll pastry.* J Texture Stud 24, 27–44, doi: 10.1111/j.1745-4603.1993.tb01275.x (1993).
14. Wang, H. H. et al. *Food commensal microbes as a potentially important avenue in transmitting antibiotic resistance genes.* FEMS Microbiol Lett 254, 226–231, doi: 10.1111/j.1574-6968.2005.00306.x (2006).
15. Al-Kharousi, Z. S., Guizani, N., Al-Sadi, A. M., Al-Bulushi, I. M. & Shaharoona, B. *Hiding in fresh fruits and vegetables: Opportunistic pathogens may cross geographical barriers.* Int J Microbiol 2016, 4292417, doi: 10.1155/2016/4292417 (2016).
16. Li, L. et al. *Antimicrobrial resistance and resistance genes in aerobic bacteria isolated from pork at slaughter.* J food prot 79, 589–597, doi: 10.4315/0362-028X.JFP-15-455 (2016).
17. Aydin, A. et al. Prevalence and antibiotic resistance of foodborne Staphylococcus aureus isolates in Turkey. Foodborne Pathog Dis 8, 63–69, doi: 10.1089/fpd.2010.0613 (2011).
18. Balaban, N. & Rasouly, A. Staphylococcal enterotoxins. Int J Food Microbiol 61, 1–10 (2000).
19. Fijalkowska, K., Peitler, D. & Karakulak, J. Staphylococci isolated from ready-to-eat meat - Identification, antibiotic resistance and toxin gene profile. Int J Food Microbiol 238, 113–120, doi: 10.1016/j.ijfoodmicro.2016.09.001 (2016).
20. Chiang, Y. C., Chang, L. T., Lin, C. W., Yang, C. Y. & Tsien, H. Y. PCR primers for the detection of staphylococcal enterotoxins K, L, and M and survey of staphylococcal enterotoxin types in Staphylococcus aureus isolates from food poisoning cases in Taiwan. J Food Prot 69, 1072–1079 (2006).
21. Wu, S., de Lencastre, H. & Tomasz, A. Genetic organization of the mecA region in methicillin-susceptible and methicillin-resistant strains of Staphylococcus sciuri. J Bacteriol 180, 236–242 (1998).
22. Tsubakishita, S., Kuwahara-Arai, K., Sasaki, T. & Hiramatsu, K. Origin and molecular evolution of the determinant of methicillin resistance in staphylococci. Antimicrob Agents Chemother 54, 4352–4359, doi: 10.1128/AAC.00356-10 (2010).
23. García-Alvarez, L. et al. Metcillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 11, 595–603, doi: 10.1016/s1473-3099(11)70126-8 (2011).
24. Hauschild, T. & Schwarz, S. Differentiation of Staphylococcus sciuri strains isolated from free-living rodents and insectivores. J Vet Med B Infect Dis Vet Public Health 50, 241–246 (2003).
25. Papamani, E., Kotezkidou, P., Tzanetakis, N. & Litopoulou-Tzanetaki, E. Characterization of Micrococccaeae isolated from dry fermented sausage. Food Microbiol 19, 441–449, doi: 10.1006/fmic.503 (2002).
26. Kloo, W. E., Schleifer, K. H. & Smith, R. F. Characterization of Staphylococcus sciuri sp. nov. and its subspecies. Int J Syst Evol Microbiol 26, 22–37, doi: 10.1099/00207713-26-1-22 (1976).
27. Rolo, J., de Lencastre, H. & Miranda, M. High frequency and diversity of cassette chromosome recombinases (ccr) in methicillin-susceptible Staphylococcus sciuri. J Antimicrob Chemother 69, 1461–1469, doi: 10.1093/jac/dku028 (2014).
28. Podkowik, M., Bystroni, J. & Bania, J. Genotypes, antibiotic resistance, and virulence factors of staphylococci from ready-to-eat food. Foodborne Pathog Dis 9, 91–93, doi: 10.1089/fpd.2011.0962 (2012).
29. Chajecka-Wierzchowska, W., Zadernowska, A., Nalepa, B., Sierpinska, M. & Laniewska-Trokenheim, L. Coagulase-negative staphylococci (CoNS) isolated from ready-to-eat food of animal origin–phenotypic and genotypic antibiotic resistance. Food Microbiol 46, 222–226, doi: 10.1016/j.fm.2014.08.001 (2015).
30. Huang, Y. H. et al. Clonal spread of SCCmec type IV methicillin-resistant Staphylococcus aureus between community and hospital. Clin Microbiol Infect 13, 717–724, doi: 10.1111/j.1469-0691.2007.01718.x (2007).
31. Lo, W. Y. et al. Community-acquired methicillin-resistant Staphylococcus aureus in children, Taiwan. Emerg Infect Dis 12, 1267–1270, doi: 10.3201/eid1208.051570 (2006).
32. Li, S. et al. Novel types of staphylococcal cassette chromosome mec elements identified in clonal complex 398 methicillin-resistant Staphylococcus aureus strains. Antimicrob Agents Chemother 55, 3046–3050, doi: 10.1128/AAC.01475-10 (2011).
33. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. Antimicrob Agents Chemother 55, 4961–4967, doi: 10.1128/AAC.00579-09 (2009).
34. Descloux, S., Rossano, A. & Perreten, V. Characterization of new staphylococcal cassette chromosome mec (SCCmec) and topoisomerase genes in fluorquinolone- and methicillin-resistant Staphylococcus pseudintermedius. J Clin Microbiol 46, 1818–1823, doi: 10.1128/JCM.02255-07 (2008).
35. Wang, L. & Archer, G. L. Roles of CcrA and CcrB in excision and integration of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. Antimicrob Agents Chemother 55, 4961–4967, doi: 10.1128/AAC.00579-09 (2009).
36. Descloux, S., Rossano, A. & Perreten, V. Characterization of new staphylococcal cassette chromosome mec (SCCmec) and topoisomerase genes in fluoroquinolone- and methicillin-resistant Staphylococcus pseudintermedius. J Clin Microbiol 46, 1818–1823, doi: 10.1128/JCM.02255-07 (2008).
37. Shah, M. M. et al. dnal gene sequence-based assay for species identification and phylogenetic grouping in the genus Staphylococcus. Int J Syst Evol Microbiol 57, 25–30, doi: 10.1099/ijs.0.64205-0 (2007).
38. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: Twenty-fifth informational supplement. Document M100-S25 CLSI, Wayne, PA (2015).
39. Tseng, S. P. et al. Genotypes and phenotypes of Staphylococcus lugdunensis isolates recovered from bacteremia. J Microbiol Immunol Infect 48, 397–405, doi: 10.1016/j.jmii.2013.11.006 (2015).
40. Dice, L. R. Measures of the amount of ecologic association between species. Ecology 26, doi: 10.2307/1932409 (1945).
41. Rizzotti, L. et al. Contribution of enterococci to the spread of antibiotic resistance in the production chain of swine meat commodities. J food Prot 68, 955–965 (2005).
42. Omoe, K., Hu, D. L., Takahashi-Omoe, H., Nakane, A. & Shinagawa, K. Comprehensive analysis of classical and newly described staphylococcal superantigenic toxin genes in Staphylococcus aureus isolates. FEMS Microbiol Lett 246, 191–198, doi: 10.1016/j.femsle.2005.04.007 (2005).
43. Li, R. et al. De novo assembly of human genomes with massively parallel short read sequencing. Genome Res 20, 265–272, doi: 10.1101/gr.097261.109 (2010).
44. Li, R., Li, Y., Kristiansen, K. & Wang, J. SOAP: short oligonucleotide alignment program. Bioinformatics 24, 713–714, doi: 10.1093/ bioinformatics/btn205 (2008).
45. Kumar, S., Stecher, G. & Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol, 1870–1874, doi: 10.1093/molbev/msw054 (2016).

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Author Contributions
Conceived and designed the experiments: S.P.T. Performed the experiments: T.Y.Y. and W.W.H. Analyzed the data: L.L. Contributed reagents/materials/analysis tools: W.C.H. Contributed to the writing of the manuscript: T.Y.Y. and S.P.T.

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