Original Article

Distribution of antibiotic resistance genes among *Staphylococcus* species isolated from ready-to-eat foods

Yu-Ting Wang [a,b], Yu-Tzu Lin [a], Tsai-Wen Wan [a], Der-Yuan Wang [b], Hsu-Yang Lin [c], Che-Yang Lin [b], Yu-Chih Chen [b], Lee-Jene Teng [a,d,*]

[a] Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University College of Medicine, Taipei, Taiwan
[b] Division of Research and Analysis, Food and Drug Administration, Ministry of Health and Welfare, Taipei, Taiwan
[c] Division of Food Safety, Food and Drug Administration, Ministry of Health and Welfare, Taipei, Taiwan
[d] Department of Laboratory Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan

A B S T R A C T

We investigated antibiotic resistance of staphylococci isolated from 1128 samples of high-circulating RTE foods in Taiwan. A total of 111 *Staphylococcus aureus* and 709 coagulase-negative staphylococci (CoNS) comprising 23 species were isolated. The prevalence of *S. aureus* differed in various category of RTE foods, highest in fresh-cut fruits/vegetables (20.5%) and lowest in low-water activity (LWA) foods (0.7%). The overall staphylococcal contamination was highest in fresh-cut fruits/vegetables (62.2%), in which multiple isolates (up to 10) or species (up to 6) in single sample were frequently found. Distinct distribution of species contributed to unique feature in each category. Prevalence of antibiotic-resistant *S. aureus* was higher in fresh-cut fruits/vegetables samples (14.2% in 127) compared to other food categories (0–7.1%). A total of 4 MRSA carrying SCC*mec* type IV or V_T were identified (3.6% in 111), in which 3 belonged to sequence type ST59 and one was ST5. Among CoNS, *S. epidermidis* and *S. warneri* exhibited higher non-intrinsic antibiotic resistance than other species. Of 41 methicillin-resistant CoNS (5.8% in 709) isolates, SCC*mec* type IV ($n = 16$) and type V_T ($n = 6$) were most frequent. Isolates of *S. saprophyticus*, *S. xylosus* and *S. sciuri* displayed high rates of resistance to fusidic acid. Novel *fusB*-family determinants were identified in *S. xylosus*, *S. sciuri* and *S. kloosii*, which may contribute to their intrinsic resistance to fusidic acid. Compared to other food categories, fresh-cut fruits/vegetables were more contaminated by staphylococci carrying non-intrinsic resistance determinants including methicillin resistance. This nation-wide study demonstrated that some categories may have potential risk for transmitting antibiotic resistance, in which *S. epidermidis* and *S. warneri* should be gotten more attention.

1.   Introduction

Staphylococci are commonly distributed in the environment, animals and humans. *Staphylococcus aureus* is known as a food-borne pathogen producing heat-stable enterotoxins that cause food poisoning symptoms [1]. It displays high pathogenicity and often develops multiple drug resistance in clinical settings [2]. Coagulase-negative staphylococci (CoNS) are the major commensal microbes of human skin. Some of the CoNS species are recognized as opportunistic pathogens [3], and some are used as starters in meat fermentation or other food processing [4]. Rare cases of food poisoning have been caused by CoNS species, but CoNS may serve as reservoirs of antibiotic resistance genes for S. *aureus* [5]. Until now, many measures have been applied to avoid transmission of S. *aureus* in clinical settings, food processing and the livestock industry. Several official programs of long-term systemic surveillance have been implemented to monitor the prevalence of antibiotic resistance from farm to table [6,7], and most of them focus on food-borne pathogens such as S. *aureus*. The risks of CoNS in food safety have not been taken as seriously.

It was found that food-isolated S. *aureus* and CoNS carrying mobile elements such as the staphylococcal cassette chromosome *mec* (SCC*mec*) conferring methicillin resistance and reduced susceptibility to multiple antimicrobial agents [8], suggesting that the food production chain could provide a substantial transmission route for resistance genes [9]. Apart from the studies focused on livestock and animal-based products that reflect the need to improve policies regarding animal drug usage, an increasing number of reports on ready-to-eat (RTE) foods directly demonstrate the risks of food contamination [10]. Previous studies about staphylococci in RTE foods mainly focused on animal-based foods such as products of meat and dairy [10,11]. Other RTE categories were also investigated in some studies [12,13], most of which concentrated on characteristics of S. *aureus*.

Discussion of the role of staphylococci in food safety is ongoing. This study was aimed not only animal-based products but also other RTE food categories with high consumption in Taiwan, including fresh-cut fruits/vegetables, ice desserts, rice products and low-water activity (LWA) foods, and each RTE food category had over 100 samples for analysis. By the full view of staphylococcal species found in highly consumed RTE foods in Taiwan, we tried to discover the unique characteristics of staphylococcal species with antibiotic resistance genes in various RTE food categories to reveal the potential risks and to further explore the feasible strategies to enhance food safety.

2.   Methods

2.1.   *Sample collection, isolation and identification of staphylococci*

In consideration of the consumption habits and wide acceptance of RTE food products in Taiwan, only the most popular, high-circulating RTE food categories were collected. A total of 1128 samples were collected from convenience stores, supermarkets, hypermarkets, traditional markets, delis, restaurants and other food-selling sites in 16 densely populated cities and counties in Taiwan from February to November in 2014, including animal-based products (n = 492), fresh-cut fruits/vegetables (without dressing) (n = 127), ice desserts (n = 187), rice products (n = 183) and LWA foods (n = 139) (Supplementary Table 1). Food sampling, preparation of sample homogenate and detection of staphylococci were followed by the U.S. Food and Drug Administration's *Bacteriological Analytical Manual* (BAM-FDA) [14]. Species identification was conducted by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) (Biotyper, Bruker Daltonics, Bremen, Germany) and/or *dnaJ* genes PCR-RFLP or 16S rRNA gene sequencing [15].

2.2.   *Antimicrobial susceptibility testing*

Antimicrobial susceptibility testing (AST) was initially performed by VITEK 2 Compact (Biomérieux, Marcy l'Etoile, France) using AST card P592 and double-checked by the disk diffusion method. The minimal inhibitory concentrations (MICs) for antimicrobial drugs were determined by agar dilution methods described by the Clinical and Laboratory Standards Institute (CLSI) [16]. The breakpoints used to indicate antibiotic resistance were according to the guidelines of the CLSI except fusidic acid. Isolates displaying MIC >1 μg/mL for fusidic acid were considered resistant as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [17]. S. *aureus* ATCC 29213 was used as the reference strain.

2.3.   *Detection of antimicrobial resistance genes by PCR*

Presence of resistance genes in staphylococci was detected by PCR. Bacterial DNA was extracted using QIAGEN DNeasy Blood and Tissue Kits (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The resistance determinants included those to penicillin (*blaZ*) [18], tetracycline (*tetK*, *tetL*, *tetM*, *tetO*, *tetS*, *tetW*) [19], oxacillin (*mecA*) [18], erythromycin (*ermA*, *ermB*, *ermC*, *msrA/B*, *ermT*) [20], aminoglycoside (*aacA-aphD*) [21], fusidic acid (*fusB*, *fusC*, *fusD*, *fusF*) [22] and trimethoprim (*dfrA*, *dfrD*, *dfrG*, *dfrK*) [18]. *Staphylococcus* strains with known antimicrobial resistance genes previously isolated in our laboratory were used as positive controls.

2.4. Molecular typing of S. aureus

The *spa* repeat region was amplified and sequenced using Sa0122-F and Sa0122-R [23]. Multilocus sequence typing (MLST) was conducted to determine the sequence types (STs) of *S. aureus* isolates. STs were assigned using the *S. aureus* MLST database (www.mlst.net) [24].

2.5. Identification of SCCmec in methicillin-resistant staphylococcal isolates

Multiplex PCR of SCC*mec* was applied for methicillin-resistant *S. aureus* [25]. SCC*mec* types in methicillin-resistant CoNS were determined by *mec* and *ccr* gene complexes described in the previous study [26].

3. Results

3.1. Staphylococcal contamination in each RTE food category

From 1128 RTE food samples, a total of 111 *S. aureus* (9.8%) and 709 CoNS comprising 23 species were isolated (Supplementary Table 2). The prevalence of *S. aureus* was highest in fresh-cut fruits/vegetables (20.5%, 26/127) followed by rice products (11.5%, 21/183), animal-based products (10.0%, 49/492), ice desserts (7.5%, 14/187) and LWA foods (0.7%, 1/139). The overall staphylococcal contamination was highest in fresh-cut fruits/vegetables (62.2% in 127), relatively low (10.8% in 139) in LWA foods and 38.0–45.9% in others (Table 1). Two or more (up to 10) isolates may be identified in each sample, which was relatively prominent in fresh-cut fruits/vegetables samples (31.5%) compared to other categories (2.9–21.3%). Contamination of multiple (3 or more, up to 6) species in single sample was obviously higher in fresh-cut fruits/vegetables (15.7% in 127) compared to others (0.7–8.7%). These data presented characteristics of staphylococcal contamination in each food category. Polymicrobial or multispecies contamination implied high degree of complexity in composition of staphylococcal community in RTE foods.

The prevalence of staphylococcal species was distinct in RTE foods (Table 1) (Supplementary Table 2). Except LWA foods, more than 10 species were identified in each RTE foods, in which *S. saprophyticus*, *S. warneri*, *S. epidermidis*, *S. xylosus* and *S. carnosus* appeared in all categories. The overall top 5 CoNS species were *S. saprophyticus* (17.9%), followed by *S. warneri* (10.0%), *S. epidermidis* (8.1%), *S. sciuri* (7.4%) and *S. xylosus* (7.4%) (Supplementary Table 2). *S. saprophyticus* was dominant in animal-based products (25.4%) and rice products (20.2%) but less frequent in ice products (8.6%). Conversely, *S. epidermidis* contamination was significant in fresh-cut fruits/vegetables (21.3%) but uncommon in animal-based products (4.1%). *S. warneri* was prominent in fresh-cut fruits/vegetables (29.9%) and ice desserts (21.4%) but rare in animal-based products (2.4%).

3.2. Antibiotic resistance in S. aureus

Among 111 *S. aureus* isolates, 65 (58.6%) were resistant to at least one antibiotic, of which 15 (13.5%) were multidrug resistant (resistant to 3 or more antibiotic class) (Table 2) (Supplementary Table 3). Penicillin resistance was common in *S. aureus* (55.0%), followed by tetracycline (31.5%). Resistance to erythromycin (10.8%), gentamicin (9.0%), oxacillin (3.6%), fusidic acid (1.8%) and trimethoprim/sulfamethoxazole (0.9%) was relatively low. MRSA isolates were identified from animal-based products ($n = 2$) and fresh-cut fruits/vegetables ($n = 2$) (Table 3). No vancomycin resistance was found.

Fresh-cut fruits/vegetables samples had slightly higher contamination rate of antibiotic-resistant *S. aureus* (14.2%, 18/127) followed by rice products (7.1%, 13/183), ice desserts (6.4%, 12/187) and animal-based products (4.5%, 22/492). The rate of multidrug-resistant *S. aureus* was also higher in fresh-cut fruits/vegetables (3.9%, 5/127) compared to rice products (2.2%, 4/183), animal-based products (1.0%, 5/492) and ice desserts (0.5%, 1/187). According to these data, we suggested greater risks of fresh-cut fruits/vegetables in transmitting antibiotic-resistant *S. aureus* compared to other food categories.

3.3. Antibiotic resistance in CoNS

Except for *S. condimenti*, *S. vitulinus* and *S. succinus*, 599 isolates of 20 species were found resistant to at least one antibiotic (84.5%, 599/709), in which 65 isolates (9.2%, 65/709) of 11 CoNS species displaying multidrug resistance. Resistance to fusidic acid was the most common (51.5%), followed by penicillin (31.3%), tetracycline (20.3%) and erythromycin (14.7%). Resistance to gentamicin (7.2%), oxacillin (5.8%) and trimethoprim/sulfamethoxazole (3.2%) was relatively low. MR-*S. epidermidis* was prominent ($n = 21$) followed by *S. saprophyticus* ($n = 10$), *S. warneri* ($n = 4$), *S. sciuri* ($n = 2$), *S. cohnii* ($n = 2$), *S. haemolyticus* ($n = 1$) and *S. pasteuri* ($n = 1$) (Table 4). MR-*S. epidermidis* had a broad range of susceptibility (2–128 μg/mL), but other species displayed moderate or high resistance to oxacillin (64–>256 μg/mL). No vancomycin-resistant CoNS were found. Among species contributing to the major staphylococcal community (over 10% of prevalence in any of RTE food categories), all *S. sciuri*, over 90% of *S. saprophyticus* and over half portion of *S. warneri*, *S. epidermidis*, *S. xylosus* and *S. pasteuri* isolates displayed antibiotic resistant, in which *S. epidermidis* (26.4%) had significantly high prevalence of multidrug resistance (Table 2). High rates of resistance to fusidic acid were found in *S. sciuri* (98.8%), *S. saprophyticus* (93.6%) and *S. xylosus* (78.3%). Penicillin resistance was common in *S. epidermidis* (78.0%), *S. warneri* (67.3%), and *S. pasteuri* (61.0%). Tetracycline resistance was frequent in *S. xylosus* (27.7%), *S. epidermidis* (24.2%) and *S. saprophyticus* (22.8%). Erythromycin and gentamicin were frequently found in *S. epidermidis* (31.9%, 20.9%) and *S. warneri* (26.5%, 20.4%) compared to other species. Few isolates displayed trimethoprim/sulfamethoxazole (0–12.1%), which were highest in *S. epidermidis*. Moreover, MR-CoNS appeared in various RTE foods, whereas the prevalence was higher in fresh-cut fruits/vegetables (11.8%, 15/127) compared to rice products (6.6%, 12/183), ice desserts (4.8%, 9/187) and animal-based products (1.0%, 5/492) (Table 4). Our data demonstrated that each species had unique patterns of antibiotic resistance. Of them, *S. epidermidis* and *S. warneri* exhibited higher resistance rates of various antibiotic resistance.

Table 1 – Characteristics of staphylococcal contamination on each RTE food category.

Food categories	No. of samples	Staphylococcal contamination rate % (no. of samples)	Rate of samples containing 2 or more isolates % (no. of samples)	Percentage of samples containing 3 or more species % (no. of samples)	Top 3 CoNS species (prevalence)
Animal-based products	492	42.5 (209)	16.1 (79)	3.7 (18)	S. saprophyticus (25.4%) S. sciuri (9.6%) S. xylosus (7.9%)
Fresh-cut fruits/vegetables	127	62.2 (79)	31.5 (40)	15.7 (20)	S. warneri (29.9%) S. epidermidis (21.3%) S. saprophyticus (15.0%)
Ice desserts	187	38.0 (71)	12.3 (23)	3.2 (6)	S. warneri (21.4%) S. epidermidus (9.1%) S. saprophyticus (8.6%)
Rice products	183	45.9 (84)	21.3 (39)	8.7 (16)	S. saprophyticus (20.2%) S. warneri (12.0%) S. epidermidis (11.5%)
Low water activity foods	139	10.8 (15)	2.9 (4)	0.7 (1)	S. epidermidis (4.3%) S. saprophyticus (3.6%) S. captis (2.2%)
Total	1128	40.6 (458)	16.4 (185)	5.4 (61)	S. saprophyticus (17.9%) S. warneri (10.0%) S. epidermidis (8.1%)

Table 2 – Antibiotic resistance and major determinants in staphylococci from RTE foods.

	Resistance rates in Staphylococcus species[b] (Major determinant)[c]						
	S. aureus (n = 111)	S. saprophyticus (n = 202)	S. warneri (n = 113)	S. epidermidis (n = 91)	S. sciuri (n = 84)	S. xylosus (n = 83)	S. pasteuri (n = 41)
Antibiotics (%)							
Oxacillin	3.6 (mecA)	5.0 (mecA)	3.5 (mecA)	23.1 (mecA)	2.4 (mecA)	0	2.4 (mecA)
Penicillin	55.0 (blaZ)	5.9 (blaZ)	67.3 (blaZ)	78.0 (blaZ)	2.4 (N.D.)[d]	3.6 (blaZ)	61.0 (blaZ)
Tetracycline	31.5 (tetK)	22.8 (tetK)	18.6 (tetK)	24.2 (tetK)	15.5 (tetK)	27.7 (tetK)	17.1 (tetK)
Erythromycin	10.8 (msrA/B)	6.9 (msrA/B)	26.5 (msrA/B)	31.9 (msrA/B)	1.2 (ermA)	4.8 (msrA/B)	17.1 (msrA/B)
Gentamicin	9.0 (aacA-aphD)	0	20.4 (aacA-aphD)	20.9 (aacA-aphD)	0	1.2 (aacA-aphD)	7.3 (aacA-aphD)
Fusidic acid	1.8 (fusC)	93.6 (fusD)	0.9 (fusB)	14.3 (fusB)	98.8 (novel)	78.3 (novel)	0
Trimethoprim/sulfamethoxazole	0.9 (dfrG)	3.5 (dfrG)	0	12.1 (dfrA)	1.2 (dfrG)	4.8 (dfrG)	0
Rate of resistant to at least one antibiotic (%)	58.6	96.5	87.6	84.6	100.0	84.3	61.0
Multidrug resistant rate[a] (%)	13.5	5.4	9.7	26.4	2.4	6.0	4.9

[a] Isolates resistant to 3 or more antibiotic class.

[b] Only CoNS species with >10% of prevalence in any of RTE food categories were listed.

[c] The details of antibiotic resistance determinants were listed in Supplementary Table 4.

[d] Two isolates displayed resistant to both penicillin and oxacillin, but only mecA was found.

Table 3 — Characteristics of MRSA isolates.

Isolate	Source	MICs of oxacillin (µg/mL)	Molecular typing			Antibiotic resistance[a]	
			ST[b]	*spa*	SCC*mec* type	Profiles	Genes
M-01	Roasted pork	128	ST59	t437	IV	OX-P	*mecA, blaZ*
#163	Braised dishes	256	ST5	t002	IV	OX-P-TE-E-SXT	*mecA, blaZ, tetM, ermC, dfrG*
S-125	Vegetable salad	32	ST59	t437	V_T	OX-P-TE-E	*mecA, blaZ, tetK, ermB*
#181	Vegetable salad	128	ST59	t437	IV	OX-P-E-CN	*mecA, blaZ, ermB, aacA-aphD*

[a] OX, oxacillin; P, penicillin; TE, tetracycline; E, erythromycin; CN, gentamicin; SXT, trimethoprim/sulfamethoxazole.
[b] Sequence type.

Table 4 — Profile of MR-CoNS isolates.

Species (no. of isolates)	MIC (µg/mL)	*mec* Complex class	*ccr* Gene	SCC*mec* type	No. of isolates	Food source (No. of isolate)
S. epidermidis (21)	2–128	A	ccrA4B4	VIII	1	Ice desserts (1)
		B	ccrA2B2	IV	13	Animal-based products (2) Fresh-cut fruits/vegetables (5) Ice desserts (3) Rice products (3)
		C2	ccrC1	V_T	4	Fresh-cut fruits/vegetables (1) Ice desserts (1) Rice products (2)
		C2	ccrC1, ccrA2B2	V_T	1	Fresh-cut fruits/vegetables (1)
		A	NT[a]	NT	2	Fresh-cut fruits/vegetables (1) Ice desserts (1)
S. saprophyticus (10)	64–>256	A	ccrA3B3	III	3	Animal-based products (1) Fresh-cut fruits/vegetables (1) Rice products (1)
		A	ccrA1B1	NT	1	Animal-based products (1)
		NT	ccrC1	NT	2	Fresh-cut fruits/vegetables (1) Rice products (1)
		A	NT	NT	4	Fresh-cut fruits/vegetables (2) Rice products (2)
S. warneri (4)	64–256	B	ccrA2B2	IV	2	Ice desserts (1) Rice products (1)
		C2	ccrA1B1	IX	2	Fresh-cut fruits/vegetables (1) Rice products (1)
S. sciuri (2)	>256	A	NT	NT	2	Animal-based products (1) Fresh-cut fruits/vegetables (1)
S. cohnii (2)	128	A	NT	NT	2	Ice desserts (1) Rice products (1)
S. haemolyticus (1)	128	C2	ccrC1	V_T	1	Fresh-cut fruits/vegetables (1)
S. pasteuri (1)	64	B	ccrA2B2	IV	1	Ice desserts (1)
Total (41)	2–>256	–	–	III	3	Animal-based products (5)
				IV	16	Fresh-cut fruits/vegetables (15)
				V_T	6	Ice products (9)
				VIII	1	Rice products (12)
				IX	2	
				NT	13	

[a] NT, untypable.

3.4. Antibiotic resistance determinants

The *blaZ* was detected in most penicillin-resistant staphylococcal isolates (272/283) (Supplementary Table 4). All 45 oxacillin-resistant isolates (4 MRSA and 41 MR-CoNS) carried *mecA*. Isolates resistant to gentamicin ($n = 61$) carried *aacA-aphD*. The genes *tetK*, *tetL* and *tetM* were identified in tetracycline resistant isolates, with *tetK* (157/179) the most frequent. The *msrA/B* was found in large portion of erythromycin-resistant isolates (92/116), and the *ermA*, *ermB*, *ermC* and *ermT* were also found in few isolates. The *dfrG* (18/24) and *dfrA* (11/24) were identified in isolates resistant to trimethoprim/sulfamethoxazole.

However, distribution of resistance determinants was diverse among staphylococcal species (Supplementary Table 4). Compared to other species, S. epidermidis more frequently carried *msrA/B*, *mecA*, *aacA-aphD*, *fusB* and *dfrA*. S. warneri and S. aureus frequently harbored *msrA/B* and *aacA-aphD* as well.

A total of 367 isolates were resistant to fusidic acid. But only *S. aureus*, *S. epidermidis*, *S. lugdunensis* and *S. warneri* carried acquired (non-intrinsic) resistant gene *fusB* or *fusC*. *S. saprophyticus* and *S. cohnii* were known as intrinsically resistant to fusidic acid due to the presence of *fusD* and *fusF*, respectively. Most *S. sciuri* (83/84) and *S. xylosus* (65/83) and all *S. kloosii* ($n = 5$) isolates displayed low-level resistance but lacked known *fusB* family genes (*fusB*, *fusC*, *fusD* or *fusF*), which was also seen in previous studies [11,27,28]. For this, we conducted PCR amplification using a pair of degenerate primers of *fusB* family conserved region designed by our laboratory [22] and obtained PCR products from *S. xylosus* and *S. kloosii* isolates. Sequence of the above products matched (92—97% identity) to the gene encoding fibronectin-binding protein in *S. xylosus* (Sx-FBP, GenBank: CP007208.1) and the gene encoding elongation factor G-binding protein in *S. kloosii* (Sk-EFGBP, GenBank: CP027846.1), respectively. The genes show 75% and 67% amino acid similarity to FusD (GenBank: AP008934.1) (Supplementary Table 5). From the published genome sequence of *S. sciuri* (GenBank: CP022046.2), we found the gene encoding elongation factor G-binding protein *S. sciuri* (Sc-EFGBP) displaying 46% amino acid similarity to FusC (GenBank: NC_002953.3). Conserved domains such as the cysteines residues were shared among Sx-FBP, Sk-EFGBP, Sc-EFGBP and known FusB family protein [29] (Supplementary Fig. 1). From the above data, we suggested that these novel determinants from *S. xylosus*, *S. kloosii*, and *S. sciuri* could confer intrinsic resistance of fusidic acid, but may not contribute to the risks of transferring resistance.

Ratio of non-intrinsic determinants in each species was listed in Fig. 1. The non-intrinsic determinants were carried highest in *S. warneri* (86.7%), followed by *S. epidermidis* (84.6%), *S. pasteuri* (61.0%) and *S. aureus* (54.7%). Owing to unique features of staphylococcal community in each food category, we suggested that the food category with high prevalence of *S. warneri*, *S. epidermidis*, *S. pasteuri* and *S. aureus* could be riskier in transmitting antibiotic resistance. Therefore, we analyzed prevalence of non-intrinsic determinants by food category. N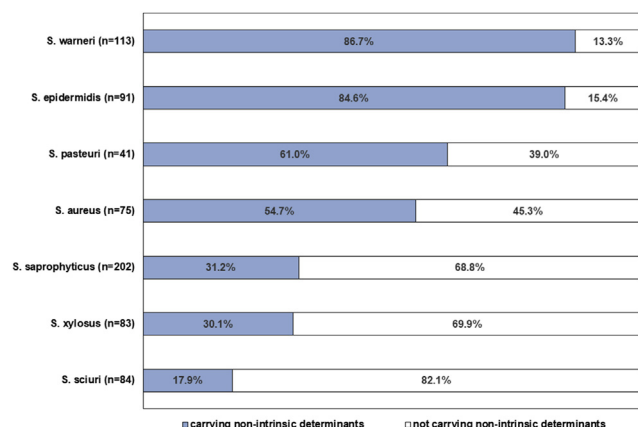on-intrinsic determinants were found higher in fresh-cut fruits/vegetables (44.9%), rice products (29.5%) and ice desserts (28.9%) than other categories (Fig. 2). In addition, the ratio of samples contaminated by 2 or more staphylococci carrying non-intrinsic determinants was significantly higher in fresh-cut fruits/vegetables (18.1%).

3.5. Molecular profiles of methicillin-resistant staphylococci

The molecular profiles of MRSA were listed in Table 3. Three MRSA carried SCC*mec* type IV and displayed high MIC to oxacillin (MIC: 128—256 μg/mL), belonging to ST59/t437 ($n = 2$) or ST5/t002 ($n = 1$). One carried SCC*mec* type V$_T$ (MIC: 32 μg/mL) and belonged to ST59/t437.

Among 41 MR-CoNS, SCC*mec* types were successfully identified in 28 isolates, in which SCC*mec* type IV was most prominent ($n = 16$) followed by type V$_T$ ($n = 6$), III ($n = 3$), IX ($n = 2$) and VIII (1). *S. epidermidis* harboring SCC*mec* type IV was significantly dominant (13/41). SCC*mec* types were not identified in most MR-*S. saprophyticus* (7/10) and all of MR-*S. sciuri* ($n = 2$) and MR-*S. cohnii* ($n = 2$) isolates (Table 4).

4. Discussion

In the present study, we conducted nation-wide and systemic surveillance of RTE foods in Taiwan, focusing on the incidence of staphylococcal isolates carrying non-intrinsic resistance determinants. Compared to previous studies in Taiwan [26,30], sample size (1128 samples, over 100 samples for each food category) and sample volume (50 g for each food sample) in the present study were greater and food items were more diverse. Our data successfully demonstrated that composition of different staphylococcal species in various RTE food categories contributed to diverse risks of potentially transmitting antibiotic resistance. Considering transmission of antibiotic resistance is getting more attentions from the public, we believe our findings would be helpful to food safety assessment.
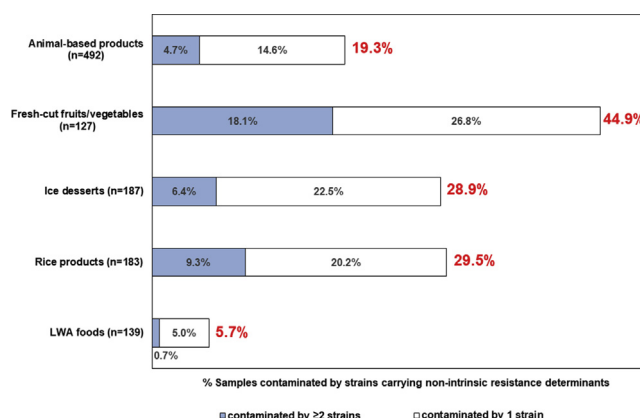

Fig. 1 — Percentage of isolates carrying (dark bar) or not carrying (light bar) non-intrinsic resistance determinants in the species contributing the majority of staphylococcal community in RTE foods (species had >10% of prevalence in any of RTE food categories).

Fig. 2 — Percentage of samples contaminated staphylococci carrying non-intrinsic resistance determinants in each food category, including samples contaminated by 2 or more stains (dark bar) or by only 1 strain (light bar) carrying non-intrinsic resistance determinants.

Critical points of RTE food safety are mainly determined by sanitary quality of ingredients, the way of food processing and hygiene during transport and marketing. Factors such as water activity largely determine microbial survival, explaining lower rates of microbial contamination found in LWA foods compared to other categories. Unlike other countries [10,11], popular animal-based RTE products in Taiwan are usually well-cooked or sold at high temperatures, which could confine the composition of staphylococcal community. On the contrary, fresh-cut fruits/vegetables are composed of multiple, uncooked ingredients from various sources and processed by frequent hand contact, indicating high risk of being contaminated by microbes from human and environmental sources. Some reports indicated that S. aureus was frequently isolated from salad vegetables, fruits and sprouts [31,32]. We have tested several cleaning methods usually utilized by retailers and housewives. For leafy vegetables and sprouts, only a limited bacterial volume of S. aureus was removed (data not shown). In the present study, we found that fresh-cut fruits/ vegetables were significant in staphylococcal contamination by S. aureus, multispecies, methicillin-resistant or other kinds of antibiotic-resistant staphylococci. The species frequently carrying various non-intrinsic antibiotic resistance such as S. warneri and S. epidermidis composed the majority of staphylococcal community in fresh-cut fruits/vegetables. These data indicated fresh-cut vegetables/fruits had higher risk in transmitting antibiotic resistance. To improve sanitation of fresh-cut fruits/vegetables, it is need to call for better control in the selection of raw materials, storage and processing steps. Furthermore, we recommend conducting routine monitoring of RTE foods to track the level of staphylococcal species with high potential for antibiotic resistance to enhance food safety.

Compared to S. aureus, the main hazards from food-related CoNS were considered limited to the presence of antibiotic resistance owing to their significantly lower toxigenic potentials [3]. Previous studies showed that the incidence and the kinds of antibiotic resistance were usually species-specific but not source-dependent [11]. Among CoNS, S. epidermidis is known as frequently isolated in infection cases and has ability to transmit resistance determinants to S. aureus [33]. In agreement with previous studies, we found S. epidermidis from RTE foods significantly displayed resistance to various antibiotics and carrying corresponding determinants. Furthermore, S. warneri was commonly isolated from various RTE foods, and its rate of non-intrinsic antibiotic resistance was as high as that of S. epidermidis. S. warneri is phenotypically similar to S. pasteuri [34] belonging to S. epidermidis-like group based on phylogenetic studies [3]. It frequently appeared in foods and human skin, animals, environments [35,36]. The ability of S. warneri to produce biofilm and enterotoxin has been reported [37]. Multidrug resistance has been reported in S. warneri isolated from many sources, but little was known about its genomic structure conferring resistance [38]. More studies are needed to explore the characteristics of S. warneri concerning antibiotic resistance and its correlation with food safety.

Molecular typing of four MRSA in the present study revealed three ST59 and one ST5. ST59/SCCmec type IV or V_T are the major community-acquired MRSA in Taiwan, whereas ST5 is frequently found in hospital-acquired MRSA in many Asia countries [39]. MRSA is the major concern of public health. The MRSA contamination rate in RTE foods in the present study was 3.6% (4/111), similar to the data from China [13], Korea [32], Malaysia [40] and Japan [12]. CoNS is recognized as the origin and reservoirs of SCCmec. Our data showed that SCCmec types IV and V_T were also identified in MR-CoNS. Therefore, we believe that continuous surveillance of both MRSA and MR-CoNS from RTE foods is important.

5. Conclusion

This study provides the systemic profile of prevalence and antibiotic resistance of staphylococci in various RTE foods popular in Taiwan. Composition of the staphylococci community in RTE foods was category-specific. Among them, fresh-cut fruits/vegetable were more contaminated by staphylococci carrying non-intrinsic resistance determinants including methicillin resistance. Therefore, it was recommended to routinely trace resistance characteristics of Staphylococcus species in RTE foods to make a complete assessment of the impacts of staphylococci on RTE food safety to review hazards and critical control points during food processing. Another new finding is that high rates of resistance to fusidic acid in S. xylosus and S. sciuri were due to carriage of novel intrinsic determinants, thus possibly will not contribute to the risk of transferring resistance.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfda.2019.05.003.

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