Original Article

Investigation into antimicrobial resistance, enterotoxin and cassette chromosome gene of *Staphylococcus aureus* isolates from humans, cows and goats in Taiwan

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Abstract

Multidrug-resistant *Staphylococcus aureus* (MDR *S. aureus*) is predominately present in humans and animals. Staphylococcal enterotoxin (SE) is one of the representative toxins produced by *S. aureus*. The drug resistance patterns, presence of eighteen SE genes and staphylococcal cassette chromosome (SCC*mec*) in *S. aureus* strains isolated from human cellulitis, septicemia, mastitis and farmed ruminant mastitis were investigated and analyzed. Methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) accounted for 51.35% (76/148) and 48.65% (72/148), respectively. Both MSSA and MRSA carried *sea* (15%), *seb* (30.8%) and *sek* (27.5%) genes. Eighty percent of the MRSA strains (52/65) belonged to SCC*mec* types I, III and IV while the MSSA strains were mostly untypable. Molecular examination of SE genes showed that *sec* might be highly crucial for goat *S. aureus* mastitis while *sel* might play a role in human, goat and cow mastitis. Enterotoxin A gene displayed the highest rate of appearance in the isolates from cow *S. aureus* mastitis as the enterotoxin was also found in human cellulitis, septicemia and mastitis, showing its key role in bovine mastitis and cross-species implications between humans and cows. MDR *S. aureus* widely appeared in human hospitals and community, and goat mastitis, whereas the strains accounted for much lower prevalence in cow mastitis. The data indicate that the patterns of SE gene are probably host species-specific and source-associated; therefore, the identification of SE genes coupled with SCC*mec* typing might be a useful method to trace the sources of infection in farmed ruminants and humans.

Keywords: multidrug-resistant *Staphylococcus aureus*, staphylococcal enterotoxin, staphylococcal cassette chromosome, mastitis

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Introduction

Multidrug-resistant *Staphylococcus aureus* (MDR *S. aureus*) predominates in gram-positive bacteria, principally since antimicrobial agents have been widely used in humans and animals. Methicillin, the semisynthetic β-lactam antimicrobial, was first used in clinical treatment in 1959. Within two years, the first Methicillin-resistant *S. aureus* (MRSA) was recognized in a hospital in the United Kingdom. The genetic sources of resistance to anti-β lactam drugs in MRSA are from two mechanisms. One is the *blaZ* gene on the plasmids encoding the β-lactamase, which hydrolyzes the β lactam ring. The other is the *mecA* gene on SCC*mec* mobile element in the chromosome of MRSA, which encodes Penicillin-binding protein 2a (PBP2a) and decreases the susceptibility of MRSA to β-lactam antimicrobials, particularly to methicillin. MRSA has been considered a highly important issue in the hygienic aspect as well as an emerging pathogen in livestock that is readily transferable to humans in contact with the animals (Kreausukon et al., 2012). Pathogenic and infectious MRSA isolates have been identified in humans (Strommenger et al., 2003), dogs (Rao et al., 1987), geriatric wards (Scott et al., 1988) and cattle (Lee et al., 2003; Rao et al., 1987). Furthermore, the prevalence of MDR-MRSA has gradually increased in humans, domestic fowl, domestic animals and pets (Cuteri et al., 2003), further highlighting the importance of MRSA/MDR-MRSA in public health.

Staphylococcal enterotoxin (SE) is a crucial toxic factor produced by *S. aureus*. As an essential cause of food poisoning, up to 18 types of SE (SEA-SEE, SEG-SER and SEU) dispersing to humans, animals and many kinds of food have been confirmed. The associations between SEs and pathogenic characteristics of *S. aureus* have been revealed. SEs have been shown to likely play a key role in respiratory diseases like allergy, asthma and chronic obstructive pulmonary disease (COPD) (Bachert et al., 2007).

SCC*mec* genes consist of *mec* gene complex and cassette chromosome recombinase gene complex (*ccr* gene complex). *mecA* gene and its regulating genes, *mecI* and *mecR1*, are involved in *mec* gene complex (Ito et al., 2001; Ito et al., 2004). Based on the *mecI-mecR1* polymorphism, five types of *mec* gene complex have been recognized: A, B, C1, C2 and D. *ccrA* and *ccrB*, which are composed of the *ccr* gene complex, are responsible for the mobility of SCC*mec*. Also, the additional type *ccrC* has been uncovered. There are eight types of *ccr* gene complex: type 1 (*ccrA1B1*), type 2 (*ccrA2B2*), type 3 (*ccrA3B3*), type 4 (*ccrA4B4*), type 7 (*ccrA1B6*), type 8 (*ccrA1B3*), and type 5 (*ccrC1*), an allotype of *ccrC*. Based on the combinations of *mec* and *ccr* gene complex, 11 types (SCC*mec* types I-XI) are recognized (Turlej et al., 2011). MRSA are crucial pathogens in community- and hospital/healthcare-associated infections (Carleton et al., 2004; Furuno et al., 2008) as MRSA are classified into two categories: community-associated MRSA (CA-MRSA) and healthcare-associated MRSA (HA-MRSA) (Kang et al., 2012). The correlations between SCC*mec* types and HA-MRSA/CA-MRSA were previously studied. Several reports revealed that MRSA with SCC*mec* types I, II, III and VI are predominant in HA-MRSA while SCC*mec* types IV, V and VII are found in CA-MRSA (Ito et al., 2004; Valsesia et al., 2010). With slight differences in categorization, SCC*mec* types IV and V have been linked with CA-MRSA, whereas HA-MRSA is associated with SCC*mec* types I, II and III in recent studies (Huang and Chen, 2011; Kang et al., 2015). Nonetheless, findings in the Middle East, which were different from classical classifications, showed that SCC*mec* types IV and V were common in hospitals but rare in the community, implicating the possibility of regional differences in SCC*mec* typing and the spread of CA-MRSA to hospitals (Mamishi et al., 2015; Shitrit et al., 2015).

The transmission routes of *S. aureus* to humans and animals include respiratory invasion, secondary infection after soft tissue damages and ingestion of contaminated food. *S. aureus* also causes mastitis in cattle and goats. However, the distribution of SEs among different *S. aureus* sources remains unclear. Characterization of SCC*mec* typing and production of enterotoxin in *S. aureus* have been recently studied in bovine mastitis in Asia (Havaei et al., 2015). Investigation into the relationship between SEs and *S. aureus* on different sources may be helpful to realize the associations between SEs and specific diseases. In the current study, comparison of the drug resistance and SE distribution with different sources of MRSA or MSSA using SCC*mec* typing in humans and farmed ruminants can provide pivotal information on hygienic monitoring of MRSA and MSSA in Taiwan and neighboring countries. These results may also contribute to clearer understanding of epidemiological data on MRSA and MSSA in farmed ruminants and humans in Taiwan.

Materials and Methods

Sample collection and bacterial storage: A total of 148 *S. aureus* isolates were investigated in this study. Forty-two cow and 35 goat clinical mastitis isolates were collected from 10 dairy cow and 6 dairy goat farms, respectively, in central-southern Taiwan (Fig. 1) from January 2010 to July 2012. All the farmed ruminants were kept and cared by the farm owners in this study. All sample collections carried out on the farm were approved by the respective farm owners. Thirty human cellulitis isolates, 16 human mastitis isolates and 25 human septicemia isolates (10 hospital infections and 15 community infections) were collected from Ditmanson Medical Foundation Chiayi Christian Hospital. This study was approved by the institutional review board of Ditmanson Medical Foundation Chiayi Christian Hospital (IRB number 097032 and 101051). As no patient contact or intervention was completed, the need for informed consent was waived. The collected bacteria were stored at -80°C (200 µL) in brain heart infusion broth (BHIB) with glycerol and cultured at 37°C for 18-24 hr.

Molecular analysis for bacterial identification and genetic detection: Bacterial storage broths with glycerol were used for nucleic acid extraction (QIAamp® DNA Mini Kit, Qiagen, Hilden, Germany). Traditional polymerase chain reaction or multiplex polymerase chain reaction (Multiplex PCR) was

performed to identify *S. aureus* and detect the presence of SE gene, and also to differentiate SCC*mec* types. Thermostable nuclease, encoded by *nuc* gene, is produced only by *S. aureus*. PCR was conducted to detect *nuc* gene for identification of *S. aureus* in the human and animal samples. The primers used to amplify *nuc* gene, SE genes and SCC*mec* genes are listed in Table 1. For *S. aureus* identification, the common reagents in three multiplex PCRs including 5 µL of DNA template, 4 µL dNTP mixture, 2.5 µL of 10x buffer and 0.7 µL of Taq DNA polymerase (2 Unit/µL) as well as 1 µL of *nuc*-1 and *nuc*-2 primers were added to the reaction. The PCR was carried out under the following condition: at 95°C for 10 min; 40 cycles at 94°C for 1 min; 53.5°C for 1 min and 72°C for 1 min, followed by 72°C for 10 min. For SE gene detection, four primer pools were used: U1 (*seh*, *seq*, *seg* and *sec*), V1 (*sek*, *seo*, *sem* and *sen*), W1 (*sep*, *seb*, *sel*, *sea*, *sei* and *seu*) and X1 (*see*, *ser*, *sed* and *sej*). For each SE gene, forward and reverse primers with concentration varying from 5-20 µM were used (Fischer et al., 2009). The PCR amplification comprised at 94°C for 2 min; 35 cycles at 94°C for 15 sec; 55°C for 20 sec and 72°C for 40 sec, followed by 72°C for 10 min. For SCC*mec* typing, each type with corresponding *ccr* and *mec* gene complex was targeted by the primers (Table 2). The reaction was preheated at 94°C for 4 min and then 30 cycle reaction was as follows: at 94°C for 4 min; 55°C for 30 sec and 72°C for 1 min, followed by 72°C for 4 min (Boye et al., 2007). Five µL of PCR products were electrophoresed on 2% agarose gel at 100V for 40 min. Bands were observed under ultraviolet light.

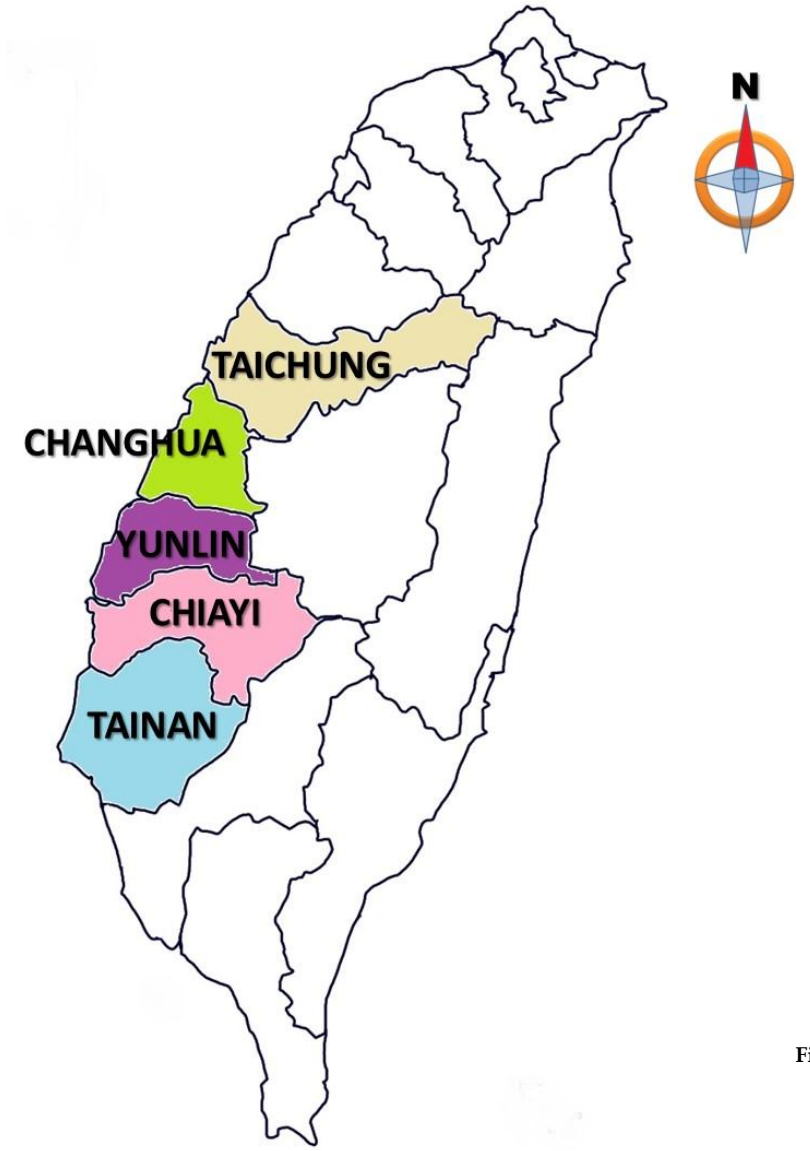

Figure 1 The sampling regions (Taichung city, Changhua county, Yunlin county, Chiayi county and Tainan city) in central-southern Taiwan

Antimicrobial susceptibility test: Antimicrobial disc diffusion susceptibility test was performed by dipping a sterile cotton swab into the broth suspension followed by inoculation on the surface of the BBLTM Muller Hinton II Agar. The procedure was repeated twice with rotation of the plate at 120 degrees each time. Antimicrobial discs (Oxoid, Basingstoke, Hants, UK) were then used for the test. The 14 antibiotics examined in this study were Penicillin G (PEN; 10 units), Ampicillin (AMP; 10 µg), Cloxacillin (CLO; 5 µg), Methicillin (MET; 5 µg), Cephalothin (CEP; 30 µg), Cefuroxime (CXM; 30 µg), Bacitracin (BAC; 10 units),

Streptomycin (STR; 10 µg), Gentamicin (GEN; 10 µg), Neomycin (NEO; 30 µg), Enrofloxacin (ENR; 5 µg), Tetracycline (TET; 30 µg), Oxytetracycline (OXY; 30 µg) and Sulfamethoxazole/Trimethoprim (SxT; 23.75 µg for Sx and 1.25 µg for T). The disc diffusion test and the guidelines 2009 of Clinical and Laboratory Standards Institute standards (CLSI, 2009) were used to determine the susceptibility and resistance of each isolate.

Statistical analysis: Categorical variables of the samples were presented as numbers and percentages in each group. Unpaired t test was performed to compare the grouped data. The analyses were two-tailed as P value ≤ 0.05 was indicated as statistical significance. All statistical analyses were conducted by STATA software.

Table 1 Primers used for amplification of *nuc* gene, staphylococcal enterotoxin genes and SCC*mec* genes

Gene	Primers	Oligonucleotide sequence (5′-3′)	Product size (bp)	Reference
nuc	*nuc*-1	GCGATTGATGGTGATACGGTT	270	(Louie et al., 2002)
	nuc-2	AGCCAAGCCTTGACGAACTAAAGC		
sea	SEA F	TTATGGTTATCAATGTGCGG	342	(Jones and Saleem, 1986)
	SEA R	TACTGTCCTTGAGCACCAAA		
seb	SEB F	ATTGGCGGTGTCTTTTGAAC	206	(Nema et al., 2007)
	SEB R	TTCGGGTATTTGAAGATGGT		
sec	SEC F	TTTTTGGCACATGATTTAATTT	541	(Xu and Zhang, 2006)
	SEC R	CAACCGTTTTATTGTCGTTG		
sed	SED F	CGTTAAAGCCAATGAAAACA	684	(Bayles and Iandolo, 1989)
	SED R	TGAAGGTGCTCTGTGGATAA		
see	SEE F	GGAGGCACACCAAATAAAAC	285	(Couch et al., 1988)
	SEE R	GGACCCTTCAGAAGAATGAA		
seg	SEG F	TGAGGTTAAAACTGAATTAGAAAA	484	(Abe et al., 2000)
	SEG R	AGAATCAACWACTTTATTATCTCCGT		
seh	SEH F	TTCACATCATATGCGAAAGC	107	(Holden et al., 2004)
	SEH R	TTTTCTTTAATGAATGGGTGA		
sei	SEI F	ACMGGTAYCAATGATTTGAT	455	(Blaiotta et al., 2004)
	SEI R	CTTACAGGCASWCCATSTCC		
sej	SEJ F	CTGATTTTCTCCCTGACGTT	731	(Zhang et al., 1998)
	SEJ R	TCGATATGCATGTTTTCAGA		
sek	SEK F	TGGACATAACGGCACTAAAA	149	(Kenny et al., 2009)
	SEK R	TTGGTARCCCATCATCTCCT		
sel	SEL F	AGACAAAAATTCACCAGAATCA	312	(Kuroda et al., 2001)
	SEL R	TTGACATCTATTTCTTGTGCG		
sem	SEM F	TTTAGTATCAATTTCTTGAGCTGTT	401	(Herron-Olson et al., 2007)
	SEM R	AAAATCATATCGCAACCGC		
sen	SEN F	ATGAGATTGTTCTACATAGCTGCAAT	680	(Jarraud et al., 2002)
	SEN R	AACTCTGCTCCCACTGAAC		
seo	SEO F	AGTTTGTGTAAGAAGTCAAGTGTAGA	180	(Jarraud et al., 2002)
	SEO R	ATCTTTAAATTCAGCAGATATTCCATCTAAC		
sep	SEP F	CTGAATTGCAGGGAACTGCT	187	(Holtfreter et al., 2004)
	SEP R	ATTGGCGGTGTCTTTTGAAC		
seq	SEQ F	ATACCTATTAATCTCTGGGTCAATG	226	(Fischer et al., 2009)
	SEQ R	AATGGAAAGTAATTTTTCCTTTGT		
ser	SER F	GTGCTAAACCAGATCCAAGG	616	(Letertre et al., 2003)
	SER R	AAGGGAACCAAATCCTTTTTA		
seu	SEU F	ATGGAGTTGTTGGAATGAAGT	796	(Letertre et al., 2003)
	SEU R	TTTTTGGTTAAATGAACTTCTACA		
ccrA1B1	β	ATTGCCTTGATAATAGCCYTCT	695	(Kondo et al., 2007)
	α1	AACCTATATCATCAATCAGTACGT		
ccrA2B2	β	ATTGCCTTGATAATAGCCYTCT	937	(Ito et al., 2001)
	α2	TAAAGGCATCAATGCACAAACACT		
ccrA3B3	β	ATTGCCTTGATAATAGCCYTCT	1791	(Kondo et al., 2007)
	α3	AGCTCAAAAGCAAGCAATAGAAT		
ccrA4B4	α4.2	GTATCAATGCACCAGAACTT	1287	(Kondo et al., 2007)
	β4.2	TTGCGACTCTCTTGGCGTTT		
ccrC	ccrCF	CGTCTATTACAAGATGTTAAGGATAAT	518	(Ito et al., 2001)
	ccrCR	CCTTTATAGACTGGATTATTCAAAATAT		
mecA-mecI	mI6	CATAACTTCCCATTCTGCAGATG	1963	(Kondo et al., 2007)
	mA7	ATATACCAAACCCGACAACTACA		
IS1272	1272F1	GCCACTCATAACATATGGAA	415	(Boye et al., 2007)
	1272R1	CATCCGAGTGAAACCCAAA		
mecA-IS431	5RmecA	TATACCAAACCCGACAACTAC	359	(Boye et al., 2007)
	5R431	CGGCTACAGTGATAACATCC		
mecA	mA1	TGCTATCCACCCTCAAACAGG	286	(Kondo et al., 2007)
	mA2	AACGTTGTAACCACCCCAAGA		

Table 2 SCC*mec* types with corresponding *ccr* and *mec* gene complex and primers used for PCR analysis

SCC*mec*	*ccr* gene complex	Primers		*mec* gene complex	Primers	
Type I	*ccrA1B1*	β	α1	Class B *mec*	1272F1	1272R1
Type II	*ccrA2B2*	β	α2	Class A *mec*	mI6	mA7
Type III	*ccrA3B3* *SCCmercury*	β ccrCF	α3 ccrCR	Class A *mec*	mI6	mA7
Type IV	*ccrA2B2*	β	α2	Class B *mec*	1272F1	1272R1
Type V	*ccrC1*	ccrCF	ccrCR	Class C2 *mec*	5RmecA	5R431
Type VI	*ccrA4B4*	α4.2	β4.2	Class B *mec*	1272F1	1272R1
Type VII	*ccrC1*	ccrCF	ccrCR	Class C1 *mec*	5RmecA	5R431
Type VIII	*ccrA4B4*	α4.2	β4.2	Class A *mec*	mI6	mA7

Results

Rate of MRSA and MSSA in human, cow and goat isolates: Among totally 148 *S. aureus* isolates, MRSA accounted for 47.3% and MSSA accounted for 52.7%. MRSA in both human (67.6%) and goat (65.7%) *S. aureus* isolates accounted for higher rates than that in cow *S. aureus* isolates (11.9%). MRSA and MSSA isolates occupied 67.6% and 32.4%, respectively, in the human isolates. In contrast, MRSA and MSSA isolates displayed 11.9% and 88.1%, respectively, in the cow mastitis isolates. MRSA and MSSA isolates were presented with 65.7% and 34.3%, respectively, in the goat mastitis isolates (Table 3).

Table 3 Drug resistance rate of *Staphylococcus aureus* isolates from humans, cows and goats

Antimicrobials	Humans				Humans (%)	Cows (%)	Goats (%)	*P* value
	Cellulitis (%)	Hospital (%)	Community (%)	Mastitis (%)				
Penicillin G	100.0 (30/30)	100.0 (10/10)	100.0 (15/15)	93.8 (15/16)	98.6 (70/71)	90.5(38/42)	88.6 (31/35)	
Ampicillin	100.0 (30/30)	100.0 (10/10)	100.0 (15/15)	93.8 (15/16)	98.6 (70/71)	90.5 (38/42)	88.6 (31/35)	
Cloxacillin	80.0 (24/30)	80.0 (8/10)	93.3 (14/15)	37.5 (6/16)	73.2 (52/71)	7.1 (3/42)	28.6 (10/35)	***, ###, ††
Methicillin	53.3 (16/30)	100.0 (10/10)	100.0 (15/15)	43.8 (7/16)	67.6 (48/71)	11.9 (5/42)	65.7 (23/35)	***, †††
Cephalothin	46.7 (14/30)	70.0 (7/10)	33.3 (5/15)	0.0 (0/16)	36.6 (26/71)	0 (0/42)	2.9 (1/35)	***, ###
Cefuroxime	100.0 (30/30)	100.0 (10/10)	100.0 (15/15)	68.8 (11/16)	93 (66/71)	7.1 (6/42)	11.4 (4/35)	***, ###
Bacitracin	0.0 (0/30)	0.0 (0/10)	0.0 (0/15)	0.0 (0/16)	0 (0/71)	2.4 (1/42)	22.9 (8/35)	##, †
Streptomycin	90.0 (27/30)	100.0 (10/10)	93.3 (14/15)	87.5 (14/16)	91.5 (65/71)	52.4 (22/42)	54.3 (19/35)	***, ###
Gentamicin	50.0 (15/30)	70.0 (7/10)	20.0 (3/15)	12.5 (2/16)	38 (27/71)	2.4 (1/42)	8.6 (3/35)	***, ###
Neomycin	100.0 (30/30)	100.0 (10/10)	93.3 (14/15)	62.5 (10/16)	90.1 (64/71)	9.5 (4/42)	5.7 (2/35)	***, ###
Enrofloxacin	43.3 (13/30)	70.0 (7/10)	33.3 (5/15)	0.0 (0/16)	35.2 (25/71)	0.0 (0/42)	2.9 (1/35)	***, ###
Tetracycline	76.7 (23/30)	90.0 (9/10)	60.0 (9/15)	50.0 (8/16)	69 (25/71)	11.9 (5/42)	82.9 (29/35)	**, ###, †††
Oxytetracycline	76.7 (23/30)	90.0 (9/10)	60.0 (9/15)	50.0 (8/16)	69 (25/71)	11.9 (5/42)	80 (28/35)	**, ###, †††
Sulfamethoxazole/ Trimethoprim	36.7 (11/30)	60.0 (6/10)	6.7 (1/15)	0.0 (0/16)	25.4 (49/71)	2.4 (1/142)	11.4 (4/35)	***, ###

*Humans vs Cows; #Humans vs Goats; †Cows vs Goats; *, #, † $P \leq 0.05$; **, ##, †† $P < 0.01$; and ***, ###, ††† $P < 0.00$

Antimicrobial resistance of MRSA and MSSA from humans and farmed ruminants: The antimicrobial resistance of *S. aureus* isolates from humans, cows and goats is shown in Table 3. The *S. aureus* isolated from human infections were fully resistant to β-lactam antimicrobials (100%) and neomycin (90.14%) as nearly one quarter of the human isolates were resistant to the 13 tested antibiotics, except for bacitracin (23.94%). Over 90% of the isolates from cow mastitis showed resistance to penicillin and ampicillin (90.5%) and, similarly, the isolates from goat mastitis exhibited high resistance to penicillin and ampicillin (88.57%). The goat isolates showed high resistance to tetracycline (82.86%) and oxytetracycline (80%), whereas the cow isolates merely presented 11.9% and 7.1% resistance to these two antibiotics, respectively. On the whole, the *S. aureus* isolates from farmed ruminants were largely resistant to penicillin and ampicillin, and moderately to streptomycin, whereas they were susceptible to bacitracin, cephalothin and enrofloxacin (Table 3). Resistance rates of the goat mastitis isolates to methicillin and cloxacillin were much higher than those of the cow mastitis isolates. The methicillin resistance rates of the goat mastitis *S. aureus* isolates (65.7%) and human *S. aureus* specimens (67.6%) were both higher than 65%, indicating that MRSA widely appear not only in humans but also in goats. Moreover, most MRSA possessed resistance to 5-13 antimicrobials while MSSA had resistance to 2-7 antimicrobials (data not shown).

Molecular detection and identification of SE genes: The SE gene identification showed that *sea*, *seb* and *sek* were the three prominent SE genes among MRSA and MSSA, accounting for 16.2%, 25% and 22.3%, respectively. This pattern could be observed in the *S. aureus* from all three human infections (cellulitis, mastitis and septicemia), whereas *sea* (14.3%), *sei* (9.5%), *sek* (4.8%), *sel* (21.4%), *seq* (4.8%) and *seu* (2.4%) were found in the *S. aureus* from cow mastitis. The *S. aureus* isolated from goat mastitis carried *sec* (17.1%), *sei* (2.9%), *sel* (8.6%) and *seu* (2.9%) genes in their genome. Comparing the *S. aureus* strains from human hospital infection with those from community infection, *sea* (30%), *seb* (20%), *sek* (30%) and *sep* (20%) genes were detected in the hospital infection strains,

whereas *seb* (80%), *sec* (6.7%), *sen* (13.3%), *sep* (6.7%) and *seu* (13.3%) were found in the community infection strains (Table 4).

Table 4 Distribution of staphylococcal enterotoxin in *S. aureus* from human, cow and goat infections

Staphylococcal enterotoxin	MSSA	MRSA	No. of isolates (%)	Humans			Humans (%)	Cows (%)	Goats (%)	*P* value
				Cellulitis (%)	Septicemia (%)	Mastitis (%)				
sea	10	14	16.2 (24/148)	40 (12/30)	12 (3/25)	18.8 (3/16)	25.4 (18/71)	14.3 (6/42)	0 (0/35)	***, ###, †
seb	18	19	25 (37/148)	46.7 (14/30)	56 (14/25)	56.3 (9/16)	52.1 (37/71)	0 (0/42)	0 (0/35)	***, ###
sec	7	3	6.8 (10/148)	6.7 (2/30)	4 (1/25)	6.3 (1/16)	5.6 (4/71)	0 (0/42)	17.1 (6/35)	*, †
sed	0	0	0 (0/148)	0 (0/30)	0 (0/25)	0 (0/16)	0 (0/71)	0 (0/42)	0 (0/35)	
see	0	0	0 (0/148)	0 (0/30)	0 (0/25)	0 (0/16)	0 (0/71)	0 (0/42)	0 (0/35)	
seg	0	0	0 (0/148)	0 (0/30)	0 (0/25)	0 (0/16)	0 (0/71)	0 (0/42)	0 (0/35)	
seh	0	0	0 (0/148)	0 (0/30)	0 (0/25)	0 (0/16)	0 (0/71)	0 (0/42)	0 (0/35)	
sei	5	0	3.4 (5/148)	0 (0/30)	0 (0/25)	0 (0/16)	0 (0/71)	9.5 (4/42)	2.9 (1/35)	*
sej	0	0	0 (0/148)	0 (0/30)	0 (0/25)	0 (0/16)	0 (0/71)	0 (0/42)	0 (0/35)	
sek	15	18	22.3 (33/148)	80 (24/30)	12 (3/25)	25 (4/16)	43.7 (31/71)	4.8 (2/42)	0 (0/35)	***, ###
sel	4	13	11.5 (17/148)	6.7 (2/30)	4 (1/25)	6.3 (1/16)	5.6 (4/71)	23.8 (10/42)	8.6 (3/35)	*
sem	1	1	1.4 (2/148)	3.3 (1/30)	0 (0/25)	6.3 (1/16)	2.8 (2/71)	0 (0/42)	0 (0/35)	
sen	1	4	3.4 (5/148)	6.7 (2/30)	8 (2/25)	6.3 (1/16)	7 (5/71)	0 (0/42)	0 (0/35)	*, #
seo	0	0	0 (0/148)	0 (0/30)	0 (0/25)	0 (0/16)	0 (0/71)	0 (0/42)	0 (0/35)	
sep	5	3	5.4 (8/148)	13.3 (4/30)	8 (2/25)	0 (0/16)	8.5 (6/71)	0 (0/42)	0 (0/35)	*, #
seq	3	4	4.7 (7/148)	16.7 (5/30)	0 (0/25)	0 (0/16)	7 (5/71)	4.8 (2/42)	0 (0/35)	#
ser	0	0	0 (0/148)	0 (0/30)	0 (0/25)	0 (0/16)	0 (0/71)	0 (0/42)	0 (0/35)	
seu	2	5	4.7 (7/148)	6.7 (2/30)	8 (2/25)	6.3 (1/16)	7 (5/71)	2.4 (1/42)	2.9 (1/35)	#

*Humans vs Cows; #Humans vs Goats; †Cows vs Goats; *, #, † $P \leq 0.05$; **, ##, †† $P < 0.01$; and ***, ###, ††† $P < 0.001$

Molecular examination and verification of SCCmec gene group: Of all isolates, SCC*mec* types I-V accounted for 53.4% while the others were unable to be typed. MRSA mostly belonged to types I, III and IV (80%). Among the ruminant mastitis isolates, 2 cow mastitis isolates were verified as type III while 9, 2 and 1 goat mastitis isolates were identified as types III, II and I, respectively. Most MSSA were not able to be typed as they did not carry the *mecA* gene (Tristan et al., 2007). The human cellulitis strains were mostly categorized into types III, IV and V (83%) while the human mastitis strains belonged to types IV and V (68.8%). The hospital strains mostly belonged to types I and III (80%) while most community strains were grouped into types I and IV (93.3%) (Table 5).

Table 5 Distribution of different SCC*mec*-typed *S. aureus* in MRSA/MSSA from human, cow and goat infections

SCC*mec*	MSSA	MRSA	No. of isolates	Humans				Cows	Goats
				Cellulitis	Hospital	Community	Mastitis		
I	1 (goat)	14	15	0	3	9	2	0	1
II	0	6	6	2	1	1	0	0	2
III	1 (goat)	21	22	8	5	0	0	0	9
IV	1 (human)	18	19	6	1	5	5	2	0
V	13	4	17	11	0	0	6	0	0
VI	0	0	0	0	0	0	0	0	0
VII	0	0	0	0	0	0	0	0	0
VIII	0	0	0	0	0	0	0	0	0
Unable typed	62	7	69	3	0	0	3	40	23
Total	78	70	148	30	10	15	16	42	35

Table 6 Analysis of Staphylococcal enterotoxin (SE) genes and SCC*mec* types in community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) and hospital-acquired MRSA (HA-MRSA)

SE genes	CA-MRSA SCCmec type				HA-MRSA SCCmec type			
	I (n = 9)	II (n = 1)	IV (n = 5)	Total (n = 15)	I (n = 5)	II (n = 1)	III (n = 3)	IV (n = 1)
sea	0	0	0	0	0	0	2	1
seb	8	0	4	12 (80%)	2	0	0	0
sec	0	1	0	1 (6.7%)	0	0	0	0
sek	0	0	0	0	2	0	1	0
sen	0	1	1	2 (13.3%)	0	0	0	0
sep	0	0	1	1 (6.7%)	1	1	0	0
seu	0	1	1	2 (13.3%)	0	0	0	0

Associations between SE genes and SCCmec types in CA-MRSA and HA-MRSA in humans: The correlations between SE genes and SCC*mec* types in CA-MRSA and HA-MRSA in the human isolates were further analyzed (Table 6). The enterotoxin *seb* accounted for 80% in the CA-MRSA isolates. Among the 12 *seb* isolates, 8 belonged to SCC*mec* type I while 4 were type IV. The remaining CA-MRSA carried *seu* (13.3%), *sen* (13.3%), sec (6.7%) and *sep* (6.7%). As for the human HA-MRSA isolates, SCC*mec* types I (50%) and III (30%) were verified the most, coupling with even appearance of *sea* and *sek* (both 30%). Unlike the presence of dominant *seb* gene in the human CA-MRSA, quite various enterotoxin types were found in the human HA-MRSA isolates of the respective SCC*mec* type. SCC*mec* type I HA-MRSA carried enterotoxin *seb* (40%), *sek* (40%) and *sep* (20%), whereas type III HA-MRSA had *sea* (66.7%) and *sek* (33.3%).

Discussion

The current study investigated and examined the presence and distribution of SE genes, SCC*mec* types and antimicrobial resistance in *S. aureus* strains isolated from human cellulitis, septicemia and mastitis as well as cow and goat mastitis using multiplex PCR and antimicrobial disc diffusion susceptibility test. In addition to *mecA* gene on SCC*mec* mobile element in the chromosome of *S. aureus* which encodes the PBP2a, *blaZ* gene against β-lactams, *aac* and *aph* gene against aminoglycosides and *sulA* gene against sulfonamides were found to be present on the plasmid which can be transmitted to other bacteria (Lowy, 2003). In this study, the *S. aureus* strains from hospital infections were recognized as the highest severity in MDR, which may be due to the adaption to multiple uses of antibiotics in hospitals. The cellulitis and community infection strains came with less severe MDR compared with the hospital strains. The human mastitis strains were the least severe in MDR among all human infections, while dominantly resistant to penicillin, ampicillin and cefuroxime (93.75%) (Table 3). This could be associated with the cross-resistance against other β-lactams like amoxicillin/clavulanate, cephalexin and dicloxacillin, which have been used to treat human mastitis (Spencer, 2008). Penicillin and ampicillin have been widely used to treat bacterial diseases in farmed animals in Taiwan for years, so high resistance against the 2 antibiotics is predictable. The cross-resistance between methicillin and cloxacillin is well known and cloxacillin has been used to treat mastitis in farmed ruminants, which may lead to the resistance to methicillin in cow and goat. However, the rate of MRSA in goat was much higher than that in cow, indicating different routine selection of methicillin or cloxacillin when treating bacterial diseases in cows and goats in Taiwan. The low detection rate of MRSA in the *S. aureus* bovine mastitis in our study is similar to the results of low MRSA rate estimated from bulk tank milk samples in the US (Haran et al., 2012). Moreover, it should be particularly noted that the *S. aureus* from goat mastitis isolates showed generally higher resistance rates against the antibiotics compared with those from cow mastitis samples; particularly the resistance possessed by *S. aureus* from goat mastitis against methicillin, bacitracin, streptomycin, tetracycline and oxytetracycline was much higher than from cow isolates. The findings in goats are similar to those shown in our previous report in Taiwan (Chu et al., 2012), providing important and consistent notice and therapeutic notes on selection of goat mastitis antimicrobial agents for veterinarians. The reduction in cloxacillin use in mastitis treatment should be disseminated. This is due to the fact that agricultural antibiotics have been a threatening menace to human health (Chang et al., 2015) and that there are limited information on antibiotic consumption in various animal species and limited surveillance programs to screen and trace the appearance of resistance in animals (Perron et al., 2008). Resistant clones of *S. aureus* are one of the most essential concerns in this aspect (Chang et al., 2015). Recently, in order to control MDR bacteria or avoid MDR-associated gene spread across humans and other animal species, especially to prevent the transmission of MDR-related genes from farmed animals to humans, the European Union has banned all nonmedicinal antibiotics in animals by 2006. Antibiotic regulations have meanwhile become stricter in USA (Chang et al., 2015). This is coherent that single drug program, shuttle program or rotation programs can be used to prevent the worsening of antimicrobial resistance (Chapman, 2007). In Taiwan, related strategies and prohibition have been addressed. Moreover, related approaches such as guidelines and recommendations on the use of antibiotics in food animals, urgently addressing barriers to the collection and analysis of antimicrobial use data have been highlighted to control the increasing hygienic crisis of antibiotic resistance (Landers et al., 2012).

The profiles of SEs principally varied between the *S. aureus* isolated from different species despite the exception of respective appearance. Since *sec* production in animal strains as well as *sea* in human strains were observed, de Silva et al. (2005) demonstrated that production of SEs could act as an indicator of the source of *S. aureus*. It is noteworthy that the possession of *sec* (17.1%) and *sel* (8.6%) in the goat

mastitis strains in the current study are in accordance with the results in a previous study, highlighting the importance of these two enterotoxin genes for *S. aureus* mastitis in goats (Morandi et al., 2007). In particular, the enterotoxin gene *sec* in the current study was also shown to correlate with goat *S. aureus* mastitis in our previous investigation (Chu et al., 2012). These results suggest that the enterotoxin gene *sec* may be highly crucial for *S. aureus* mastitis in goats. On the other hand, the relatively multiple presence of SEs showing *sea* (14.3%), *sei* (9.5%), *sek* (4.8%), *sel* (21.4%), *seq* (4.8%) and *seu* (2.4%) genes carried in the cow mastitis strains (Table 5) was quite different from the findings found in previous studies. However, the highest percentage of *sea* gene is consistent with three previous studies, strongly suggesting that *sea* gene may play an essential role in bovine mastitis caused by *S. aureus* (Chu et al., 2012; Havaei et al., 2015). Meanwhile, *sea* gene was also present in the isolates from human *S. aureus* cellulitis, septicemia, mastitis, underlining cross-species existence of identical enterotoxin gene in humans and cows and its hygienic implication. In previous findings, *sea* has also been shown to induce overexpression of the shock-related inflammation-mediated substance, so it has been indicated as a crucial virulent factor in *S. aureus*-induced septicemia (Ferry et al., 2005; Tristan et al., 2007). In addition, among the numerous SEs, *sel* and *seu* genes were found not only in humans, but also in cow and goat mastitis isolates, suggesting that the 2 enterotoxin genes could be associated with mastitis in humans and farmed ruminants in Taiwan. In particular, in the current work *sel* exhibited over 5% emergence in the mastitis of the investigated species, implying that this enterotoxin may be essential in cross-species mastitis. CA-MRSA from humans mostly carried *seb* and these strains were predominately classified into SCC*mec* types I and IV. On the other hand, most HA-MRSA isolates belonged to SCC*mec* types I and III as they possessed enterotoxins *sea*, *seb*, *sek* and *sep* evenly (Table 7). The distribution was similar to that found in a previous study (Huang et al., 2007). Based on the current and previous data, the differences in SCC*mec* typing and produced SE patterns by *S. aureus* may be source-associated; therefore, further research is required to verify this hypothesis.

On the other hand, many studies have pointed out that CA-MRSA is an important cause of hospital infections, which means the appearance of healthcare-associated CA-MRSA infections (Gonzalez et al., 2006; Huang et al., 2007). CA-MRSA-infected patients staying in hospitals for long-term treatment may lead to CA-MRSA spread in medical environments (Gonzalez et al., 2006). However, this phenomenon was not seen in the current study. Instead, community-associated HA-MRSA infection strains were recognized, accounting for 66.7%. These strains were isolated from community infections but were typed as SCC*mec* type I. Reasons for the epidemiological distribution are probably that patients got latent infection with HA-MRSA in hospitals but HA-MRSA did not reactivate until the patients went home. More specifically, it is proposed that patients are infected in the hospital and then HA-MRSA becomes latent in their bodies. After they returned home, HA-MRSA reactivates under certain situations favorable for MRSA transmission or suffering stress, and then spreads in the community. The results of the current study showed that most of the hospital infection isolates belonged to HA-MRSA (SCC*mec* types I-III), which is principally consistent with the SCC*mec* typing distributions validated by Huang and Chen (2011) and Kang et al. (2015) in Taiwan. Moreover, these strains obviously presented more severity in MDR than CA-MRSA, indicating that they may be the dominant strains in hospital and community infection. It should be noted that in the current study 9 SCC*mec* type I and 1 SCC*mec* type II were found in the CA-MRSA strains, indicating possible transmission of MRSA strain from HA-MRSA to CA-MRSA. Examinations such as pulse-field gel electrophoresis (PFGE) or multilocus sequence typing (MLST) will be helpful to further analyze the correlations between SCC*mec* types and sources of infection.

CA-MRSA-caused skin and soft tissue infections (SSTIs) in humans at increasing frequency has been reported. In this study, among the thirty *S. aureus* strains isolated from human cellulitis, 56.7% belonged to SCC*mec* types IV and V (community-associated infection types). In fact, CA-MRSA has been one of the most common causes of SSTIs in community (Moran et al., 2005). As one of the SSTIs, human mastitis mostly occurs in woman 8 weeks postpartum. *S. aureus* is not only the common cause (account for 30-50% of cases), but also one of the normal flora in healthy woman breast milk (30% of isolation rate) (Holmes and Zadoks, 2011). Pregnant women and newborns infected with CA-MRSA were reported. CA-MRSA has been the main cause of human postpartum mastitis, and mostly belongs to SCC*mec* types III and IV (Reddy et al., 2007). Also, in our study, two cow mastitis strains were identified as SCC*mec* type IV, implying that CA-MRSA could be associated with cow mastitis. The results are in agreement with a previous study (Nam et al., 2011). On the other hand, previous reports about MRSA-caused clinical mastitis in goats are limited. Aras et al. (2012) showed 2 isolates from 42 *S. aureus* strains causing goat mastitis (Aras et al., 2012). In the present study, 23 of 35 goat mastitis *S. aureus* strains were MRSA, in which 2 were SCC*mec* type II and 8 were type III, whereas the others were unable to be typed. Concerns about public health should be aroused not only since the rate of MRSA isolated from goat mastitis here is obviously higher than previous data, but also because the SCC*mec* types of goat mastitis MRSA in the current study were types III and II. These new findings indicate that the rising rate of MRSA from goat mastitis might have somewhat hygienic association with HA-MRSA in Taiwan. Some possible reasons have been demonstrated. Chu et al. (2012) analyzed both SCC*mec* types and pulsotypes of *S. aureus* from dairy goats and revealed that the genetically diverse MRSA strains might be acquired from humans or transferred from different goat breeding farms. On the other hand, although the percentage of MRSA in bovine mastitis is lower than previous data in Iran, our data is in accordance with those found previously which also showed that SCC*mec* type IV was the major type in bovine mastitis (Havaei et al., 2015). In the current study, 69 *S. aureus*

strains were unable to be typed. Two of them carried the *mecA* gene; it is presumed that these two strains may not belong to SCC*mec* types I-VIII. Among the 69 strains, 40 and 23 were isolated from the cow and goat mastitis, respectively, showing that numerous *S. aureus* isolates from cow and goat mastitis neither carry the *mecA* gene, nor are resistant to methicillin (Table 5). This distribution of methicillin resistance may be associated with the differences in types of antibiotic constantly selected by veterinarians of farmed ruminants and doctors of humans. Further investigation should be performed to monitor whether the *mecA* gene is transferred to farmed ruminants or not.

Taken together, MDR *S. aureus* and MRSA have been widely distributed in human hospital and community environment as well as in goat mastitis, whereas less appeared in cow mastitis in Taiwan. In accordance with our previous findings, the increasing rate of MRSA in goat mastitis and its possible association with HA-MRSA in Taiwan should be particularly noticed. The SCC*mec* types of *S. aureus* may be related to the environment (hospital or community) and source of infection. In addition, the SCC*mec* typing data showed that HA-MRSA strains were found in the goat mastitis samples, whereas CA-MRSA was present in the cow mastitis, suggesting a possible link between humans and farmed ruminants. Moreover, the data suggested that the patterns of SE genes produced by *S. aureus* could be host species-specific and source-associated. Therefore, the identification of SE gene and SCC*mec* typing may be an approach to track the source of *S. aureus* infections. The current results not only provide crucial antimicrobial resistance and hygienic information on the screening and control of *S. aureus* in human and veterinary medicine, but also arouse attention and vigilance to cautious use of antibiotics for goat mastitis.

References

Abe J, Ito Y, Onimaru M, Kohsaka T and Takeda T. 2000. Characterization and distribution of a new enterotoxin-related superantigen produced by *Staphylococcus aureus*. Microbiol Immunol. 44(2): 79-88.

Aras Z, Aydin I and Kav K. 2012. Isolation of methicillin-resistant *Staphylococcus aureus* from caprine mastitis cases. Small Rumin Res. 102(1): 68-73.

Bachert C, Gevaert P, Zhang N, van ZT and Perez-Novo C. 2007. Role of staphylococcal superantigens in airway disease. Chem Immunol Allergy 93: 214-236.

Bayles KW and Iandolo JJ. 1989. Genetic and molecular analyses of the gene encoding staphylococcal enterotoxin D. J Bacteriol. 171(9): 4799-4806.

Blaiotta G, Ercolini D, Pennacchia C, Fusco V, Casaburi A, Pepe O and Villani F. 2004. PCR detection of staphylococcal enterotoxin genes in *Staphylococcus* spp. strains isolated from meat and dairy products. Evidence for new variants of *seg* and *sei* in *S. aureus* AB-8802. J Appl Microbiol. 97(4): 719-730.

Boye K, Bartels MD, Andersen IS, Møller JA and Westh H. 2007. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCC*mec* types I-V. Clin Microbiol Infect. 13(7): 725-727.

Carleton HA, Diep BA, Charlebois ED, Sensabaugh GF, Perdreau-Remington F. 2004. Community-adapted methicillin-resistant Staphylococcus aureus (MRSA): population dynamics of an expanding community reservoir of MRSA. J Infect Dis.190(10):1730-1738.

Chang Q, Wang W, Regev-Yochay G, Lipsitch M and Hanage WP. 2015. Antibiotics in agriculture and the risk to human health: how worried should we be? Evol Appl. 8(3): 240-247.

Chapman HD. 2007. Rotation programmes for coccidiosis control. Int Poultry Prod. 15(1): 7-9.

Chu C, Yu C, Lee Y, and Su Y. 2012. Genetically divergent methicillin-resistant *Staphylococcus aureus* and *sec*-dependent mastitis of dairy goats in Taiwan. BMC Vet Res. 8: 39.

Colombari V, Mayer MD, Laicini ZM, Mamizuka E, Franco BD, Destro MT and Landgraf M. 2007. Foodborne outbreak caused by *Staphylococcus aureus*: phenotypic and genotypic characterization of strains of food and human sources. J Food Prot. 70(2): 489-493.

Couch JL, Soltis MT and Betley MJ. 1988. Cloning and nucleotide sequence of the type E staphylococcal enterotoxin gene. J Bacteriol. 170(7): 2954-2960.

Cuteri V, Mezzasoma P and Valente C. 2003. Application of biomolecular methods to *Staphylococcus aureus* strains from dairy cows. Vet Res Commun. 27 Suppl 1: 335-338.

de Silva RE, do Carmo LS and de Silva N. 2005. Detection of enterotoxins A, B and C genes in Staphylococcus aureus from goat and bovine mastitis in Brazilian dairy herds. Vet Microbiol. 106(1-2): 103-107.

Ferry T, Thomas D, Genestier AL, Bes M, Lina G, Vandenesch F and Etienne J. 2005. Comparative prevalence of superantigen genes in *Staphylococcus aureus* isolates causing sepsis with and without septic shock. Clin Infect Dis. 41(6): 771-777.

Fischer A, Francois P, Holtfreter S, Broeker B and Schrenzel J. 2009. Development and evaluation of a rapid strategy to determine enterotoxin gene content in *Staphylococcus aureus*. J Microbiol Methods. 77(2): 184-190.

Furuno JP, Hebden JN, Standiford HC, Perencevich EN, Miller RR, Moore AC, Strauss SM and Harris AD. 2008. Prevalence of methicillin-resistant *Staphylococcus aureus* and Acinetobacter baumannii in a long-term acute care facility. Am J Infect Control. 36(7): 468-471.

Gonzalez BE, Rueda AM, Shelburne III SA, Musher DM, Hamill RJ and Hultén KG. 2006. Community-associated strains of methicillin-resistant *Staphylococccus aureus* as the cause of healthcare-associated infection. Infect Control Hosp Epidemiol. 27(10): 1051-1056.

Haran KP, Godden M, Boxrud D, Jawahir S, Bender JB and Sreevatsan S. 2012. Prevalence and Characterization of *Staphylococcus aureus*,

Including Methicillin-Resistant *Staphylococcus aureus*, Isolated from Bulk Tank Milk from Minnesota Dairy Farms. J Clin Microbiol. 50(3): 688-695.

Havaei SA, Assadbeigi B, Esfahani BN, Hoseini NS, Rezaei N and Havaei SR. 2015. Detection of mecA and enterotoxin genes in *Staphylococcus aureus* isolates associated with bovine mastitis and characterization of Staphylococcal cassette chromosome mec (SCCmec) in MRSA strains. Iran J Microbiol. 7(3): 161-167.

Herron-Olson L, Fitzgerald JR, Musser JM and Kapur V. 2007. Molecular correlates of host specialization in *Staphylococcus aureus*. PLoS One 2(10): e1120.

Holden MT, Feil EJ, Lindsay JA, Peacock SJ, Day NP, Enright MC, Foster TJ, Moore CE, Hurst L, Atkin R, Barron A, Bason N, Bentley SD, Chillingworth C, Chillingworth T, Churcher C, Clark L, Corton C, Cronin A, Doggett J, Dowd L, Feltwell T, Hance Z, Harris B, Hauser H, Holroyd S, Jagels K, James KD, Lennard N, Line A, Mayes R, Moule S, Mungall K, Ormond D, Quail MA, Rabbinowitsch E, Rutherford K, Sanders M, Sharp S, Simmonds M, Stevens K, Whitehead S, Barrell BG, Spratt BG and Parkhill J. 2004. Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. Proc Natl Acad Sci. 101(26): 9786-9791.

Holmes MA and Zadoks RN. 2011. Methicillin resistant *S. aureus* in human and bovine mastitis. J Mammary Gland Biol Neoplasia. 16(4): 373-382.

Holtfreter S, Bauer K, Thomas D, Feig C, Lorenz V, Roschack K, Friebe E, Selleng K, Lövenich S, Greve T, Greinacher A, Panzig B, Engelmann S, Lina G and Bröker BM. 2004. egc-Encoded superantigens from Staphylococcus aureus are neutralized by human sera much less efficiently than are classical staphylococcal enterotoxins or toxic shock syndrome toxin. Infect Immun. 72(7): 4061-4071.

Huang YC and Chen CJ. Community-associated meticillin-resistant *Staphylococcus aureus* in children in Taiwan, 2000s. 2011. Int J Antimicrob Agents. 8(1): 2-8.

Huang YH, Tseng SP, Hu JM, Tsai JC, Hsueh PR and Teng LJ. 2007. Clonal spread of SCCmec type IV methicillin-resistant *Staphylococcus aureus* between community and hospital. Clin Microbiol Infect. 13(7): 717-724.

Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C and Hiramatsu K. 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 45(5): 1323-1336.

Ito T, Ma XX, Takeuchi F, Okuma K, Yuzawa H and Hiramatsu K. 2004. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. Antimicrob Agents Chenmother. 48(7): 2637-2651.

Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Forey F, Nesme X, Etienne J and Vandenesch F. 2002. Relationships between *staphylococcus aureus* genetic background, virulence factors, agr groups (alleles), and human disease. Infect Immun. 70(2): 1040-1048.

Jones CL and Saleem AK. 1986. Nucleotide sequence of the enterotoxin B gene from *Staphylococcus aureus*. J Bacteriol. 166(1): 29-33.

Kang YC, Hsiao CH, Yeh LK, Ma DH, Chen PY, Lin HC, Tan HY, Chen HC, Chen SY and Huang YC. 2015. Methicillin-Resistant *Staphylococcus aureus* Ocular Infection in Taiwan: Clinical Features, Genotying, and Antibiotic Susceptibility. Medicine. 94(42): e1620.

Kang YC, Tai WC, Yu CC, Kang JH and Huang YC. 2012. Methicillin-resistant *Staphylococcus aureus* nasal carriage among patients receiving hemodialysis in Taiwan: Prevalence rate, molecular characterization and de-colonization. BMC Infect Dis.12: 284.

Kenny JG, Ward D, Josefsson E, Jonsson IM, Hinds J, Rees HH, Lindsay JA, Tarkowski A and Horsburgh MJ. 2009. The *Staphylococcus aureus* response to unsaturated long chain free fatty acids: survival mechanisms and virulence implications. PLoS one. 4(2): E4344.

Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J and Hiramatsu K. 2007. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* tyoe assignment: rapid identification system for *mec*, *ccr*, and major differences in Junkyard regions. Antimicrob Agent Chemother. 51(1): 264-274.

Kreausukon K, Fetsch A, Kraushaar B, Alt K, Müller K, Krömker V, Zessin KH, Käsbohrer A and Tenhagen BA. 2012. Prevalence, antimicrobial resistance, and molecular characterization of methicillin-resistant *Staphylococcus aureus* from bulk tank milk of dairy herds. J Dairy Sci. 95(8): 4382-4388.

Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, Cui L, Oguchi A, Aoki K, Nagai Y, Lian J, Ito T, Kanamori M, Matsumaru H, Maruyama A, Murakami H, Hosoyama A, Mizutani-Ui Y, Takahashi NK, Sawano T, Inoue R, Kaito C, Sekimizu K, Hirakawa H, Kuhara S, Goto S, Yabuzaki J, Kanehisa M, Yamashita A, Oshima K, Furuya K, Yoshino C, Shiba T, Hattori M, Ogasawara N, Hayashi H and Hiramatsu K. 2001. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. Lancet. 357(9264): 1225-1240.

Landers TF, Cohen B, Wittum TE and Larson EL. 2012. A review of antibiotic use in food animals: perspective, policy, and potential. Public Health Rep. 127(1): 4-22.

Lee JH. 2003. Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl Environ Micribiol. 69(11): 6489-6494.

Letertre C, Perelle S, Dilasser F and Fach P. 2003. Identification of a new putative enterotoxin SEU

encoded by the egc cluster of *Staphylococcus aureus*. J Appl Microbiol. 95(1): 38-43.

Louie L, Goodfellow J, Mathieu P, Glatt A, Louie M and Simor AE. 2002. Rapid detection of methicillin-resistant staphylococci from blood culture bottles by using a multiplex PCR assay. J Clin Microbiol. 40(8): 2786-2790.

Lowy FD. 2003. Antimicrobial resistance: the example of *Staphylococcus aureus*. J Clin Invest. 111(9): 1265-1273.

Mamishi S, Mahmoudi S, Bahador A, Matini H, Movahedi Z, Sadeghi RH and Pourakbari B. 2015. Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* in an Iranian referral paediatric hospital. Br J Biomed Sci. 72(2): 47-51.

Moran GJ, Amii RN, Abrahamian FM and Talan DA. 2005. Methicillin-resistant *Staphylococcus aureus* in community-acquired skin infections. Emerg Infect Dis. 11(6): 928-930.

Morandi S, Brasca M, Lodi R, Cremonesi P and Castiglioni B. 2007. Detection of classical enterotoxins and identification of enterotoxin genes in *Staphylococcus aureus* from milk and dairy products. Vet Microbiol. 124(1-2): 66-72.

Nam HM, Lee AL, Jung SC, Kim MN, Jang GC, Wee SH and Lim SK. 2011. Antimicrobial Susceptibility of *Staphylococcus aureus* and Characterization of Methicillin-Resistant *Staphylococcus aureus* Isolated from Bovine Mastitis in Korea. Foodborne Pathog Dis. 8(2): 231-238.

Nema V, Agrawal R, Kamboj DV, Goel AK and Singh L. 2007. Isolation and characterization of heat resistant enterotoxigenic *Staphylococcus aureus* from a food poisoning outbreak in Indian subcontinent. Int J Food Microbiol. 117(1): 29-35.

Perron GG, Quessy S and Bell G. 2008. A reservoir of drug-resistant pathogenic bacteria in asymptomatic hosts. PLoS One. 3(11): e3749.

Rao PN, Naidu AS, Rao PR and Rajyalakshmi K. 1987. Prevalence of staphylococcal zoonosis in pyogenic skin infections. Zentbl Bakteriol Mikrobiol Hyg A. 265(1-2): 218-226.

Reddy P, Qi C, Zembower T, Noskin GA and Bolon M. 2007. Postpartum mastitis and community-acquired methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis. 13(2): 29-301.

Scott GM, Thomson R, Malone-Lee J and Ridgway GL. 1988. Cross-infection between animals and man: possible feline transmission of *Staphylococcus aureus* infection in humans? J Hosp Infect. 12(1): 29-34.

Seybold U, Kourbatova EV, Johnson JG, Halvosa SJ, Wang YF, King MD, Ray SM and Blumberg HM. 2006. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of health care—associated blood stream infections. Clin Infect Dis. 42(5): 647-656.

Shitrit P, Openhaim M, Reisfeld S, Paitan Y, Regev-Yochay G, Carmeli Y and Chowers M. 2015. Characteristics of SCCmec IV and V Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Israel. Isr Med Assoc J. 17(8): 470-475.

Spencer JP. 2008. Management of mastitis in breastfeeding women. Am Fam Physician. 78: 727-732.

Strommenger B, Kettlitz C, Werner G and Witte W. 2003. Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. J Clin Microbiol. 41(9): 4089-4094.

Thomas D1, Chou S, Dauwalder O and Lina G. 2007. Diversity in *Staphylococcus aureus* enterotoxins. Chem Immunol Allergy. 93: 24-41.

Tristan A1, Ferry T, Durand G, Dauwalder O, Bes M, Lina G, Vandenesch F and Etienne J. 2007. Virulence determinants in community and hospital meticillin-resistant *Staphylococcus aureus*. J Hosp Infect. 65 Suppl 2: 105-109.

Turlej A, Hryniewicz W and Empel J. 2011. Staphylococcal cassette chromosome *mec* (SCC*mec*) classification and typing methods: an overview. Pol J Microbiol. 60(2): 95-103.

Valsesia G, Rossi M, Bertschy S and Pfyffer GE. 2010. Emergence of SCCmec type IV and SCCmec type V methicillin-resistant Staphylococcus aureus containing the Panton-Valentine leukocidin genes in a large academic teaching hospital in central Switzerland: external invaders or persisting circulators? J Clin Microbiol. 8(3): 720-727.

Wielders CL, Fluit AC, Brisse S, Verhoef J and Schmitz FJ. 2002. mecA gene is widely disseminated in Staphylococcus aureus population. J Clin Microbiol. 40(11): 3970-3975.

Xu MK and Zhang CG. 2006. Gene expression and function study of fusion immunotoxin anti-Her-2-scFv-SEC2 in Escherichia coli. Appl Microbiol Biotechnol. 70(1): 78-84.

Zhang S, Iandolo JJ and Stewart GC. 1998. The enterotoxin D plasmid of *Staphylococcus aureus* encodes a second enterotoxin determinant (*sej*). FEMS Microbiol Lett. 168(2): 227-233.

บทคัดย่อ

การตรวจสอบยาต้านจุลชีพ, enterotoxin และ cassette chromosome gene ของเชื้อ *Staphylococcus aureus* ที่แยกได้จากมนุษย์ วัว และแพะในไต้หวัน

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เชื้อ *Staphylococcus aureus* ดื้อยาหลายขนาน (Multidrug-resistant *Staphylococcus aureus*; MDR *S. aureus*) เป็นเชื้อก่อโรคที่พบมากในมนุษย์และสัตว์ Staphylococcal enterotoxin (SE) เป็นสารพิษที่ผลิตโดยเชื้อ *S. aureus* การศึกษาในครั้งนี้ได้ทำการตรวจวิเคราะห์รูปแบบการดื้อยา ยีน SE 18 ยีน และ staphylococcal cassette chromosome (SCC*mec*) ของเชื้อ *S. aureus* ที่แยกได้จากผู้ป่วยที่มีภาวะเซลล์เนื้อเยื่ออักเสบ ผู้ป่วยที่ติดเชื้อในกระแสเลือด และผู้ป่วยที่มีภาวะเต้านมอักเสบ รวมถึงสัตว์เคี้ยวเอื้องที่มีภาวะเต้านมอักเสบ การศึกษาพบเชื้อ *S. aureus* ที่ดื้อต่อยา Methicillin (Methicillin-resistant *S. aureus*; MRSA) และเชื้อที่ไวต่อยา Methicillin (methicillin-sensitive *S. aureus*; MSSA) 51.35 เปอร์เซนต์ (76/148) และ 48.65 เปอร์เซนต์ (72/148) ตามลำดับ ทั้งเชื้อ MSSA และ MRSA มียีน *sea* 15 เปอร์เซนต์ ยีน *seb* 30.8 เปอร์เซนต์ และยีน *sek* 27.5 เปอร์เซนต์ โดย 80 เปอร์เซนต์ ของเชื้อ MRSA (52/65) อยู่ในกลุ่มของ SCC*mec* ชนิดที่ I, III และ IV ในขณะที่เชื้อ MSSA ไม่สามารถแยกกลุ่มได้ การตรวจระดับโมเลกุลของยีน SE พบว่า ยีน *sec* อาจเป็นยีนที่สำคัญของเชื้อ *S. aureus* ที่ก่อให้เกิดภาวะเต้านมอักเสบในแพะ ในขณะที่ยีน *sel* อาจมีบทบาทสำคัญในมนุษย์ แพะ และวัวที่มีภาวะเต้านมอักเสบ ยีน enterotoxin A เป็นยีนที่พบมากที่สุดในเชื้อ *S. aureus* ที่แยกได้จากวัวที่มีภาวะเต้านมอักเสบ เช่นเดียวกันกับที่พบในผู้ป่วยที่มีภาวะเซลล์เนื้อเยื่ออักเสบ ผู้ป่วยที่ติดเชื้อในกระแสเลือด และผู้ป่วยที่มีภาวะเต้านมอักเสบ ซึ่งแสดงให้เห็นว่า ยีน enterotoxin A มีบทบาทสำคัญและมีความสัมพันธ์ระหว่างมนุษย์และวัวที่มีภาวะเต้านมอักเสบ ความชุกของเชื้อ *S. aureus* ที่ดื้อยาหลายขนานถูกพบอย่างกว้างขวางในผู้ป่วยตามโรงพยาบาลและชุมชน รวมถึงในแพะที่มีภาวะเต้านมอักเสบ ส่วนในวัวที่มีภาวะเต้านมอักเสบถูกพบน้อยมาก ข้อมูลจากการศึกษารูปแบบยีน SE อาจจะใช้เป็นข้อมูลที่แสดงความสัมพันธ์ระหว่างความจำเพาะของสายพันธุ์โฮสต์และแหล่งที่มาของเชื้อ ดังนั้นการจำแนกยีน SE และการจัดกลุ่ม SCC*mec* สามารถใช้เป็นเครื่องมือในการตรวจสอบแหล่งที่มาของการติดเชื้อในสัตว์เคี้ยวเอื้องและมนุษย์ได้

คำสำคัญ: เชื้อ *Staphylococcus aureus* ดื้อยาหลายขนาน, staphylococcal enterotoxin, staphylococcal cassette chromosome, ภาวะเต้านมอักเสบ

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