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High prevalence and molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from retailed meats, south Thailand

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Abstract

Methicillin-resistant *Staphylococcus aureus* is an important pathogenic bacterium playing crucial roles in healthcare institutions including the communities. Moreover, food-borne illnesses caused by MRSA were also reported. In this study, 50 meat samples were investigated for the presence of MRSA. Suspected *S. aureus* colonies were selected and confirmed as *S. aureus* by 16S rRNA gene amplification using *S. aureus* specific primers. Investigation of *mecA* and other virulence genes, were carried out by PCR approach. Of 474 suspected isolates, 185 (39.02%) were confirmed to be *mecA*$^+$ *S. aureus*. All *mecA*$^+$ *S. aureus* were isolated from 34 meat samples (68%). Of these 185 isolates, 103, 42 and 40, were obtained from beef, pork and chicken, respectively. Two (1.08%) *mecA*$^+$ *S. aureus* from beef, PSU123 and PSU124, and two (1.08%) from chicken, PSU125 and PSU126, posessed von Willebrand factor binding protein gene. One (0.54%) of *mecA*$^+$ *S. aureus* obtained from chicken, PSU127, revealed the posession of *spa* gene. MRSA typing by SCC*mec* type revealed that PSU127 belonged to type IV while PSU123 to PSU126, were thought to be classified as SCC*mec* type other than type I to VI. Pulsed-field gel electrophoresis exhibited the distantly relationship among five *mecA*$^+$ *S. aureus* strains. The majority of pathogenic strains exhibited the resistance to penicillin G, erythromycin, ceftazidime, clindamycin and fusidic acid while PSU125 revealed the resistance to extra antimicrobial agent, tetracycline. This is the first report of MRSA contamination in retailed meats sold in south of Thailand.

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most frequent pathogens causing nosocomial infections worldwide (de Sousa *et al*., 2003; Johnson *et al*., 2005; Witte *et al*., 2007). The reported cases of MRSA infections have been gradually increased in several countries along a past decade. In Songklanagarind hospital, Hat-Yai city, Thailand, the nosocimial infection caused by MRSA reached epidemic circumstances in late December, 1986 (Jamulitrat *et al*., 1988). At that moment, 6 of 90 patients died within 14 months and the outbreak could not be attenuated although the infected people were immediately quarantined. The outbreak and sporadic cases by MRSA in this area were continually reported to date. Despite the fact that MRSA is frequently transmitted from person-to-person in hospitals or infections may be occurred in the polulations which are related to the healthcare institutions, food-borne illnesses by MRSA have also been reported (Jones *et al*., 2002). Resistance to methicillin is mediated through the *mec* operon which is a part of the staphylococcal cassette chromosome *mec* (SCC*mec*) (El Karamany *et al*., 2013). The *mecA* gene codes for an altered penicillin-binding protein, PBP2a, which has a lower affinity for binding β-lactam antibiotics (Mostafa, 2013). *S. aureus* possesses several virulence factors responsible for its pathogenicity to the host. It produces many virulence factors such as coagulase, γ-hemolysin, Panton-Valentine leukocidin (PVL), staphylococcal protein A. These virulence factors are responsible for the host attachment, host invasion and destruction of host defense system. Moreover, it may possess the staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1), the superantigens (Hwang *et al*., 2007). Both the SEs and TSST-1 belong to the pyrogenic toxin superantigen (PTSAg) family (Hwang *et al*., 2007). These superantigens (SAg) are able to bind to the major histocompatibility complex (MHC) class II and form a complex with Vβ chain of a T-cell receptor, resulting in a nonspecific manner stimulation of T-cell proliferation. Many of SAg genes

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are located in the mobile genetic elements, plasmids, prophages and staphylococcal pathogenicity islands (Baba *et al*., 2002). Although the additional types of SEs have newly been described, the classical SEs, staphylococcal enterotoxin A (SEA) to staphylococcal enterotoxin E (SEE), are the common causes of staphylococcal food poisoning.

During slaughtering processes, MRSA can be contaminated on carcasses (Boost *et al*., 2013), contributing to the high contamination rates of MRSA on retailed meats in fresh markets. The superantigens can be found in those MRSA strains. Therefore, meats and meat products act as the vehicle in transmission of MRSA to the buchers and consumers (Boost *et al*., 2012). The surveillance of MRSA in retailed meats was thought to be important. Although several studies of MRSA in meats have been documented in various countries (Jones *et al*., 2002; Katai *et al*., 2005; van Loo *et al*., 2007 ; Weese *et al*., 2010), insufficient evidences of MRSA in meats were documented in Thailand. Thus, we aimed to investigate the prevalence of MRSA strains from retailed meats in Southern Thailand, which may be contaminated with pathogenic MRSA, capable of causing food poisoning, including the examination of their cumulative virulence factors which contribute to the severe human infections. Furthermore, the antimicrobial resistant patterns were elucidated, providing the informations about potential drug of choice useful for the therapy in either sporadic or outbreak cases by MRSA isolated from the environment.

Materials and Methods

Sample collection

A total of 50 meat samples (pork = 16, chicken = 14, beef = 20) were collected from various meat retailers in Hat-Yai city, Thailand between April and September, 2013. Five to ten different meat samples from different markets were randomly selected. The samples were collected once a week to ensure that the meats were from different batches. The meats were brought to the laboratory within two hours for processing. Ten grams of meats were mixed with 90 ml of tryptic soy broth (TSB) and homogenized for 1 min. The liquid portion was incubated at 37ºC without shaking for 6 h. Subsequently, one ml of bacterial culture was diluted as dilution of 10^{-1} and 10^{-2} and plated on Mannitol Salt agar (MSA) (Difco, USA). The plates were incubated 37ºC for 18 h. Ten yellow colonies per sample were picked up and grown in TSB for 6 h before kept stock at -80ºC.

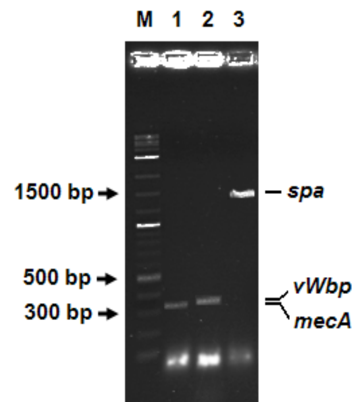

Figure 1. PCR assay detecting 310 bp of *mecA*, 320 bp of *vWbp*, and 1,500 bp of *spa* gene. Lane M, 2 log DNA ladder. Lane 1-3, representative of positive sample to each gene.

DNA template preparation

A single bacterial colony was inoculated into 3 ml of TSB and incubated at 37ºC for 6 h with 150 rpm shaking. The culture was subjected to boiling for 10 min and immediately on ice for 5 min. After centrifugation at 11,000×*g* for 10 min, ten-fold dilution of the supernatant portion was performed to obtain the DNA template for PCR.

Virulence genes detection

Seven virulence genes were determined by uniplex PCR method, *mecA*, *sea*, *coa*, *luk-PV*, *vWbp*, *femB* and *spa* genes. The sequences of oligonucleotide primers and annealing temperature used in this study were shown in Table 1. *vWbp* oligonucleotide primers were designed based upon the consensus sequences of *vWbp* genes complete sequence obtained from National Center for Biotechnology Information (NCBI). The amplification was carried out using *GoTaq*® Flexi system (Promega, USA), consisting of 3.0 mM $MgCl_2$, 0.1 mM dNTPs, 0.4 µM each primer pair, 0.5 unit of *GoTaq* DNA polymerase, 1X *GoTaq* Flexi buffer and 2 µl of boiled DNA template. The total reaction volume was 25 µl. The amplicons were analyzed in 1.0% agarose gel electrophoresis, stained with ethidium bromide before image capture by Gel documentation system (SYNGENE, USA) (Figure 1).

Identification of S. aureus by 16S rRNA gene

To confirm whether the isolates were *S. aureus*, amplification of 16S rRNA gene specific to *S. aureus* was carried out by PCR method using oligonucleotide primers described by Monday and Bohach (1999) (Table 1). The amplicons were analyzed by 1.0% agarose gel electrophoresis. The gel was stained with ethidium bromide and image captured.

Table 1. Oligonucleotide primers used in this study

Target gene	Name	Sequences (5' to 3')	Annealing temperature	Amplicon size (bp)	References
mecA	mecA1	GTAGAAATGACTGAACGTCCGATAA	50ºC	310	Geha *et al*., 1994
	mecA2	CCAATTCCACATTGTTTCGGTCTAA			
luk-PV	luk-PV1	ATCATTAGGTAAAATGTCTGGACATGATCCA	57ºC	433	Lina *et al*., 1999
	luk-PV2	GCATCAASTGTATTGGATAGCAAAAGC			
coa	COA1	CGAGACCAAGATTCAACAAG	51ºC	730	Wichelhaus *et al*., 2001
	COA2	AAAGAAAACCACTCACATCAGT			
spa	SPA1	ATCTGGTGGCGTAACACCTG	55ºC	1,500	Wichelhaus *et al*., 2001
	SPA2	CGCTGCACCTAACGCTAATG			
femB	FemB1	TTACAGAGTTAACTGTTACC	48ºC	651	Kobayashi *et al*., 1994
	FemB2	ATACAAATCCAGCACGCTCT			
vWbp	vWbp-F	GCTGGATTAAATGGTGAAAGTCATG	50ºC	320	This study
	vWbp-R	GTTTATTAAAACGTTTTTGATGACC			
sea	SEA-F	GCAGGGAACAGCTTTAGGC	52ºC	520	Monday and Bohach, 1999
	SEA-R	GTTCTGTAGAAGTATGAAACACG			
16S rRNA	16S SA-F	GTAGGTGGCAAGCGTTATCC	50ºC	228	Monday and Bohach, 1999
	16S SA-R	CGCACATCAGCGTCAG			
J1 region (type I)	CIF F2	TTCGAGTTGCTGATGAAGAAGG	53ºC	495	Milheiriço *et al*., 2007.
	CIF R2	ATTTACCACAAGGACTACCAGC			
ccr complex (type V)	*ccr*C F2	GTACTCGTTACAATGTTTGG	50ºC	449	Milheiriço *et al*., 2007.
	*ccr*C R2	ATAATGGCTTCATGCTTACC			
J3 region (type III)	RIF5 F10	TTCTTAAGTACACGCTGAATCG	50ºC	414	Milheiriço *et al*., 2007.
	RIF5 R13	GTCACAGTAATTCCATCAATGC			
J1 region (type V)	SCCmecVJ1F	TTCTCCATTCTTGTTCATCC	50ºC	377	Milheiriço *et al*., 2007.
	SCCmecVJ1R	AGAGACTACTGACTTAAGTGG			
J3 region (type I, II, IV, VI)	dcs F2	CATCCTATGATAGCTTGGTC	50ºC	342	Milheiriço *et al*., 2007.
	dcs R1	CTAAATCATAGCCATGACCG			
ccr complex (type II, IV)	*ccr*B2 F2	AGTTTCTCAGAATTCGAACG	50ºC	311	Milheiriço *et al*., 2007.
	*ccr*B2 R2	CCGATATAGAAWGGGTTAGC			
J1 region (type II)	kdp F1	AATCATCTGCCATTGGTGATGC	53ºC	284	Milheiriço *et al*., 2007.
	kdp R1	CGAATGAAGTGAAAGAAAGTGG			
J1 region (type III)	SCCmecIIIJ1F	CATTTGTGAAACACAGTACG	50ºC	243	Milheiriço *et al*., 2007.
	SCCmecIIIJ1R	GTTATTGAGACTCCTAAAGC			
mec complex (type II and III)	mecI P2	ATCAAGACTTGCATTCAGGC	50ºC	209	Milheiriço *et al*., 2007.
	mecI P3	GCGGTTTCAATTCACTTGTC			

MRSA typing

Staphylococcal cassette chromosome *mec* (SCC*mec*) (type I to VI) was investigated by PCR method as described by Milheiriço *et al*. (2007) (Table 1). PCR was performed as a simplex PCR. After amplification, the amplicons were analyzed by 1.2% agarose gel electrophoresis. The gel was stained with ethidium bromide and image captured. PCR amplification of *mecA* gene was also performed to be used as an internal control. In addition, pulsed-field gel electrophoresis was also carried out. Briefly, MRSA strains were treated with 50 µg/ml of lysostaphin (Sigma-Aldrich, USA) for 30 min to obtain spheroplast before plug preparation. The genomes of *S. aureus* were cleaved by FastDigest *Sma* I restriction enzyme (Thermo Scientific, USA.) at 37ºC for 1 h. The digested DNA fragments were separated in 1.0% agarose gel (Invitrogen, USA) using 0.5× TBE buffer by CHEF DR III system (Bio-rad, USA). Electrophoresis was performed at 6V/cm, field angle 120º at 14ºC. The initial and final switch times were 5.0 and 40.0 s, respectively. Overall run time was 21 h. After electrophoresis, the gel was stained by ethidium bromide and the image result was eventually captured. The 48.5 kb Lambda marker was used as a molecular size standard. Dendrogram was constructed using unweighted pair-group method of arithmetic average (UPGMA) (BioNumerics software version 7.0, Appied maths, Belgium).

Antimicrobial susceptibility testing

All *S. aureus* samples were determined for the antimicrobial susceptibility by disk diffusion method (CLSI, 2013). Fifteen antimicrobial agents were used in this experiment: amikacin (30 µg), erythromycin (15 µg), gentamicin (10 µg), chloramphenicol (30 µg), cephalothin (30 µg), ceftazidime (30 µg), penicillin G (10 µg), tetracycline (30 µg), norfloxacin (10 µg), clindamycin (2 µg), fosfomycin (50 µg), fusidic acid (10 µg), cefoxitin (30 µg), oxacillin (1 µg), and vancomycin. Clear zone was measured by Vernier caliper. Antimicrobial agents were purchased from Oxoid (UK). Vancomycin susceptibility was performed by E-test method. The susceptibility breakpoint for vancomycin was ≤4.

Results

Bacterial isolation and screening for mecA gene

In order to investigate MRSA in meats, three common meat types were purchased from fresh markets in Hat-Yai city, Thailand. A total of 474 isolates suspected to be *S. aureus*, were collected from such 50 meat samples. *mecA* gene were first examined in all isolates by PCR. Of 474 isolates, the results revealed the possession of *mecA* gene in

Table 2. Contamination of *mecA*+ *S. aureus* in retailed meats sold in Hat-Yai city area

Sample	No. of MRSA positive sample (%)	No. of isolated MRSA
Beef (*n* = 20)	18 (90%)	103
Pork (*n* = 16)	8 (50%)	42
Chicken (*n* = 14)	8 (57.14%)	40

Table 3. Virulence genes pattern of a total *mecA*+ *S. aureus* isolates from meats

Isolate (*n*)	Virulence gene (%)					
	coa	*vWbp*	*spa*	*luk-PV*	*sea*	*femB*
Beef (103)	0 (0)	2 (1.94)	0 (0)	0 (0)	0 (0)	0 (0)
Pork (42)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chicken (40)	0 (0)	2 (5)	1 (2.5)	0 (0)	0 (0)	0 (0)
Total (185)	0 (0)	4 (2.16)	1 (0.54)	0 (0)	0 (0)	0 (0)

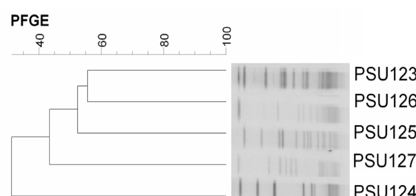

Figure 2. PFGE-based dendrogram of *mecA*+ *S. aureus* isolated from meat samples

185 isolates (39.02%) (average number of detected *mecA*+ *S. aureus* was 3.9 isolates per sample). These *mecA*+ isolates belonged to 34 meat samples (68%) (Table 2). All 185 isolates were further confirmed to be *S. aureus* by amplification of 16S rRNA gene using primers specific to *S. aureus.* When focused on each group of meat, it was obvious that the highest *mecA*+ *S. aureus* contamination was found in beef, 18 of 20 samples (90%) (Table 2). Chicken exhibited the *mecA*+ *S. aureus* contamination as a second rank, 8 of 14 samples (57.14%).

Other virulence genes detection

In order to examine the presence of other six virulence genes, *coa*, *vWbp*, *sea*, *spa*, *femB* and *luk-PV*, in *mecA*+ *S. aureus*, PCR method using specific oligonucleotide primers to each gene, was performed. In this study, of 185 *mecA*+ *S. aureus* isolates, none of the strains exhibited the presence of *coa* gene. Thus, another protein responsible for the plasma clotting, von Willebrand factor binding protein, *vWbp*, was further investigated. Of 185 MRSA, 4 were shown to carry *vWbp* (2 from chicken and 2 from beef) (Table 3). *spa* gene was detected in one chicken sample (2.5%). The presence of *mecA* together with the absence of *femB*, are the indicator of coagulase negative MRSA (Jonas *et al*., 2002). In this study, all *mecA*+ isolates revealed the lack of *femB*, demonstrating that these *S. aureus* were coagulase negative MRSA. This result was corresponded to the lack of *coa* gene in these MRSA strains (Table 3). Staphylococcal enterotoxin A gene, one of the common superantigens responsible for food poisoning, was absence in all *mecA*+ *S. aureus* tested. Panton-Valentine leukocidin gene was also negative (Table 3).

MRSA typing

SCC*mec* typing was initially performed to distinguish *mecA*+ *S. aureus* strains. In this study, investigation of SCC*mec* type was carried out by PCR method using oligonucleotide primers specific to SCC*mec* type I to VI as previously described by Milheiriço *et al.* (2007). The results revealed that PSU123 to PSU125 and PSU127 may be classified as SCC*mec* type other than type I to VI because no specific DNA band was observed. *mecA* gene that used as internal control could be normally observed. While PSU126 belonged to SCC*mec* type IV. PFGE clearly demonstrated the distantly relationship among *mecA*+ *S. aureus* strains. At 80% similarity, all *mecA*+ *S. aureus* were classified to be five distinguishable types, with PSU124 being located in the most distantly related (Figure2).

Antimicrobial susceptibility testing

Antimicrobial susceptibility test was performed by disk diffusion method using 15 antimicrobial agents. Five *mecA*+ *S. aureus* strains that positive for *vWbp* and *spa*, were subjected to the test. One pathogenic strains, PSU127 resisted to penicillin G, erythromycin, ceftazidime and fusidic acid while three pathogenic strains, PSU123, PSU124, and PSU126, revealed the same antimicrobial resistance pattern as PSU127 except they resisted to one extra antimicrobial agent, clindamycin. In addition, PSU125 exhibited the resistance to 6 antimicrobial agents, penicillin G, erythromycin, ceftazidime, tetracycline, clindamycin, and fusidic acid. All *mecA*+ *S. aureus* were susceptible to vancomycin (Minimal Inhibitory Concentration ranged from 1.0 to 4.0).

Discussion

Various rates of MRSA contamination in meats were reported from many areas of the world. de Boer *et al.* (2009) investigated MRSA in various kinds of meat samples in retail trades, Netherlands. MRSA was found in beefs, porks, and chickens as 10.6%, 10.7%, and 16%, respectively. In addition, our study was in accordance with the work from Weese *et al.* (2010). They performed the MRSA investigation in four provinces in Canada. They also found the less contamination of MRSA in pork (9.6%), ground beef (5.6%), and chicken (1.2%). Particularly, no MRSA was detected in pork and beef from Saskatchewan province. Interesting report from Rhode Island, it was demonstrated that 0% of MRSA contamination was found in beef, pork, and chicken meat samples (Chan *et al*., 2008). Our results were contrasted to the

works from many countries. In this study, high rates of *mecA*$^+$ *S. aureus* contamination were discovered in all meat types especially beef (90%) (Table 2).

Despite the fact that selective and differential media such as CHROMagar MRSA supplemented with cefoxitin can be employed for isolation of MRSA, in our previous experiment, we demonstrated the high detection rate of *mecA*$^+$ *S. aureus* in meat samples (unpublished data). Thus, based upon this information, general enrichment media such as tryptic soy broth followed by Mannitol salt agar, were thought to effectively amplify *mecA*$^+$ *S. aureus* from the samples. Pu *et al.* (2009) used trypticase soy broth supplemented with 10% NaCl and 1% sodium pyruvate in enrichment process for obtaining MRSA from retailed meats in Louisiana. Moreover, Weese *et al.* (2010) applied enrichment broth composed of 1% tryptone, 7.5% NaCl, 1% mannitol, and 0.25% yeast extract for MRSA isolation from meat marketed in four provinces in Canada. These works have been shown that the ordinary enrichment methods were efficiently capable of isolating MRSA from meat samples. This study emphasized the notion that in the samples obtained from low hygienic environments or where the high prevalence of MRSA has already been reported, the ordinary enrichment and selection methods followed by the indispensable screening, the detection of *mecA*, can be employed for the isolation of MRSA.

Coagulase is the polypeptide that converts fibrinogen to fibrin, led to the plasma clot (McAdow *et al*., 2011). Coagulase is encoded by *coa* gene. Approximately 670 amino acids of coagulase are varied among *S. aureus* strains (McAdow *et al*., 2012). All *mecA*$^+$ *S. aureus* in this study exhibited the lack of *coa* gene. Therefore, it has been thought that another type of coagulase, von Willebrand factor binding protein, may be haboured by them. von Willebrand factor (vWF) is a glycoprotein synthesized by endothelial cells and megakaryocytes (Ruggeri , 1999). It is a large glycoprotein composed of 2,050 amino acids. vWF plays a pivotal role in the maintenance of haemostasis especially in the situation of rapid blood flow. Bjerketorp *et al.* (2002) described the novel virulence factor of *S. aureus* called von Willebrand factor binding protein which functions as coagulase. In this study, *vWbp* gene was detected by the primers designed based upon the complete sequence of *vWbp* of seven *S. aureus* strains. This primer pair was thought to effectively detect *vWbp* from various *S. aureus* strains. We also applied this primer pair to the confirmed clinical MRSA strains containing *vWbp* (unpublished data) and it was found that the *vWbp* gene could also be detected clearly. Thus, this primer pair was suggested to be useful for the detection of pathogenic MRSA from various sources. Although *vWbp* was thought to play a role as coagulase in MRSA in this study, the subsequent investigation of *vWbp* gene revealed that only four strains, PSU123 to PSU126, possessed this gene. Even though these two main virulence factors responsible for plasma clotting were absence in most isolates, other virulence factors responsible for clotting phenomenon such as clumping factor may be exist and plays a role in pathogenesis.

Staphylococcal protein A is a 40 k Da antiphagocytic protein that covalently anchored to the peptidoglycan of bacterial cell wall. Approximately 90% of protein A are present in the cell wall. However, the remaining 10% are located in the bacterial cytoplasm (Shakeri *et al.*, 2010). Protein A of *S. aureus* is encoded by *spa* gene. Shakeri *et al.* (2010) reported the prevalence of *spa* gene in *S. aureus.* Of 208 *S. aureus*, 8 (3.8%) lacked *spa* gene detected by PCR method. However, *spa* gene could be found in only 3.4% of MRSA. Our investigation was corresponded to the work from Shakeri *et al.* (2010). It was found that only one MRSA strain, PSU127, possessed *spa* gene.

Community-acquired MRSA (CA-MRSA) has been reported to cause an outbreak and gradually increase in playing the pivotal roles in human infections. The cases resulted from CA-MRSA infections were reported to be associated with necrotizing pneumonia or pulmonary abscesses and sepsis. The cases were considered severe and rapidly fatal. Gordon and Lowy (2008) showed that there was a strong epidemiological correlation between CA-MRSA infections and the presence of Panton-Valentine leukocidin. In addition, these CA-MRSA strains were found to habour SCC*mec* type IV. Therefore, the presence of SCC*mec* type IV and Panton valentine leukocidin are the important makers of CA-MRSA. In this study, although PSU126 haboured SCC*mec* type IV, this strain did not contain Panton Valentine leukocidin. Thus, we consider PSU126 as non-CA-MRSA strain.

Various MRSA strains either from clinical samples or raw meats, were reported to resist many antimicrobial agents (Lee *et al*., 2008; O'Brien *et al.*, 2012; Boost *et al*., 2013). O'Brien *et al.* (2012) investigated MRSA from retail pork products. They found that 65.4% of identified MRSA isolates were resistant to tetracycline, 38.5% were resistant to erythromycin and 34.6% were resistant to clindamycin. These resistant patterns were also found in our MRSA in this study. Recently, one study from Boost *et al.* (2013) which examined MRSA from

Table 4. Virulence genes pattern and antibiogram pattern of *mecA*$^+$ *S. aureus* isolated from retailed meats

Isolate	Meat type	Virulence genes							[a]Antibiogram pattern
		mecA	*sea*	*coa*	*vWbp*	*luk-PV*	*spa*	*femB*	
PSU123	Beef	+	-	-	+	-	-	-	P, E, CAZ, DA, FD
PSU124	Beef	+	-	-	+	-	-	-	P, E, CAZ, DA, FD
PSU125	Chicken	+	-	-	+	-	-	-	P, E, CAZ, TE, DA, FD
PSU126	Chicken	+	-	-	+	-	-	-	P, E, CAZ, DA, FD
PSU127	Chicken	+	-	-	-	-	+	-	P, E, CAZ, FD

[a]erythromycin 15 μg, E; ceftazidime 30 μg, CAZ; penicillin G 10 μg, P; tetracycline 30 μg, TE; clindamycin 2 μg, DA; fusidic acid 10 μg, FD.

retail meats in Hong Kong also demonstrated the multiple drug resistance.

The *mecA*$^+$ *S. aureus* in this study displayed the susceptibility to oxacillin and cefoxitin. This phenomenon was not surprising because there have been studies documented the oxacillin-sensitive MRSA. O'Brien *et al.* (2012) has demonstrated that 4 of 26 (15.38%) MRSA isolated from pork products, were susceptible to oxacillin by broth microdilution despite habouring the *mecA* gene. Furthermore, the earlier report from Leedom Larson *et al.* (2011) supported O'Brien *et al.* (2012) and our works. They sought for MRSA in pork production shower facilities in Iowa and Illinois. Eight MRSA were subjected to antimicrobial susceptibility testing and it was found that two (25%) from swine's nasal and one (12.5%) from shower floor did not resist to oxacillin. This suggested the presence of oxacillin-sensitive MRSA in the environment. To our comprehension, it was thought that these MRSA strains haboured *mecA* gene but not produce PBP2a, referred as cryptically methicillin resistant strains (Kobayashi *et al*., 1994). The primers detecting *mecA* gene in this study were thought to be reliable in *mecA* detection because this primer pair has been used for the screening of MRSA from 500 isolates obtained from Mayo Clinic patients, Minnesota, United States (Geha *et al*., 1994) and 439 patients swabs (nose, throat, groin, perineum, wound, and drainage) from German tertiary care hospital (Jonas *et al.*, 2002). Furthermore, this primer pair was applied for confirmation of many oxacillin-resistant *S. aureus* from Songklanagarind and VachiraPhuket hospital (data not shown). In addition, *mecA* gene detection by PCR method was considered indispensable for MRSA screening. Despite the fact that these MRSA were resistant to multiple drugs such as tetracycline, clindamycin and fusidic acid, fortunately, they were still susceptible to vancomycin (Table 4).

MRSA is not generally considered as a food-borne pathogen. However, in this decade, several reports described the presence of enterotoxin-producing MRSA and toxic shock syndrome toxin, the superantigens in meats and other food samples (Jones *et al.*, 2002; Hwang *et al.*, 2007; Wang *et al.*, 2008; de Boer *et al*., 2009; O'Brien *et al*., 2012). Thus, the presence of MRSA in meats and food samples are important and able to play a role in food poisoning. The presence of staphylococcal enterotoxins is important for food safety aspect. The classical SEs are known as the common causes of staphylococcal food poisoning. One report from the Rebublic of Korea, 143 *S. aureus* isolates obtained from pork and chicken meats were characterized for the presence of SE type by multiplex PCR (Hwang *et al*., 2007). They showed that 72 of 143 possessed at least one toxin gene. In their work, *sea* gene was found in only 10 (7%). Moreover, *seb*, *sec*, *sek*, *sel* and *seq* genes were not detected. In our study, we used *sea* gene, one of the common enterotoxins involved in staphylococcal food poisoning, as a sole representative of enterotoxin genes in the investigation of retailed meats. All *mecA*$^+$ *S. aureus* isolates revealed the lack of *sea* gene (Table 3). Nevertheless, although *sea* was not observed in *mecA*$^+$ *S. aureus*, another type of SE-encoding genes were probably exist and may capable of posing a health risk to the populations in this area.

Conclusion

The presence of MRSA in retailed meats is essential in the aspect of food safety. In this study, high numbers of *mecA*$^+$ *S. aureus* were observed in all three common meat types sold in this Southern Thai area. Even though one of the common superantigen genes, *sea*, was not detected in all meat samples, other type of superantigens was probably existed. Moreover, MRSA virulence factors such as *vWbp* and *spa* were also detected in several *mecA*$^+$ *S. aureus* strains and may contribute to the human pathogenesis. In addition, these strains revealed the resistance to several antimicrobial agents. These finding emphasizes the presence of pathogenic MRSA in Southern Thai environment. Frequent surveillance of MRSA in retailed meats sold in this area, including the attentive hygiene of buchers and consumers, were cautioned to be strictly performed to prevent the outbreaks of this organism.

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