CHARACTERIZATION OF *STAPHYLOCOCCUS* SPP FROM MEAT AND READY-TO-EAT FOOD, HAT YAI CITY, SONGKHLA, THAILAND

Pharanai Sukhumungoon[1], Natthakul Bunnueang[1], Natnicha Butsabong[1], Aphisara Sae-lim[1] and Pattamarat Rattanachuay[2]

[1]Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla; [2]Department of Pre-clinic, Faculty of Science and Technology, Prince of Songkla University, Pattani campus, Pattani, Thailand

Abstract. *Staphylococcus aureus* contamination in food is considered important for food safety as its virulence factors are able to cause illnesses in humans. Screening of 100 meat and 30 ready-to-eat (RTE) food samples from open markets, Hat Yai city, Songkhla Province, Thailand during June 2015 to January 2017 revealed 11 samples contaminated with 27 methicillin-susceptible *S. aureus* (MSSA) isolates, of which 52% and 67% carried *spa* and *femB*, respectively, with only a minority (3 and 4 strains) carrying *sec* and *vWbp*, respectively. Staphyloxanthin production on tryptic soy agar was observed in 7 strains but not auto-aggregation. All but two strains were resistant to tetracycline, the exception being strains resistant to both tetracycline and gentamicin and another sensitive to all 8 antimicrobial agents tested. Biofilm formation using crystal violet binding assay showed an average biofilm formation for all strains ranging from A_{570} nm 0.35 (strain PSN-27) to 3.08 (strain PSN-4) ($p < 0.05$). Virulence as determined by a whole-blood phagocytosis assay demonstrated that bacterial number after incubated in whole blood varied from -0.5 fold to 82 folds comparing to their controls. In addition, one strain each of *mecA*$^+$ *S. epidermidis* and *S. haemolyticus*, is capable of producing yellow pigment, high biofilm formation, and of auto-aggregation, were isolated from RTE foods. These staphylococci have the potential to cause illness in humans and should be included in any survey of bacterial contamination in foods.

Keywords: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *mecA*, food, Thailand

Correspondence: Pattamarat Rattanachuay, Department of Pre-clinic, Faculty of Science and Technology, Prince of Songkla University, Pattani campus, Pattani 94000, Thailand.
Tel: +66 (0) 73 313928; Fax: +66 (0) 73 335130
E-mail: pattamarat_1@hotmail.com
Pharanai Sukhumungoon, Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.
Tel: +66 (0) 74 288322; Fax: +66 (0) 74 446661
E-mail: pharanai82@gmail.com

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) carries *mecA* encoding penicillin binding protein 2a (PBP2a), which is responsible for low binding affinity to β-lactam antibiotics (Utsui and Yokota, 1985). This β-lactam antibiotic-resistant ability reinforces *S. aureus* to be more virulent, thereby exacerbating the severity of infected patients and prolong-

ing the therapeutic period (Carbon, 1999). Surveillance of MRSA in patients, including in food sources, remains indispensable as the pathogen often carries other virulence genes that cause a broad range of symptoms, ranging from diarrhea to such devastating illnesses as endocarditis, respiratory tract infection, and bacteremia, which if left untreated result in high rates of mortality (Martineau *et al*, 1998). Severity of illness can be worsened in elderly and immunocompromised patients (Bunnueang *et al*, 2016).

In addition to its role in nosocomial infection, *S. aureus* also is involved in intestinal tract infections by producing staphylococcal enterotoxins (SEs) (Bunnueang *et al*, 2016). MRSA was responsible for an outbreak of acute community-acquired gastroenteritis, which involved infection of staphylococcal enterotoxin C-producing MRSA and the consumption of coleslaw from a delicatessen (Jones *et al*, 2002). Thus, food items are considered as important vehicles of MRSA. Previous studies indicated that meat and ready-to-eat (RTE) food are chiefly responsible as food sources of MRSA and methicillin-sensitive *S. aureus* (MSSA) (Hammad *et al*, 2012; Alibayov *et al*, 2014).

Staphylococcus epidermidis is the most important member of coagulase-negative staphylococci group, responsible for community- and hospital-acquired infections, causing bacteremia, osteomyelitis, urinary tract infection (Chaieb *et al*, 2007; Eftekhar and Raei, 2011). The presence at high frequencies of *mecA* in *S. epidermidis* from various geographical regions of the world has been reported for decades (McDonald *et al*, 1995; Chaieb *et al*, 2007; Eftekhar and Raei, 2011). In addition to *mecA*, *S. epidermidis* is equipped with many other virulence factors, *eg*, biofilm formation ability that mainly supports bacterial defense system and delta- toxin responsible for hemorrhagic enterocolitis in humans (Vuong and Otto, 2002). In coagulase-negative staphylococci, the second most frequently isolated from human blood cultures is *S. haemolyticus* (Takeuchi *et al*, 2005), which usually is found as normal microbiota of skin isolated from axillae, perineum and human inguinal, and due to contamination of external surface of the medical devices is able to cause infection in the circulatory system (Viale and Stefani, 2006). Thus, *S. haemolyticus* carrying *mecA* is a common hospital pathogen as reported in many regions of the world (Ubukata *et al*, 1990; Pereira *et al*, 2010; Barros *et al*, 2012). However, the presence of this bacterial species in RTE food has not been reported in Thailand.

Thus, the present study reports the prevalence, virulence profiles, and antimicrobial susceptibilities of MSSA from meats and RTE foods in southern Thailand. In addition, the presence of *mecA*$^+$ *S. epidermidis* and *mecA*$^+$ *S. haemolyticus* in RTE foods are also addressed. This information should be of importance to public health of Thailand.

MATERIALS AND METHODS

Sample collection

One hundred and thirty food samples [100 raw meat samples (33 beef, 34 pork, and 33 chicken) and 30 RTE food samples] were collected from open markets throughout Hat Yai city, Songkhla Province, southern Thailand during June 2015-January 2017. Samples were brought within 2 hours to the laboratory for processing as previously described by Bunnoeng *et al* (2014). In short, for raw meat samples, 10 g of sample were mixed with 90 ml of tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD) and homogenized

for 1 minute in a CIR-400 stomacher (Seward, West Sussex, UK) and the liquid portion was incubated at 37ºC for 6 hours. One hundred µl aliquot of 10^{-3} dilution of the broth was spread on Baird Parker-egg yolk tellurite agar (Becton Dickinson) and incubated at 37ºC for 18 hours. Five to 10 typical colonies (black colony surrounded by clear zone) were randomly selected for Gram staining and further molecular analyses.

Ten samples of each of the three types of RTE food commonly consumed, namely, fried rice, soup, and green papaya salad, sold at food courts adjacent to a hospital in Hat Yai were collected approximately three hours after being available for purchase. Ten gram of solid or 10 ml of liquid portion of a sample were homogenized with 90 ml of TSB and processed as described above.

PCR identification of *mecA* and *nuc*

A single colony was cultured in 3 ml of TSB for 3 hours at 37ºC with shaking. One ml aliquot of culture solution was subjected to boiling for 10 minutes, then, placed on ice for 5 minutes, centrifuged at 11,000*g* for 10 minutes and the supernatant was diluted 10 folds with sterile deionized water, as source of DNA. Amplification of *mecA* and *nuc* were performed as described by Bunnueang *et al* (2016) using specific primer pairs (Table 1). PCR was carried out in a 25-µl reaction mixture comprising of 3.0 mM $MgCl_2$, 0.1 mM dNTPs, 0.4 µM each primer pair, 0.5 U Go*Taq* DNA polymerase (Promega, Madison, WI), 1X Go*Taq* Flexi green buffer, and 2 µl of DNA solution. Thermocycling (conducted in T100™ Thermal Cycler; Bio-Rad, Hercules, CA) condition was as follows: 95ºC for 3 minutes; followed by 35 cycles of 94ºC for 1 minute, 50ºC for 1 minute (for both *mecA* and *nuc*) and 72ºC for 1 minute; with a final step at 72ºC for 5 minutes. Amplicons were analyzed using 1.0 % agarose gel-electrophoresis, stained with ethidium bromide and recorded using WSE-5200 Printpraph 2M gel imaging system (ATTO, Tokyo, Japan).

Strain that carried both *mecA* and *nuc* was classified as *mecA*$^+$ *S. aureus* and that carried *nuc* alone as *mecA*$^-$ *S. aureus*. MRSA was confirmed by cefoxitin resistance phenotype using a disk diffusion assay (CLSI, 2014) while MSSA by cefoxitin susceptibility. Strain that carried *mecA* alone was classified as *mecA*$^+$ *Staphylococcus* sp.

Virulence gene assay

Detection of 9 virulence genes (*coa*, *femB*, *luk-PV*, *sea*, *seb*, *sec*, *sed*, *spa*, and *vWbp*) was carried out using uni-plex PCR. The 25-µl reaction was consisted of 3.0 mM $MgCl_2$, 0.1 mM dNTPs, 0.4 µM each primer pair (listed in Table 1), 0.5 U Go*Taq* DNA polymerase (Promega), 1X Go*Taq* Flexi buffer, and 2 µl of DNA solution. Thermocycling conditions were as follows: 95ºC for 3 minutes; followed by 35 cycles of 94ºC for 1 minute, 48ºC for 1 minute (*femB*) or 50ºC for 1 minute (*coa*, *vWbp*, *sea*, *seb*, *sec*, *sed*) or 55ºC for 1 minute (*spa*) or 57ºC for 1 minute (*luk-PV*), and 72ºC for 1 minute or 1.5 minutes for *spa*; and a final step at 72ºC for 5 minutes. Amplicons were analyzed as described above.

Biofilm formation assay

Crystal violet binding assay was carried out as previously described (Yarwood *et al*, 2004) with slight modification. In brief, an individual colony was cultured in 5 ml of TSB supplemented with 1.0% (w/v) glucose and 2.0% (w/v) NaCl for 18 hours as a starter culture. Subsequently, bacterial cell number was quantified to 0.5 McFarland turbidity unit (approximately 1.5×10^8 CFU/ml) using a densitometer (Biosan, Riga, Latvia) with the same medium as

diluent. Then, 200-µl aliquot of bacterial suspension was placed into a 96-well flat-bottom polystyrene microtiter plate (NEST Biotech, Shanghai, China) and incubated at 37°C for 18 hours under static condition. After planktonic cells were discarded, the well was washed once with distilled water and air-dried. Attached biofilm was stained with 200 µl of 0.5% (w/v) crystal violet for 5 minutes, rinsed twice with distilled water and air-dried. Cell-bound crystal violet was released by adding 200 µl of absolute ethanol and $A_{570\,nm}$ measured using a microplate reader (Biotek, Winooski, VT). Each experiment was performed in triplicate.

Staphyloxanthin production and auto-aggregation assays

Production of staphyloxanthin was determined in cells grown on tryptic soy agar (TSA) plate (Becton Dickinson) at 37°C for 48 hours as described by Morikawa *et al* (2001) with slight modifications. In brief, bacteria were picked up and cultured in 3 ml of Luria-Bertani (LB) broth (Becton Dickinson) at 37°C for 48 hours. Bacterial cultures were centrifuged at 11,000*g* for 10 minutes and pellet examined for presence of staphyloxanthin yellow pigment.

Auto-aggregation was investigated by inoculating a single bacterial colony in 3 ml of LB broth (Becton Dickinson) and culturing at 37°C for 48 hours without shaking. Auto-aggregation was identified from the settlement of bacteria to the bottom of the culture tube.

Whole-blood phagocytosis assay

The assay was performed as previously described by Liu *et al* (2005) with slight modification. In short, a 3 ml aliquot of 3-hour bacterial culture in TSB broth was sedimented, washed twice with phosphate-buffered saline pH 7.4 (PBS). The cells were adjusted to approximately 1.5 × 10^4 cells in 25 µl of PBS and suspended in 75 µl of fresh human blood with 0.38% (w/v) citric acid as an anticoagulant. The solution was incubated at 37°C for 4 hours with gentle agitation. Enumeration of surviving *S. aureus* cells was carried out in triplicate by surface plate counting on mannitol salt agar (Becton Dickinson). Negative control was the bacteria incubated in PBS. Phagocytosis efficiency was measured as fold increase in bacterial number compared to its control.

Antibiogram profiling

All *S. aureus* strains were examined for antimicrobial susceptibility using the disk diffusion method (CLSI, 2014). Eight antimicrobial agents used in this assay were cefoxitin (30 µg), chloramphenicol (30 µg), clindamycin (2 µg), fosfomycin (50 µg), fusidic acid (10 µg), gentamicin (10 µg), tetracycline (30 µg), and trimethoprim/sulfamethoxazole (23.75/1.25 µg) (Oxoid, Hampshire, UK). Clear zone was measured by Vernier caliper.

Molecular identification and characterization of *mecA*$^+$ staphylococci strains

Unidentified *mecA*$^+$ staphylococci strains were subjected to 16S rDNA sequencing. Approximately 1,500 bp fragment of 16S rDNA was PCR amplified using eubacterial universal primer pair, BSF8/20 and REVB (Table 1) (Kanokratana *et al*, 2004). A 25-µl reaction mixture was composed of 3.0 mM $MgCl_2$, 0.1 mM dNTPs, 0.4 µM primer pair, 0.5 U Go*Taq* DNA polymerase (Promega), 1X Go*Taq* Flexi green buffer, and 2 µl of DNA solution. Thermocycling was conducted in T100™ Thermal Cycler (Bio-Rad, Hercules, CA) as follows: 95°C for 3 minutes; followed by 35 cycles of 94°C for 1 minute, 50°C for 1 minute and 72°C for 1 minute; with a final step at 72°C for 5 minutes. Am-

Table 1
Primers used in the study.

Target gene	Name	Sequence (5′ to 3′)	Amplicon size (bp)	Reference
mecA	mecA1	GTAGAAATGACTGAACGTCCGATAA	310	Geha *et al* (1994)
	mecA2	CCAATTCCACATTGTTTCGGTCTAA		
luk-PV	luk-PV1	ATCATTAGGTAAAATGTCTGGACATGATCCA	433	Lina *et al* (1999)
	luk-PV2	GCATCAASTGTATTGGATAGCAAAAGC		
coa	COA1	CGAGACCAAGATTCAACAAG	730	Wichelhaus *et al* (2001)
	COA2	AAAGAAAACCACTCACATCAGT		
vWbp	vWbp-F	GCTGGATTAAATGGTGAAAGTCATG	320	Bunnoeng *et al* (2014)
	vWbp-R	GTTTATTAAAACGTTTTTGATGACC		
femB	FemB1	TTACAGAGTTAACTGTTACC	651	Kobayashi *et al* (1994)
	FemB2	ATACAAATCCAGCACGCTCT		
spa	SPA1	ATCTGGTGGCGTAACACCTG	1,500	Wichelhaus *et al* (2001)
	SPA2	CGCTGCACCTAACGCTAATG		
sea	SEA-F	GCAGGGAACAGCTTTAGGC	520	Monday and Bohach (1999)
	SEA-R	GTTCTGTAGAAGTATGAAACACG		
seb	SEB-SEC-F	ATGTAATTTTGATATTCGCAGTG	643	Monday and Bohach (1999)
	SEC-R	TGCAGGCATCATATCATACCA		
sec	SEC-F	CTTGTATGTATGGAGGAATAACAA	283	Monday and Bohach (1999)
	SEC-R	TGCAGGCATCATATCATACCA		
sed	SED-F	GTGGTGAAATAGATAGGACTGC	384	Monday and Bohach (1999)
	SED-R	ATATGAAGGTGCTCTGTGG		
nuc	nuc1	GTAGGTGGCAAGCGTTATCC	279	Zhang *et al* (2004)
	nuc2	CGCACATCAGCGTCAG		
16S rRNA gene	BSF8/20	AGAGTTTGATCCTGGCTCAG	~1,500	Kanokratana *et al* (2004)
	REVB	GGTTACCTTGTTACGACTT		

plicons were gel-purified using Gel/PCR DNA fragment extraction kit (Geneaid, Taipei, Taiwan) and sequenced using an ABI Prism 337 instrument (Applied Biosystems, Waltham, MA). Sequences were compared with known staphylococci strains in GenBank database using nucleotide BLAST program and were deposited as GenBank accession no. MG577057 (for *S. epidermidis*) and MG577058 (for *S. haemolyticus*). Biofilm formation, yellow pigment production, and auto-aggregation of the *mecA*$^+$ strains were examined as described above.

Statistical analysis

Data were analyzed using SPSS for Windows, version 11.0 (SPSS, Chicago, IL). One-way ANOVA was employed to compare biofilm formation ability and whole-blood phagocytosis assay among MSSA strains. Significance was set at p-value < 0.05.

RESULTS

Prevalence and virulence profile of *S. aureus* isolates from raw meat and RTE samples

Of 1,244 colonies from 100 meat and 30 RTE samples, 27 (2.2%) colonies were MSSA, the majority of which carried *femB* (67%) and *spa* (52%), with 4 and 3 strains carrying *vWbp* and *sec*, respectively (Table 2). Staphyloxanthin production was observed in 7 (26%) strains on TSA plate and not by the culture method.

Table 2
Characteristics of *Staphylococcus aureus* isolated from raw meat and ready-to-eat food, Hat Yai city, Songkhla Province, Thailand, June 2015-January 2017.

Meat type	Sample no. (number of isolates)	Strain	Virulence gene						Staphylococcal enterotoxin gene				Staphyloxanthin production	Auto-aggregation	Drug resistance*	Biofilm assay**	WBP assay***
			mecA	*coa*	*spa*	*vWbp*	*lukPV*	*femB*	*sea*	*seb*	*sec*	*sed*					
Pork	8 (1)	PSN-1	-	-	+	-	-		-	-	-	-	-	-	TE	1.35^{de}	16^{bcd}
	23 (2)	PSN-2	-	-	-	-	-	-	-	-	+	-	+	-	TE	0.66^{ab}	28^{cd}
		PSN-3	-	-	-	-	-	-	-	-	+	-	+	-	TE	0.66^{ab}	2^{a}
	25 (1)	PSN-4	-	-	+	+	-	+	-	-	-	-	-	-	TE	3.08^{f}	2^{a}
	29 (8)	PSN-5	-	-	+	+	-	+	-	-	-	-	-	-	TE	0.59^{ab}	12^{abcd}
		PSN-6	-	-	+	-	-	+	-	-	-	-	-	-	TE	0.71^{abc}	7^{ab}
		PSN-7	-	-	+	-	-	+	-	-	-	-	-	-	TE	0.71^{abc}	82^{d}
		PSN-8	-	-	+	-	-	+	-	-	-	-	-	-	TE	0.77^{abc}	4^{a}
		PSN-9	-	-	+	+	-	+	-	-	-	-	-	-	TE	0.60^{ab}	4^{a}
		PSN-10	-	-	+	-	-	+	-	-	-	-	-	-	TE	0.70^{abc}	25^{abc}
		PSN-11	-	-	+	-	-	+	-	-	-	-	-	-	TE	0.75^{abc}	7^{a}
		PSN-12	-	-	+	-	-	+	-	-	-	-	-	-	TE	0.71^{abc}	61^{abcd}
	30 (1)	PSN-13	-	-	+	-	-	+	-	-	-	-	-	-	TE	0.88^{abcd}	3^{a}
Chicken	9 (1)	PSN-14	-	-	-	-	-	+	-	-	-	-	-	-	TE	0.88^{abcd}	7^{abc}
	20 (5)	PSN-15	-	-	-	-	-	-	-	-	-	-	+	-	TE	1.08^{bcde}	-0.5^{a}
		PSN-16	-	-	-	-	-	-	-	-	-	-	+	-	TE	1.10^{bcde}	6^{abc}
		PSN-17	-	-	-	-	-	-	-	-	-	-	+	-	TE	1.05^{bcde}	5^{abcd}
		PSN-18	-	-	-	-	-	+	-	-	-	-	-	-	TE	0.75^{abc}	2^{a}
		PSN-19	-	-	-	-	-	-	-	-	-	-	+	-	TE	1.10^{bcde}	3^{ab}
	22 (1)	PSN-20	-	-	+	+	-	+	-	-	-	-	-	-	CN,TE	0.70^{abc}	0.5^{a}
Beef	6 (3)	PSN-21	-	-	+	-	-	+	-	-	-	-	-	-	TE	0.70^{abc}	5^{a}
		PSN-22	-	-	-	-	-	+	-	-	+	-	-	-	TE	0.84^{abcd}	24^{ab}
		PSN-23	-	-	-	-	-	+	-	-	-	-	-	-	TE	1.26^{cde}	10^{a}
	10 (1)	PSN-24	-	-	-	-	-	-	-	-	-	-	+	-	SA	1.06^{bcde}	24^{bcd}
Green papaya salad	1 (3)	PSN-25	-	-	-	-	-	+	-	-	-	-	-	-	TE	0.85^{abcd}	2^{a}
		PSN-26	-	-	-	-	-	-	-	-	-	-	-	-	TE	0.61^{ab}	3^{a}
		PSN-27	-	-	+	-	-	+	-	-	-	-	-	-	TE	0.35^{a}	2^{a}

*CN, gentamicin; SA, susceptible to all antibiotics tested; TE, tetracycline. **Biofilm assay, different lowercase letters indicate significant difference among groups (p-value < 0.05). ***Fold increase in bacterial number compared to its control determined by whole-blood phagocytosis assay, different lowercase letters indicate significant difference among groups (p-value < 0.05).

Table 3
Characteristics of *mecA*+ *Staphylococcus epidermidis* and *S. haemolyticus* isolated from ready-to-eat foods, Hat Yai city, Songkhla Province, Thailand, June 2015-January 2017.

Isolate no.	*Staphylococcus* sp	Food type	Biofilm formation ($A_{570\ nm}$)	Yellow pigment production	Auto-aggregation
PSN-28	*S. epidermidis*	Green papaya salad	3.02 (high)[a]	+	+
PSN-29	*S. haemolyticus*	Soup	1.62 (high)	+	+

[a]Based on Gad *et al* (2009).

Auto-aggregation was not observed in all MSSA strains.

Antibiograms

Twenty-six (96%) MSSA strains were resistant to tetracycline, with MSSA strain PSN-20 isolated from chicken resistant to both tetracycline and gentamicin (Table 2).

Biofilm formation assay

Biofilm formation was performed using a crystal violet binding assay. Average $A_{570\ nm}$ of all strains ranged between 0.35 (strain PSN-27) and 3.08 (PSN-4) (Table 1). Positive control clinical MRSA strain PSU-20 exhibited $A_{570\ nm}$ of 0.759 (data not shown).

Whole-blood phagocytosis assay

Bacterial number of most of *S. aureus* after incubated in whole blood varied from -0.5 fold to 82 folds compared to their controls (Table 2). Eight MSSA strains, namely, PSN-1, PSN-2, PSN-5, PSN-7, PSN-10, PSN-12, PSN-22, and PSN-24, survived in whole blood, resulting in increases in bacterial numbers (>10 folds). Lower bacterial propagation (< 5 folds) was observed in 11 MSSA strains, namely, PSN-3, PSN-4, PSN-8, PSN-9, PSN-13, PSN-18, PSN-19, PSN-20, PSN-25, PSN-26, and PSN-27. In particular, a decrease in MSSA strain PSN-15 numbers was observed, suggesting a lower ability to survive in human blood (Table 2). Clinical MRSA strain PSU-85 (*spa*$^+$, *vWbp*$^+$, *femB*$^+$, *coa*$^+$, *sea*$^+$) used as virulence strain control exhibited a 2-fold increase in bacterial number (data not shown).

Molecular identification and characterization of *mecA*$^+$*nuc*$^-$ *Staphylococcus* strains

Two *mecA*$^+$*nuc*$^-$ *Staphylococcus* strains (from RTE foods) were identified from their 16S rDNA sequences as *S. epidermidis* with similarity of 99% (PSN-28, GenBank accession no. MG577057) and *S. haemolyticus* with similarity of 93% (PSN-29, GenBank accession no. MG577058). PSN-28 and PSN-29 were able to produce biofilm and staphyloxanthin, and showed auto-aggregation property (Table 3).

DISCUSSION

Occurrence of *S. aureus* contamination in raw meat and meat products are probably the results of poor food hygienic handling or contamination directly from the animals themselves (Normanno *et al*, 2005). Prevalence of MSSA in raw meats in this current study was considered relatively low compared to studies from the United States (ranging from 37% to 77%) and no MRSA was detected, in agreement

with our survey (Waters *et al*, 2011; Abdalrahman *et al*, 2015). There are several factors affecting the presence of *S. aureus* in different areas of the world, *eg*, differences among processing facilities, sampling and isolation methods, and collection times (Abdalrahman *et al*, 2015).

As for RTE food, Hammad *et al* (2012) reported 87% of *S. aureus* contamination in sashimi (Japanese raw fish) in Hiroshima Prefecture, Japan in 2010. Similarly, in the present study, *S. aureus* were found in green papaya salad, a Thai traditional food, that is consumed raw. It is worth noting that one of the strains (PSN-27) carries *spa* coding for staphylococcal protein A (SpA), which induces B lymphocyte superantigen activity, leading to proliferative supraclonal expansion and apoptotic collapse of activated B lymphocytes (Goodyear and Silverman, 2003). Thus, this suggests the plausibility of microbiological hazard introduced to consumers.

MSSA resistance to tetracycline in all MSSA strains is not surprising given its widespread use in clinical practice and veterinary medicine in Thailand (Ketpanyapong, 2007). Resistance to tetracycline in MSSA in this present study is consistent with the previous study from USA in 2011 showing the majority of *S. aureus* in meat are resistant to this antibiotic (Waters *et al*, 2011). Resistance to tetracycline by *S. aureus* strains isolated from RTE food (sashimi) has also been reported (Hammad *et al*, 2012). Nevertheless, the detection of an MSSA strain with additional resistance to gentamicin is a worrying observation, which will need close monitoring.

Investigation of MSSA virulence genes in the present study revealed the absence of *coa*, *luk-PV* and the classical staphylococcal enterotoxin genes *sea*, *seb*, and *sed*, results consistent with the previous study of Bunnoeng *et al* (2014) investigating 185 *mecA*$^+$ *S. aureus* isolates from meat sold in the same study area. However, *vWbp* encoding von Willebrand factor-binding protein and *spa* could be detected in some extent. These results suggest a correlation of possession of virulence gene between MSSA and *mecA*$^+$ *S. aureus* from meat in this particular area.

Biofilm formation is one of the important weapons of *S. aureus* employed during chronic infection, allowing *S. aureus* to persist in many host's tissues such as bone and heart valves, causing osteomyelitis and endocarditis, respectively, or on implanted devices such as catheter and cardiac pace maker (Lister and Horswill, 2014). A sessile microbial community protected by this extracellular polymeric substance is resistant to killing by antimicrobial agents at concentrations sufficient to kill cells in the planktonic state (Kaplan *et al*, 2012). Thus, it is more difficult for treating patients infected by biofilm-producing *S. aureus* and thereby prolonging therapeutic treatment. Based on Gad *et al* (2009), optical density of stained adherent bacteria at 570 nm of more than 0.240 is considered high biofilm formation. Thus, in this present study, all MSSA demonstrated A_{570} ranged from 0.35 to 3.08, indicating high biofilm-formation ability. This may suggest the problem for therapeutic processes of these strains.

In addition to biofilm formation, autoaggregation is a bacterial virulence factor facilitating adherence of *S. aureus* to host's tissues (Kuroda *et al*, 2008). One of *S. aureus* important cell wall-anchored proteins is protein G (SasG), postulated to form a homo-oligomer causing intercellular aggregation (auto-aggregation). Thus,

SasG-dependent aggregation might lead to an increase in cell population, resulting in adherence to host cells (Kuroda *et al*, 2008). Sung *et al* (2008) noted that *sasG* is found in (most of) clinical *S. aureus* isolates but not those from animal sources, consistent with the present study that all MSSA isolates did not possess auto-aggregation ability.

In our whole-blood phagocytosis assay, the increase in bacterial numbers higher than 10 folds (highest being 82 folds) is not surprising as it has also been reported by others (Voyich *et al*, 2005; Kobayashi *et al*, 2010). Although the mechanisms used by *S. aureus* to circumvent killing by the human immune system are incompletely defined, however, it is accepted that *S. aureus* possesses numerous virulence factors to combat the immune system, such as prevention of recognition and binding of neutrophils, protection against reactive oxygen species produced within phagosome, alteration of neutrophil turnover rates resulting in bacterial lysis and dissemination, and toxins production (DeLeo *et al*, 2009). Voyich *et al* (2005) investigating the survival ability of different *S. aureus* virulence strains during phagocytosis by human neutrophil and discovered that phagocytosis of each strain occurs rapidly and is essentially complete within 15 minutes; nevertheless, a significant number of the ingested *S. aureus* strains survives within the neutrophils, with community-associated MRSA exhibiting a higher degree of survival. Furthermore, some *S. aureus* strains exhibited increase in bacterial number (2 to 8 folds) within 3-6 hours after ingested by neutrophils. These results are in agreement with the findings in our study.

Staphyloxanthin, a golden carotenoid pigment produced by *S. aureus*, is one of virulence factors involved in protection against oxidative stress imposed by the host (Lee *et al*, 2012). Staphyloxanthin is typically a secondary metabolite that is produced through 4,4'-diaponeurosporene, through enzymes encoded by genes organized in the *crtOPQMN* operon (Katzif *et al*, 2005). As staphyloxanthin is produced by most clinical *S. aureus* strains (Duthie and Lorenz, 1952), this is consistent to our results showing that only 26% of *S. aureus* from animal sources produced staphyloxanthin. This might be due to the mutation in *crt* (Pelz *et al*, 2005) and/or from the lack of cold shock protein A (CspA), resulting in decreased expression of genes involved in staphyloxanthin biosynthesis (Katzif *et al*, 2005). In addition to *S. aureus*, in this current study, yellow pigment was also observed in both *S. epidermidis* and *S. haemolyticus*. This phenomenon is not surprising since several studies have reported the production of yellow pigments by many staphylococci (Brown, 1966; Becker *et al*, 2014).

The presence of *mecA*$^+$ *S. epidermidis* and *mecA*$^+$ *S. haemolyticus* in RTE food (green papaya salad and soup, respectively) is of importance as these types of food are consumed without further cooking. The presence of *mecA*$^+$ *Staphylococcus* spp from food sources has seldom been documented (Hammad *et al*, 2012; Bunnoeng *et al*, 2014). The *mecA*$^+$ *S. epidermidis* and *mecA*$^+$ *S. haemolyticus* strains produced yellow pigment and auto-aggregation. Thus, survey of staphylococci contamination in food should not be restricted to *S. aureus*.

In summary, pathogenesis by *S. aureus* is a multifactorial process that is dependent on the expression of several virulence factors. Moreover, some *S. aureus* strains show antimicrobial resistance

ability. Thus, contamination of this bacterial species including other *Staphylococcus* spp in food, especially RTE food, should not be overlooked. Surveillance of staphylococci in food and dissemination of such knowledge should be performed on a regular basis for the benefit of consumers in the study area and elsewhere in Thailand.

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