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Molecular Assessment of Staphylococcal Bacteria in Highly Educated Communities

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Abstract

Background Emergence of resistant strains of *staphylococcus aureus*, namely methicillin-resistant *S. Aureus* (mrsa) in all levels of urban and rural societies has become a haunting problem for the recent world.

Objectives This study assesses and explores the transfer of resistant *Staphylococcus aureus* bacteria in certain high social class of community focusing on nail as reservoir for transmitting the infection.

Methods One hundred swabs taken from nails were collected from college students in Malaysia. Assays for identification and differentiating *Staphylococcus aureus* were conducted to identify target bacteria. Moreover, this study compared the efficacy of the different identification tests with gold standard, PCR assay. The tests used were tube coagulase, DNase agar test, antibiogram, several routine biochemical identification tests and PCR assays. PCR assay used specific primers for resistance or species-related genes: *mecA, ermA, ermB, ermC, msrA, linA, femA,* and *nuc* genes.

Results A total of 155 bacterial isolates were isolated from college students' nails, non-PCR assays of identification and resistance detection revealed presence and spread of MRSA in nails of 3 college students. PCR-amplification of the *nuc* gene was used as a baseline test to detect *Staphylococcus aureus*. 20 isolates were detected as *Staphylococcus aureus* using traditional tests while PCR showed only 4 isolates are *S. aureus*, only 3 of them are MRSA. Sensitivity of antibiogram ranged from 88.9 to 100% but its specificity was very low (0-100%). For tube coagulase, sensitivity was 36.4-100%) while specificity was also not so high (66.7-100%).

Conclusion Collectively, nails proved to have potential for the transfer of MRSA in community of college students in South East Asia. Moreover, PCR assay for identification of *S. aureus* resistance proved to be superior on other methods.

Keywords *Staphylococcus aureus*, MRSA, PCR, antibiotic resistance

Introduction

Staphylococcus aureus is a normal colonizer in human and various animal species [1]. It normally colonizes the nares, hands, the rectum or vagina in human and causes infections. However, in the past 50 years, the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) which confer resistance toward β-lactam antibiotics had become prevalent in many parts of the world and in turns causes infections that were extremely difficult to treat [1,2]. The resistance in MRSA is due to the presence of the resistance gene, *mec* gene which encodes for the low affinity penicillin-binding protein 2a (PBP2a), or in rare cases, the hyperproduction of β-lactamase [3-7].

Recently, another concern about MRSA is the spread of MRSA to the community, community-acquired MRSA (CA-MRSA) that have caused infections and death in normal people without any healthcare-associated risk factors [8-10]. CA-MRSA infections commonly occur as skin and soft tissue infections such as impetigo, furuncles, or abscesses in healthy individuals [2,11-13].

However, it also can lead to the rare but life threatening disease like necrotizing pneumonia, necrotizing fasciitis or septicemia [13-26].

The CA-MRSA infections raised the concern of the public. These infections are transmitted among healthy persons. The recent data shows that the skin-skin contact and the skin-formite contact are important routes of transmission when compared to the nasal colonization [9]. The repeated close physical contact especially between broken skins during sport and games along with poor personal hygiene is the main contribute to the outbreak of MRSA [10]. However, environmental hygiene is also very important. Formites and commonly touched household objects such as soaps, doorknobs, toilet handles and kitchen sinks can serve as the reservoir for the MRSA and involved in the recurrent infections [9].

A person may accidentally acquire MRSA through scratches into their nail areas. In the case of poor personal hygiene, MRSA may reside under the nail as this place provides a suitable niche for their survival. Due to severe shortage in researching MRSA survival in and/or under nail, the current study aimed at investigating the presence of MRSA under the nail of college students; moreover, this study explored the circulation of MRSA with nails using different identification techniques. In addition, the identification techniques in the current study were also evaluated and compared with each other regarding their capability and efficiency in detecting and diagnosing MRSA from nails.

Methods

Subjects of the current study were 100 college students of University Putra Malaysia Kuala Lumpur, Malaysia except those who were involved in healthcare assignments such as medical and veterinary students. The research project was approved by the Research Ethics Committee of their institution, according to the Declaration of Helsinki. One hundred swabs of students' nail were collected in the period between October and December 2010; age, gender, field of study and history of cold/flu for each student were recorded during sample collection. The collected swabs were incubated in nutrient broth (Merck, KGaA, Germany) for 4 to 6 hours at 37^{o}C for pre-enrichment. Then, the cultures were streaked onto the 5% human defibrinated blood agar plates (Merck, KGaA, Germany) and incubated at 37^{o}C for 18-24 hours. The isolates obtained were identified by standard procedure using colony morphology, Gram's stain characteristic, catalase test and glucose oxidation and fermentation test. Of the one hundred students, 27 were males and 73 were female (71 Malay, 26 Chinese, and 3 Indian), which age between 19 to 34 years old.

Media

All Gram-positive cocci isolates were cultured on mannitol salt agar (MSA) (Oxoid, Cambridge, UK) and incubated at 37 ^{o}C for 18 to 24 hours. The ability to ferment mannitol was confirmed by the growth of yellow colonies on MSA surrounding by yellow zone after incubation, which indicates a positive result [27]. All of the mannitol- positive isolates were the presumptive of *Staphylococcus aureus*.

DNase Test

Single colonies of mannitol positive isolates were streaked on DNase agar (Oxoid, Cambridge, UK) and incubated at 37 ^{o}C for 18 to 24 hours. The agar was flooded with 1 N HCl after incubation and the presence of clearing zone indicates a positive result [27].

Tube Coagulase Test

Bacterial suspensions of a 0.5-2.0 McFarland standard of mannitol-positive isolates were prepared by suspending the isolates in 3mL Phosphate-Buffer Solution (PBS). Then, 350 µL of bacterial suspension was added to 150 µL of citrated human and rabbit plasma in sterile glass test-tubes. The tubes were incubated at 37 ^{o}C for 4 hours and observed for clot formation in every 30 minutes interval. If clotting did not occur, all coagulase-negative tubes containing citrated rabbit plasma, were further incubated at room temperature for 18 hours for citrated rabbit plasma while citrated human plasma coagulase-

negative tubes were further incubated at 37 °C for 18 hours. Agitation of the tubes was avoided when observation was made to prevent disruption of partially formed clots. The formation of clots indicates a positive result [27].

Antibiogram Typing

The susceptibility of all mannitol-positive isolates to antimicrobial agents (Oxoid, Cambridge, UK) was determined by using Mueller-Hinton agar (Merck, KGaA, Germany) disk diffusion method according to the guidelines of the CLSI. The bacteria suspension turbidity was adjusted to a 0.5 McFarland standard. The plates were incubated at 37 °C for 24 hours. Eleven antibiotic discs at the specific absolute concentration were as follow: cefoxitin (30 µg), chloramphenicol (30 µg), oxacillin (1 µg), vancomycin (30 µg), erythromycin (30 µg), trimethopterin (1.25 µg), penicillin G (1 µg), ampicillin (10 µg), methicillin (10 µg), tetracycline (30 µg), and gentamycin (10 µg) [28,29].

DNA Extraction

Eleven presumptive *Staphylococcus aureus* isolates which shows resistant toward cefoxitin, oxacillin, erythromycin, and methicillin were cultured in Luria-Bertani broth (Merck, KGaA, Germany) at 37 °C for 24 hours. DNA extraction was done by using the GeneJET™ Genomic DNA Purification Kit (Fermentas, #K0721). The purified DNA was stored at -20°C for further DNA typing.

Genomic Determination of the Resistant Genes

The specific genes responsible for antimicrobial resistance were determined by using polymerase chain reaction (PCR). In this study, the genes to be amplified are *mecA*, *ermA*, *ermB*, *ermC*, *msrA*, *linA*, *nuc*, and *femA*. The sequences, primers, PCR conditions for each gene are shown in table 1 (30-36). The amplified PCR products were analyzed and detected by ethidium bromide staining following 1.0% agarose gel electrophoresis at 80V for 45 minutes.

Table 1: Sequences primers and PCR conditions used in amplification of *mecA* gene *ermA* gene *ermB* gene *ermC* gene *msrA* gene *linA* gene *nuc* gene and *femA* gene

Target gene	Primer sequences	PCR Condition	Size (bp)	Thermocycler	Reference
mecA	5'-TCCAGATTACAACTTCACCAGG-3' 5'-CCACTTCATATCTTGTAACG-3'	32 cycles of 94 °C for 30 s 53 °C for 30 s and 72 °C for 50 s	162	Techne	30
ermA	5'-GTTCAAGAAC AATCAATACA GAG-3' 5'-GGATCAGGAA AAGGACATTT TAC-3'	32 cycles of 94 °C for 30 s 52 °C for 30 s and 72 °C for 60 s	421	Eppendorf	31
ermB	5'-CCGTTTACGAAATTGGAACAGGTAAAGGGC-3' 5'-GAATCGAGAC TTGAGTGTGC-3'	32 cycles of 94 °C for 30 s 55 °C for 30 s and 72 °C for 60 s	359	Bio Rad	32
ermC	5'GCTAATATTG TTTAAATCGT CAATTCC-3' 5'-GGATCAGGAA AAGGACATTT TAC-3'	32 cycles of 94 °C for 30 s 52 °C for 30 s and 72 °C for 60 s	572	Bio Rad	31
msrA	5'-GGCACAATAA GAGTGTTTAA AGG-3' 5'-AAGTTATATC ATGAATAGAT TGTCCTGTT-3'	30 cycles of 94 °C for 60 s 50 °C for 60 s 72 °C for 90 s	940	Bio Rad	33
linA	5'-GGTGGCTGGG GGGTAGATGT ATTAACTGG-3' 5'-GCTTCTTTTGAAATACATGGTATTTTCGATC-3'	32 cycles of 94 °C for 30 s 57 °C for 30 s and 72 °C for 60 s	323	Bio Rad	34
nuc	5'-GCGATTGATGGTGATACGGTT-3' 5'-AGCCAAGCCTTGACGAACTAAAGC-3'	32 cycles of 94 °C for 35 s 52 °C for 35 s and 72 °C for 50 s	276	Eppendorf	36
femA	5'-CTTACTTACTGCTGTACCTG-3' 5'-ATCTCGCTTGTTATGTGC-3'	32 cycles of 94 °C for 40 s 48 °C for 40 s and 72 °C for 50 s	684	Techne	35

Statistical analysis

The data of the current study was processed using Microsoft EXCEL 2007 (Microsoft, Corp., USA). P value less than 0.05 was considered significant.

Results

From 100 nail swabs, 155 bacterial isolates were obtained. Out of 155 bacterial isolates, 132 (85.2%) were Gram-positive cocci and 23 isolates (14.8%) were non Gram-positive cocci. Only Gram-positive isolates were subjected to other tests. For catalase test, 129 out of 132 Gram-positive isolates (97.7%) were catalase-positive. In glucose oxidation and fermentation test, 113 isolates (85.6%) can utilize glucose in both aerobic and anaerobic conditions, 19 isolates (14.4%) cannot utilize glucose or utilize in one condition only (aerobically or anaerobically). Acid production from the fermentation of mannitol in the mannitol salt agar (MSA) produces yellow colonies and changes the surrounding media into yellow color which was indicated as a positive result. The high salt content in the MSA inhibit the growth of most, but not all, bacteria other than staphylococci (59). A total of 70 isolates (53.0%) showed a positive result in mannitol salt agar test. The mannitol- positive isolates acted as presumptive of *Staphylococcus aureus (S. aureus)*. Based on the result of DNase test, 20 isolates (28.6%) out of the 70 presumptive *S. aureus* isolates gave positive test results as indicated by the presence of clearing zone on the surrounding of the colonies when flooded with 1 N HCl. In tube coagulase test, the positive results were determined by the formation of clot within the glass tube. Both citrated human plasma and citrated rabbit plasma gave the same number of isolates showing positive results, which were 13 isolates (18.6%) out of the 70 isolates tested. These 13 positive isolates were considered as *S. aureus* while the rest were considered as coagulase-negative staphylococci (CoNS). However, the activity of fibrinolysin was absent in this test. The details of each test result are shown in Table 2.

Table 2. Identification of *S. aureus* with various tests

Results	MSA (N=132)	Catalase (N=132)	GO & FT (N=132)	DNase (N=70)	Tube Coagulase (N=70)	
					CHP	CRP
Positive	70 (53.0%)	129 (97.73%)	113 (85.6%)	20 (28.6%)	13 (18.6%)	57 (81.4%)
Negative	62 (47.0%)	3 (2.3%)	19 (14.4%)	50 (71.4%)	13 (18.6%)	57 (81.4%)

GO = glucose oxidation, FT = fermentation test, CHP = citrated human plasma, CRP = citrated rabbit plasma

Antibiogram Typing

The susceptibility data of all presumptive *S. aureus* was presented in Table 3. From the results, all isolates (100%) were susceptible toward vancommycin (30 µg) and gentamycin (10 µg). There was one isolate (1.4%) showing resistance and 1 isolate (1.4%) had intermediate resistance toward methicillin (10 µg). The number of the isolates which were resistant toward other antimicrobial agents were: Cefoxitin (30 µg) 1 (1.4%), Chloramphenicol (30 µg) 1(1.4%), Oxacillin (1 µg), 1(1.4%), Erythromcin (30 µg) 10(14.3%), Trimethoprim (1.25 µg) 5(7.2%), Tetracyclin (30 µg) 2(2.9%), PenicillinG (1 µg) 58(82.9%), and Ampicillin (10 µg) 59(84.3%).

Based on the result, all coagulase-positive isolates (100%) were resistant toward penicillin G (1 µg) and ampicillin (10 µg), while all were susceptible toward vancomycin (30 µg) and gentamycin (10 µg). There was only one isolate (7.7%) resistant toward tetracycline and one isolate (7.7%) was intermediate toward erythromycin.

Genomic Determination of Resistant Genes

By using PCR amplification, only four isolates (36.4%) were found to be positive for the presence of *nuc* gene while the other seven isolates (63.6%) were negative for the presence of *nuc* gene. *mecA* gene was present in 9 isolates (81.8%), including the coagulase isolates and the CoNS. Ten isolates (90.9%) possess the *ermC* gene, *femA* gene, and *msrA* gene. All of the isolates tested (100%) did not possess *ermA* and *ermB* gene. In contrast, all of the tested isolates (100%) conferred resistance to lincosamides as they possess *linA* gene. None of the isolates possessed all the three combinations of the *erm* genes. The *erm* gene is mostly found in the isolates that possess the *mecA* gene. The prevalence of the resistant genes among the 11 isolates is shown in table 4. Moreover, the resistant genes within each isolate were shown in table 5.

In comparison with the coagulase test (Table 3), only one coagulase isolate tested showed the presence of the *nuc* gene. However, three CoNS isolates possess the *nuc* gene. The *mecA* gene which confers resistance to methicillin was detected in nine isolates (81.8%) whereas only two isolates showed resistance to methicillin in antibiogram typing.

Table 3. Antibiogram typing among coagulase-positive isolates and coagulase-negative isolates

Antimicrobial Agents	Coagulase Positive (N=13)			Coagulase Negative (N=57)			General For All Isolates (N=70)		
	S	I	R	S	I	R	S	I	R
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Cefoxiton	13(100)	-	-	56(98.2)	-	1 (1.8)	69(98.6)	-	1(1.4)
Chloramphenicol	13(100)	-	-	56(98.2)	1(1.8)	-	69(98.6)	1(1.4)	-
Oxacillin	13(100)	-	-	56(98.2)	-	1(1.8)	69(98.6)	-	1(1.4)
Vancomycin	13(100)	1(7.7)	-	57(100)	-	-	70(100)	-	-
Erythromycin	12(92.3)	-	-	48(84.2)	5(8.8)	4(7.0)	60(85.7)	6(8.6)	4(5.7)
Trimethoprim	13(100)	-	-	52(91.2)	2(3.5)	3(5.3)	65(92.8)	2(2.9)	3(4.3)
Penicillin G	-	-	13(100)	12(21.1)	-	45(78.9)	12(17.1)	-	58(82.9)
Ampicillin	-	-	13(100)	11(19.3)	-	46(80.7)	11(15.7)	-	59(84.3)
Methicillin	13(100)	-	-	55(96.4)	1(1.8)	1(1.8)	68(97.2)	1(1.4)	1(1.4)
Tetracycline	12(92.3)	-	1(7.7)	56(98.2)	-	1(1.8)	68(97.1)	-	2(2.9)
Gentamycin	13(100)	-	-	57(100)	-	-	70(100)	-	-

Table 4. Prevalence of resistant genes in 11 isolates

Resistant genes	Coagulase-positive isolates (n=1)		Coagulase-negative isolates (n=10)		General for all isolates (n=11)	
	Positive	Negative	Positive	Negative	Positive	Negative
nuc	1 (100%)	-	3 (30%)	7 (70%)	4 (36.4%)	7 (63.6%)
mecA	-	1 (100%)	9 (90%)	1 (10%)	9 (81.8%)	2 (18.2%)
ermA	-	1 (100%)	-	10 (100%)	0	11 (100%)
ermB	-	1 (100%)	-	10 (100%)	0	11 (100%)
ermC	1 (100%)	-	9 (90%)	1 (10%)	10 (90.9%)	1 (9.1%)
femA	1 (100%)	-	9 (90%)	1 (10%)	10 (90.9%)	1 (9.1%)
msrA	1 (100%)	-	9 (90%)	1 (10%)	10 (90.9%)	1 (9.1%)
linA	1 (100%)	-	10 (100%)	-	11 (100%)	0

Table 5. The distribution of resistant genes among each isolate

Isolates No.	Resistant Genes							
	nuc	*mecA*	*ermA*	*ermB*	*ermC*	*femA*	*msrA*	*linA*
4	-	+	-	-	+	+	+	+
17b	-	+	-	-	+	+	+	+
22b	-	-	-	-	-	+	+	+
24	+	-	-	-	+	+	+	+
27a	-	+	-	-	+	+	+	+
39a	+	+	-	-	+	+	+	+
47a	+	+	-	-	+	+	+	+
50	+	+	-	-	+	+	+	+
81a	-	+	-	-	+	+	+	+
91b	-	+	-	-	+	+	+	+
95b	-	+	-	-	+	-	-	+

Based on the molecular results, four isolates were confirmed as *S. aureus*, as they were positive for *nuc* gene. However, only one of them was coagulase positive isolate. From 100 nail swab samples collected, these four *S. aureus* were isolated from 4 students. Out of these four isolates, three were confirmed as MRSA as they were positive for *mecA* gene, indicating that MRSA was isolated from 3 students. One of the isolates that possesses *nuc* gene did not contain *mecA* gene but contained other resistant genes (*ermC, msrA, linA* and *and femA*). Therefore, it was categorized as multi-resistant *Staphylococcus aureus*. Six of the CoNS also possessed *mecA* gene; thus, they were categorized as methicillin-resistant coagulase-negative staphylococci. One of the CoNS was with neither *erm* genes nor *mecA* gene detected. Thus, it was non methicillin-resistant CoNS.

Comparison of antibiogram and other tests with PCR assay

Discrepant results were observed in our study. The antibiogram typing failed to detect methicillin resistance in seven isolates (77.8%), eight isolates (88.9%) for oxacillin resistance, one isolate (10% with *erm* genes and 11.1 with *msrA* gene) for erythromycin resistance. The sensitivity and specificity of the antibiogram typing compared with the PCR identification is shown in table 6 while the findings of comparison (sensitivity and specificity) other test compared to PCR assay (Golden standard) are shown in table 7.

Table 6. Sensitivity and specificity of the antibiogram typing compared to PCR detection of resistant genes in the isolates

Antibiogram Typing Result		PCR results		% Sensitivity	% Specificity
		Positive	Negative		
Methicillin	**Positive**	2	0	100	22.2
	Negative	7	2		
Oxacillin[a]	**Positive**	1	0	100	20
	Negative	8	2		
Erythromycin[b]	**Positive**	9	1	90	0
	Negative	1	0		
Erythromycin[c]	**Positive**	8	1	88.9	50
	Negative	1	1		

[a]: molecular detection of oxcillin resistant is based on the detection of *mecA* gene, [b]: comparison of erythromycin-resistant with the *erm* genes, [c]: comparison of erythromycin-resistant with the *msrA* gene

Table 7. Identification of *S. aureus* with the common tests compared with the PCR detection of *nuc* gene.

Tube Coagulase Test		PCR detection of *nuc* Gene		% Sensitivity	% Specificity
		Positive	Negative		
Human Plasma	**Positive**	1	0	100	70
	Negative	3	7		
Rabbit Plasma	**Positive**	1	0	50	66.7
	Negative	3	7		
DNase	**Positive**	1	1	36.4	100
	Negative	3	6		
MSA	**Positive**	4	7	50	66.7
	Negative	0	0		
MSA/DNase/rabbit plasma	**Positive**	1	1	50	66.7
	Negative	3	6		

Discussion

Recently, the emergence of MRSA infections in the community among healthy individuals without any risk factors had increased steadily and become a great concern of the community [10,37]. In this study, all the three MRSA isolates originated from female students. In addition, all of these three did not have history of cold/flu. The resistant *mecA* gene was predominantly present in the female isolates (8 persons, 88.9%) and seldom present in male isolate (1 person, 11.1%). All of the multiresistant CoNS and *S. aureus* were mainly isolated from female, 13.7% (10 out of 73 females) and 3.7% (1 out of 27 males) from male. Figure 9 shows the distribution of *mecA* gene among the students with different gender.

It was mentioned that the presence of *mec A* gene does not always mean that the *Staphylococcus spp.* show resistance to methicillin as shown in the susceptibility test of E-test and disk diffusion test. This may be due to the presence of the incomplete regulator genes (*mecI* and/or *mecRI*) or maybe because of the inability to express the *mecA* gene [38]. The current study proposed that MRSA can colonize nail just like skin and anterior nares (unpublished data). During physical contact, the MRSA may attach to the skin but they also retain underneath nail of the person. In addition, MRSA may transmit from our daily use item such as cell phones, coins, keys, doorknobs and others (unpublished data).

The resistance to erythromycin was mainly due to the presence of either, *ermA, ermB, ermC* or combination of the *erm* genes. It was indicated that *ermA* and *ermC* genes are responsible for most of the erythromycin resistance in *S. aureus* which is similar to the result obtained from the current study. All of the isolates contain only *ermC* gene. The *ermA* is part of the transposon Tn554 in the chromosome, while the *ermC* is located on the plasmid [39]. *ermC* gene is the most prevalent form where it was found in all eight isolates. None of the isolates contained *ermA* and *ermB* genes. As described previously, *ermB* gene is commonly found on animal strains [39]. The results also coincide with the research done by Eady *et al.;* who documented that *ermC* is predominant in clinical and commensal coagulase-negative staphylococci [39].

Resistance towards macrolides is due to the presence of *msr* gene. In this study, *msrA* instead of *msrB* was choosen. Ten of 11 isolates (90.9%) tested contain *msr A* gene. Previous studies reported that no *S. aureus* contains both *erm* and *msrA* genes [40]. However, all of the *S. aureus* (100%) in the current study possessed combination of these two genes. Most of the CoNS also contain both *erm* and *msrA* genes.

There were only two isolates showing either *msrA* or *erm* gene. *lin A/linA'* gene is responsible to confer resistance to lincosamides only. It was found in the current study that all of the isolates (100%) contained this *linA* gene. It is uncommon for staphylococci to confer resistance only to lincosamides [41]. Although the incidence of *linA* gene to appear alone is low, there was one isolate (9.1%) with this condition, which contains *linA* gene alone without *msr* genes or *erm* genes. Most of the isolates contained *linA* gene in conjugation with *mrsA* gene, *erm* genes or both.

The *S. aureus* specific gene, *femA* which does not cross react with other bacteria such as *S. epidemidis* was used to identify pure *S. aureus*. Although *femA* sequences are phylogenetically conserved to staphylococci; however, *femA* for *S. aureus* is 78% homologous to the *femA* of *S. epidemidis* [42]. Therefore, there is a possibility of giving false positive *S. aureus*. In order to confirm that isolates were *S. aureus*, another gene, *nuc* gene was used together with the *femA* gene. In our study, ten of 11 isolates (90.9%) contained *femA* gene. However, only four of 11 isolates contained *nuc* gene.

Discrepant results were observed in our study. The antibiogram typing failed to detect methicillin resistance in 7 isolates (77.8%), 8 isolates (88.9%) for oxacillin resistance, 1 isolate (10% with *erm* genes and 11.1% with *msrA* gene) for erythromycin resistance. The sensitivity and specificity of the antibiogram typing compared with the PCR identification is shown in table 6. The methicillin resistance is attributed to the expression of *mecA* gene which produces low affinity penicillin binding protein 2a (PBP2a), or in rare cases, attributed to the hyperproduction of the β-lactamase enzymes or production of altered binding capacity proteins [3,5-7]. However, the presence of *mecA* gene does not always mean that *S. aureus* confer resistance to methicilln, as it can be explained by the incomplete regulator genes (*mecI* and/or *mecRI*) or inability to express *mecA* gene. Therefore, many isolates in the current study were susceptible to methicillin in antibiogram typing but possessed *mecA* gene. The discrepant results in our study could be explained by this mechanism as well. Therefore, the sensitivity of methicillin was 100% with specificity of 22.2%. The oxacillin resistance also expressed *mecA* gene. In this case, the sensitivity of oxacillin disk was 100% but with 20% only specificity. Erythromycin resistance in the isolates was encoded by *erm* and *msrA* genes. The erythromycin disk diffusion method showed sensitivity of 90% and specificity of 0% when compared with the PCR results for *erm* genes and sensitivity of 88.9% and specificity of 50% when compared with *msrA* gene.

Tube coagulase is one of the most reliable methods to identify *S. aureus*. There are 2 types of methods to detect the production of coagulase from *S. aureus*, tube coagulase test (TCT) and slide coagulase test (SCT). The SCT works by detecting the bound coagulase, which is also known as "clumping factor" that react directly to the fibrinogen in plasma, causing rapid cell agglutination. Negative SCT should reconfirm with TCT because they might produce extracellular coagulase. The extracellular coagulase detects a substance in the plasma known as coagulase reacting factor (CRF) to form a complex, which later reacts (clot formation) with the fibrinogen to form fibrin (form clot) [27].

In the current study, both human and rabbit plasma were used in the TCT. A previous study had showed that human plasma gives discordant results [43]. Rabbit plasma was the standard in performing the coagulase test. On the other hand, the current study reports that human and rabbit plasma give the same results. There were no difference in the TCT result using human and rabbit plasma. This might due to the fact that fresh human plasma was used. Both TCT using human and rabbit plasma gave the similar sensitivity and specificity, namely, 100% and 70%, respectively, as shown in Table 7. Three of the coagulase-negative isolates (30%) had *nuc* gene, indicating that some of the isolates were misidentified as CoNS. It was reported that these coagulase-negative *S. aureus* may probably react weakly or negatively with the TCT [27]. In our

study, all the methicillin-resistant *S. aureus* were negative for coagulase test.

DNase test was also used to identify *S. aureus*. In our study, DNase test gave a sensitivity of 50% and specificity of 66.7%, which have a lower value compared to other studies [27]. Two of the 11 isolates were positive for DNase test. However, 3 of the 4 *S. aureus* isolates were DNase negative, which were also methicillin resistant.

MSA test also aids in the identification of *S. aureus.* The MSA gave a sensitivity of 36.4% only and specificity of 100%. Four of 11 MSA positive isolates (36.4%) were confirmed as *S, aureus* with the presence of *nuc* gene. The other 71 isolates gave a false positive by the absence of *nuc* gene.

After comparing individual test to identify the *S. aureus*, none of the single phenotypic tests can accurately identify *S. aureus*. Detecting *S. aureus* using the TCT gave the highest sensitivity of 100%, followed by the DNase 50% and lastly the MSA with 36.4%. Meanwhile, MSA test showed the highest specificity, 100%, followed by TCT with 70% and lastly the DNase test with 66.7%. Data of the current study was same as the previous studies in which the sensitivity of TCT reached 94-100% [27]. In contrast to the other studies, the sensitivity and specificity in our study were much lower [44]. Among the 3 phenotypic tests, TCT was shown to be more suitable to identify *S. aureus* (100% sensitivity and 70% specificity). Our finding was different from another study where DNase test was superior to TCT [44]. Due to the low sensitivity and specificity, MSA and DNase were used in routine identification of *S. aureus* at the initial stage [43].

The current study showed that there were three MRSA isolates (3%) from 100 nail swab samples. This also indicates that MRSA can be found in and colonize the nail of the healthy individuals. This provided evidence that MRSA do exist actively in population of high social rank such as university students. The level of MRSA existence in university students might be much lower than other sectors of the community as known that university students maintain better personal hygiene than others, which is an important precaution step to be away from the dangerous MRSA. Therefore, detection of MRSA in university students can be considered as dangerous indicator for the given community. Furthermore, methicillin-resistant coagulase-negative staphylococci (MRCoNS) were also isolated from the student's nail swabs. Six MRCoNS (6%) were isolated from swabs of 100 student's nail. There was one isolate (1%) showing multi-resistance but susceptible to methicillin. All of these 11 isolates were also resistant to other antimicrobial agents such as erythromycin and lincosamides.

Taken together, it was found that MRSA do not only found in mails of highly educated people but can circulate between nails and other parts of the body, Besides, we have proven the existence of MRSA and methicillin-resistant CoNS among college students' nail. Furthermore, screening of resistance genes was shown to be superior on all other modes of detection/identification of MRSA. Attention should be raised for MRSA identified in community. Good personal hygiene and environmental hygiene are important to prevent the colonization of MRSA. Besides, there is no single phenotypic test can be used to adequately identify *S. aureus*. Initially tested isolates with MSA and DNase, followed by TCT can improve the efficiency and accuracy. However, final confirmation with the "golden standard", PCR should be performed to identify *S. aureus* and their antibiotics resistance. Hence, it is recommended doing larger study on the circulation of MRSA among university students in other regions of the world. Moreover, it is recommended that MRSA containing protocols should cover all sectors of the community even these composed of high socioeconomic individuals.

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