Characterization of resistance to selected antibiotics and Panton-Valentine leukocidin-positive *Staphylococcus aureus* in a healthy student population at a Malaysian University

Zarizal Suhaili^{1*}, Putri 'Amira Rafee², Norhidayah Mat Axis³, Chew Chieng Yeo⁴, Syafinaz Amin Nordin⁵, Abdul Rachman Abdul Rahim⁶, Mazen M. Jamil Al-Obaidi⁷, Mohd Nasir Mohd Desa⁸

Abstract

Introduction This study aims to assess the antimicrobial susceptibility profiles of *Staphylococcus aureus* strains isolated from university students and to determine the prevalence of constitutive and inducible clindamycin resistance, the latter being able to cause therapeutic failure due to false in vitro clindamycin susceptibility.

Methods *S. aureus* strains were isolated from the nasal swabs of 200 health sciences students of a Malaysian university. Twelve classes of antibiotics were used to evaluate the antimicrobial susceptibility profiles with the macrolide-lincosamide-streptogramin B (MLS_B) phenotype for inducible clindamycin resistance determined by the double-diffusion test (D-test). Carriage of resistance and virulence genes was performed by PCR on *S. aureus* isolates that were methicillin resistant, erythromycin resistant and/or positive for the leukocidin gene, *pvl* (n=15).

Results Forty-nine isolates were viable and identified as *S. aureus* with four of the isolates characterized as methicillin-resistant *S. aureus* (MRSA; 2.0%). All isolates were susceptible to the antibiotics tested except for penicillin (resistance rate of 49%), erythromycin (16%), oxacillin (8%), cefoxitin (8%) and clindamycin (4%). Of the eight erythromycin-resistant isolates, iMLS_B was identified in five isolates (three of which were also MRSA). The majority of the erythromycin-resistant isolates harbored the *msrA* gene (four iMLS_B) with the remaining iMLS_B isolate harboring the *ermC* gene.

Conclusion The presence of MRSA isolates which are also iMLS_B in healthy individuals suggests that nasal carriage may play a role as a potential reservoir for the transmission of these pathogens.

Keywords Leukocidin, *pvl*-positive MSSA, MLS_B, *pvl*-positive MRSA.

Introduction

Staphylococcus aureus is a Gram positive and coagulase producing bacterium usually

considered as a normal flora or commensal of the skin and nasal cavities of humans and various animal species.¹ Previous studies have shown that

Received: 09 November 2017; revised: 17 December 2017; accepted: 25 December 2017.

¹MSc, BSc, Dip. Med. Lab Tech. Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, School of Animal Science, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia; ²MSc, Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; ³MSc, Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; ⁴PhD, Faculty of Medicine, Universiti Sultan Zainal Abidin, Medical Campus, Kuala Terengganu, Terengganu, Malaysia; ⁵MPath, Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor,

Malaysia; ⁶PhD, Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; ⁷PhD, Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; ⁸PhD, Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Laboratory of Halal Science Research, Halal Products Research Institute, Universiti Putra Malaysia, 43400 Serdang, Malaysia.

*Corresponding author: Suhaili Zarizal, MSc, BSc, Dip. Med. Lab Tech. Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, School of Animal Science, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia. zarizalsuhaili@yahoo.com

20-50% of the human population are often colonized with *S. aureus*.^{2,3} Methicillin-resistant *S. aureus* (MRSA) is one of the major risk pathogens associated with evolution of antimicrobial resistance. Additionally, antibiotics from the macrolide-lincosamide-streptogramin B (MLS_B) family are usually drugs of choice for the treatment of staphylococcal infections. However, resistance to erythromycin (a macrolide) is usually associated with resistance to clindamycin (a lincosamide) and to type B streptogramin, and this cross-resistance is normally mediated by the erythromycin ribosomal methylase (*erm*) genes.⁴ Three MLS_B phenotypes are typically identified in staphylococci: a constitutive resistant (cMLS_B) phenotype, an inducible clindamycin resistant (iMLS_B) phenotype, and a clindamycin-susceptible, macrolide-streptogramin B-resistant (MS) phenotype. It is difficult to detect the iMLS_B phenotype in routine laboratory testing as they appear clindamycin-susceptible and erythromycin resistant in vitro. This false susceptibility to clindamycin in strains with the iMLS_B phenotype may lead to therapeutic failure.⁴

PVL is a bi-component pore-forming toxin with the ability to destroy and lyse leukocytes.⁵ The presence and emergence of *pvl*-positive MRSA has been previously described in clinical isolates or hospital-associated MRSA (HA-MRSA).⁶ A key feature of community-acquired (CA) MRSA is the presence of SCCmec types IV and V, as well as (in some cases) their co-existence with the *pvl* gene.⁷ Recently, MRSA infection is no longer confined to patients with known risk factors or exposed to healthcare settings. Emergence of CA-MRSA has been documented from several regions across the globe and imposes important clinical implications especially among patients without traditional risk factors.⁸

Previous studies reported that *pvl*-positive MSSA strains have been associated with outbreaks of skin and soft tissue infections (SSTI) among communities as well as from nosocomial

infection. The *pvl* prevalence in MSSA infections varies between countries and is reported with higher prevalence in African countries.⁹ Interestingly, there is evidence showing that *pvl*-positive *S. aureus* strains, particularly MSSA, may emerge and become *pvl*-positive MRSA by acquiring the SCCmec elements comprising the *mecA* gene through horizontal gene transfer, thus conferring the methicillin-resistance capability.¹⁰

Thus, the presence and emergence of MSSA-*pvl*-positive isolates among healthy nasal carriers should not be neglected. Unfortunately, reports on the prevalence of *pvl* among *S. aureus* isolated from healthy individuals in the community in Malaysia are lacking. Here, we described the antimicrobial susceptibility profiles and carriage of resistance and virulence determinants in a collection of *S. aureus* strains isolated from healthy students in a major tertiary university in Malaysia. Interestingly we observed the occurrence of *S. aureus* isolates that showed inducible MLS_B phenotype, were *pvl*-positive and harbored several other virulence genes. Selected isolates were characterized by SCCmec typing, staphylococcal protein A (*spa*) typing and accessory regulator (*agr*) typing for comprehending their genetic background.

Methods

Bacterial culture and growth maintenance

A total of 49 *S. aureus* isolates, previously collected from the anterior nares of 200 healthy undergraduate students from the Health Sciences program, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia in 2012 (n=30) and 2013 (n=19) were viable for further analysis in this study. The isolates had been kept in 15% glycerol stock at -20°C and revived on mannitol salt phenol red agar (MSA, Oxoid, Lenexa, KS, USA) and incubated at 37°C for 24 hours. Presumptive *S. aureus* colonies with yellowish colonies were further subcultured onto nutrient agar supplemented with 3.5% NaCl prior to further phenotypic identification. Confirmation of presumptive *S. aureus* strains was performed by Gram staining, determination of the presence of coagulase and catalase activity as well as genotypic

identification by using *nuc*-PCR.¹¹ All *nuc*-positive *S. aureus* were further characterized as MRSA by PCR detection of *mecA* gene as described previously.¹¹ Positive controls were used for genotypic identification including MRSA ATCC 33591, MSSA ATCC 22932, IMR S5-*pvl*-positive and an in-house MSSA KT/312045 isolate that was previously characterized as *pvl*-positive.¹²

Antibiotic susceptibility test

Kirby-Bauer disc diffusion was used to identify the susceptibility profiles of the *S. aureus* isolates using the panel of antibiotics as suggested previously by Magiorakos et al.¹³ (Table 1) and interpreted using the breakpoints set by the Clinical and Laboratory Standards Institute (CLSI, 2016)¹⁴ and British Society for Antimicrobial Chemotherapy (BSAC).¹⁵ Determination of the MLS_B phenotype for the erythromycin resistant isolates was performed using the double-diffusion test (D-test) based on the CLSI (2016) guidelines.

Molecular detection of genes encoding virulence and antimicrobial resistance

The presence of several resistance and virulence genes in the *S. aureus* isolates was determined by PCR. The following genes conferring resistance to the respective antibiotics were screened: *blaZ* (penicillin), *mupA*, *ileS* (mupirocin), *aac(6')-aph(2'')* (gentamicin), *ermA*, *ermB*, *ermC*, *msrA* (erythromycin), *vanA*, *vanB* (vancomycin), *cfr* (linezolid), *tetA*, *tetB* (tetracycline), and *fusB*, *fusC*, *fusD* (fusidic acid).^{16,17} Additionally PCR was also performed to detect 17 different virulence genes for enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *sei*, *sej*), exfoliative-toxins (*eta*, *etb*, *etd*), cytotoxin (*lukS/lukF*), and adhesins (*hla*, *hlg*, *ica*, *efb*, *sasX*).^{6,16,18} Amplification products were separated by electrophoresis in a 1.5% (w/v) agarose gel (Promega, USA) in a 1X tris-borate-EDTA (TBE) running buffer (Bioline, London, UK). Following staining in a 1 µg/mL GelRed solution (10 mg/mL) (Biothium, Fremont, CA, USA), the PCR products were visualized and documented using an Alpha Imager gel

documentation system (Thermo Fisher Scientific, Waltham, MA, USA). A 100 bp molecular weight marker (Bioline Hyperladder) was electrophoresed alongside samples to enable the size of the PCR products to be compared and determined.

Molecular typing of isolates

Four MRSA isolates were further characterized for SCC_{mec} typing using a multiplex PCR method which generated a clear and easily discriminated pattern for the 5 main SCC_{mec} types (I to V) with specific primers as described by Boye et al.¹⁹ The isolates that showed unrelated patterns were classified as non-typeable (NT). Meanwhile, the same four MRSA with eleven MSSA isolates with either *pvl*-positive or MLS_B phenotypes were further characterized for *spa* and *agr* group typing. The polymorphic X-region of the *spa* gene was amplified and submitted to 1st Base Laboratory, Malaysia, for sequencing services and assembled using CLC Workbench Version 5.0 (CLCbio, Boston, MA, USA) prior to being imported and analyzed with DNAgear.²⁰ *Spa* types were determined using the *spa* database website (<http://spaserver.ridom.de>). Meanwhile typing of the accessory gene regulator (*agr*) group was also performed as previously described using a multiplex PCR primer set, comprising a common forward primer (Pan) and reverse primers (*agr1*, *agr2*, *agr3* and *agr4*) that were specific to each *agr* group.²¹

Statistical analysis

The frequencies of *S. aureus* isolates as well as susceptibility towards antibiotics were counted and the resistance percentages were calculated by 95% confidence interval. The correlation between isolates and both resistance and virulence genes was determined by Chi-square and Fisher's exact test. Statistical significance was identified as $p < 0.05$. All statistical analysis was performed by using JMP 10.1 (SAS Institute, Cary, NC, USA).

Ethical approval

Informed written and signed consents were obtained from recruited volunteers. The study

and method of obtaining consent were approved by the University Research Ethics Committee of the Universiti Putra Malaysia (JKEUPM) Ref: UPM/FPSK/100-9/2-JKEUP(JSB(U)_Dec (12)07).

Results

Bacterial characterization and antibiotics susceptibility testing

All forty-nine presumptive *S. aureus* isolates that produced yellow colonies were confirmed as *S. aureus* genotypically by *nuc*-PCR identification. The percentage of *S. aureus* carriers among healthy undergraduate students was (24.5%). Four out of 49 (8%) isolates were positive for the *mecA* gene and thus categorized as MRSA, while the remaining 45 isolates (95%) were MSSA. Antimicrobial susceptibility testing indicated that all isolates were resistant to penicillin whereas resistance rates to cefoxitin and oxacillin were much lower, at 8% each (or 4/49 isolates).

All isolates were susceptible to vancomycin, teicoplanin, linezolid, ciprofloxacin, norfloxacin, mupirocin, fusidic acid, rifampicin, tigecycline, doxycycline, tetracycline, gentamicin, trimethoprim/sulfamethoxazole, moxifloxacin, chloramphenicol, quinupristin/dalfopristin and fosfomycin (Table 1). Five (11%) out of 45 MSSA isolates were erythromycin resistant followed by clindamycin at 4% (2/45 isolates). Among five erythromycin-resistant MSSA isolates, iMLS_B was observed in two isolates, cMLS_B also in two isolates, and MS in the remaining isolate (Table 2). Two (4%) MSSA isolates were multidrug-resistant (MDR) with resistance towards 3 antimicrobial classes (penicillin, erythromycin and clindamycin). Meanwhile for MRSA, three (75%) out of four isolates were erythromycin resistant with iMLS_B phenotypes, and there were no MDR isolates detected.

Molecular typing

In this study, four methicillin-resistant *S. aureus* and 11 MSSA isolates with either erythromycin-resistant MLS_B phenotypes (iMLS_B or cMLS_B) or *pvl*-positive, or both MLS_B and *pvl*-positive, were selected. As shown in Table 2, the four MRSA isolates showed only two SCC_{mec}

Table 1. List of antibiotics used and susceptibility pattern of 49 isolates of *S. aureus*

Antibiotic agents		Resistant n (%)	Susceptible n (%)
PEN	Penicillin	49 (100%)	0 (0%)
FOX	Cefoxitin	4 (8%)	45 (92%)
OX	Oxacillin	4 (8%)	45 (92%)
E	Erythromycin	8 (16%)	41 (84)
DA	Clindamycin	2 (4%)	47 (96%)
DO	Doxycycline	0 (0%)	49 (100%)
TE	Tetracycline	0 (0%)	49 (100%)
CN	Gentamicin	0 (0%)	49 (100%)
SXT	Trimethoprim/ sulfamethoxazole	0 (0%)	49 (100%)
CIP	Ciprofloxacin	0 (0%)	49 (100%)
MXF	Moxifloxacin	0 (0%)	49 (100%)
TEC	Teicoplanin	0 (0%)	49 (100%)
VA	Vancomycin	0 (0%)	49 (100%)
C	Chloramphenicol	0 (0%)	49 (100%)
QD	Quinupristin/ dalfopristin	0 (0%)	49 (100%)
LZD	Linezolid	0 (0%)	49 (100%)
*TGC	Tigecycline	0 (0%)	49 (100%)
*FD	Fusidic acid	0 (0%)	49 (100%)
*FOS	Fosfomycin	0 (0%)	49 (100%)
MUP	Mupirocin	0 (0%)	49 (100%)
Total		49 isolates	

All antimicrobial susceptibility breakpoint interpretation was performed according to CLSI 2016 except for *Breakpoint guideline BSAC (2014).

types, whereas three of the MRSA isolates harbored SCC_{mec} I (75%) and the remaining one had SCC_{mec} III (25%). Furthermore, a total of 11 different *spa* types were identified in both MSSA and MRSA isolates, where only two MSSA isolates were of the same *spa* type (t548) albeit from different *agr* types (type I and IV). Nevertheless, three isolates (two MSSA and one MRSA) were non-typeable. Meanwhile, three *agr* genotypes were observed where the most frequent *agr* type was *agr* III (54%), followed by *agr* I (33%) and *agr* IV (13%) (Table 2). No *agr* type II was observed in whole isolates. The results also indicated that three of the MRSA isolates that were typed with SCC_{mec} I were of *agr* type III while the remaining MRSA isolate which

Table 2. Details of phenotypic, virulence and resistance determinants of 15 selected isolates of *S. aureus*

<i>S. aureus</i> isolate	Year	Phenotypic resistance profiles	MLS _B phenotype	AMR genes	Virulence genes	<i>spa</i> type	SCC <i>mec</i> type	<i>agr</i> type
MRSA								
1.	2012	PEN, FOX, OX	-	<i>mecA</i> , <i>blaZ</i>	<i>efb</i> , <i>ica</i> , <i>seg</i> , <i>hla</i> , <i>hlg</i>	t186	III	IV
2.	2012	PEN, FOX, OX, E	iMLS _B	<i>mecA</i> , <i>blaZ</i> , <i>msrA</i>	<i>efb</i> , <i>ica</i> , <i>sec</i> , <i>sei</i> , <i>hla</i> , <i>hlg</i>	t701	I	III
3.	2012	PEN, FOX, OX, E	iMLS _B	<i>mecA</i> , <i>blaZ</i> , <i>msrA</i>	<i>pvl</i> , <i>efb</i> , <i>ica</i> , <i>sec</i> , <i>sei</i> , <i>hla</i> , <i>hlg</i>	t145	I	III
4.	2013	PEN, FOX, OX, E	iMLS _B	<i>mecA</i> , <i>blaZ</i> , <i>msrA</i>	<i>efb</i> , <i>ica</i> , <i>seg</i> , <i>hla</i> , <i>hlg</i>	NT	I	III
MSSA								
1.	2012	PEN	-	<i>blaZ</i>	<i>pvl</i> , <i>efb</i> , <i>ica</i> , <i>sec</i> , <i>sei</i> , <i>hla</i> , <i>hlg</i>	t548	-	IV
2.	2012	PEN	-	<i>blaZ</i>	<i>pvl</i> , <i>efb</i> , <i>ica</i> , <i>sec</i> , <i>seg</i> , <i>hla</i> , <i>hlg</i>	t159	-	III
3.	2012	PEN	-	<i>blaZ</i>	<i>pvl</i> , <i>efb</i> , <i>ica</i> , <i>sea</i> , <i>sei</i> , <i>hla</i> , <i>hlg</i>	t14331	-	I
4.	2012	PEN	-	<i>blaZ</i>	<i>pvl</i> , <i>efb</i> , <i>ica</i> , <i>sec</i> , <i>sei</i> , <i>hla</i> , <i>hlg</i>	t336	-	I
5.	2012	PEN	-	<i>blaZ</i>	<i>pvl</i> , <i>efb</i> , <i>ica</i> , <i>sec</i> , <i>seg</i> , <i>hla</i> , <i>hlg</i>	t548	-	I
6.	2012	PEN	-	<i>blaZ</i>	<i>pvl</i> , <i>efb</i> , <i>ica</i> , <i>sec</i> , <i>hla</i> , <i>hlg</i>	t026	-	III
7.	2012	PEN, E	iMLS _B	<i>blaZ</i> , <i>msrA</i>	<i>pvl</i> , <i>efb</i> , <i>ica</i> , <i>hla</i> , <i>hlg</i>	t6290	-	I
8.	2013	PEN, E, DA	cMLS _B	<i>blaZ</i> , <i>msrA</i>	<i>efb</i> , <i>ica</i> , <i>hla</i> , <i>hlg</i>	NT	-	III
9.	2013	PEN, E	MS	<i>blaZ</i>	<i>efb</i> , <i>ica</i> , <i>hla</i> , <i>hlg</i>	t1381	-	III
10.	2013	PEN, E, DA	cMLS _B	<i>blaZ</i> , <i>msrA</i>	<i>efb</i> , <i>ica</i> , <i>hla</i> , <i>hlg</i>	t14503	-	III
11.	2013	PEN, E	iMLS _B	<i>blaZ</i> , <i>ermC</i>	<i>efb</i> , <i>ica</i> , <i>hla</i> , <i>hlg</i>	NT	-	I

AMR – antimicrobial resistance gene; cMLS_B – constitutive macrolide-lincosamide-streptogramin B resistant; DA – clindamycin; E – erythromycin; FOX – ceftiofur; iMLS_B – inducible macrolide-lincosamide-streptogramin B resistant; MS – macrolide-streptogramin B-resistant; NT – non-typeable; OX – oxacillin; PEN – penicillin.

contained SCC*mec* III was of *agr* type IV. Nevertheless, *agr* types I and III were predominant among MSSA isolates (33% each) and one MSSA isolate was of *agr* type IV.

Prevalence of antimicrobial resistance and virulence determinants

The 15 isolates subjected earlier to molecular typing were further screened for resistance and virulence determinants using PCR as indicated in Table 3. All isolates were positive for the *blaZ* gene, which was not surprising as all the isolates were penicillin resistant, there were no significant differences between MRSA and MSSA ($p=1$).

For the eight erythromycin-resistant isolates, most of them (6/8) harbored the *msrA* gene (four iMLS_B and two cMLS_B). Only one erythromycin-

resistant isolate, an iMLS_B MSSA, was positive for the *ermC* gene. Intriguingly, the single erythromycin-resistant MSSA strain that displayed the MS phenotype did not show any positive results for the macrolide-resistance genes that were screened by PCR in this study (i.e., *ermA*, *ermB*, *ermC* and *msrA*) (Table 2). All 15 isolates screened were negative for the other antibiotic resistance genes, which correlated well with their susceptibility phenotypes: vancomycin (*vanA* and *vanB* genes), mupirocin (*mupA*, *ileS*), gentamicin (*aac(6')*-*aph(2'')*), linezolid (*cfr*), tetracycline (*tetA*, *tetB*), and fusidic acid (*fusB*, *fusC*, *fusD*). There were no significant differences between MRSA and MSSA for *blaZ* ($p=1.000$), *ermC* ($p=1.000$) and *msrA* ($p=0.235$).

Table 3. Antimicrobial resistance and virulence gene distribution among MRSA and MSSA isolates

Gene	Antimicrobial resistance genes			OR	*95%CI	p-value
	MRSA n=4	MSSA n=11	Total n=15			
<i>mecA</i>	4	0	4 (27%)	-	--	<0.05
<i>blaZ</i>	4	9	13 (87%)	-	--	1
<i>ermA</i>	0	0	0	-	--	-
<i>ermB</i>	0	0	0	-	--	-
<i>ermC</i>	0	1	1 (6%)	-	--	1
<i>msrA</i>	3	3	6 (40%)	8	0.58-110	0.24
<i>aac(6')-aph</i>	0	0	0	-	-	-
<i>cfr</i>	0	0	0	-	-	-
<i>fusB</i>	0	0	0	-	-	-
<i>fusC</i>	0	0	0	-	-	-
<i>fusD</i>	0	0	0	-	-	-
<i>ileS</i>	0	0	0	-	-	-
<i>tetA</i>	0	0	0	-	-	-
<i>tetB</i>	0	0	0	-	-	-
<i>vanA</i>	0	0	0	-	-	-
<i>vanB</i>	0	0	0	-	-	-

Gene	Virulence genes			OR	*95%CI	p-value
	MRSA n=4	MSSA n=11	Total n=15 (%)			
<i>pvl</i>	1	7	8 (53)	0.58	0.04-7.66	1
<i>sea</i>	0	1	1 (7)	0	--	1
<i>seb</i>	0	0	0	-	-	-
<i>sec</i>	2	2	4 (27)	4.5	0.37-54	0.52
<i>sed</i>	0	0	0	-	-	-
<i>see</i>	0	3	3 (20)	0	--	0.52
<i>seg</i>	2	2	4 (27)	4.5	0.37-54	0.52
<i>sei</i>	2	3	5 (33%)	2.7	0.25-28	0.56
<i>sej</i>	0	0	0	-	-	-
<i>efb</i>	4	11	15 (100)	-	--	1
<i>ica</i>	4	11	15 (100)	-	--	1
<i>hla</i>	4	11	15 (100)	-	--	1
<i>hlg</i>	4	11	15 (100)	-	--	1
<i>eta</i>	0	0	0	-	-	-
<i>etb</i>	0	0	0	-	-	-
<i>etd</i>	0	0	0	-	-	-
<i>sasX</i>	0	0	0	-	-	-

95%CI – 95% confidence interval; OR – odds ratio.

Eight (16%) *S. aureus* isolates harbored the cytotoxin *pvl* gene, of which only one was MRSA and the other seven were MSSA isolates. All 15 isolates were positive for four (*efb*, *ica*, *hla* and *hlg*) out of six adhesion genes as shown in Table 2. Additionally, for enterotoxin genes, ten (67%) out of 15 isolates harbored at least one or two

staphylococcal enterotoxin (SE) genes. Most of the SE genes were *sei* (33% or 5/15 isolates) followed by *sec* (27% or 4/15 isolates), *seg* (27% or 4/15 isolates), *see* (20% or 3/15 isolates) and *sea* (7% or 1/15 isolates). Among the four MRSA, two isolates harbored only *seg* whereas the other two isolates harbored both the *sec* and

sei genes. Meanwhile, one MSSA isolate (ND19) was positive for only the *see* gene, while five other MSSA isolates were positive for a combination of either *sea*+*sei* (ND11), *sec*+*sei* (ND6 and ND13) or *see*+*seg* (ND10 and ND15) genes. Furthermore, all MSSA isolates were negative for all SEs genes. Nevertheless, no adhesion (*sasX*), exfoliative-toxin (*eta*, *etb* and *etd*) and enterotoxin (*tsst*) genes were detected among all 15 isolates tested.

Discussion

S. aureus is a major pathogen with a high virulence that causes both HA and CA infections globally, including skin and soft tissue infection with varying severity, from mild to life-threatening infections such as sepsis and bacteremia.¹⁸ This study showed that the MRSA proportion was only 8%, which is similar to a recent study at a similar setting in nasal isolates.² The proportion of MRSA carriage in our collection was in concordance with other worldwide studies investigating the prevalence of MRSA carriage that varied within 0.34 to 12% rates in medical students and healthcare centres.^{2,3,22}

Among the four MRSA strains, three isolates were detected as SCCmec I and one isolate was identified as SCCmec III. Although the number of MRSA in this study is low, the predominant occurrence of SCCmec I from this study is consistent with a previous study in a similar setting by Mat Azis et al., indicating the persistency of MRSA with SCCmec I.² Similarly, another previous study reported a high prevalence of SCCmec I (68%) among all MRSA isolates from nares of healthcare workers in Nepal.²³ Additionally, *spa* typing showed a diverse genetic background of the isolates in this collection. Of the 11 different *spa* types identified, *spa* type t548 was the predominant clone involving two MSSA isolates with different *agr* types and *pvl* positive character. The associated sequence type (ST) for the *spa* type t548 was ST-5 and ST-97. Similarly, other clinical MRSA isolates positive for the *pvl* gene also shared the same *spa* type, t548.²⁴ Furthermore, one of our *pvl*-positive MRSA isolates with *spa* type t701 (SCCmec I) was also

reported as the most frequently identified MRSA in Iran, except for the SCCmec III, which is different from our isolates.²⁵ Meanwhile another study in Malaysia, reported the same *spa* type, t701, among nasal *S. aureus* isolates from persistent carriers.² On the other hand, three non-typeable (NT) *spa* strains were detected in this study, either due to *spa* mutations or due to absent *spa*. However, NT *spa* strains may still have the potential to induce invasive disease.²⁶

The predominant *agr* genotype in this collection of isolates among MRSA and MSSA was *agr* type III (54%), the majority of this *agr* were *pvl*-positive, which is in agreement with other reports indicating the predominance of the same *agr* type among *S. aureus*.²¹ Most of our *agr* type I MSSA isolates were positive for the *pvl* gene and this is in contrast with a previous study conducted by Afroz et al., indicating that the presence of *pvl* among MSSA was clustered within *agr* type II and III.²⁷ Our results also revealed that *agr* type IV only existed in two isolates (13%) of MRSA and MSSA strains. Likewise, a previous study showed that this type of *agr* IV is an uncommon feature in *S. aureus* isolates.²⁸

Our isolates collection showed the presence of *pvl*-positive isolates (16%), where one out of eight were MRSA, while the remaining were harbored in MSSA. Our findings showed a higher proportion of *pvl*-positive isolates compared with previous studies in Malaysia that reported 5.5% *pvl*-positive MSSA among pigs and pigs handler samples, conducted by Neela et al.²⁹ Neela et al. also stated that the prevalence of *pvl*-positive isolates in Malaysian carriage displayed an increasing pattern, from 5.5% to 16%, indicating that the community-*pvl* is able to cause MSSA infections worldwide. Community-associated *S. aureus* surveillance must not be underestimated, since a high prevalence of *pvl*-positive MSSA has been reported in our nearest neighboring countries such as Indonesia and Thailand, meanwhile a low prevalence of *pvl* among MRSA was reported in Indonesia.^{8,30}

Moreover, in this collection of isolates, the classical SEs (*sea* - *see*) as well as *seg*, *sei* and *sej*

were screened in MRSA and MSSA. Four MRSA isolates harbored at least one or two SEs, while six MSSA harbored two SEs and only one isolate carried a single SE. The presence of SEs among nasal isolates become a public health concern as these genes are often associated with foodborne poisoning, toxic shock syndrome and various other toxin-mediated diseases.¹⁶ In this present study, the highest rate of SE was *sei* with 33%, which differed from other studies that reported that the *sea* gene was common among *S. aureus* isolates from nasal samples, while another report found a high prevalence of the *sei* gene from invasive strains of both MRSA and MSSA.³¹ The occurrence of the *sea* gene in this collection was low at 7% compared with other studies previously reported elsewhere.^{18,32} Additionally, our results also showed that eight (53%) (4 MRSA and 4 MSSA) isolates were positive for either *sec* or *seg*. Previous reports also showed the presence of *sec* among the clinical MRSA isolates from Terengganu referral Hospital, east coast of Malaysia, involving 2 MRSA and 3 MSSA isolates.¹⁸ Although both *seg* and *sei* genes are located in the same enterotoxin gene cluster (*egc*) operon, all of our isolates did not harbor both genes simultaneously, which is similar to data reported by other researchers.³¹ Likewise, none of the *eta*, *etb* and *etd* genes were detected among all tested isolates; similar results were also reported elsewhere.³³

The major limitation of this study was that the bacterial sample collection was not constructed in a systematic consecutive manner, and some volunteers did not allow the use of further details on demographic as well as retrospective data examination. Therefore, this study does not represent a prevalence study but it shows the characterization of the 49 isolates with a further analysis on 15 isolates that carried antimicrobial resistance and virulence genes of interest only. Nevertheless, as inclusion of isolates is random and no bias can be reasonably expected, the proportion may reflect some epidemiological perspective to a certain extent in providing insight into the distribution of antimicrobial susceptibility occurrences with both resistance as well as virulence genes

determinants in MRSA and MSSA. Thus, data obtained here can be used for monitoring the presence of virulence and resistance determinant genes in *S. aureus* isolated from nasal carriers.

Conclusions

Interestingly, the presence of *pvl*-positive MSSA carriage and MLS_B phenotypes in our study suggests that nasal carriage may play an important role as potential reservoir and transmission of these pathogens in posing disease threat regardless of methicillin resistance. Therefore, although the risk of human infection with both MRSA and MSSA from nasal carriage is often considered rare, its potential risk to the public health should not be undermined.

Authors' contributions statement: MNMD conceived and designed the study. ZS, NMA, ARAR and PAR performed the experiment, samples collection, data analysis and wrote the manuscript. CCY, SAN, MMJA worked on data arrangement, commented and revised the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to disclose.

Funding: This work was supported by the Research University Grant Scheme, Universiti Putra Malaysia [grant number: 9455000]; the Fundamental Research Grant Scheme, Ministry of Higher Education, Malaysia [grant number: 5524924] and Universiti Sultan Zainal Abidin Seed Fund Research [grant numbers: UDM/09/BR (009)].

Acknowledgement: The authors would like to thank Mr Sabri in helping and providing on laboratory preparation in Universiti Putra Malaysia. Mrs Syaidatul Najiah for their help in samples preparation in Universiti Sultan Zainal Abidin, Besut campus.

References

1. Grema HA, Geidam YA, Gadzama GB, Ameh JA, Suleiman A. Methicillin resistant *Staphylococcus aureus* (MRSA): a review. *Adv Anim Vet Sci* 2015;3:79-98. [\[Crossref\]](#)
2. Mat Azis N, Pung HP, Abdul Rachman AR, et al. A persistent antimicrobial resistance pattern and limited methicillin-resistance-associated genotype in a short-term *Staphylococcus aureus* carriage isolated from a student population. *J Infect Public Health* 2017;10:156-64. [\[Crossref\]](#)
3. Mat Azis N, Hamid AAB, Pung HP, et al. *Staphylococcus aureus* infection risk in a population of health sciences students at a public university. *Iran J Public Health* 2014;43:112-6.

4. Jarajreh D, Aqel A, Alzoubi H, Al-Zereini W. Prevalence of inducible clindamycin resistance in methicillin-resistant *Staphylococcus aureus*: the first study in Jordan. *J Infect Dev Ctries* 2017;11: 350-354. [\[Crossref\]](#)
5. Boyle-Vavra S, Daum RS. Community-acquired methicillin-resistant *Staphylococcus aureus*: the role of Panton-Valentine leukocidin. *Lab Invest* 2007;87:3-9. [\[Crossref\]](#)
6. Lina G, Piémont Y, Godail-Gamot F, et al. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* 1999;29:1128-32. [\[Crossref\]](#)
7. Rong D, Wu Q, Xu M, Zhang J, Yu S. Prevalence, virulence genes, antimicrobial susceptibility, and genetic diversity of *Staphylococcus aureus* from retail aquatic products in China. *Front Microbiol* 2017;8:714. [\[Crossref\]](#)
8. Nickerson EK, Wuthiekanun V, Kumar V, et al. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* carriage in children in Cambodia. *Am J Trop Med Hyg* 2011;84:313-7. [\[Crossref\]](#)
9. Breurec S, Fall C, Pouillot R, et al. Epidemiology of methicillin-susceptible *Staphylococcus aureus* lineages in five major African towns: high prevalence of Panton-Valentine leukocidin genes. *Clin Microbiol Infect* 2011;17:633-9. [\[Crossref\]](#)
10. Rasigade J, Laurent F, Lina G, et al. Global distribution and evolution of Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus*, 1981-2007. *J Infect Dis* 2010;201:1589-97. [\[Crossref\]](#)
11. Saiful AJ, Mastura M, Zarizal S, Mazurah MI, Shuhaimi M, Ali AM. Detection of methicillin-resistant *Staphylococcus aureus* using *mecA/nuc* genes and antibiotic susceptibility profile of Malaysian clinical isolates. *World J Microbiol Biotechnol* 2006;22:1289-94. [\[Crossref\]](#)
12. Suhaili Z, Lean SS, Mohamad NM, Rachman AR, Desa MN, Yeo CC. Draft genome sequence of *Staphylococcus aureus* KT/312045, an ST1-MSSA PVL positive isolated from pus sample in East Coast Malaysia. *Genom Data* 2016;9:111-2. [\[Crossref\]](#)
13. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18:268-81. [\[Crossref\]](#)
14. The Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing CLSI supplement M100S. Clinical and Laboratory Standards Institute, Wayne, PA. 2016.
15. Andrews JM, BSAC Working Party on Susceptibility Testing. BSAC standardized disc susceptibility testing method (version 7). *J Antimicrob Chemother* 2008;62:256-78. [\[Crossref\]](#)
16. Ma Y, Zhao Y, Tang J, Tang C, Chen J, Liu J. Antimicrobial susceptibility and presence of resistance & enterotoxins/enterotoxin-like genes in *Staphylococcus aureus* from food. *CyTA - J Food* 2017;16:76-84. [\[Crossref\]](#)
17. Castanheira M, Watters AA, Bell JM, Turnidge JD, Jones RN. Fusidic acid resistance rates and prevalence of resistance mechanisms among *Staphylococcus* spp. isolated in North America and Australia, 2007-2008. *Antimicrob Agents Chemother* 2010;54:3614-7. [\[Crossref\]](#)
18. Lim KT, Hanifah YA, Mohd Yusof MY, Thong KL. Investigation of toxin genes among methicillin-resistant *Staphylococcus aureus* strains isolated from a tertiary hospital in Malaysia. *Trop Biomed* 2012;29:212-9.
19. Boye K, Bartels MD, Andersen IS, Møller JA, Westh H. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCCmec types I-V. *Clin Microbiol Infect* 2007;13:725-7. [\[Crossref\]](#)
20. AL-Tam F, Brunel AS, Bouzinbi N, Corne P, Bañuls AL, Shahbazkia HR. DNAGear-a free software for *spa* type identification in *Staphylococcus aureus*. *BMC Res Notes*. 2012;5:642. [\[Crossref\]](#)
21. Ben Ayed A, Boutiba-Ben Boubaker I, Ennigrou S, Ben Redjeb S. Accessory gene regulator (*agr*) typing of *Staphylococcus aureus* isolated from human infections. *Arch Inst Pasteur Tunis* 2008;85:3-8.
22. Orlin I, Rokney A, Onn A, Glikman D, Peretz A. Hospital clones of methicillin-resistant *Staphylococcus aureus* are carried by medical students even before healthcare exposure. *Antimicrob Resist Infect Control* 2017;6:15. [\[Crossref\]](#)
23. Joshi PR, Acharya M, Aryal R, et al. Emergence of staphylococcal cassette chromosome *mec* type I with high-level mupirocin resistance among methicillin-resistant *Staphylococcus aureus*. *Asian Pac J Trop Biomed* 2017;7:193-7. [\[Crossref\]](#)
24. Vali L, Dashti AA, Mathew F, Udo EE. Characterization of heterogeneous MRSA and MSSA with reduced susceptibility to chlorhexidine in Kuwaiti hospitals. *Front Microbiol* 2017;8:1359. [\[Crossref\]](#)
25. Mohammadi S, Sekawi Z, Monjezi A, et al. Emergence of SCCmec type III with variable antimicrobial resistance profiles and *spa* types among methicillin-resistant *Staphylococcus aureus* isolated from healthcare- and community-acquired infections in the west of Iran. *Int J Infect Dis* 2014;25:152-8. [\[Crossref\]](#)
26. Baum C, Haslinger-Löffler B, Westh H, et al. Non-*spa*-typeable clinical *Staphylococcus aureus* strains are naturally occurring protein A mutants. *J Clin Microbiol* 2009;47:3624-9. [\[Crossref\]](#)
27. Afroz S, Kobayashi N, Nagashima S, et al. Genetic characterization of *Staphylococcus aureus* isolates carrying Panton-Valentine leukocidin genes in Bangladesh. *Jpn J Infect Dis* 2008;61:393-6.
28. Manago K, Nishi J, Wakimoto N, et al. Biofilm formation by and accessory gene regulator typing of methicillin-resistant *Staphylococcus aureus* strains

-
- recovered from patients with nosocomial infections. Infect Control Hosp Epidemiol 2012;27:187-90.
29. Neela V, Ehsanollah GR, Zambari S, Van Belkum A, Mariana NS. Prevalence of Panton-Valentine leukocidin genes among carriage and invasive *Staphylococcus aureus* isolates in Malaysia. Int J Infect Dis 2009;13:e131-2. [\[Crossref\]](#)
 30. Santosaningsih D, Santoso S, Budayanti NS, et al. Epidemiology of *Staphylococcus aureus* harboring the *mecA* or Panton-Valentine leukocidin genes in hospitals in Java and Bali, Indonesia. Am J Trop Med Hyg 2014;90:728-34. [\[Crossref\]](#)
 31. Becker K, Friedrich AW, Lubritz G, Weilert M, Peters G, Von Eiff C. Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. J Clin Microbiol 2003;41:1434-9. [\[Crossref\]](#)
 32. Chmielarczyk A, Pomorska-Wesołowska M, Szczypta A, Romaniszyn D, Pobiega M, Wójkowska-Mach J. Molecular analysis of methicillin-resistant *Staphylococcus aureus* strains isolated from different types of infections from patients hospitalized in 12 regional, non-teaching hospitals in southern Poland. J Hosp Infect 2017;95:259-67. [\[Crossref\]](#)
 33. Koosha RZ, Fooladi AA, Hosseini HM, Aghdam EM. Prevalence of exfoliative toxin A and B genes in *Staphylococcus aureus* isolated from clinical specimens. J Infect Public Health 2014;7:177-85. [\[Crossref\]](#)

Please cite this article as:

Suhaili Z, Rafee PA, Mat Azis N, Yeo CC, Nordin SA, Abdul Rahim AR, Al-Obaidi MMJ, Mohd Desa MN. Characterization of resistance to selected antibiotics and Panton-Valentine leukocidin-positive *Staphylococcus aureus* in a healthy student population at a Malaysian University. GERMS 2018;8(1):21-30. doi: 10.18683/germs.2018.1129