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Distribution of *sasX*, *qacA/B* and *mupA* genes and determination of genetic relatedness of methicillin-resistant *Staphylococcus aureus* among clinical isolates and nasal swab samples from the same patients in a hospital in Malaysia

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ABSTRACT

Introduction: This study determined the distribution of *sasX*, *qacA/B* and *mupA* genes from methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from clinical samples and nasal swab samples of the same patients and analysed their genetic relatedness.

Methods: Polymerase chain reaction (PCR) was used to detect the presence of *sasX*, *qacA/B* and *mupA* genes from 47 paired MRSA isolates. A paired isolate was defined as one nasal swab (colonising) isolate and clinical isolate that caused infection in the same patient. 22 selected paired isolates were subjected to multilocus sequence typing (MLST). The genetic relatedness among the isolates and association between the putative genes with epidemic sequence types (STs) were investigated.

Results: 7 (14.9%, n = 14) paired isolates were positive for the *sasX* gene. *qacA/B* genes were positive in 7.4% (n = 7) of the isolates, from three paired isolates and one clinical isolate whose paired colonising isolate was negative. The paired sample of three patients were positive for both genes. The *mupA* gene was not detected in all the isolates. MLST revealed two epidemic STs, ST22 and ST239, and a novel ST4649. *sasX* and *qacA/B* genes were found in ST239 in 29.5% (n = 13) and 13.6% (n = 6) of cases, respectively. Gene co-existence occurred in 13.6% (n = 6) of MRSA ST239 and 2.3% (n = 1) of MRSA ST4649.

Conclusion: *sasX* and *qacA/B* genes were present in the MRSA isolates, while the *mupA* gene was undetected. ST22 and ST239 were the major MRSA clones. The circulating MRSA genotypes conferred different virulence and resistance determinants in our healthcare settings.

Keywords: clones, MRSA, nucleotide gene, sequence type

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen of global concern, causing both healthcare-associated and community-acquired infections. Anterior nares have been discovered as the main reservoir for MRSA.⁽¹⁾ Colonising strains may serve as endogenous reservoirs for overt clinical infections or may spread to other patients and lead to infection.⁽²⁾ In relation to this, the emerging virulent factor of the *sasX* gene in MRSA strains may encourage MRSA colonisation in the anterior nares. *sasX* is among the newly described surface-anchored *S. aureus*-binding proteins that interface with human matrix molecules, MSCRAMMs (microbial surface components recognising adhesive matrix molecules).⁽³⁾ Besides nasal colonisation, *sasX* contributes to biofilm formation and immune evasion mechanism. *sasX*-positive MRSA was reported to be a potential cause of serious diseases such as lung disease and abscess formation.⁽⁴⁾ Chlorhexidine gluconate is one of the chemical agents used for decolonisation of MRSA.⁽⁵⁾ The presence of the *qacA/B* gene is related to elevated minimum bactericidal concentrations for chlorhexidine and failures in MRSA decolonisation protocols.⁽⁶⁾ Another drug that has been widely used for MRSA decolonisation is mupirocin ointment for local nasal application. The presence of the *mupA* gene is related to high-level mupirocin resistance and has been linked to the failure of mupirocin therapy for patients infected with MRSA.

The presence of both virulence and resistance genes in MRSA-specific clones has not been extensively evaluated. Hence, multilocus sequence typing (MLST) has been performed to study genetic relatedness in MRSA isolates from clinical specimens. One of the sequence types (STs) that was reported to have disseminated to other European countries and developed itself as the dominant clone is MRSA ST22. ST22 has replaced the Brazilian and Iberian clones, which were the predominant clones in Europe.⁽⁵⁾

MRSA ST239 is another strain that can produce exotoxins, causing a broad range of life-threatening infections.⁽⁷⁾ MRSA ST239 is commonly associated with nosocomial infections and

was also reported to be circulating in different countries with distinctive clone identities such as Hungarian, Brazilian, Portuguese and Viennese.⁽⁸⁾ The detection of *sasX*, *qacA/B* and *mupA* genes, as well as the discovery of the circulating MRSA strains carrying specific genes, is significant for treatment plans in the healthcare setting and could intensify currently practised decontamination protocols.

METHODS

A total of 94 MRSA isolates (i.e. paired isolates of nasal swabs and clinical specimens of 47 patients) retrieved from archived isolates from the Microbiology Department, Hospital University Sains Malaysia, in Kelantan, Malaysia, from January 2016 to December 2017 were included in the study. A non-purposive sampling method was used in this study. All confirmed, colonising and clinical MRSA isolates from the same patients within the study period were included in this study. The isolates were from various specimens (Fig. 1).

The isolates were reconfirmed using phenotypic methods such as Gram staining, lysis formation on blood agar and a few biochemical tests including catalase, DNase, tube coagulase and fermentation of mannitol. MRSA isolates were phenotypically confirmed using the cefoxitin susceptibility test according to the standard protocol established by USM Microbiology laboratory diagnostics, while inhibition zone diameter interpretation was based on Clinical and Laboratory Standards Institute (CLSI) 2017 criteria.⁽⁹⁾ The amount of cefoxitin used was 30 mcg with an inhibition zone of ≥ 22 mm, and a minimum inhibitory concentration value of ≤ 4 mcg/mL was read as sensitive.

Genomic DNA extraction from clinical isolates was conducted using the QIAamp DNeasy Blood and Tissue Kit (QIAGEN, Hilden, North Rhine-Westphalia, Germany), according to the manufacturer's instruction with slight modification. MRSA isolates were molecularly confirmed by polymerase chain reaction (PCR) amplification of *femA* and *mecA* genes.⁽¹⁰⁾ ATCC

25923 and ATCC 33591 *S. aureus* strains were used as controls for *femA* and *mecA* gene detection, respectively.

Detection of virulence and resistance genes was conducted using published protocols.⁽¹¹⁻¹³⁾ Positive controls for *sasX* (MK509012) and *qacA/B* (MK542001) genes were obtained from local MRSA isolates. These isolates were characterised and confirmed by sequence analysis. ATCC BAA 1708 was used as the positive control for *mupA* gene detection according to M100, CLSI 2017 (28th edition).⁽⁹⁾

DNA amplification was performed in 25 μ L of PCR mixture (Thermo Fisher Scientific, Vilnius, Vilniaus, Lithuania) consisting of 20 μ M each of forward and reverse primers, 1 U/ μ L *Taq* Polymerase, 160 μ M dNTP mix, PCR water, 10 \times *Taq* buffer with $(\text{NH}_4)_2\text{MgCl}_2$, 25 mM MgCl_2 and 2 μ L DNA template (20 ng/ μ L). The oligonucleotides primers used in this study have been previously described and were synthesised commercially (Integrated DNA Technologies, Singapore). The PCR reaction was performed using the Mastercycler nexus gradient thermal cycler (Eppendorf, Hamburg, Germany). PCR amplification for the respective targets was conducted using published primers (Table I). The gel was stained with Florosafe (Apical Scientific Sdn Bhd, Selangor, Malaysia) and visualised using a gel imaging system (AlphaImager; Alpha Innotec, Kasendorf, Germany).

A total of 22 paired MRSA isolates were selected for MLST study based on sample types and clinical presentations. Seven *S. aureus* housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL*) were amplified using the Mastercycler nexus gradient thermal cycler (Eppendorf). PCR reaction was performed with an initial denaturation at 95°C for five minutes, followed by 30 cycles of denaturation at 95°C for one minute, annealing at 55°C for one minute and extension at 72°C for one minute; and a final extension at 72°C for five minutes. All primer sequences and PCR protocols used were synthesised based on the previous study.⁽¹⁴⁾ The protocol and PCR condition for the respective genes used are shown in Table I. The PCR products were sent for

sequencing analysis (Apical Scientific Sdn Bhd, Selangor, Malaysia), performed bidirectionally. Each sequence contig was submitted to the *Staphylococcus aureus* webpage on PubMLST (<https://pubmlst.org/organisms/staphylococcus-aureus>) for allelic profiles and ST characterisation. The phylogenetic tree was constructed using MEGA 7 software to demonstrate the distribution of STs and correlation of MRSA isolated from nasal swab and clinical sources.⁽¹⁵⁾

Data was analysed using descriptive analysis. All graphs related to the study were constructed and described in terms of percentage and frequency. Ethical clearance was obtained and approval was granted for implementation by the Human Research Ethics Committee (JEPeM) of Universiti Sains Malaysia (study protocol code USM/JEPeM/17010056).

RESULTS

A total of 47 paired MRSA isolates were analysed. The distribution of the isolates from various specimens is shown in Fig. 1. PCR showed that seven paired isolates (14.9%, $n = 14$) were positive for the *sasX* gene. Meanwhile, *qacA/B* genes were positive in 7.4% of the isolates, consisting of three paired isolates and one clinical isolate for which the respective paired colonising isolate was negative. The paired sample of three patients were positive for both genes. None of the isolates was positive for the *mupA* gene. Among 22 selected paired isolates that were subjected to MLST, three distinctive STs including ST22 (68.2%, $n = 30$), ST239 (29.5%, $n = 13$) and a novel ST, ST4649 (2.3%, $n = 1$), were identified. The gene distributions are shown in Tables II and III. The *mupA* gene was absent in all STs. Co-existence of the *sasX* and *qacA/B* genes occurred exclusively in 13.6% ($n = 6$) of MRSA ST239 and 2.3% ($n = 1$) of MRSA ST4649. The association of virulence-related, chlorhexidine resistance and mupirocin resistance genes with different STs of MRSA is shown in Table IV.

Table II. Distinctive sequence types with gene distribution patterns of 44 paired isolates.

Pattern of genes	ST (% , n)
<i>sasX</i> + <i>qacA/B</i> - <i>mupA</i> -	ST239 (15.9%, n = 7)
<i>sasX</i> + <i>qacA/B</i> + <i>mupA</i> -	ST239 (13.6%, n = 6) ST4649 (2.3%, n = 1)
<i>sasX</i> - <i>qacA/B</i> - <i>mupA</i> -	ST22 (68.2%, n = 30)

ST: sequence type

Table III. Gene distribution of MRSA isolates in different sequence types.

ST*	No. (%)	Gene (No. [%])		
		<i>sasX</i>	<i>qacA/B</i>	<i>mupA</i>
ST22	30 (68.2)	0 (0)	0 (0)	0 (0)
ST239	13 (29.5)	13 (29.5)	6 (13.6)	0 (0)
ST4649	1 (2.3)	1 (2.3)	1 (2.3)	0 (0)

MRSA: methicillin-resistant *Staphylococcus aureus*

Table IV. Association of virulence-related *sasX* gene, chlorhexidine resistance *qacA/B* gene and mupirocin resistance *mupA* gene with different sequence types of selected 22 paired MRSA isolates.

Paired sample	Clinical/colonising isolates*	Type of sample	<i>sasX</i>	<i>qacA/B</i>	ST
1	Clinical	Blood	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
2	Clinical	Pus	Detected	Detected	239
	Colonising	Nasal swab	Detected	Detected	239
3	Clinical	ETT	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
4	Clinical	Pleural fluid	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
5	Clinical	ETT	Detected	Detected	239
	Colonising	Nasal swab	Detected	Not detected	239
6	Clinical	Pus	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
7	Clinical	Blood	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
8	Clinical	Blood	Detected	Detected	ST4649a
	Colonising	Nasal swab	Detected	Detected	239
9	Clinical	Sputum	Detected	Not detected	239
	Colonising	Nasal swab	Detected	Not detected	239
10	Clinical	Blood	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
11	Clinical	Tissue	Detected	Not detected	239
	Colonising	Nasal swab	Detected	Not detected	239
12	Clinical	ETT	Detected	Not detected	239
	Colonising	Nasal swab	Detected	Not detected	239
13	Clinical	Wound swab	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22

14	Clinical	Tracheal aspirate	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
15	Clinical	Tracheal aspirate	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
16	Clinical	Tissue	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
17	Clinical	Blood	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
18	Clinical	Tissue	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
19	Clinical	Slough tissue	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
20	Clinical	Sputum	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
21	Clinical	ETT	Not detected	Not detected	22
	Colonising	Nasal swab	Not detected	Not detected	22
22	Clinical	Tracheal aspirate	Detected	Detected	239
	Colonising	Nasal swab	Detected	Detected	239

**Mupirocin resistance gene mupA was not detected in all the tested isolates. Colonising isolates were all from nasal swab sample. ETT: endotracheal tube; ST: sequence type*

The phylogenetic relationship analysis revealed genetic variability among MRSA isolates, which was grouped into two major clades (Clade I and II), as shown in Fig. 2. Two predominant clones identified in this study were ST22 and ST239, while ST4649 was found to be novel, with a unique ST. The phylogenetic tree indicated that the MRSA isolates discovered from both nasal and clinical samples were clonal.

DISCUSSION

Patients who have MRSA nasal colonisation are at high risk of developing subsequent infections.⁽²⁾ The most frequent MRSA isolation in our study was from endotracheal tube (ETT) fluid secretions (17.0%, n = 16). The pathogen was able to colonise ETTs and thrive in biofilm formed on the ETTs of intubated patients. This colonisation could increase the risk of developing ventilator-associated pneumonia following intubation, especially in intensive care units, and

increase the mortality rates of hospitalised patients.⁽¹⁶⁾ Aside from ETT secretions, 7.4% of the MRSA isolated were from blood samples and swab samples (n = 7). In a 2018 study, Lin et al reported that six out of ten patients had paired colonising and clinical isolates (wound culture) from MRSA with one positive methicillin-sensitive *S. aureus* isolated from a patient.⁽¹⁷⁾ Previous studies of MRSA colonisation causing subsequent infections demonstrated that the most common paired clinical isolates were from wounds or abscesses (48 out of 85)⁽¹⁸⁾ and blood (31 out of 217).⁽¹⁹⁾ However, the data is not comparable due to differences in study design.

Involvement of various MRSA virulence genes potentially contributes to its infection severity and endemicity. In our study, 14.9% (n = 14) of the MRSA isolates carried the *sasX* gene. A study done in a China hospital detected the *sasX* gene in 36.7% of cases.⁽²⁰⁾ However, the reported outcomes cannot be used to compare with the current study due to differences in study approach and sample size (94 vs. 610).⁽²⁰⁾ In the present study, the *sasX* gene was detected in isolates from various sample types such as wound swab, ETT secretions, blood, sputum, tracheal aspirate and tissue. One *sasX* gene was detected in ST4649, while others (n = 13) were exclusively from ST239. The finding paralleled those from other reports.^(21,22) Despite the common occurrence of the *sasX* gene in ST239, Nair et al reported in 2013 that the *sasX* gene was not detected in all MRSA isolates, including MRSA ST239, from a Mongolian hospital. However, the contrasting finding might be due to its low prevalence in their hospital.⁽²³⁾

The present study found *qacA/B* genes in 7.4% (n = 7) of the MRSA isolates. In contrast, a recent study conducted in Gansu Provincial Hospital, China, showed that all 85 MRSA isolates were positive for the *qacA/B* gene.⁽²⁴⁾ Additionally, another previous study also reported a high frequency (83.3%, 50 out of 60) of *qacA/B* genes in Malaysia-collected MRSA isolates.⁽²⁵⁾ The association between the *qacA/B* genes with ST was also observed in this study. Our findings showed that *qacA/B* genes were found mostly in ST239 (13.6%, n = 6), with 2.3% (n = 1) in ST4649. This finding is supported by Lu et al, who in 2014 reported 4.7% *qacA/B*-positive

MRSA in ST239.⁽²⁶⁾ However, Ho et al earlier reported a contradictory finding in which a high frequency of *qacA/B* genes (88.9%) was detected in ST239.⁽²⁷⁾

A previous study reported the increased incidence of mupirocin resistance in MRSA and the failure of decolonisation treatments.⁽²⁶⁾ Fortunately, the *mupA* gene was not detected in all MRSA isolates of this study. A similar finding was reported by Nejabat et al.⁽²⁸⁾ However, a previous study conducted in Malaysia found that 70% (11 out of 16) of ST239 isolates were positive for the *mupA* gene.⁽²⁹⁾

Our study showed the advantages of MLST data analysis in the determination of genetic variability among MRSA isolates, genetic relationship between nasal swab and clinical samples from MRSA strains, association between virulence and resistance genes with different STs, and genetic relatedness among the MRSA strains from neighbouring countries.

Phylogenetic analysis reported genetic diversity among MRSA isolates, with two major clades consisting of three different STs. The most frequent MRSA clone circulating in this healthcare setting was ST22 (68.2%, n = 30), which is similar to the findings of Espadinha et al in 2013.⁽³⁰⁾ The first isolation of ST22 in Malaysia was reported in 2009 and the emergence of multidrug-resistant ST22-MRSA-IV in a tertiary hospital in Malaysia was reported a few years later.⁽²⁹⁾ Thus, this study is significant, as it revealed that ST22 is gaining prominence in a Malaysian hospital.

The second predominant ST found in this study was ST239 (29.5%, n = 13). The high prevalence of MRSA ST239 in the Asian region might be due to the dissemination of a few epidemic clones. ST239-MRSA-III was a major MRSA clone found in Asian hospitals (i.e. healthcare-acquired MRSA) particularly in China and some Southeast Asian countries.⁽³¹⁾ A study conducted by Sit et al in 2017 showed the predominance of ST239-MRSA-III circulating in a tertiary teaching hospital in Malaysia.⁽³²⁾

The two major STs (ST22 and ST239) circulating in this hospital from different localities

were nosocomial strains. This indicates that the infection control measures in the hospital setting should be further enhanced. Furthermore, the same major genotypes were reported in an Australian hospital.⁽³²⁾ However, in Singaporean hospitals, ST22 remained the predominant genotype in circulation. In addition, a new nosocomial strain, ST45, was reported to be gradually replacing ST239.^(32,33) Hence, this study concluded that surveillance is essential for the monitoring and control of infectious diseases.

A novel ST was discovered in this study and assigned as ST4649. It is a single locus variant of ST239 that is distinguished by one mutation in the *arcC* gene allele. On the phylogenetic tree, ST4649 was clustered in the same clade as ST239, denoting their genetically close relationship. It is noteworthy that genetic diversity can be attributed to horizontal virulence gene transfer; the occurrence of novel STs reflects the ongoing dynamic process of genetic recombination and clonal expansion of the bacterial population.⁽³²⁾

In terms of genetic relatedness, MRSA isolates from nasal swabs and clinical samples of 22 patients were highly related and clonal. Hence, nasal colonisation of MRSA might subsequently lead to infection. A similar study has demonstrated that two major STs, ST239 and ST5, had a genetic correlation between nasal and clinical isolates.⁽³⁴⁾ Studies by Tilahun et al and Kao et al also revealed that the same MRSA genotypes originated from both colonisation and infection sites.^(35,36)

Based on the association of virulence and resistance genes in different ST and their distribution patterns, this study revealed the co-existence of *sasX* and *qacA/B* genes in MRSA isolates. Gene co-existence occurred exclusively in 13.6% (n = 6) of ST239 and merely 2.3% (n = 1) of ST4649, which was comparable to previous studies.^(20,37) To the best of our knowledge, this is the first report of the co-existence of *sasX*- and *qacA/B*-positive ST239 in a Malaysian tertiary hospital.

This study was not without limitations. Correlation validation was not performed between

the laboratory results and the clinical outcomes. Hence, the correlation between clinical outcomes and the study outcome provides a good prospect for future research. As molecular typing using MLST was only performed on selective MRSA strains, molecular characterisation of entire MRSA strains may describe the high genetic variability of the MRSA being studied and the higher frequency of genetic relatedness between nasal and clinical isolates.

In conclusion, the *sasX* gene and *qacA/B* gene were present in MRSA isolates in our healthcare setting, while the *mupA* gene was not detected. ST22 and ST239 were the major MRSA clones that predominated our healthcare setting, and a new ST4649 was discovered. We have demonstrated that the circulating MRSA genotypes conferred different virulence and resistance determinants.

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REFERENCES

1. Abad CL, Pulia MS, Safdar N. Does the nose know? An update on MRSA decolonization strategies. *Curr Infect Dis Rep* 2013; 15:455-64.
2. Albrecht VS, Limbago BM, Moran GJ, et al. Staphylococcus aureus colonization and strain type at various body sites among patients with a closed abscess and uninfected controls at US emergency departments. *J Clin Microbiol* 2015; 53:3478-84.
3. Li M, Du X, Villaruz AE, et al. MRSA epidemic linked to a quickly spreading colonization and virulence determinant. *Nat Med* 2012; 18:816-9.

4. Dhiman M, Bhatt N, Chau-Han V, Farooq U, Khan A. Emergence of methicillin resistant *Staphylococcus aureus* (MRSA) isolates from north India harboring a novel *sasX* gene: further analyzing its role in biofilm formation. *Ann Med Biomed Sci* 2017; 3:46-50.
5. Aires de Sousa MA, Sanches IS, Ferro ML, et al. Intercontinental spread of a multidrug-resistant methicillin-resistant *Staphylococcus aureus* clone. *J Clin Microbiol* 1998; 36:2590-6.
6. Warren DK, Prager M, Munigala S, et al. Prevalence of *qacA/B* genes and mupirocin resistance among methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in the setting of chlorhexidine bathing without mupirocin. *Infect Control Hosp Epidemiol* 2016; 37:590-7.
7. Abimanyu N, Murugesan S, Krishnan P. Emergence of methicillin-resistant *Staphylococcus aureus* ST239 with high-level mupirocin and inducible clindamycin resistance in a tertiary care center in Chennai, South India. *J Clin Microbiol* 2012; 50:3412-3.
8. Feil EJ, Nickerson EK, Chantratita N, et al. Rapid detection of the pandemic methicillin-resistant *Staphylococcus aureus* clone ST 239, a dominant strain in Asian hospitals. *J Clin Microbiol* 2008; 46:1520-2.
9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 27th edition. CLSI supplement M100. Pennsylvania: Clinical and Laboratory Standards Institute, 2017.
10. Al-Talib H, Yean CY, Al-Khateeb A, Hasan H, Ravichandran M. Rapid detection of methicillin-resistant *Staphylococcus aureus* by a newly developed dry reagent-based polymerase chain reaction assay. *J Microbiol Immunol Infect* 2014; 47:484-90.

11. Monecke S, Müller E, Dorneanu OS, Vremeră T, Ehricht R. Molecular typing of MRSA and of clinical *Staphylococcus aureus* isolates from Iași, Romania. *PLoS One* 2014; 9:e97833.
12. Noguchi N, Suwa J, Narui K, et al. Susceptibilities to antiseptic agents and distribution of antiseptic-resistance genes *qacA/B* and *smr* of methicillin-resistant *Staphylococcus aureus* isolated in Asia during 1998 and 1999. *J Med Microbiol* 2005; 54(Pt 6):557-65.
13. Yun HJ, Lee SW, Yoon GM, et al. Prevalence and mechanisms of low- and high-level mupirocin resistance in staphylococci isolated from a Korean hospital. *J Antimicrob Chemother* 2003; 51:619-23.
14. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000; 38:1008-15.
15. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol* 2018; 35:1547-49.
16. Percival SL, Suleman L, Vuotto C, Donelli G. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. *J Med Microbiol* 2015; 64:323-34.
17. Lin SY, Lin NY, Huang YY, Hsieh CC, Huang YC. Methicillin-resistant *Staphylococcus aureus* nasal carriage and infection among patients with diabetic foot ulcer. *J Microbiol Immunol Infect* 2018; pii: S1684-1182(18)30155-5.
18. Eko KE, Forshey BM, Carrel M, et al. Molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) nasal colonization and infection isolates in a Veterans Affairs hospital. *Antimicrob Resist Infect Control* 2015; 4:10.
19. Robicsek A, Suseno M, Beaumont JL, Thomson RB Jr, Peterson LR. Prediction of methicillin-resistant *Staphylococcus aureus* involvement in disease sites by concomitant nasal sampling. *J Clin Microbiol* 2008; 46:588-92.

20. Song Y, Du X, Li T, Zhu Y, Li M. Phenotypic and molecular characterization of *Staphylococcus aureus* recovered from different clinical specimens of inpatients at a teaching hospital in Shanghai between 2005 and 2010. *J Med Microbiol* 2013; 62(Pt 2):274-82.
21. Eshaghi M, Bibalan M, Pournajaf A, Gholami M, Talebi M. Detection of new virulence genes in *mecA*-positive *Staphylococcus aureus* isolated from clinical samples: the first report from Iran. *Infect Dis Clin Pract* 2017; 25:310-3.
22. Zarizal S, Yeo CC, Faizal GM, et al. Nasal colonisation, antimicrobial susceptibility and genotypic pattern of *Staphylococcus aureus* among agricultural biotechnology students in Besut, Terengganu, east coast of Malaysia. *Trop Med Int Health* 2018; 23:905-13.
23. Nair R, Hanson BM, Kondratowicz K, et al. Antimicrobial resistance and molecular epidemiology of *Staphylococcus aureus* from Ulaanbaatar, Mongolia. *PeerJ* 2013; 1:e176.
24. Chen X, Wu Z, Zhou Y, et al. Molecular and virulence characteristics of methicillin-resistant *Staphylococcus aureus* in burn patients. *Front Lab Med* 2017; 1:43-7.
25. Shamsudin MN, Alreshidi MA, Hamat RA, et al. High prevalence of *qacA/B* carriage among clinical isolates of methicillin-resistant *Staphylococcus aureus* in Malaysia. *J Hosp Infect* 2012; 81:206-8.
26. Lu Z, Chen Y, Chen W, et al. Characteristics of *qacA/B*-positive *Staphylococcus aureus* isolated from patients and a hospital environment in China. *J Antimicrob Chemother* 2014; 70:653-7.
27. Ho J, Branley J. Prevalence of antiseptic resistance genes *qacA/B* and specific sequence types of methicillin-resistant *Staphylococcus aureus* in the era of hand hygiene. *J Antimicrob Chemother* 2012; 67:1549-50.

28. Nejabat M, Khashei R, Bazargani A, Ebrahim-Saraie HS, Motamedifar M. Evaluation of high-level of mupirocin resistance among clinical isolates of methicillin-resistant *Staphylococcus aureus* from Shiraz, Iran (2008-2009). *Pharm Sci* 2015; 21:225-8.
29. Ghasemzadeh-Moghaddam H, van Belkum A, Hamat RA, van Wamel W, Neela V. Methicillin-susceptible and -resistant *Staphylococcus aureus* with high-level antiseptic and low-level mupirocin resistance in Malaysia. *Microb Drug Resist* 2014; 20:472-7.
30. Espadinha D, Faria NA, Miragaia M, et al; Médicos Sentinela Network. Extensive dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) between the hospital and the community in a country with a high prevalence of nosocomial MRSA. *PLoS One* 2013; 8:e59960.
31. Chen CJ, Huang YC. New epidemiology of *Staphylococcus aureus* infection in Asia. *Clin Microbiol Infect* 2014; 20:605-23.
32. Sit PS, Teh CS, Idris N, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infection and the molecular characteristics of MRSA bacteraemia over a two-year period in a tertiary teaching hospital in Malaysia. *BMC Infect Dis* 2017; 17:274.
33. Teo J, Tan TY, Hon PY, et al; Network for Antimicrobial Resistance Surveillance (Singapore). ST22 and ST239 MRSA duopoly in Singaporean hospitals: 2006-2010. *Epidemiol Infect* 2013; 141:153-7.
34. Jeremiah C, Kandiah JP, Spelman DW, et al. Differing epidemiology of two major healthcare-associated methicillin-resistant *Staphylococcus aureus* clones. *J Hosp Infect* 2016; 92:183-90.
35. Tilahun B, Faust AC, McCorstin P, Ortegon A. Nasal colonization and lower respiratory tract infections with methicillin-resistant *Staphylococcus aureus*. *Am J Crit Care* 2015; 24:8-12.

36. Kao KC, Chen CB, Hu HC, et al. Risk factors of methicillin-resistant *Staphylococcus aureus* infection and correlation with nasal colonization based on molecular genotyping in medical intensive care units: a prospective observational study. *Medicine (Baltimore)* 2015; 94:e1100.
37. Kaczmarek M, Mullett MS, McDonald JE, Denman S. Multilocus sequence typing provides insights into the population structure and evolutionary potential of *Brenneria goodwinii*, associated with acute oak decline. *PLoS One* 2017; 12:e0178390.

Table I. List of primer sequences and polymerase chain reaction (PCR) thermal cycle conditions for each target gene.

Target gene	Primer set	Primer sequence (5' to 3' end)	PCR product size (bp)	Step	No. of cycles	Temperature (°C), duration	Reference, yr
<i>femA</i> and <i>mecA</i>	<i>femA</i> -F	CGATCCATATTTACCATATCA	450	Initial denaturation	1	95°C, 3 min	Al-Talib et al, 2014 ⁽¹⁰⁾
	<i>femA</i> -R	ATCACGCTCTTCGTTTGTAGTT		Denaturation	35	95°C, 30 s	
	<i>mecA</i> -F	ACGAGTAGATGCTCAATATAA	Annealing	50°C, 30 s			
	<i>mecA</i> -R	CTTAGTTCTTTAGCGATTGC	Extension	72°C, 30 s			
				Final extension	1	72°C, 5 min	
<i>sasX</i>	<i>sasX</i> -F	ATTGAAGCTCAGACTCCTAG	120	Initial denaturation	1	95°C, 3 min	Monecke et al, 2017 ⁽¹¹⁾
				Denaturation	35	95°C, 30 s	
	Annealing	55°C, 15 s					
<i>sasX</i> -R	GTTATCAGTTGTAGCAGTAGT	Extension	72°C, 12 s				
<i>mupA</i>	<i>mupA</i> -F	TATATTATGCGATGGAAGGTTGG	456	Initial denaturation	1	95°C, 5 min	Yun et al, 2003 ⁽¹³⁾
				Denaturation	35	95°C, 30 s	
	Annealing	50°C, 15 s					
<i>mupA</i> -R	AATAAAATCAGCTGGAAAGTGTTG	Extension	72°C, 10 s				
<i>qacA/B</i>	<i>qacA/B</i> -F	GCAGAAAGTGCAGAGTTTCG	361	Initial denaturation	1	95°C, 3 min	Noguchi et al, 2005 ⁽¹²⁾
				Denaturation	35	95°C, 30 s	
	Annealing	50°C, 20 s					
	<i>qacA/B</i> -R	CCAGTCCAATCATGCCTG	Extension	72°C, 30 s			
				Final extension	1	72°C, 5 min	

FIGURES

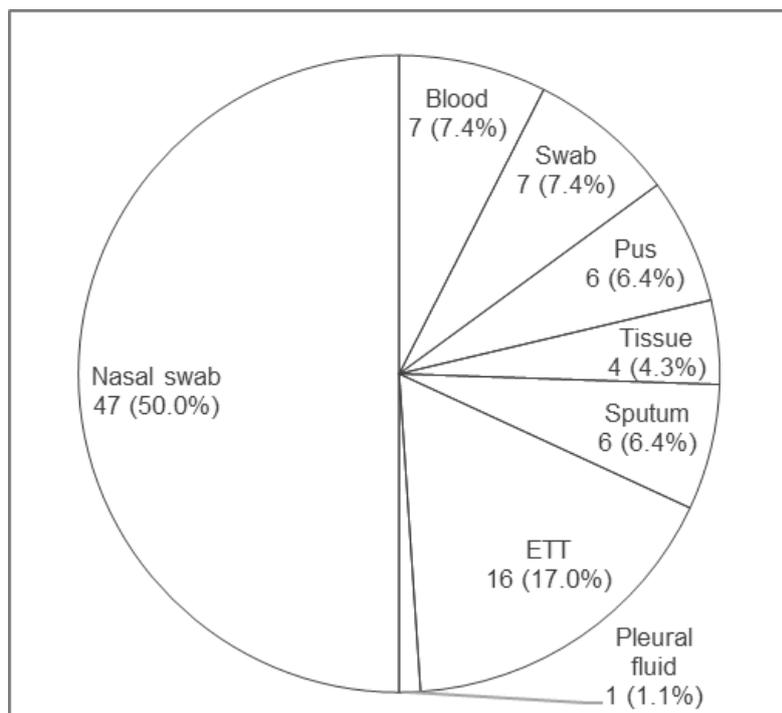


Fig. 1 Chart shows the distribution of methicillin-resistant *Staphylococcus aureus* from 47 paired isolates. ETT: endotracheal tube

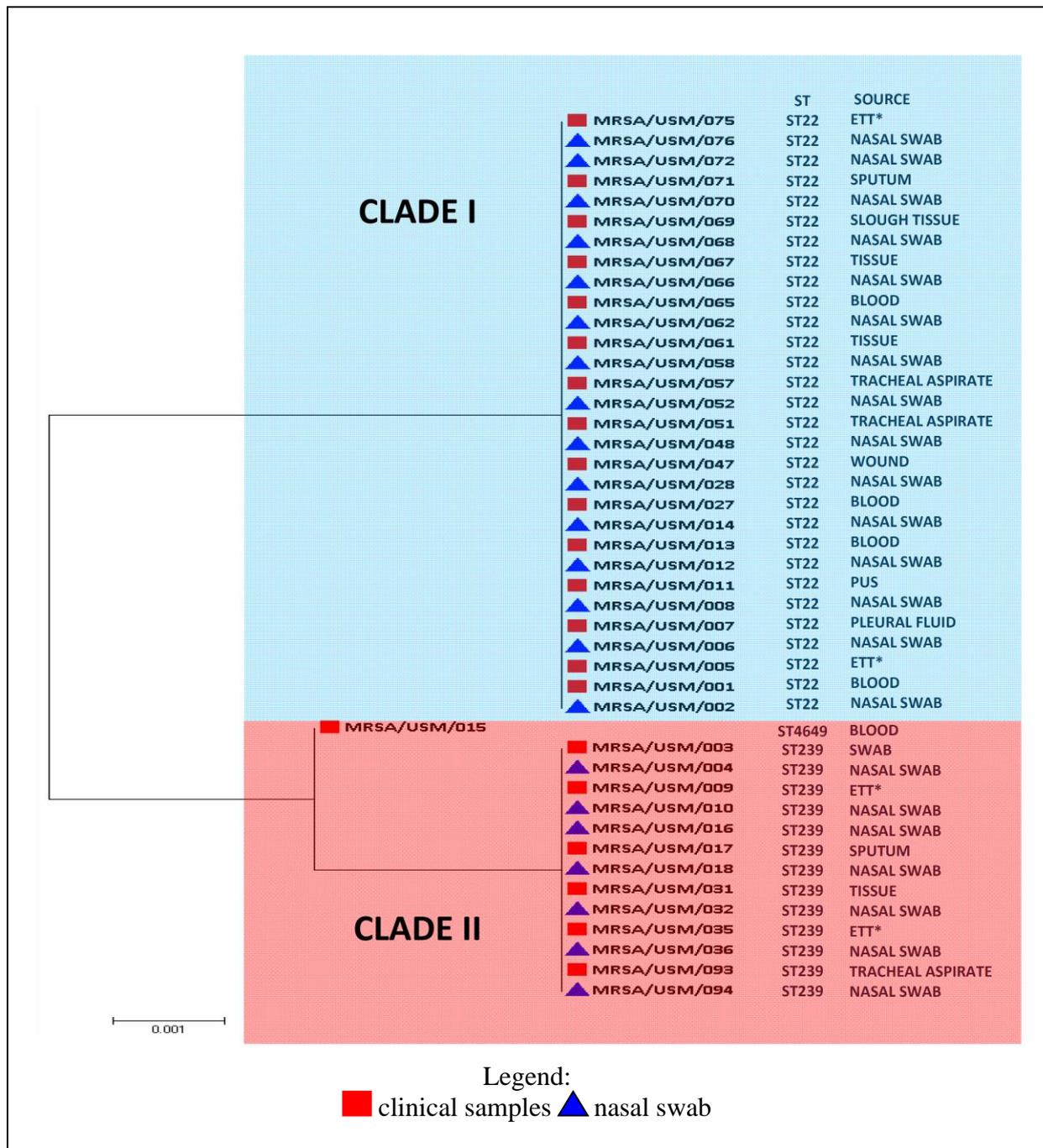


Fig. 2 Phylogenetic tree shows the genetic relatedness of paired isolates (n = 47). Paired samples were arranged according to the patient sequence in Table IV.