Journal of Infection and Public Health (2016) xxx, xxx–xxx



http://www.elsevier.com/locate/jiph

# A persistent antimicrobial resistance pattern and limited methicillin-resistance-associated genotype in a short-term *Staphylococcus aureus* carriage isolated from a student population

**Norhidayah Mat Azis[a], Hui P. Pung[a], Abdul R. Abdul Rachman[a], Syafinaz Amin Nordin[b], Seri N.E. Sarchio[a], Zarizal Suhaili[c], Mohd N. Mohd Desa[a,d,*]**

[a] *Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Malaysia*
[b] *Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Malaysia*
[c] *School of Animal Sciences, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Tembila Campus, Besut, Malaysia*
[d] *Laboratory of Halal Science Research, Halal Products Research Institute, Universiti Putra Malaysia, Serdang, Malaysia*

Received 6 November 2015; received in revised form 11 February 2016; accepted 24 February 2016

**KEYWORDS**
*Staphylococcus aureus*;
MRSA;
Antimicrobial;
*mecA* gene

**Summary** The aim of the present study was to assess and compare the antimicrobial susceptibility pattern against a panel of antibiotics and molecular and methicillin resistance-associated genotypes of 120 carriage *S. aureus* isolates previously isolated from a student population at two isolation events within a one-month interval. The antibiotic susceptibility of isolates was determined using the Kirby-Bauer disc-diffusion method (cefoxitin by Etest). The MRSA was screened using polymerase chain reaction for the presence of the *mecA* gene. The *mecA-positive* isolates were subjected to staphylococcal cassette chromosome (SCC) *mec*

* Corresponding author at: Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Malaysia. Tel.: +60 389472344; fax: +60 389436178.
*E-mail address:* mnasir@upm.edu.my (M.N. Mohd Desa).

http://dx.doi.org/10.1016/j.jiph.2016.02.013


Please cite this article in press as: Mat Azis N, 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 (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.013

typing, multilocus sequence typing (MLST) and eBURST analysis. All isolates were characterized for the presence of the Panton–Valentine leukocidin (PVL) gene, an enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) pattern and the *spa* type. For the two occasions where *S. aureus* was isolated, the highest frequency of resistance was observed for penicillin (70% and 65%, respectively), with a lower rate against erythromycin and tetracycline (<12%). All isolates were susceptible to ciprofloxacin and gentamycin. As for methicillin resistance, eight isolates had minimum inhibitory concentrations (MIC) of resistant categories, but 10 isolates (8.33%) were positive for the *mecA* gene. The *mecA*-positive isolates belonged to SCC*mec* types I ($n = 9$) and V ($n = 1$). MLST was resolved for only three MRSAs, ST508 ($n = 1$), ST88 ($n = 1$) and ST96 ($n = 1$). The results of the eBURST analysis showed that the MRSA isolates analyzed in the present study were potentially related to MRSA identified in other countries. Approximately half of the persistent *S. aureus* carriers harbored *S. aureus* of a similar *spa* type in the respective individuals during both isolation events. A persistent antimicrobial pattern and limited distinct MRSAs were observed over the short study period. The latter frequently exhibited SCC*mec* type I, commonly associated with hospital-acquired (HA) characteristics, but further delineation is needed to justify the origins of these bacteria.


## Introduction

*Staphylococcus aureus* is a well-known opportunistic pathogen that frequently colonizes the skin and nasal mucosa of humans. Although multiple body sites can be colonized in human beings, the anterior nares have been identified as the primary reservoirs of *S. aureus*, with carrier rates ranging between 20 and 30% in healthy populations [1]. It has been reported that the colonization of *S. aureus* in the nose is a potential risk factor for acquiring subsequent staphylococcal infections [2,3]. In addition, this opportunistic pathogen shows increasing resistance to certain antimicrobial agents, causing difficulties in managing staphylococcal infections [4]. In recent years, methicillin-resistance *S. aureus* (MRSA) strains have emerged as a major cause of health care-associated and community-associated infections worldwide. The incidence of *S. aureus* and MRSA is now growing at an alarming rate, with a high incidence of community-associated MRSA infections in both children and adult populations [5]. Therefore, the continuous surveillance of *S. aureus* and evaluation of the antibiotic susceptibility patterns of these bacteria are crucial to monitor the emerging trend. Moreover, analysis of the phenotypic and genotypic characteristics of *S. aureus* isolates carried in a population over a period of time might also provide further insight into the persistent pattern of antimicrobial resistance and associated genotypes.

The present study utilized a collection of *S. aureus* isolates recently isolated from the anterior nares of a health science student population obtained over a one-month period [6] to compare the antimicrobial susceptibility pattern against a panel of commonly used antibiotics and identify the molecular characteristics pertaining to methicillin resistance-associated genotypes, sequence types (ST), ERIC-based DNA fingerprint patterns and surface protein A (*Spa*) types. This comparative analysis might provide a model for understanding the dissemination and epidemiological patterns of *S. aureus* over a short-term transition in a population.

## Materials and methods

### Isolates

In a recent study [6], 192 health science undergraduate students at the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, participated in a nasal swab collection, with 60 students (31.3%) isolated for *S. aureus*. In the following month, 180 of these students participated in a second sampling, with 60 students (33.3%) positive for *S. aureus*. During the one-month period, 39 participants were defined as persistent carriers of *S. aureus* during both first and second sampling events. Methicillin resistance was preliminarily screened using the cefoxitin-disc diffusion method and polymerase chain reaction (PCR) for the presence of the 147-bp *mecA* gene. All 120 isolates were preserved at −80 °C in Luria Bertani broth supplemented with 20% glycerol.

Please cite this article in press as: Mat Azis N, 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 (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.013

In the present study, the bacterial stocks were revived on blood agar and re-identified based on Gram staining, growth on Mannitol Salt Agar (MSA) (Merck KGaA, Frankfurter Straβe, Darmstadt, Germany) and coagulase reactions. All isolates were subjected to genomic DNA extraction for molecular analysis using the Exgene™ Cell SV Extraction Kit (AITbiotech, Science Park Drive, Singapore). *S. aureus* American Type Culture Collection (ATCC) 700699 and 25923 were used as reference strains in all analyses.

### Antibiotic susceptibility of *S. aureus*

The susceptibility of *S. aureus* isolates to various antibiotics was determined according to Clinical and Laboratory Standards Institute guidelines [7]. The Etest method was used to examine susceptibility against cefoxitin, while the disc diffusion method was used for erythromycin (15 μg), gentamicin (10 μg), ciprofloxacin (5 μg), rifampin (5 μg), penicillin (10 units) and tetracycline (30 μg) (all from Oxoid, Altrincham, Cheshire, England).

### Detection of methicillin-resistance gene

The detection of the *mecA* gene was re-certified through PCR using the following primers: *mecA*-F (5′-AAA ACTAGGTGTTGGTGAAGATATACC-3′) and *mecA*-R (5′-GAAAGGATCTGTACTGGG TTAATCAG-3′), according to Suhaili et al. [8]. Isolates with amplicons of the expected size (147 bp) were categorized as MRSA strains. A few representatives of the PCR products were sequenced for gene identity confirmation (data not shown).

### SCC*mec* typing for methicillin-resistant *S. aureus* (MRSA)

All MRSA isolates were subjected to SCC*mec* typing through multiplex PCR according to Boye et al. [9], targeting genes *ccrA2-B*, *ccrC*, *IS1272* and *mecA—IS431* to differentiate SCC*mec* types I (415 bp), II (937 bp), III (518 bp), IV (937 and 415 bp) and V (518 and 359 bp).

### Multilocus sequence typing (MLST)

Only *mec*A-positive isolates were subjected to MLST analysis. The nucleotide sequences of seven housekeeping genes, carbamate kinase (*arc*) (456 bp), shikimate dehydrogenase (*aroE*) (456 bp), glycerol kinase (*glpF*) (465 bp), guanylate kinase (*gmk*) (429 bp), phosphate acetyltransferase (*pta*) (474 bp), triosephosphate isomerase (*tpi*) (402 bp) and acetyl coenzyme A acetyltransferase (*yqiL*) (516 bp), were amplified through PCR according to Enright et al. [10]. The PCR products were sequenced at the 1st BASE lab (Serdang, Selangor, Malaysia). The allelic profiles and sequence types (ST) were determined using the MLST database at http://www.mlst.net. The eBURST algorithm was also generated at http://eburst.mlst.net/.

### Detection of Panton—Valentine Leukocidin (PVL) gene

All isolates were screened for PVL gene through PCR using the following primers: *luk-PV-1* (5′-ATCATTAGGTAAAATGTCTGGACATGATCCA-3′) and *luk-PV-2* (5′-GCATCAASTGTATTGGATAGCAAAAGC-3′) and running parameters according to Lina et al. [11]. Isolates with a band fragment of 433 bp were sequenced for gene identity confirmation (data not shown) and reported as positive for the PVL gene.

### Spa typing

The polymorphic X region of the *spa* gene in the genomic DNA of *S. aureus* was amplified through PCR using the following primers; 1095F (5′-AGACGATCCTTCGGTGAGC-3′) and 1517R (5′-GCTTTTGCAATGTCATTTACTG-3′) and running parameters according to Japoni-Nejad et al. [12]. The PCR products were sequenced at the 1st BASE lab (Serdang, Selangor, Malaysia) using the same primers. *Spa* types were determined using DNAgear software at http://w3.ualg.pt/hshah/DNAGear/.

### Enterobacterial repetitive intergenic consensus (ERIC)-PCR

ERIC-PCR was performed according to Ye et al. [13], using primers ERIC1R (5′-ATGTAAG-CTCCTGGGGATTCAC-3′) and ERIC2 (5′-AAGTAAGTG ACTGGGGTGAGCG-3′). Images of the DNA band patterns were analyzed to generate a dendrogram using GelComparII version 6.6.11 software (Applied Maths, Sint-Martens-Latem, Belgium) and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

## Results and discussion

### Antimicrobial susceptibility pattern

All isolates survived preservation and matched the characteristics of *S. aureus*. Upon antimicrobial assay, the highest frequency of resistance was observed for penicillin in both isolations (70%

Please cite this article in press as: Mat Azis N, 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 (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.013

**Table 1** Antimicrobial susceptibility patterns of *S. aureus* isolated from the first and second isolation events.

| Types of antibiotics | The first isolation event ($n = 60$) | | The second isolation event ($n = 60$) | |
|---|---|---|---|---|
| | I | R | I | R |
| [a]Cefoxitin (30 μg) | 0 (0%) | 4 (6.67%) | 0 (0%) | 4 (6.67%) |
| Erythromycin (15 μg) | 2 (3.33%) | 7 (11.67%) | 3 (5%) | 4 (6.67%) |
| Gentamicin (10 μg) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Ciprofloxacin (5 μg) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Rifampin (5 μg) | 0 (0%) | 0 (0%) | 1 (1.67%) | 0 (0%) |
| Penicillin (10 units) | 0 (0%) | 42 (70%) | 0 (0%) | 39 (65%) |
| Tetracycline (30 μg) | 0 (0%) | 7 (11.67%) | 0 (0%) | 7 (11.67%) |

R, resistant; I, intermediate.
[a] Etest method.

and 65%, respectively) (Table 1). This finding is consistent with other studies involving healthy communities showing a high resistance to penicillin (>60%) [14–17]. Penicillin is one of the most commonly used antibiotics to treat bacterial infections. Since the 1950s, penicillin has become the most useful therapeutic agent in treating staphylococcal infections [18]. Reflecting the wide usage of this antibiotic, a high level of resistance of *S. aureus* toward penicillin can be expected. In addition, a low resistance rate of the *S. aureus* isolates to tetracycline and erythromycin (<12%) in both isolation events was also observed. These data are consistent with other studies reporting a low rate of tetracycline resistance (<12%) among healthy individuals [14,17,19]. A low resistance rate to erythromycin (<12%) was also reported in other local surveillance studies [14,20,21]. However, the efficacy of ciprofloxacin, gentamycin and rifampin remains exceptional, and the *S. aureus* isolates examined in the present study remained susceptible to these antibiotics. Similarly, a study at the same locality in 2006 also reported a high sensitivity of *S. aureus* nasal isolates to ciprofloxacin (86.4%), gentamycin (97.5%) and rifampin (98.8%) [14]. These findings indicate that certain antibiotics remain effective over time for treating *S. aureus* infections. Therefore, the use of these drugs should be properly managed to minimize the emergence of resistance.

Cefoxitin was used to infer methicillin resistance at the phenotypic level, and eight isolates (four each from first and second isolation, respectively) were identified based on the Etest. Four MRSA isolates (two each from first and second sampling, respectively) were isolated from two persistent carrier individuals. One of the MRSAs (from second sampling of persistent carrier) was also multidrug-resistant (MDR), showing co-resistance to three antibiotics of different classes [22,23]: erythromycin (macrolide), penicillin (β-lactam) and tetracycline (tetracycline). There were two other MDRs, but these isolates were characterized as MSSA strains from the first sampling. Although a low prevalence of MRSA was observed in the study population, previous findings at the same locality in 2009 and 2012 reported no individuals screened for *S. aureus* in those studies were colonized by MRSA [21,24]. In addition, the results of the present study indicate the persistence of MRSAs over a short period, with one strain associated with MDR. This result might represent the current status, likely indicating a turning point for further dissemination if no precautionary measures are taken.

## Distribution of *mecA* gene and *SCCmec* type

Among the 120 isolates of *S. aureus*, 10 (8.33%) isolates (four and six isolates from first and second isolation events, respectively) were positive for the *mecA* gene, as these isolates showed the expected amplicon size. The *mecA*-positive detection was consistent with the eight isolates showing resistance toward cefoxitin in the Etest, while the other two *mecA*-positive isolates (from second event: one persistent carrier and one non persistent carrier) were cefoxitin-susceptible according to Etest methods. This result potentially reflects the presence of phenotypic heterogeneity among the drug resistant (heteroresistance) strains of *S. aureus* in the present study, therefore screening *S. aureus* isolates for the presence of *mecA* is warranted to avoid the misclassification of MRSA as MSSA, highlighting the need for PCR screening to monitor the dissemination of potential MRSA. SCC*mec* typing of the 10 *mecA*-positive isolates identified SCC*mec* type I for nine isolates and SCC*mec* type V for one isolate. SCC*mec* type I has been previously reported to be carried by hospital acquired-methicillin resistant *S. aureus* (HA-MRSA) [9,25]. The presence of MRSA with HA-associated SCC*mec* type I in this population might suggest that the isolates originated from

Please cite this article in press as: Mat Azis N, 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 (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.013

**Table 2** Results of the MLST analysis.

| Isolates code | Sequence type | *arcC* | *aroeE* | *glpF* | *gmk* | *pta* | *tpi* | *yqiL* |
|---|---|---|---|---|---|---|---|---|
| [a]A04_P03 | ST508 | 10 | 40 | 8 | 6 | 10 | 3 | 2 |
| [a]B23_P03 | NT | 3 | 36 | 1 | 14 | 161 | 170 | 13 |
| [a]A35_P24 | ST88 | 22 | 1 | 14 | 23 | 12 | 4 | 31 |
| [a]B13_P24 | NT | 31 | 31 | 1 | 14 | 12 | 170 | 11 |
| B58_NP | ST96 | 12 | 1 | 1 | 15 | 11 | 1 | 40 |
| A02_NP | NT | 3 | 1 | 6 | 15 | 161 | 170 | 40 |
| A38_NP | NT | 20 | 20 | 23 | 6 | 161 | 170 | 40 |
| B16_NP | NT | 12 | 4 | 1 | 4 | 44 | 57 | 40 |
| B06_NP | NT | 31 | 31 | 1 | 1 | 12 | 170 | 11 |
| B55_NP | NT | 1 | 1 | 1 | 15 | 233 | 99 | 40 |

NT-non typable (isolate does not have a sequence type).
[a] Isolates A04_P03 and B23_P03 were obtained from a single individual, while isolates A35_P24 and B13_P24 were obtained from another individual.

hospital settings. Considering that the participants in the present study were students of the health science program living in the vicinity of the hospital and mixing with colleagues from the medical program, such exposure might result in the spread of MRSA from the hospital to the student community, or vice versa.

## ST and eBURST in comparison with *SCCmec* type and presence of PVL gene

Only the 10 *mecA*-positive isolates were subjected to MLST, whereby the respective amplicons from the isolates matching the expected DNA band size were successfully sequenced. Nevertheless, submission in the MLST database only assigned three isolates for ST, while the remaining isolates could not be typed (Table 2). The three MRSAs were ST508 (1st sampling), ST88 (1st sampling) and ST96 (2nd sampling), and all MRSAs were SCC*mec* type I. In the eBURST analysis, the MRSA isolate with ST508 belonged to a single-locus variant [SLV] of clonal complex (CC) 45 (Fig. 1A). CC45 was previously reported as one of the major lineages of MRSA clones [26,27]. Interestingly, this MRSA isolate was PVL negative, consistent with a report that PVL positivity was less detected among CC45-MRSA isolates [27]. Previous studies from Malaysia reported that MLST type ST508 of CC45 was present among MSSA strains isolated from the hospital [28] and community [26]. However, in the present study, ST508 of CC45 was identified in a MRSA isolate. The presence of ST508-MRSA in the present study might infer that the MRSA strain evolved from ST508-MSSA strains after acquiring the SCC*mec* mobile element. However, the ST508-MRSA isolate examined in the present study carried SCC*mec* type I and might also be related to ST45-MRSA sporadically detected in Hong Kong, which also carried SCC*mec* type I [27,29]. Another MRSA isolate examined in the present study resides in CC88 (Fig. 1B). This isolate had ST88 and was PVL negative. Fan et al. [25] reported that ST88 was largely identified among community isolates in China. The other MRSA in the present study clustered in CC96, as shown in Fig. 1C. This isolate had ST96 and was positive for the PVL gene. This isolate was the only MRSA (from second isolation) detected among the five isolates with the PVL gene, in the present study but the latter were all MSSAs (two isolates from each isolation). Nevertheless, ST96 has not been widely reported. Recently, one strain of CC96-MRSA with SCC*mec* type III was reported in Russia [30]. The presence of a PVL-positive MRSA strain associated with SCC*mec* type I (commonly carried by HA) is troublesome, as PVL has been associated with high mortality and morbidity, reflecting the virulence property of this gene.

## ERIC-PCR, spa type and comparative analysis

Considering that the sampling was performed twice in a same population over a short period of time, there could be dissemination of the related isolates within the population, particularly in persistent carriers in which the same isolate could have persisted over the one-month period in the respective individuals, particularly the MRSA and MDR strains. The ERIC assay yielded two to eight bands ranging approximately 100—4000 base pairs, which were subsequently subjected to dendrogrammatic analysis. Based on the generated dendrogram, the majority of the isolates were genetically distinct (Fig. 2), with only a limited potential related dissemination. All isolates were segregated into eight clusters: clusters A to H, at only 29% similarity. *Spa* typing was also performed to correlate the

Please cite this article in press as: Mat Azis N, 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 (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.013

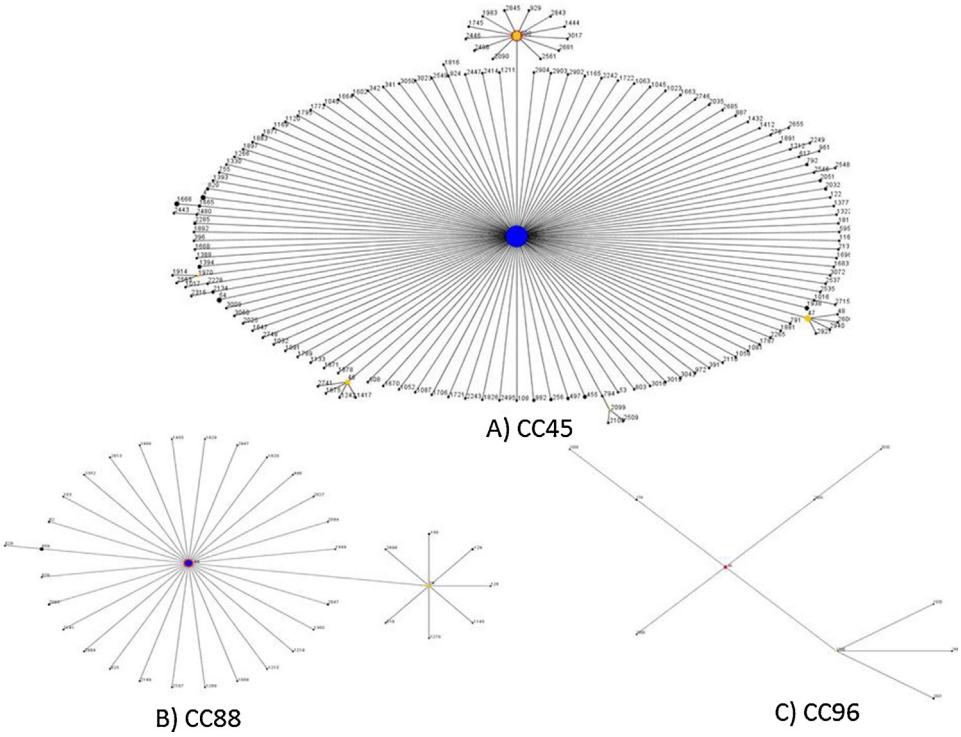


**Figure 1** eBURST diagram of the three clonal complexes (CC), CC45, CC88 and CC96, associated with the three MRSA isolates (in red) examined in the present study, respectively.

distribution of the isolates in the dendrogram, but only 76 (63.3%) isolates were successfully typed. Nineteen (48.72%) of the 39 persistent carriers carried *S. aureus* of a single *spa* type in the respective individual in the two sampling events; four persistent carriers carried isolates of *spa* type t701 ($n = 8$), while other individuals carried types t267 ($n = 4$), t008 ($n = 4$) and t127 ($n = 4$). Isolates of *spa* types t505 ($n = 2$), t1236 ($n = 2$), t164 ($n = 2$), t050 ($n = 2$), t002, ($n = 2$) t024 ($n = 2$), t2883 ($n = 2$), t4720 ($n = 2$) and t346 ($n = 2$) were each carried by a single different individual, respectively, in both isolation events. A majority of isolates of a single *spa* type carried in the respective persistent carriers (15/19) also showed similar antibiotic susceptibility patterns. Furthermore, there were five persistent carriers, each with isolates of a similar *spa* type, antibiotic susceptibility pattern and ERIC based-DNA fingerprint in the respective individual, suggesting that the respective carriers potentially carried related strains over the one-month period; two of these persistent carriers had isolates (A39_P26 and B28_P26; A45_P29 and B36_P29) grouped in cluster E, while the remaining carriers had isolates (A22_P14 and B7_P14; A24_P15 and B3_P15; A50_P32 and B10_P32) grouped in cluster F. To a certain extent, these findings are consistent with the statement that persistent carriers might carry one type of strain over time [31].

Nonetheless, most of the MDR and all 10 *mecA*-positive strains were non-typable, and one isolate failed in the PCR detection for the *spa* gene. Two (one MRSA and one MSSA) out of the three MDR isolates were positioned in the same cluster (cluster F), while the other MDR (MSSA) isolate was in cluster A. In addition, the MRSAs were distinctly distributed among the clusters: two MRSA isolates were in cluster A (A02_NP and A04_P03), three MRSA isolates in clusters E (A35_P24, A38_P25 and B16_P05) and F (B06_P17, B13_P24 and B55_P11), respectively, and one MRSA isolate was in clusters C (B23_P03) and G (B58_NP), respectively. For MRSAs from persistent carriers, two MRSAs from a single individual harbored a similar SCC*mec* type I, with a non-typable *spa* type and an antibiotic susceptibility pattern, but both isolates were not clustered in the same group (A04_P03; Cluster A and B23_P03; Cluster C). Another pair of MRSA in a persistent carrier (A35_P24; Cluster E and B13_P24; Cluster F), showed similar patterns, except for susceptibility against erythromycin (the isolate from the first isolation event was resistant, while the isolate from the second isolation event showed an intermediate result). These results suggest that most of the MRSAs were potentially of a diverse genetic background.

Please cite this article in press as: Mat Azis N, 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 (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.013

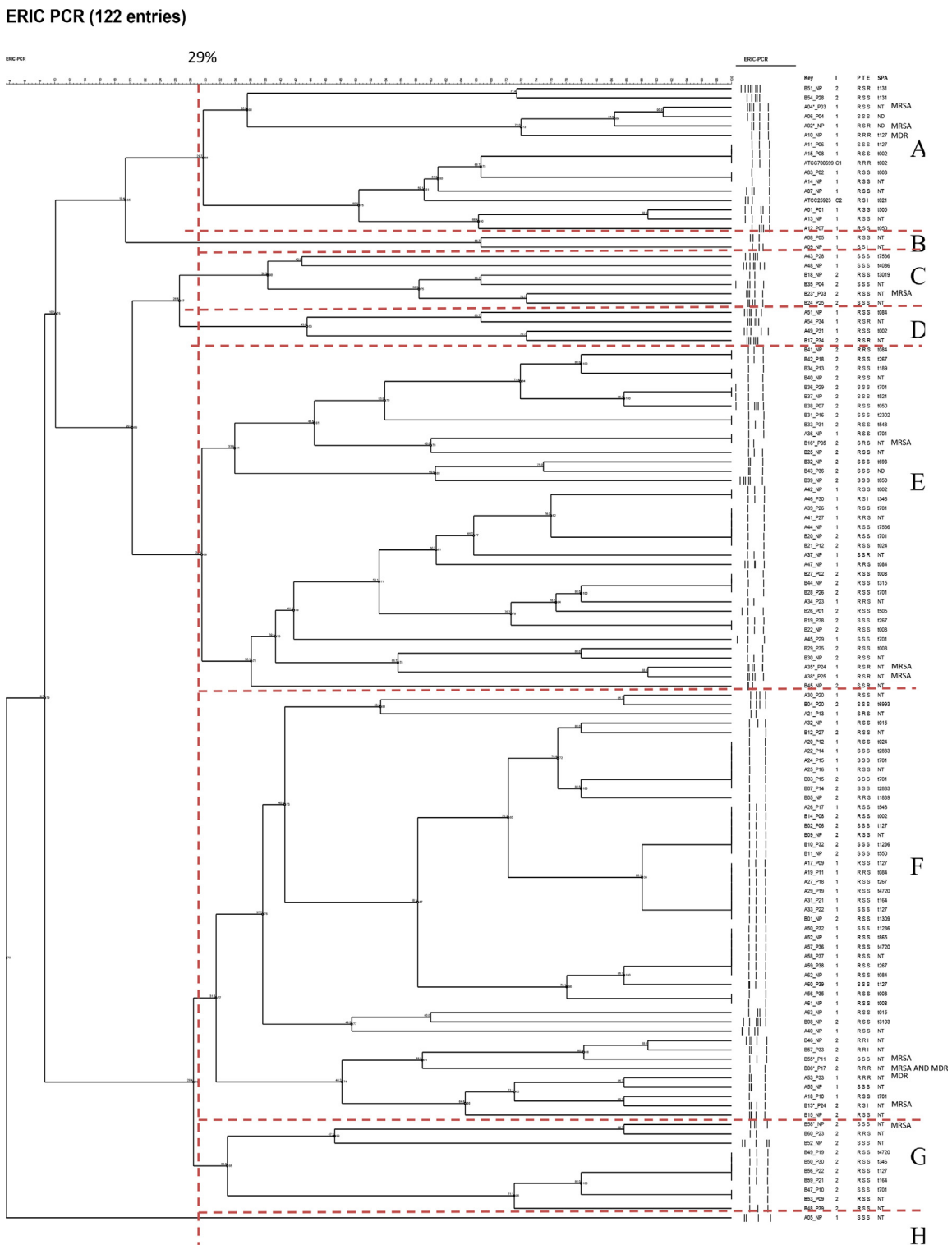


**Figure 2** Dendrogram of the ERIC-based DNA fingerprint analysis using GelcomparII. *MRSA and Multi-drug resistance (MDR) are shown with abbreviations. 1st column: Isolate Code; 2nd column: Isolation event (1: 1st event; 2: 2nd event); 3rd column: Resistance pattern (P: Penicillin; T: Tetracycline; E: Erythromycin); and 4th column: *Spa* type. The identity of the isolates is labeled with 'A' for isolates from the first isolation and 'B' for isolates from the second isolation, followed by numbers. Additional labeling includes 'NP' for isolates from non-persistent carriers and 'P' from persistent carriers, followed by a number. The latter will have the same number for isolates obtained from the same individual.

Please cite this article in press as: Mat Azis N, 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 (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.013

## Conclusion

As a whole, a persistent antimicrobial pattern and limited methicillin resistance-associated genotypes were observed over the short study period, with the potential persistence of similar isolates in some of the respective carriers. The number of MRSA strains was low, but those strains identified in different individuals were largely distinct. Interestingly, some strains had genotypes associated with a clonal complex comprising isolates identified in other countries. The source of these MRSAs is of interest, but whether the MRSAs in the present study are of community or hospital origin cannot be discriminated. Although most of the isolates carried SCC*mec* type I, a previously prominent characteristic of HA-MRSA, these bacteria could not be easily differentiated, as both HA- and community-acquired (CA) MRSA have merged and evolved and the earlier genotype associated with either HA- or CA-MRSA is disappearing [32].

## Funding

This research was partially funded through grants no. 04-02-12-1764RU and ERGS/1-2013/5527167. This study was approved through the Ethics Committee for Research involving Human Subjects (JKEUPM), Universiti Putra Malaysia (UPM/TNCPI/RMC/1.4.18.1 (JKEUPM)/F1).

## Competing interests

None declared.

## Ethical approval

None required.

## Acknowledgments

The authors would also like to acknowledge the technical staff at the Department of Biomedical Science and Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, for assistance and support in the present study.

## References

[1] Shibabaw A, Abebe T, Mihret A. Antimicrobial susceptibility pattern of nasal *Staphylococcus aureus* among Dessie Referral Hospital health care workers, Dessie, Northeast Ethiopia. Int J Infect Dis 2014;25:22—5.

[2] Chen CJ, Wang SC, Chang HY, Huang YC. Longitudinal analysis of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* carriage in healthy adolescents. J Clin Microbiol 2013;51:2508—14.

[3] Gualdoni GA, Lingscheid T, Tobudic S, Burgmann H. Low nasal carriage of drug-resistant bacteria among medical students in Vienna. GMS Krankenhaush Interdiszip 2012;7:1—4.

[4] Chen C-J, Huang YC. New epidemiology of *Staphylococcus aureus* infection in Asia. Clin Microbiol Infect 2014;20:605—23.

[5] Seybold U, Kourbatova EV, Johnson JG, Halvosa SJ, Wang YF, King MD, et al. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of health care-associated blood stream infections. Clin Infect Dis 2006;42:647—56.

[6] Mat Azis N, Ab Hamid A, Pung HP, Abdul Rafee PA, Yahya FA, Amin Nordin S, 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.

[7] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement. CLSI document M100-S23. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.

[8] Suhaili Z, Johari SA, Sajili MH, Yahya A, Zakaria ZA, Mohd Desa MN, et al. In silico PCR verification and simplex real-time PCR detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from east coast Malaysian clinical isolates. Walailak J Sci Technol 2013;10:237—46.

[9] Boye K, Bartels MD, Andersen IS, Møller JA, Westh H. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCC*mec* types I-V. Clin Microbiol Infect 2007;13:725—7.

[10] Enright MC, Day NPJ, 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.

[11] Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter M-O, Gauduchon V, et al. Involvement of Panton-Valentine Leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 1999;29:1128—32.

[12] Japoni-Nejad A, Rezazadeh M, Kazemian H, Fardmousavi N, van Belkum A, Ghaznavi-Rad E. Molecular characterization of the first community-acquired methicillin-resistant *Staphylococcus aureus* strains from Central Iran. Int J Infect Dis 2013;17:e949—54.

[13] Ye Y, Jiang Q, Wu Q, Zhang J, Lu J, Lin L. The characterization and comparison of *Staphylococcus aureus* by antibiotic susceptibility testing, enterobacterial repetitive intergenic consensus-polymerase chain reaction, and random amplified polymorphic DNA-polymerase chain reaction. Foodborne Pathog Dis 2012;9:168—71.

[14] Choi CS, Yin CS, Bakar AA, Sakewi Z, Naing NN, Jamal F, et al. Nasal carriage of *Staphylococcus aureus* among healthy adults. J Microbiol Immunol Infect 2006;39:458—64.

[15] Du J, Chen C, Ding B, Tu J, Qin Z, Parsons C, et al. Molecular characterization and antimicrobial susceptibility of nasal *Staphylococcus aureus* isolates from a Chinese medical college campus. PLoS ONE 2011;6:1—5.

[16] Prates KA, Torres AM, Garcia LB, Yamada Ogatta SF, Cardoso CL, Bronharo Tognim MC. Nasal carriage of methicillin-resistant *Staphylococcus aureus* in university students. Braz J Infect Dis 2010;14:316—8.

Please cite this article in press as: Mat Azis N, 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 (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.013

[17] Zanelli G, Sansoni A, Zanchi A, Cresti S, Pollini S, Rossolini GM, et al. *Staphylococcus aureus* nasal carriage in the community: a survey from central Italy. Epidemiol Infect 2002;129:417—20.
[18] Ng ST, Lim CY, Tan CS, Abd Karim A, Haron H, Ahmad N, et al. Emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA). Webmed Central Infect Dis 2011;2:1—11.
[19] Bischoff WE, Wallis ML, Tucker KB, Reboussin A, Sherertz RJ, Wake S. *Staphylococcus aureus* nasal carriage in a student community: prevalence, clonal relationships, and risk factors. Infect Control Hosp Epidemiol 2014;25:485—91.
[20] Shamsudin MN, Sekawi Z, Van Belkum A, Neela V. First community-acquired methicillin-resistant *Staphylococcus aureus* in Malaysia. J Med Microbiol 2008;57:1180—1.
[21] Syafinaz AM, Nur Ain NZ, Nadzirahi SN, Fatimah JS, Shahram A, Mohd Nasir MD. *Staphylococcus aureus* nasal carriers among medical students in a medical school. Med J Malays 2012;67:636—8.
[22] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, 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.
[23] Critchley IA, Draghi DC, Sahm DF, Thornsberry C, Jones ME, Karlowsky JA. Activity of daptomycin against susceptible and multidrug-resistant gram-positive pathogens collected in the SECURE study (Europe) during 2000—2001. J Antimicrob Chemother 2003;51:639—49.
[24] VasanthaKumari N, Alshrari ASD, Rad EG, Moghaddam HG, Van Belkum A, Alreshidi MA, et al. Highly dynamic transient colonization by *Staphylococcus aureus* in healthy Malaysian students. J Med Microbiol 2009;58:1531—2.
[25] Fan J, Shu M, Zhang G, Zhou W, Jiang Y, Zhu Y, et al. Biogeography and virulence of *Staphylococcus aureus*. PLoS ONE 2009;4:1—11.
[26] Ghasemzadeh-Moghaddam H, Ghaznavi-Rad E, Sekawi Z, Yun-Khoon L, Aziz MN, Hamat RA, et al. Methicillin-susceptible *Staphylococcus aureus* from clinical and community sources are genetically diverse. Int J Med Microbiol 2011;301:347—53.
[27] Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. PLoS ONE 2011;6:1—24.
[28] Lim KT, Yeo CC, Suhaili Z, Thong KL. Comparison of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* strains isolated from a tertiary hospital in Terengganu, Malaysia. Jpn J Infect Dis 2012;65:502—9.
[29] Ip M, Yung RWH, Ng TK, Luk WK, Tse C, Hung P, et al. Contemporary methicillin-resistant *Staphylococcus aureus* clones in Hong Kong. J Clin Microbiol 2005;43:5069—73.
[30] Mendes RE, Deshpande LM, Smyth D, Shopsin B, Farrell DJ, Jones RN. Characterization of methicillin-resistant *Staphylococcus aureus* strains recovered from a phase IV clinical trial for linezolid versus vancomycin for treatment of nosocomial pneumonia. J Clin Microbiol 2012;50:3694—702.
[31] Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997;10:505—20.
[32] Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. Infect Genet Evol 2008;8:747—63.



Please cite this article in press as: Mat Azis N, 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 (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.013