

Prevalent Genotypes of *Staphylococcus aureus* Strains Isolated From Healthcare Workers in Duhok City, Kurdistan Region, Iraq

Nawfal R Hussein^{1,*}

¹Department of Internal Medicine, College of Medicine, University of Duhok, Duhok, Kurdistan Region, Iraq

*Corresponding author: Nawfal R Hussein, Department of Internal Medicine, College of Medicine, University of Duhok, Duhok, Kurdistan Region, Iraq. E-mail: nawfal.hussein@yahoo.com

Received 2015 December 9; Revised 2015 December 19; Accepted 2015 December 21.

Abstract

Background: Nasal carriage of *Staphylococcus aureus* and its virulence determinants are major risk factors for subsequent infections and transmission.

Objectives: The current study aimed to determine the prevalence of panton-valentine leukocidin (PVL) gene, the SCCmec types, and the prevalent methicillin-resistant *Staphylococcus aureus* (MRSA) genotypes in strains isolated from healthcare workers (HCWs).

Materials and Methods: DNA was extracted from 114 frozen *S. aureus* strains amongst them MRSA isolates were identified by conventional methods and PVL; genotyping were determined using PCR and pulsed-field gel electrophoresis (PFGE).

Results: Fifty-nine out of 114 (51.7%) isolates were MRSA carrying *mecA*, 18/59 (31%) harboured SCCmec type I, 12/59 (20.1%) harboured SCCmec type II and 12/59 (20.1%) harboured type III. The *pvl* gene was detected in 10 *S. aureus* isolates, six MRSA and four MSSA. PFGE clustered the 114 isolates of *S. aureus* into eight predominant types (designated A - H). The most prevalent PFGE type was type A (n = 44, 37.7%).

Conclusions: More than a quarter of the strains harboured SCCmec type I. *Staphylococcus aureus* isolates were clustered into eight predominant PFGE pulsotypes. The current study should be considered preliminary and further use of molecular studies to monitor the epidemiology of MRSA and its evolution is highly recommended.

Keywords: Iraq, *mecA*, MRSA, Duhok, PVL, SCCmec

1. Background

Staphylococcus aureus is a Gram-positive bacterium, causes both community and hospital-acquired infections. The bacteria colonise skin and nasal passage in human and also cause invasive diseases such as pneumonia and sepsis (1). With the introduction of penicillin, the mortality from *S. aureus* infections decreased dramatically. However, such a decrease was short-lived due to the emergence of penicillinase producing *S. aureus* (1). Methicillin was subsequently introduced to treat *S. aureus* infections, but methicillin-resistant *S. aureus* (MRSA) strains rapidly emerged and became a major problem in hospitals in the 1960s. The *mecA* gene, which is responsible for methicillin resistance is carried on a mobile genetic element of a 21 to 67-kb which is known as the staphylococcal cassette chromosome *mec* (SCCmec) (2). This gene encodes a modified penicillin-binding protein (PBP-2a) with low affinity for beta-lactam antibiotics such as methicillin. SCCmec characterization depends upon the variations of the *mec* gene complex, cassette chromosome recombinase (*ccr*) complex, and the junkyard regions. Five SCCmec categories (I to V) were originally identified in *S. aureus* (3-5). In addition, SCCmec VI and VII types have also been discovered, but

they are not as common as the others are. PVL is a two-component *S. aureus* spore-forming protein encoded by the *lukF-PV* and *lukS-PV* genes (6). PVL toxin is responsible for the increased virulence of community acquired MRSA (CA-MRSA), since this gene is responsible for many of the severe clinical syndromes of MRSA such as severe necrotising pneumonia (3-5).

2. Objectives

Studying CA-MRSA and its genotyping was conducted in Iraq (3-5). The current study aimed to determine the prevalence of PVL-containing MRSA isolates, the SCCmec types in methicillin resistant staphylococci and the prevalent MRSA genotypes in *S. aureus* strains isolated from healthcare workers in Duhok city.

3. Materials and Methods

3.1. Bacterial Identification and Antimicrobial Susceptibility Testing

Author had access to 114 *S. aureus* strains isolated from

healthcare workers (HCWs). The frozen isolates were directly inoculated onto mannitol salt agar plates and incubated aerobically at 35°C for 48 hours. The isolates were identified as *S. aureus* based on Gram stain, catalase test, coagulase test, and mannitol salt agar fermentation. Antimicrobial susceptibility testing to oxacillin was carried out adopting the Kirby-Bauer disk diffusion and agar dilution assay methods using Muller-Hinton agar (Oxoid Limited, Hampshire, England), according to the recommendations of the clinical laboratory standards institute (CLSI) (7, 8).

3.2. DNA Extraction and PCR for *mecA* and *pvl* Genes

DNA was extracted from *S. aureus* isolates using the Qiagen DNA purification kit according to the manufacturer's instructions (DNAeasy, Qiagen). All methicillin-resistant isolates were examined for the presence of the *mecA* gene. The presence of the *mecA* gene was confirmed by PCR amplification as described by Murakami et al. (9). Thermal cycling conditions to amplify *mecA* were 95°C for 30 seconds, 58°C for one minute, and 72°C for two minutes, for a total of 35 cycles. PCR amplification of *mecA* used previously described primers (MR1: GTG GAA TTG GCC AAT ACA GG and MR2: TAG GTT CTG CAG TAC CGG AT primers), which can amplify a 1399 base pair fragment specific for *mecA* (10). The PVL was amplified using standard protocols under the following thermal cycling conditions: 35 cycles, each consisting of denaturation at 95°C for 30 seconds, annealing at 55°C for one minutes, and elongation at 72°C for two minutes. Primers Luk-PV-1 (ATCATTAGGTAATAATGTCTGGACATGATCCA) and Luk-PV-2 (GCATCAAGTGTATTGGATAGCAAAAGC) were used (11). Amplification for both *mecA* and *pvl* genes started with an initial denaturation at 95°C for 60 seconds and a final elongation step of five minutes at 72°C. Reactions were performed in 25 µL volumes containing 1 µL of genomic DNA, 1 µL primer, 0.5 µL of Taq DNA polymerase, 0.5 µL dNTPs, and 2.5 10x PCR buffer. Then 5 µL of the PCR products were electrophoresed in 1.5% wt/vol agarose gel for 40 minutes at 80 V in 1x TAE buffer. All PCR products were stained with ethidium bromide (1 mg/L) and visualized under UV light; 100bp DNA ladder (Gibco, Paisley, UK) was used as a size marker (M) in all products.

3.3. SCCmec Typing

SCCmec typing was performed according to the previously described protocol of Zhang et al. (12). The PCR was run under the following conditions: denaturation step at 94°C for five minutes followed by 10 cycles of 94°C for 45 seconds, 65°C for 45 seconds, and 72°C for 90 seconds and another 25 cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 90 seconds, ending with a final extension step at 72°C for 10 minutes and followed by a hold at 4°C. The PCR am-

plicons were visualized via UV light after electrophoresis on a 2% wt/vol agarose gel containing 0.5 µg/mL ethidium bromide.

3.4. Pulsed-Field Gel Electrophoresis

PFGE was carried out as previously described (13). DNA was digested by *Sma*I restriction enzyme (New England) and loaded into 1% agarose gel and ran at 14°C in TBE using a CHEF-DR III system (Bio-Rad). The PFGE was performed at 200 V with an initial switch time of five seconds and final switch time of 40 seconds, the included angle was 120° and the total run time was 21 hours. The generated DNA fingerprints were analysed according to the criteria proposed by Tenover et al. (14). BioNumerics software was used to determine similarities of banding patterns (version 4.5; Applied Maths). The cluster analysis was conducted via the unweighted pair group method analysis (UPGMA). Isolates with a coefficient of similarity $\geq 75\%$ were considered one pulsotype and labelled alphabetically. In addition, isolates with a coefficient of similarity $\geq 50\%$ were grouped in a separate pulsotype. Isolates with a similarity between 50% and 75% were regarded as PFGE subtypes.

3.5. Statistics

Data were transferred to the statistical package for social science (SPSS) software version 19 for analysing.

4. Results

4.1. MRSA and MSSA

Amongst the 114 strains, 59 isolates were resistant to oxacillin.

4.2. *mecA*, SCCmec and PVL

The presence of *mecA*, the genes encoding SCCmec type and PVL toxin were investigated. All MRSA strains were positive for *mecA*. Additionally, the SCCmec types I, II and III were screened among the MRSA isolates, SCCmec types I, II and III were 31% (18 isolates), 20.1% (12 isolates) and 20.1% (12 isolates), respectively. Additionally, 19.2% of the strains were type IV (10 strains). Among the 114 isolated *S. aureus*, 10 strains carried the genes encoding PVL: six for MRSA and four for MSSA.

4.3. PFGE

All 114 *S. aureus* isolates were clustered into eight predominant PFGE pulsotypes (designated types A - H) (Table 1). The most prevalent PFGE type was type A (n = 43, 37.7%). Isolates with type A had 7 subtypes named A1 - A7, and among them the most predominant subtype was A1 (10/44). Isolates with type B had three subtypes B1 - B3. Other pulsotypes, C, D, E, F, G and H were less common with fewer subtypes, C1 and C2 and D1 and D2. Pulsotypes E - H had no subtypes (Table 2).

Table 1. The Number of Strain Pulsotype in *Staphylococcus aureus* Strains Isolated From Healthcare Workers in Iraq

| Pulsotype | MRSA | MSSA | Strains, No. (%) |
|--------------|-----------|-----------|------------------|
| A | 21 | 22 | 43 (37.7) |
| B | 13 | 12 | 25 (21.9) |
| C | 5 | 5 | 10 (8.77) |
| D | 6 | 4 | 10 (8.77) |
| E | 8 | 2 | 10 (8.77) |
| F | 3 | 3 | 6 (5.26) |
| G | 1 | 4 | 5 (4.38) |
| H | 2 | 3 | 5 (4.38) |
| Total | 59 | 55 | 114 |

Abbreviation: MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin susceptible *S. aureus*.

Table 2. The Distribution of SCCmec in Methicillin-Resistant *Staphylococcus aureus* Strains According to the Pulsotype

| Pulsotype | Number of SCCmec | | | | | | | | Total | Untypable |
|--------------|------------------|-----------|-----------|----------|----------|----------|----------|----------|-----------|-----------|
| | I | II | III | IVa | IVb | IVd | IVc | V | | |
| A | 9 | 5 | 2 | 2 | 1 | 1 | 0 | 0 | 20 | 1 |
| B | 3 | 3 | 4 | 2 | 0 | 0 | 0 | 0 | 12 | 1 |
| C | 1 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 4 | 1 |
| D | 4 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 6 | 0 |
| E | 1 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 6 | 2 |
| F | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 1 |
| G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| H | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| Total | 18 | 12 | 12 | 7 | 2 | 1 | 0 | 0 | 52 | 7 |

5. Discussion

This project showed that all MRSA strains were positive for *mecA* and the rate of strains harbouring SCCmec types I, II and III were 31%, 20.1% and 20.1%, respectively. SCCmec type IV was less common than other genotypes since 19.2% of the strains were SCCmec type IV. Such a diversity was also found in Iran (15), where 3.4% of MRSA strains were found to be SCCmec type I, 13.8% SCCmec type II, 9.2% SCCmec type IVb, 4.6% SCCmec type IVd and 3.4% SCCmec type V. In the same study, there was also a high frequency of non-typeable strains as 58.6% of MRSA strains were non-typeable. However, such diversity was not found in Turkey since the study showed that 22 MRSA isolates which were tested for SCCmec types were SCCmec type III (16). The analysis of MRSA isolates in Holland showed that 8% of nosocomial isolates carry the locus for PVL. In Iran, the prevalence of MRSA, the *pvl* gene and MRSA-PVL isolates were 32%, 19%, and 10%, respectively (17). In Germany, the prevalence of MRSA isolates among HCWs was 11.3%, among which 9.1% of the isolates were positive for PVL (18). Worldwide, there is an increase in the spread of PVL-positive MRSA clones (19). The current study found that the prevalence of *pvl* gene, MSSA-PVL and MRSA-PVL

isolates were 16.9%, 6.8% and 10.1% respectively. This study should be considered preliminary and further use of molecular studies to monitor the epidemiology of MRSA and its evolution in these hospitals in the country is highly recommended. Pulsed Field Gel Electrophoresis (PFGE) is considered the gold standard typing method for epidemiological tracing of a variety of bacterial species including *S. aureus* since it is highly discriminatory, stable and reproducible (20). Newer techniques such as multilocus sequence typing (MLST), *spa*-typing and DNA sequencing allow typing equally well as the PFGE. However, the need for trained staff and highly expensive equipment made the use and the availability of these techniques difficult in Iraq. In the current study, more than 50% of *S. aureus* isolates were pulsotype A or B indicating that they might have spread from the same sources. In addition, the obtained data should be considered as baseline information and to understand the changes in the prevalence of molecular subtypes and the transmissibility pattern of such strains, further studies are needed to investigate isolates from clinical samples and compare them to strains obtained from healthcare workers. To conclude, more

than a quarter of the current studied strains harboured SCCmec type I. *Staphylococcus aureus* isolates were clustered into eight predominant PFGE pulsotypes and the most prevalent PFGE type was type A. Further use of molecular studies to monitor the epidemiology of MRSA and its transmission patterns in these hospitals in the country is highly recommended.

Footnotes

Authors' Contribution:The project was conducted by Nawfal R Hussein.

Funding/Support:This study was approved by the ethics committee in the University of Duhok, School of Medicine, Kurdistan Region, Iraq.

References

- Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. *Infect Genet Evol.* 2008;**8**(6):747-63. doi: 10.1016/j.meegid.2008.07.007. [PubMed: 18718557]
- Kim J. Understanding the Evolution of Methicillin-Resistant *Staphylococcus aureus*. *J. Clin Microbiol.* 2009;**31**(3):17-23. doi: 10.1016/j.jclinmicnews.2009.01.002.
- Assafi MS, Mohammed RQ, Hussein NR. Nasal carriage rates of *Staphylococcus aureus* and ca-methicillin resistant *Staphylococcus aureus* among university students. *Int J Microbiol Res.* 2015;**5**(4):123-7.
- Hussein NR, Alyas A, Majeed M, Assafi MS. Prevalence rate and prevalent genotypes of ca-mrsa in kurdistan region: First report from iraq. *Int. J. Pure Appl. Sci. Technol.* 2015;**27**(1):44-9.
- Hussein NR, Basharat Z, Muhammed AH, Al-Dabbagh SA. Comparative evaluation of MRSA nasal colonization epidemiology in the urban and rural secondary school community of Kurdistan, Iraq. *PLoS One.* 2015;**10**(5):e0124920. doi: 10.1371/journal.pone.0124920. [PubMed: 25932644]
- David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev.* 2010;**23**(3):616-87. doi: 10.1128/CMR.00081-09. [PubMed: 20610826]
- National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing, 14th informational supplement. 2004.
- Tenover FC, Moellering RC. The rationale for revising the Clinical and Laboratory Standards Institute vancomycin minimal inhibitory concentration interpretive criteria for *Staphylococcus aureus*. *Clin Infect Dis.* 2007;**44**(9):1208-15. doi: 10.1086/513203. [PubMed: 17407040]
- Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of *Staphylococcus* by polymerase chain reaction. *J Clin Microbiol.* 1991;**29**(10):2240-4. [PubMed: 1939577]
- Moussa I, Shibl AM. Molecular characterization of methicillin-resistant *Staphylococcus aureus* recovered from outpatient clinics in Riyadh, Saudi Arabia. *SAUDI MED J.* 2009;**30**(5):611-7. [PubMed: 19417957]
- McClure JA, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W, et al. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant *Staphylococcus aureus*. *J Clin Microbiol.* 2006;**44**(3):1141-4. doi: 10.1128/JCM.44.3.1141-1144.2006. [PubMed: 16517915]
- Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol.* 2005;**43**(10):5026-33. doi: 10.1128/JCM.43.10.5026-5033.2005. [PubMed: 16207957]
- McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. *J Clin Microbiol.* 2003;**41**(11):5113-20. [PubMed: 14605147]
- Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol.* 1995;**33**(9):2233-9. [PubMed: 7494007]
- Zeinali E, Moniri R, Musavi GH. Antibiotic resistance and molecular subtypes of clinical methicillin-resistant *Staphylococcus aureus* in a teaching hospital. *Indian J Med Microbiol.* 2011;**29**(3):318-9. doi: 10.4103/0255-0857.83926. [PubMed: 21860123]
- Mert G, Kiliç A, Bedir O, Başustaoglu AC. Clinical significance and staphylococcal cassette chromosome mec (SCCmec) characterization of coagulase-negative staphylococci isolated from blood cultures. *Turk J Med Sci.* 2011;**41**(5):859-65.
- Lari AR, Pourmand MR, Ohadian Moghadam S, Abdossamadi Z, Namvar AE, Asghari B. Prevalence of pvl-containing mrsa isolates among hospital staff nasal carriers. *Laboratory Medicine.* 2011;**42**(5):283-6. doi: 10.1309/lman7hr6vjea3nmr.
- Maltezou HC, Vourli S, Katerelos P, Maragos A, Kotsalidou S, Remoudaki E, et al. Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* outbreak among healthcare workers in a long-term care facility. *Int J Infect Dis.* 2009;**13**(6):e401-6. doi: 10.1016/j.ijid.2009.02.004. [PubMed: 19395300]
- Tristan A, Bes M, Meugnier H, Lina G, Bozdogan B, Courvalin P, et al. Global distribution of Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus*, 2006. *Emerg Infect Dis.* 2007;**13**(4):594-600. doi: 10.3201/eid1304.061316. [PubMed: 17553275]
- Faria NA, Carrico JA, Oliveira DC, Ramirez M, de Lencastre H. Analysis of typing methods for epidemiological surveillance of both methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains. *J Clin Microbiol.* 2008;**46**(1):136-44. doi: 10.1128/JCM.01684-07. [PubMed: 17989188]