High prevalence of toxic shock syndrome toxin–producing epidemic methicillin-resistant Staphylococcus aureus 15 (EMRSA-15) strains in Kuwait hospitals

E. E. Udo, S. S. Boswihi and N. Al-Sweih
Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait

Abstract

This study characterized EMRSA-15 isolates obtained from patients in Kuwait hospitals for their genotypic relatedness, antibiotic resistance and carriage of virulence genes using pulsed-field gel electrophoresis (PFGE), coagulase serotyping, SCCmec subtyping, spa typing, multilocus sequence typing and DNA microarray. The isolates were resistant to trimethoprim (75.6%), ciprofloxacin (29.7%), erythromycin and clindamycin (24.3%), tetracycline (19.0%), and gentamicin and kanamycin (21.6%). All 37 isolates belonged to sequence type (ST) 22, coagulase type XI, three PFGE types and eight subtypes, ten spa types including t223 (51.3%), t852 (13.5%), t032 (8.1%), t790 (8.1%), t3107 (5.4%) and one each of t309, t2251, t3935, t5708 and t5983. Twenty-six isolates (70.2%) carried SCCmec IV and three isolates carried SCCmec Ivh. All isolates carried agr1, cap5 and egc gene cluster (seg, sei, selm, seln, selo, and selu). tst (toxic shock syndrome toxin) was detected in 23 isolates. Eight isolates (21.6%) were positive for Panton-Valentine leukocidin (PVL). Genotypic analysis revealed that 62.1% of the isolates comprising ST22-Iva-t223 (51.3%) and ST22-Iva-t309/t2251/t3935/t5708 (10.8%) were CC22-[tst1+] UK EMRSA-15/Middle Eastern variant, 21.6% were CC22-PVL+ EMRSA-15 variant and 16.2% were CC22-UK EMRSA-15/Barnim clone. These results show that the tst1 positive-ST22-IVa-t223 (Middle Eastern variant) and the CC22-PVL+ EMRSA-15 variant were the dominant EMRSA-15 variants in Kuwait hospitals.

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) strains continue to be isolated from both healthcare- and community-associated infections in different parts of the world. The increase in the number of MRSA infections reported worldwide has been accompanied by changes in the characteristics of MRSA strains emerging in different parts of the world [1]. Consequently, epidemiologic typing using a combination of phenotypic and genotypic typing methods has contributed to our understanding of changes in the clonal distribution of MRSA isolated in different geographical locations over time. Whereas MRSA isolates obtained in the 1960s (early or classic MRSA) were usually susceptible to majority of non-β-lactam antibiotics, those isolated in the late 1970s and beyond were multiply resistant to non-β-lactam antibiotics and were described as epidemic MRSA (EMRSA) because of their capacity to spread extensively and cause serious infections among hospitalized patients [1,2]. Epidemiologic typing by Kerr et al. [3] and Aucken et al. [4] identified 17 different EMRSA clones, designated EMRSA-1 to EMRSA-17, in the United Kingdom.

The EMRSA-15 clone was first characterized in England on the basis of phage typing (a weak lysis pattern with phage 75), susceptibility to antimicrobial agents and failure to produce urease [5]. The urease-negative phenotype resulted from a single nucleotide deletion within the UreC gene, leading to a frameshift inactivation of the alpha subunit of the urease gene [6]. Subsequently, multilocus sequence typing of the EMRSA-15 strains classified them as multilocus sequence (ST) type 22 (ST22) [7].
EMRSA-15 strains are an important cause of nosocomial bacteraemia in many UK and Irish hospitals [8], and they are a major pathogen in other European healthcare facilities [9,10]. EMRSA-15 strains have also been isolated in Australia [11], Singapore [12], India [13,14], Malaysia [15], Kuwait [16], Saudi Arabia [17], United Arab Emirates [18], Qatar [19] and Oman [20]. The survival and widespread transmission of EMRSA-15 strains have been attributed to genetic changes within the bacterial genome due to the acquisition of antibiotic resistance and virulence genes and adaptation to the healthcare environment [6].

Molecular characterization of EMRSA-15 strains isolated in the Gulf Cooperative Council (GCC) countries have revealed different genetic backgrounds with different antibiotic susceptibility patterns. Whereas EMRSA-15 isolates from the United Arab Emirates belonged to spa types t032 or t005 [18], the majority of recent isolates from Qatar [19] and Oman [20] belong to t852.

In this study, we investigated EMRSA-15 isolates obtained in Kuwait public hospitals using a combination of molecular typing methods to establish their genetic relatedness to EMRSA-15 isolated in the United Kingdom and other GCC countries.

Materials and Methods

MRSA isolates
A total of 37 EMRSA-15 strains, isolated in the years 2005 (four isolates representing four pulsed-field gel electrophoresis (PFGE) patterns) and 2010 (33 isolates), were included in this study. These MRSA isolates were among clinical isolates submitted to the MRSA Reference Laboratory, Kuwait, for molecular typing. The MRSA isolates were obtained as part of routine bacteriology services in the individual hospital laboratories and were archived at the MRSA Reference Laboratory, Kuwait. The MRSA isolates were isolated at ten hospitals: Mubarak (nine isolates), Al Sabah (seven isolates), Ibn Sina (five isolates), maternity hospital (five isolates), Adan (three isolates), Al Razi (three isolates), Farwaniya (two isolates), Jahra (one isolates), Armed Forces (one isolate) and Chest Disease Hospital (one isolates). Isolates were identified by cultural characteristics, Gram staining, and positive tube coagulase and DNase tests. The isolates were preserved in glycerol 40% (v/v) in brain heart infusion broth (Oxoid, Basingstoke, UK) at −80°C. They were recovered by subculturing in brain–heart infusion broth at 37°C for 24 hours followed by two further subcultures on brain–heart infusion agar. Preliminary identification of MRSA isolates as EMRSA-15 was based on negative urease test [5] and carriage of SCCmeC type IV genetic element.

Urease production
Urease production was detected on Christensen urea agar slope after 72 hours’ incubation at 35°C as described previously [5,16].

Antibacterial susceptibility testing
Antibacterial susceptibility testing was performed by the disk diffusion method [21] with the following antimicrobial disks (Oxoid): benzyl penicillin (10 μg), cefoxitin (30 μg), kanamycin (30 μg), mupirocin (200 and 5 μg), gentamicin (10 μg), erythromycin (15 μg), clindamycin (2 μg), chloramphenicol (30 μg), tetracycline (10 μg), trimethoprim (2.5 μg), fusidic acid (10 μg), rifampicin (5 μg), ciprofloxacin (5 μg), teicoplanin (30 μg) and linezolid (30 μg). Minimum inhibitory concentration for cefoxitin, vancomycin and teicoplanin were determined with Etest strips (bioMérieux, Marcy l’Étoile, France) according to the manufacturer’s instructions. S. aureus strain ATCC 25923 was used as a quality control strain for susceptibility testing. The Dtest was used to test for inducible resistance to clindamycin.

Molecular typing of isolates
Isolates were typed by PFGE, coagulase gene typing, SCCmeC typing, spa typing and multilocus sequence typing (MLST). PFGE was performed on all 37 MRSA isolates as described previously [22]. DNA for PCR amplification was isolated and purified as described previously [20]. Coagulase typing was performed as described previously [23]. For the detection of coagulase type XI, primer pair (coaS-F) 5’-TGGGCAATTACATTTTG GAG-3’ and (coaS-XI-R) 5’-TCGTTTGGGTAGTGTGTTT-3’ (395 bp) were designed and used in this study. PCR amplification was carried out on a Gene AMP PCR System 9700 instrument (Thermo Fisher Scientific Life Sciences, Waltham, MA, USA) in a total volume of 25 μL of reaction mixture containing 12.5 μL of AmpliTaq Gold master mix (Roche, Basel, Switzerland), 50 pmol of each primer and 2 μL of extracted DNA. The PCR reaction was 95°C for 15 minutes, followed by 30 cycles with 95°C for 30 seconds, 56°C for 40 seconds, 72°C for 30 seconds and a final extension step of 72°C for 5 minutes. PCR products were analysed by agarose gel electrophoresis using 2% (w/v) agarose in Tris-EDTA buffer.

SCCmeC typing was performed by PCR assays as described previously [24,25], spa typing was performed as described by Harmsen et al. [26]. Clonal relatedness of the spa types was determined by the BURP (Based Upon Repeat Pattern) algorithm as described by Mellmann et al. [27]. MLST was performed on all 37 isolates as described by Enright et al. [7].

Detection of genes for antibiotic resistance and virulence
DNA microarray analysis using Identibac S. aureus Genotyping Kit 2 (Alere Technology, Jena, Germany) was used following protocols provided by the manufacturer. Data generated were
analysed using the ArrayMate software and the ArrayMate Reader (Alere Technology), which assigned MRSA isolates to STs and clonal complexes (CCs) by comparing each isolate to STs and CCs of previously characterized isolates in the ArrayMate database [28,29].

## Results

The MRSA isolates were obtained from nasal swabs (n = 18), skin and soft tissues (n = 10), sputum (n = 3), groin (n = 2), ear (n = 2), and vaginal swab and axilla (n = 1 each). The patients consisted of 23 men and 13 women. The sex of one patient was not specified.

All 37 isolates were urease negative and were susceptible to vancomycin and teicoplanin (minimum inhibitory concentration \( \leq 1.5 \) mg/L), linezolid, chloramphenicol, rifampicin, mupirocin and fusidic acid but resistant to trimethoprim (n = 28; 75.6%), ciprofloxacin (n = 11; 29.7%), erythromycin and clindamycin (n = 9; 24.3%), gentamicin and kanamycin (n = 8; 21.6%), and tetracycline (n = 7; 19.0%).

### Molecular typing of MRSA isolates

The isolates were grouped into three PFGE patterns, designated types A (22 isolates), B (14 isolates) and C (one isolate), as summarized in Table 1 and Fig. 1.

### DNA microarray analysis of isolates

DNA microarray analysis revealed that the 37 isolates were positive for genes encoding accessory gene regulator type I (agrI), capsular polysaccharide type 5 (cap5), staphylococcal enterotoxin egc gene cluster (seg, sei, selm, seln, selo), haemolysin beta (hlb), putative membrane protein (hl), staphylokinase (soK) and chemotaxis inhibition protein (chp) but...
differed in the carriage of sea, seb, sec, tst (toxic shock syndrome toxin), hlgA (haemolysin gamma A) and hla (haemolysin alpha). Eight isolates (21.6%) consisting of t852, t3107 and t5983 were positive for Panton-Valentine leukocidin (PVL) (Table 2). None of the isolates was positive for sed.

As shown in Table 2, DNA microarray analysis classified the isolates into three groups: CC22-UK EMRSA-15/Barnim-MRSA clone, CC22-[tst1+] UK EMRSA-15/Middle Eastern variant and CC22-PVL+ UK EMRSA-15. The CC22-UK EMRSA-15/Barnim-MRSA clone comprised six isolates, which belonged to SCCmec-IVh-t032 (three isolates) and SCCmec IVa-t790 (three isolates), that were resistant to erythromycin and clindamycin and carried ermc. The t790 isolates were positive for sec but lacked hla and hlgA, which were present in t032 isolates.

The CC22-[tst1+] UK EMRSA-15/Middle Eastern variant consisted of 23 isolates belonging to spa type t223 (n = 19) and one isolate each of t309, t2251, t3935 and t5708. These isolates harboured tst and were resistant to trimethoprim but differed in their resistance to tetracycline, erythromycin and clindamycin and carriage of virulence genes.

The CC22-PVL+ UK EMRSA-15 variant consisted of eight isolates that belonged to spa types t852 (n = 5), t3107 (n = 2) and t5983 (n = 1). These isolates were PVL positive and were resistant to gentamicin, kanamycin, tobramycin, erythromycin, clindamycin, trimethoprim and ciprofloxacin. The t852 isolates were negative for scn, hla and hlgA.

**Discussion**

The results of this study provide insight into the epidemiology of EMRSA-15 isolates in Kuwait hospitals. Similar to EMRSA-15 isolates reported elsewhere [6,7,29], the isolates investigated in this study belonged to ST22 and carried a type IV SCCmec genetic element. However, molecular subtyping and DNA microarray analysis revealed differences in their genetic backgrounds, suggesting multiple origins for EMRSA-15 in Kuwait hospitals.

The study revealed that isolates fitting the CC22-IV[tst1+] UK EMRSA-15/Middle Eastern variant, consisting of spa types t223 (51.3%), t309 (2.7%), t2251 (2.7%), t3935 (2.7%) and t5708 (2.7%), constituted the dominant EMRSA-15 variant in Kuwait hospitals in 2010. In addition, the t223 isolates widespread in the country evidenced by their isolation in six of the ten hospitals studied. The t223 isolates harboured antibiotic resistance and virulence profiles similar to PVL-negative, tst-positive ST22-IV-t223 isolates reported to have colonized children and parents in the Gaza Strip [30,31] as well as PVL-negative, tst-positive ST22-IV-MRSA-t223 recovered from healthy individuals in Jordan [32], making it the dominant EMRSA-15 clade in the Middle East.
TABLE 2. Molecular characteristics of ST22 MRSA isolates

| Year       | Strain definition  | spa type | Antibiotic resistance (n) | Antibiotic resistance genes | Toxins encoding genes | Miscellaneous genes |
|------------|--------------------|----------|---------------------------|-----------------------------|----------------------|---------------------|
| UK EMRSA-15/Barnim |                    |          |                           |                             |                      |                     |
| 2005 1     | ST22-MRSA-IVh       | t032     | E, CC, CIP                | emIC                        | sec, sec sel, egc    | hla, hili, hlgA     |
| 2010 2     | ST22-MRSA-IVh       | t032     | E (1), CC (1), CIP (2), W (1) | emIC                        | egc                   | sn, hla, hlgA       |
| 2010 3     | ST22-MRSA-IVh       | t790     | E (2), CC (2)             | emIC                        | sec, egc              | sn                  |
| UK EMRSA-15/Middle Eastern variant [txt*] |        |          |                           |                             |                      |                     |
| 2005 2     | ST22-MRSA-IVx       | t223     | E (1), CC (1), TE (2), W (2) | emIC, aacK, dfrF1            | tat, egc              | hla, hlgA           |
| 2010 17    | ST22-MRSA-IVx       | t223     | TE (4), W(4)              | dfrF1                       | tat, egc              | sn, hla             |
| 2005 1     | ST22-MRSA-IVx       | t309     | W                          | dfrF1                       | tat, egc              | sn, hla             |
| 2010 1     | ST22-MRSA-IVx       | t221     | TE                          | dfrF1                       | tat, egc              | sn, hla             |
| 2010 1     | ST22-MRSA-IVx       | t393     | dfrF1                      | tat, sec egc                | sn                    |                     |
| 2010 1     | ST22-MRSA-IVx       | t5708    | W                          | dfrF1                       | tat, egc              | sn                  |
| GCC22-MRSA-IV-PVL |              |          |                           |                             |                      |                     |
| 2010 5     | ST22-MRSA-IV         | t852     | CN (5), K (5), E (1), CC (1), TOB (4), W (3), CIP, aacA-aphD, aadD |                          |                      |                     |
| 2010 2     | ST22-MRSA-IV         | t3107    | CN (2), K (2), TOB (1), E (2), CC (2), W (2), CIP, CIP, aacA-aphD, aadD, dfrF1 |                  | PV, PVL, egc          | sn, hla             |
| 2010 1     | ST22-MRSA-IV         | t5983    | E, CC, W, CIP, CN, K, TOB | emIC, aacA-aphD, aadD, dfrF1 |                      |                     |

All isolates carried hemolysin delta (hld) egc gene cluster (seg, sei, seln, selo, selu). CC, clindamycin; chloramphenicol; CIP, ciprofloxacin; CN, gentamicin; TE, tetracycline; TOB, tobramycin; tst, toxic shock syndrome toxin; W, trimethoprim. 

The multiresistant CC22-MRSA-IV-PVL⁺ variant, consisting of spa types t852 (13.5%), t3107 (5.4%) and t5983 (2.7%), was the second most common EMRSA-15 variant in this study. ST22-IV-MRSA-t852 isolates were reported among healthy carriers and patients in Indian hospitals as early as 2008 [13,33], which may represent the origin of this EMRSA-15 variant. Since then, PVL-positive, multiresistant spa type t852 isolates have been reported among ST22-IV MRSA isolates in Saudi Arabia [17], Qatar [19] and Oman [20], suggesting an increasing transmission of this variant in the Gulf Cooperative Council countries. The t852 isolates were also reported among of ST22-IV MRSA isolated in Zurich, Switzerland, between 2012 and 2014 [34], pointing to their spread in European hospitals.

Surprisingly, the ST22-IVh-t032 isolates related to the UK EMRSA-15/Barnim MRSA clone [10] were less common in this study. Similarly, ST22-IV MRSA related to UK EMRSA-15/Barnim MRSA clone was detected only in 8.9% of MRSA in a hospital in Riyadh, Saudi Arabia [17]. Also, t032, which was the dominant spa type of ST22-IV-MRSA in a United Arab Emirates hospital in 2003, was replaced by t005 in 2008, with none of the 2008 isolates carrying t032 [18]. Furthermore, none of ST22-IV-MRSA reported recently in Qatar [19] and Oman [20] belonged to t032, suggesting a displacement of t032 isolates in Kuwait and other GCC hospitals, in contrast to its continued spread in Europe [8,10,29], Malaysia [15] and Singapore [12]. Similar to t032, c790 isolates, which were detected in small numbers in this study, constituted the dominant EMRSA-15 spa type in central Iran [35]. These studies highlight the genetic diversity of ST22-IV subtypes in different countries and the importance of molecular subtyping in understanding their epidemiology.

The UK EMRSA-15/Barnim clone has been associated with invasive infections and has been the dominant cause of bloodstream infections in European countries [3,8]. In contrast, the ST22 isolates in this study were obtained mostly from skin and soft tissue infections and colonization sites. Similarly, the majority of ST22-IV isolates reported from Indian hospitals [33] and day care centres in the United States [36] were from carriers, probably reflecting the genetic diversity observed in this study and the ability of the clones to survive and proliferate under different environments which support their global spread.

All ST22-IV-MRSA isolates in this study possessed egc (seg, sei, seln, selo and selu), as has been reported in other studies [29,37,38], implying that egc is a major virulence factor for ST22-IV-MRSA isolates. However, the PVL-positive t852 isolates lacked genes for staphylococcal complement inhibitor (scn), alpha haemolysin (hla) and the A component of haemolysins gamma (hlgA), highlighting genetic changes in the emerging variant.

The majority of the ST22-IV-MRSA isolates were resistant to trimethoprim, erythromycin and clindamycin, as has been reported in ST22-IV-MRSA isolates obtained in Ireland [39].

Conclusions

This study revealed that CC22-IV[tstI⁺] UK EMRSA-15/Middle Eastern variant is the dominant EMRSA-15 variant in Kuwait, followed by the PVL-positive, multiresistant t852 variant, with only few of the isolates related to the European EMRSA-15/Barnim variant of spa type t032. The presence of a mixed population of MRSA isolates poses unique problems for infection control. The study has enriched our...
understanding of the epidemiology of EMRSA-15 in Kuwait, emphasizing the need for continuous surveillance of MRSA in healthcare facilities to detect changes in their clonal composition and distribution.

Acknowledgements

Supported by grant YM 02/12 and Research Core Facility project SRUL02/13 from Kuwait University Research Sector. S. Boswihi received a graduate student fellowship from the College of Graduate Studies, Kuwait University, Kuwait. This study was presented as a poster at the 15th general meeting of the American Society for Microbiology, New Orleans, LA, USA, 30 May–2 June 2015.

Conflict of Interest

None declared.

References

[1] Grundman H, Aannesen DM, van den Wijngaard CC, Spratt BG, Harnsen D, Friedrich AW. Geographic distribution of Staphylococcus aureus causing invasive disease in Europe. A molecular epidemiological analysis. PLoS Med 2010;7:e1000215.

[2] Grubb WB. Genetics of MRSA. Rev Med Microbiol 1988;9:153–62.

[3] Kerr S, Kerr GE, Mackintosh CA, Marples RR. A survey of methicillin-resistant Staphylococcus aureus affecting patients in England and Wales. J Hosp Infect 1990;16:35–48.

[4] Aucken HM, Ganner M, Murchan S, Cookson BD, Johnson AP. A new UK strain of epidemic meticillin-resistant Staphylococcus aureus (EMRSA-17) resistant to multiple antibiotics. J Antimicrob Chemother 2002;50:171–5.

[5] Richardson JF, Reith S. Characterization of a strain of methicillin-resistant Staphylococcus aureus (EMRSA-15) by conventional and molecular methods. J Hosp Infect 1993;25:45–52.

[6] Holden MT, Hsu LY, Kurt K, Weinert LA, Mather AE, Harris SR, et al. A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant Staphylococcus aureus pandemic. Genome Res 2013;23:653–64.

[7] 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.

[8] Johnson AP, Aucken HM, Cavendish S, Ganner M, Wale MC, Warner M, et al. Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS). J Antimicrob Chemother 2001;48:143–4.

[9] Wite W, Enright M, Schmitz FJ, Cuny C, Braulke C, Heuck D. Characteristics of a new epidemic MRSA in Germany ancestral to United Kingdom EMRSA 15. Int J Med Microbiol 2001;290:677–82.

[10] Ghebremedhin B, Konig W, Wite W, Hardy KJ, Hawkey PM, Konig B. Subtyping of ST22-MRSA-IV (Barnim epidemic MRSA strain) at a university clinic in Germany from 2002 to 2005. J Med Microbiol 2007;56:365–75.

[11] Pearman JW, Coomb GW, Grubb WB, O’Brien F. A British epidemic strain of methicillin-resistant Staphylococcus aureus (UK EMRSA-15) in Western Australia. Med J Aust 2001;174:662.

[12] Hsu LY, Koh TH, Singh K, Kang ML, Kurup A, Tan BH. Dissemination of multiresistant methicillin-resistant Staphylococcus aureus in Singapore. J Clin Microbiol 2005;43:2923–5.

[13] Nadig S, Raju SR, Arakere G. Epidemic meticillin-resistant Staphylococcus aureus (EMRSA-15) variants detected in healthy and diseased individuals in India. J Med Microbiol 2010;59:815–21.

[14] Dhanwar B, Rao C, Udo EE, Gadepalli R, Vishnubhushana S, Kapil A. Dissemination of methicillin-resistant Staphylococcus aureus SCCmec type IV and SCCmec type V epidemic clones in a tertiary hospital: challenge to infection control. Epidemiol Infect 2015;143:343–53.

[15] Ghaznavi-Rad E, Nor Shamuddin M, Sekawi Z, Khooon LY, Aziz MN, Hamat RA, et al. Predominance and emergence of clones of hospital-acquired methicillin-resistant Staphylococcus aureus in Malaysia. J Clin Microbiol 2010;48:867–72.

[16] Udo EE, Al-Sweih N, Noronha B. Characterization of non multi-resistant methicillin-resistant Staphylococcus aureus (including EMRSA-15) in Kuwait Hospitals. Clin Microbiol Infect 2006;12:262–9.

[17] Monecke S, Skalni L, Hasani R, Ruppelt A, Ghazali SS, Hakazi A, et al. Characterisation of MRSA strains isolated from patients in a hospital in Riyadh, Kingdom of Saudi Arabia. BMC Microbiol 2012;12:146.

[18] Sonnevend A, Blair I, Alkaabi M, Jumaa P, al Haj M, Ghazawi A, et al. Change in meticillin-resistant Staphylococcus aureus clones at a tertiary care hospital in the United Arab Emirates over a 5-year period. J Clin Pathol 2012;65:178–82.

[19] El-Mahdy T S, El-Ahmady M, Goering RV. Molecular characterization of MRSA isolated over a two year period in a Qatari hospital from multinational patients. Clin Microbiol Infect 2014;20:169–73.

[20] Udo EE, Al-Lawati BAH, Al-Muharmi Z, Thukral SS. Genotyping of MRSA strains isolated from the Sultan Qaboos University Hospital, Oman reveals the dominance of a Panton-Valentine leukocidin–negative ST6-IVt304 clone. New Microbes New Infect 2014;2:100–5.

[21] Clinical and Laboratory Standard Institute. 22nd informational supplement M100-S22. Performance standards for antimicrobial susceptibility testing. Vol. 32, no. 3. Wayne, PA: CLSI; 2012.

[22] Udo EE, Farook VS, Molakas EM, Jacob LE, Sanyal SC. Molecular fingerprinting of mupirocin-resistant Staphylococcus aureus from a burn unit. Int J Infect Dis 1999;3:82–7.

[23] Hirose M, Kobayashi N, Ghosh S, Paul SK, Shen T, Urushibara N, et al. Genotyping of methicillin-resistant Staphylococcus aureus in the Sultan Qaboos University Hospital, Oman reveals the dominance of a Panton-Valentine leukocidin–negative ST6-IVt304 clone. New Microbes New Infect 2010;6:257–63.

[24] Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2002;46:2155–61.

[25] 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;43:5026–33.

[26] Harmsen D, Claus H, Wite W, Rothanger J, Claus H, Turnwald D, et al. Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol 2003;41:5542–8.

[27] Mellmann A, Weniger T, Berssenbrügge C, Rothganger J, Slickers P, Ehricht R. Assignment of mec element in Staphylococcus aureus. Jpn J Infect Dis 2010;63:257–63.

[28] Monecke S, Slickers P, Ehricht R. Assignment of Staphylococcus aureus isolates to clonal complexes based on microarray analysis and pattern recognition. FEMS Immunol Med Microbiol 2008;53:237–51.
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:e17936.

Biber A, Abuelaish I, Rahav G, Raz M, Cohen L, Valinsky L, et al. A typical hospital-acquired methicillin-resistant Staphylococcus aureus clone is widespread in the community in the Gaza strip. PLoS One 2012;7:e42864.

Al Laham N, Mediasilla JR, Chen L, Abdelateef N, Elamreen FA, Ginocchio CC, et al. MRSA clonal complex 22 strains harbouring toxic shock syndrome toxin (TSST-1) are endemic in the primary hospital in Gaza, Palestine. PLoS One 2015;10:e0120008.

Al-Bakri AG, Al-Hadithi H, Kasabri V, Othman G, Kriegeskorte A, Becker K. The epidemiology and molecular characterization of methicillin-resistant staphylococci sampled from a healthy Jordanian population. Epidemiol Infect 2013;141:2384–91.

Shambat S, Nadig S, Prabhakara S, Bes M, Etienne J, Arakere G. Clonal complexes and virulence factors of Staphylococcus aureus from several cities in India. BMC Microbiol 2012;12:64.

Seidl K, Leimer N, Marques MP, Furrer A, Holzmann-Burgel A, Senn G, et al. Clonality and antimicrobial susceptibility of methicillin-resistant Staphylococcus aureus at the University Hospital Zürich, Switzerland between 2012 and 2014. Ann Clin Microbiol Antimicrob 2015;14:14.

Japoni-Nejad A, Rezzaddeh 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.

Moritz ED, Hanson BM, Kates AE, Smith TC. Molecular characteristics of Staphylococcus aureus isolated from employees, children, and environmental surfaces in Iowa child daycare facilities. Am J Infect Control 2015;43:482–8.

Scicluna EA, Shore AC, Thurmer A, Ehricht R, Slickers P, Borg MA, et al. Characterisation of MRSA from Malta and the description of a Maltese epidemic MRSA strain. Eur J Clin Microbiol Infect Dis 2010;29:163–70.

Oksuz L, Dupieux C, Tristan A, Bes M, Etienne J, Gurler N. The high diversity of MRSA clones detected in a university hospital in Istanbul. Int J Med Sci 2013;10:1740–5.

Kinnevey PM, Shore AC, Brennan GI, Sullivan DJ, Ehricht R, Monecke S, et al. Extensive genetic diversity identified among sporadic methicillin-resistant Staphylococcus aureus isolates recovered in Irish hospitals between 2000 and 2012. Antimicrob Agents Chemother 2014;58:1907–17.