*Original Article*

# Isolation of *PVL/ACME*-Positive, Community Acquired, Methicillin-Resistant *Staphylococcus aureus* (USA300) from Iran

**Amir Azimian[1], *Seyed Asghar Havaei[2], Reza Besharati[1], Kiarash Ghazvini[3], Mahsa Khosrojerdi[4], Mahmood Naderi[5], Siamak Mirab Samiee[6, 7]**

[1]Department of Pathobiology and Laboratory Science, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran;
[2]Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran;
[3]Department of Microbiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran;
[4]Department of Pediatrics, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran;
[5]Liver and Pancreatobiliary Disease Research Center, Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran;
[6]Food and Drug Laboratory Research Center, Ministry of Health and Medical Education, Tehran, Iran;
[7]Reference Health Laboratories, Ministry of Health and Medical Education, Tehran, Iran.

**Received Aug 11, 2015; accepted Sep 28, 2015**

**Introduction:** Methicillin-Resistant *Staphylococcus aureus* (MRSA) is responsible for an increasing number of serious hospital- and community-acquired infections. USA300 is known to be the most common cause of community-acquired infections, but recently there have been some reports on hospital-acquired infections caused by this strain. **Methods:** Totally 171 isolates of *S. aureus* were collected from different clinical samples in selected university hospitals in the cities of Mashhad, Tehran, and Isfahan. Then, they were assessed by agar screening and disk diffusion methods to determine their resistance to Methicillin. The isolated MRSA strains were confirmed by detection of *mecA* gene. The staphylococcal cassette chromosome *mec* (SCC*mec*), *agr*, and *spa* typing and also detection of Panton-Valentine leukocidin (*PVL*) and arginine catabolic mobile element (*ACME*) genes were performed on *mecA* harboring isolates. Multilocus sequence typing was performed on *PVL/ACME* positive MRSA strains. **Results:** We found a *PVL/ACME* positive MRSA isolate. Genetic evaluation results for this isolate produced the following profile: positive for *mecA*, *pvl*, *arcA*, and *hla* genes, negative for *vanA, sec*, and *tst1*, and belonged to *agr* I, SCC*mec* IV, sequence type 8 (ST8), and *spa* t008. **Conclusion:** Our results suggest a finding of USA300CA-MRSA isolate in Mashhad, Iran. This is an uncommon finding, because USA300 is routinely found in areas other than Middle East. A notable point about these isolates is that they belong to American Endemic clones. *J Med Microbiol Infec Dis, 2014, 2 (3): 100-104.*

**Keywords**: *Staphylococcus aureus*, Methicillin-Resistant, Panton-Valentine leukocidin, Iran.

## INTRODUCTION

*Staphylococcus aureus* is responsible for a wide range of community- and hospital-acquired infections, ranging from simple skin and soft tissue infections to life-threatening infections, such as toxic shock syndrome, endocarditis, *etc.* [1-3]. Resistance to antimicrobial agents emerged soon after the first use of antibiotics to treat *staphylococcal* infections [1]. The mechanism of resistance to beta-lactam antibiotics includes integration of the staphylococcal cassette chromosome *mec* (SCC*mec*) into the *S. aureus* genome [4]. The antibiotic resistance associated with SCC*mec* is caused by altered penicillin binding protein 2a (PBP2a), which is encoded by the *mecA* gene. This molecule has a low affinity for beta-lactam antibiotics [5, 6]. So far, 11 different SCC*mec* elements have been identified [7, 8]. There are five predominant SCC*mec* types of Methicillin-resistant *Staphylococcus aureus* (MRSA) in Iran (types I to V). Types I to III are typically considered as hospital-associated MRSA (HA-MRSA), and types IV and V are commonly linked to community-associated MRSA (CA-MRSA) [9-12].

MRSA has been a common pathogen in health care settings since 1960 [13]. Since the late 1990s, the emergence of CA-MRSA outside health care settings has been increasingly reported [14].

Multiple CA-MRSA clones have been identified according to Pulsed-field Gel Electrophoresis (PFGE) patterns. These clones were found to be responsible for outbreaks of MRSA in the United States and also other parts of the world. Among the CA-MRSA lineages, USA1000 displays a "sporadic" phenotype, USA1100 exhibits a "local outbreak" phenotype, and USA300 displays an "epidemic" phenotype, capable of wide spread [15-17].

***Correspondence:** Seyed Asghar Havaei
Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Hezar Jarib St., Isfahan, Iran, 8174673461.
**Email:** havaei.seyedasghar@gmail.com
**Tel:** +98 (31) 37929038 **Fax:** +98 (31) 32225400

USA300 is the main type of MRSA strain causing community-acquired infections in the United States. However, it is becoming a common cause of MRSA infection in health care facilities [18, 19].

To date, isolation of USA300 CA-MRSA has been increasingly reported in different parts of the world [17, 20-24]. It has been associated with skin, soft tissue, and also invasive infections in previously healthy people [25]. USA300 typically carries Panton-Valentine leukocidin (*PVL*)-encoding genes, SCC*mec* element type IV [17], and arginine catabolic mobile element (*ACME*) [26].

USA300 isolates were initially classified based on PFGE profiles. Today, using molecular methods, they are commonly characterized as multilocus sequence type 8 (ST8) [27, 28], *spa* type t008 [27, 28] or t121 [29], SCC*mec* IV [23, 27, 28, 30, 31], *PVL*-positive [23, 27, 28, 32, 33], and possessing *ACME* [23, 27, 34]. Some previous studies proved that molecular determinant such as Multilocus ST, *Spa* type, and presence of *PVL* and *ACME* can be used for characterization of USA300 isolates with high sensitivity and specificity, similar to PFGE [27]. In the present study, we isolated USA300 CA-MRSA for the first time in Iran, and it can be speculated that this successful international clone can have been imported and be in circulation in Iran.

*S. aureus* was isolated from wound exudates of a 31-year-old woman who suffered from recurrent exudative lesions. Analysis of the isolated *S. aureus* showed that it was a *mecA* positive and *PVL*/*ACME* harboring strain.

## MATERIAL AND METHODS

In our study, all strains of *S. aureus* isolated from patients admitted in selected hospitals in Mashhad, Isfahan, and Tehran in autumn has been evaluated. These hospitals are large reference hospitals in these cities, which were chosen due to the coverage of a wide variety of patients of various races and lifestyles. A total of 171 isolates of *S. aureus* were obtained from patients between September 2011 and December 2011 at Al-Zahra hospital in Isfahan, Emam Reza Hospital in Mashhad, and Dey Hospital in Tehran, Iran. Clinical samples, such as urine, sputum, blood, abscess, eye, throat, wound, nose, and respiratory specimens were included in this study. *S. aureus* isolates were identified by Gram staining, catalase, coagulase, DNase, and mannitol fermentation tests. The classification of isolates into community- and hospital-acquired MRSA, was performed according to criteria set by Center of Disease Control and prevention (CDC) [4, 6]. Patients' data such as history of hospitalization, surgery, antibiotic use, time of MRSA isolation, *etc.* were collected using questionnaire forms.

**Screening for methicillin and vancomycin resistance.** All *S. aureus* isolates were screened for oxacillin and vancomycin resistance by agar screening method. The isolates that had grown in vancomycin agar screening medium were tested with E-Test method for MIC determination.

**Antimicrobial susceptibility test.** Antibiotic susceptibility testing was performed using oxacillin, minocyclin, levofloxacin, ciprofloxacin, tetracycline, co-trimoxazol, gentamicin, clindamycin, and rifampicin antimicrobial disks for disk diffusion method (MAST DISKS™) according to Clinical Laboratory Standards Institute (CLSI) guidelines [35, 36]. *S. aureus* ATCC 25923 was used as control.

**Genomic DNA extraction.** Genomic DNA of *S. aureus* isolates were extracted using QIAamp Blood DNA mini kit. According to the manufacturer's protocol, we added lysostaphin enzyme at a final concentration of 30 µg/mL per extraction tube.

**PCR assay.** PCR reaction was performed using a TaKaRa TP600 thermal cycler (TaKaRa, Japan) in a volume of 50 µl. We used an EmeraldAmp Max PCR Master Mix for all PCRs.

(i) **PCR identification of the *mecA* and *vanA* genes.** The presence of the *vanA* and *mecA* genes was determined by PCR as previously described [1, 37].

(ii) **Multiplex PCR for detection of toxin genes.** The presence of the *Toxic Shock Syndrome Toxin-1 (tst 1)*, *enterotoxin C* (*etc*), *alpha Hemolysin* (*hla*), and *PVL (lukS-PV and lukF-PV)* genes was determined using PCR reaction as previously described [1, 37]. These genes were selected because they represent well-characterized virulence factors that are not uniformly distributed in the *S. aureus* population.

(iii) **PCR identification of *arcA* gene (*ACME* marker).** The presence of *arcA* gene was determined by PCR reaction using the following primers: *arcA*-Forward: 5′-TCATCCACAGACACTTCATCG-3′ and *arcA*-Reverse: 5′-GGTAACGCTTTAGGACAATCG-3′. PCR was performed using the following thermal settings: 5 min at 94°C for initial enzyme activation followed by 40 cycles of amplification consisting of denaturation at 94°C for 40 s, annealing at 60°C for 40 s, and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The 731 bp PCR product was detected by 1% agarose gel electrophoresis and ethidium bromide staining.

(iv) **Multiplex PCR for SCC*mec* and *agr* typing.** SCC*mec* and agr typing were performed as previously described [1, 38].

**MLST.** Multilocus sequence typing was performed by PCR and sequencing of the internal fragments of *arc*, *aro*, *glp*, *gmk*, *pta*, *tpi*, and *yqi* genes of *S. aureus* [39].

***spa* typing.** s*pa* typing was performed by PCR and sequencing of polymorphic X region of *spa* gene as previously described [40].

**Nucleotide sequencing.** The PCR products were purified by a QIAquick Gel extraction kit. The purified PCR products were sequenced using an ABI 3730XL DNA analyzer (Applied Biosystems) in both directions.

## RESULTS

Antibiogram analysis by disk diffusion method showed that our isolate was susceptible to most of the used antibiotic disks. Susceptibility to oxacillin and vancomycin was also evaluated by agar screening method. After all phenotypic

tests for *S. aureus* reconfirmation were repeated, MICs were determined by the E-test method. The results of the antimicrobial susceptibility testing were as follows: the strain was resistant to oxacillin (MIC, 128 µg/ml), clindamycin, and rifampicin and susceptible to vancomycin (MIC, 2 µg/ml), minocycline, levofloxacin, ciprofloxacin, tetracycline, co-trimoxazol, and gentamicin. Moreover, genetic evaluation results for this strain yielded the following profile: positive for the *mecA*, *pvl*, *arcA*, and *hla* genes and negative for *vanA*, *sec* and *tst1*, and belonged to *agr* I, SCC*mec* IV, ST8, *spa* t008.

## DISCUSSION

USA300 CA-MRSA is highly virulent and associated with skin and soft tissue infections and also invasive and sometimes fatal infections, such as bacteremia, endocarditis, necrotizing pneumonia, and osteomyelitis in previously healthy people [17, 25].

Outbreaks of CA-MRSA infections have been reported all over the world and successful clones are usually associated with specific geographic areas [41-43]. Clones with multilocus ST1 (USA400) and ST8 (USA300) are mostly reported in the United States and Canada [44], multilocus ST80 in Europe [44], multilocus ST59 in the Asia-Pacific region, and multilocus ST30 worldwide, including the United States, Europe, Western pacific area, and Japan [45-47]. These five clones are responsible for most of the CA-MRSA worldwide.

Unfortunately, there is not enough information about the molecular epidemiology of CA-MRSA in Iran. Japoni Nejad *et al*. reported that the most prevalent CA-MRSA clone in their study was t790/ST22/SCC*mec* IV. St22 has previously reported as a dominant CA-MRSA clone in Germany [12]. The most predominant clones in other Asian countries includes: ST59/spa t437/Scc*mec* IV in Vietnam, Hong Kong, Sri Lanka, and Taiwan; ST30/spa t019/ Scc*mec* IV in the Philippines, and ST72/spa t324/Scc*mec* IV in Korea [12].

USA300 has been identified as the predominant CA-MRSA genetic background in North America [27] and has been reported as a cause of clinical infection in many countries [17, 48, 49]. Previous studies suggested that the most prevalent CA-MRSA clones in Asian countries including Iran were related to European clones [12], but in the present study, we could isolate USA300 CA-MRSA (an American clone) for the first time in Iran. It is alarming, because up to now USA300 clone has been only found in Japan and Korea [50, 51], and our finding could be a sign of an increasing incidence of this clone in other Asian countries. Increasing reports of USA300 isolation in various parts of the world can introduce this clone as a successful worldwide disseminated clone. Due to the very low number of studies on molecular epidemiology of CA-MRSA in Iran, more extensive studies are required in various geographic regions in Iran to determine the prevalence of MRSA clones.

Given the high transmissibility of USA300 and increasing number of reports on USA300 isolation in various parts of the world, it is possible that this clone becomes a major problem worldwide.

## ACKNOWLEDGEMENT

This study was supported by the Isfahan University of Medical Sciences (Grant number: 390256). Also, we would like to thank Stem Cell Research Center (Bone Yakhteh) for supporting technical assistance.

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

## REFERENCES

1. Azimian A, Havaei SA, Fazeli H, Naderi M, Ghazvini K, Samiee SM, Soleimani M, Peerayeh SN. Genetic Characterization of a Vancomycin-Resistant Staphylococcus aureus Isolate from the Respiratory Tract of a Patient in a University Hospital in Northeastern Iran. J Clin Microbiol. 2012; 50 (11): 3581-5.

2. Peerayeh SN, Azimian A, Nejad QB, Kashi M. Prevalence of agr Specificity Groups Among Staphylococcus aureus Isolates From University Hospitals in Tehran. Lab Medicine. 2009; 40 (1): 27-9.

3. Azimian A, Najar-pirayeh S, Mirab-Samiee S, Naderi M. Occurrence of methicillin resistant Staphylococcus aureus (MRSA) among clinical samples in tehran-iran and its correlation with polymorphism of specific accessory gene regulator (AGR) groups. Braz J Microbiol. 2012; 43 (2): 779-85.

4. Hiramatsu K, Katayama Y, Yuzawa H, Ito T. Molecular genetics of methicillin-resistant Staphylococcus aureus. Int J Med Microbiol. 2002; 292 (2): 67-74.

5. Hartman B, Tomasz A. Altered penicillin-binding proteins in methicillin-resistant strains of Staphylococcus aureus. Antimicrob Agents Chemother. 1981; 19 (5): 726-35.

6. Pinho MG, Lencastre Hd, Tomasz A. An acquired and a native penicillin-binding protein cooperate in building the cell wall of drugresistant staphylococci. Proc Natl Acad Sci U S A. 2001; 98 (19): 10886-91.

7. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. Antimicrob Agents Chemother. 2009; 53 (12): 4961-7.

8. Li S, Skov RL, Han X, Larsen AR, Larsen J, Sørum M, Wulf M, Voss A, Hiramatsu K, Ito T. Novel types of staphylococcal cassette chromosome mec elements identified in clonal complex 398 methicillin-resistant Staphylococcus aureus strains. Antimicrob Agents Chemother. 2011; 55 (6): 3046-50.

9. Japoni A, Jamalidoust M, Farshad S, Ziyaeyan M, Alborzi A, Japoni S, Rafaatpour N. Characterization of SCCmec types and antibacterial susceptibility patterns of Methicillin Resistant Staphylococcus aureus in southern Iran. Jpn J Infect Dis. 2011; 64 (1): 28-33.

10. Fatholahzadeh B, Emaneini M, Gilbert G, Udo E, Aligholi M, Modarressi MH, Nouri K, Sedaghat H, Feizabadi MM. Staphylococcal cassette chromosome mec (SCCmec) analysis and antimicrobial susceptibility patterns of methicillin-resistant Staphylococcus aureus (MRSA) isolates in Tehran, Iran. Microb Drug Resist. 2008; 14 (3): 217-20.

11. Fatholahzadeh B, Emaneini M, Aligholi M, Gilbert G, Taherikalani M, Jonaidi N, Eslampour MA, Feizabadi MM. Molecular

characterization of Methicillin Resistant Staphylococcus aureus clones from a teaching Hospital in Tehran. Jpn J Infect Dis. 2009; 62 (4): 309-11.

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 (11): e949-e54.

13. Naimi TS, Ledell KH, Como-Sabetti C, Borchardt SM, Boxrud DJ, Etienne J, Johnson SK, Vandenesch F, Fridkin S, O'Boyle C, Danila RN, Lynfield R. Comparison of community and health care-associated Methicillin Resistant Staphylococcus aureus infection. JAMA. 2003; 290 (22): 2976-84.

14. Brennan GI, Shore AC, Corcoran S, Tecklenborg S, Coleman DC, O'Connell B. Emergence of Hospital- and Community-Associated Panton-Valentine Leukocidin-Positive Methicillin-Resistant Staphylococcus aureus Genotype ST772-MRSA-V in Ireland and Detailed Investigation of an ST772-MRSA-V Cluster in a Neonatal Intensive Care Unit. J Clin Microbiol. 2012; 50 (3): 841-7.

15. Chambers HF, Deleo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol. 2009; 7 (9): 629-41.

16. Pan ES, Diep BA, Charlebois ED, Auerswald C, Carleton HA, Sensabaugh GF, Perdreau-Remington F**.** Population dynamics of nasal strains of methicillin-resistant Staphylococcus aureus–and their relation to community-associated disease activity. J Infect Dis. 2005; 192 (5): 811-8.

17. Tenover FC, Goering RV. Methicillin-resistant Staphylococcus aureus strain USA300: origin and epidemiology. J Antimicrob Chemother. 2009; 64 (3): 441-6.

18. Richter SS, Heilmann KP, Dohrn CL, Riahi F, Costello AJ, Kroeger JS, Biek D, Critchley IA, Diekema DJ, Doern GV. Activity of ceftaroline and epidemiologic trends in Staphylococcus aureus isolates collected from 43 medical centers in the United States in 2009. Antimicrob Agents Chemother. 2011; 55 (9): 4154-60.

19. Maree CL, Daum RS, Boyle-Vavra S, Matayoshi K, Miller LG. Community-associated methicillin-resistant Staphylococcus aureus isolates causing healthcare-associated infections. Emerg Infect Dis. 2007; 13 (2): 236-42.

20. Kennedy AD, Otto M, Braughton KR, Whitney AR, Chen L, Mathema B, Mediavilla JR, Byrne KA, Parkins LD, Tenover FC, Kreiswirth BN, Musser JM, et al. Epidemic community-associated methicillin-resistant Staphylococcus aureus: Recent clonal expansion and diversification. Proc Natl Acad Sci U S A. 2008; 105 (4): 1327-32.

21. Shibuya Y, Hara M, Higuchi W, Takano T, Iwao Y, Yamamoto T. Emergence of the community-acquired methicillin-resistant Staphylococcus aureus USA300 clone in Japan. J Infect Chemother. 2008; 14 (6): 439-41.

22. Nagao M, Linuma Y, Suzuki M, Matsushima A, Takakura S, Ito Y, Ichiyama S. First outbreak of methicillin-resistant Staphylococcus aureus USA300 harboring the Panton-Valentine leukocidin genes among Japanese health care workers and hospitalized patients. Am J Infect Control. 2010; 38 (9): e37-9.

23. McDougal LK, Fosheim GE, Nicholson A, Bulens SN, Limbago BM, Shearer JES, Summers AO, Patel JB. Emergence of Resistance among USA300 Methicillin-Resistant Staphylococcus aureus Isolates Causing Invasive Disease in the United States. Antimicrob Agents Chemother. 2010; 54 (9): 3804-11.

24. Lim S, Chung DR, Baek JY, Kim SH, Peck KR, Lee NY, Song JH. A third case of USA300 community-associated methicillin-resistant Staphylococcus aureus infection in Korea. Korean J Intern Med. 2013; 28 (2): 258-60.

25. 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.

26. Diep BA, Stone GG, Basuino L, Graber CJ, Miller A, des Etages SA, Jones A, Palazzolo-Ballance AM, Perdreau-Remington F, Sensabaugh GF, DeLeo FR, Chambers HF. The arginine catabolic mobile element and staphylococcal chromosomal cassette mec linkage: convergence of virulence and resistance in the USA300 clone of methicillin-resistant Staphylococcus aureus. J Infect Dis. 2008; 197 (11): 1523-30.

27. David MZ, Taylor A, Lynfield R, Boxrud DJ, Short G, Zychowski D, Boyle-Vavra S, Daum RS. Comparing pulsed-field gel electrophoresis with multilocus sequence typing, spa typing, staphylococcal cassette chromosome mec (SCCmec) typing, and PCR for Panton- Valentine leukocidin, arcA, and opp3 in methicillin-resistant Staphylococcus aureus isolates at a U.S. medical center. J Clin Microbiol. 2013; 51 (3): 814-9.

28. Tenover FC, McDougal LK, Goering RV, Killgore G, Projan SJ, Patel JB, Dunman PM. Characterization of a strain of communityassociated methicillin-resistant Staphylococcus aureus widely disseminated in the United States. J Clin Microbiol. 2006; 44 (1): 108-18.

29. Haran KP, Godden SM, Boxrud D, Jawahir S, Bender JB, Sreevatsan S. Prevalence and characterization of Staphylococcus aureus, including methicillin-resistant Staphylococcus aureus, isolated from bulk tank milk from Minnesota dairy farms. J Clin Microbiol. 2012; 50 (3): 688-95.

30. Talan DA, Krishnadasan A, Gorwitz RJ, Fosheim GE, Limbago B, Albrecht V, Moran GJ; EMERGEncy ID Net Study Group. Comparison of Staphylococcus aureus from skin and soft-tissue infections in US emergency department patients, 2004 and 2008. Clin Infect Dis. 2011; 53 (2): 144-9.

31. Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, Davidson MG, Lin F, Lin J, Carleton HA, Mongodin EF, Sensabaugh GF, Perdreau-Remington F. Complete genome sequence of USA300, and epidemic clone of community-acquired methicillin-resistant Staphylococcus aureus. Lancet. 2006; 367 (9512): 731-9.

32. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, Talan DA; EMERGEncy ID Net Study Group. Methicillin-resistant S. aureus infections among patients in the emergency department. N Engl J Med. 2006; 355 (7): 666-74.

33. Limbago B, Fosheim GE, Schoonover V, Crane CE, Nadle J, Petit S, Heltzel D, Ray SM, Harrison LH, Lynfield R, Dumyati G, Townes JM, et al. Characterization of methicillin-resistant Staphylococcus aureus isolates collected in 2005 and 2006 from patients with invasive disease: a population-based analysis. J Clin Microbiol. 2009; 47 (5): 1344-51.

34. Okano JT, Bowler S. Are correctional facilities amplifying the epidemic of community-acquired methicillin-resistant Staphylococcus aureus?. Nat Rev Microbiol. 2010; 8 (1): 83.

35. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests; Standard-eleventh edition. CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute; 2012; 32 (1).

36. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-eleventh edition. CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute; 2012; 32 (1).

37. Havaei SA, Azimian A, Fazeli H, Naderi M, Ghazvini K, Samiee SM, Masoumi Z, Akbari M. Genetic Characterization of Methicillin

Resistant and Sensitive, Vancomycin Intermediate Staphylococcus aureus Strains Isolated from Different Iranian Hospitals. ISRN Microbiol. 2012; 215275.

38. Boye K, Bartels MD, Andersen IS, Møller JA, Westh H. A new multiplex PCR for easy screening of methicillin-resistant Staphylococcus aureus SCCmec types I-V. Clin Microbiol Infect. 2007; 13 (7): 725-7.

39. 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 (3): 1008-15.

40. Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, Vogel U. 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 (12): 5442-8.

41. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME, Etienne J. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis. 2003; 9 (8): 978-84.

42. Deurenberg RH, Stobberingh EE. The evolution of Staphylococcus aureus. Infect Genet Evol. 2008; 8 (6): 747-63.

43. Tristan A, Bes M, Meugnier H, Lina G, Bozdogan B, Courvalin P, Reverdy ME, Enright MC, Vandenesch F, Etienne J. Global distribution of Panton-Valentine leukocidin-positive methicillin-resistant Staphylococcus aureus, 2006. Emerg Infect Dis. 2007; 13 (4): 594-600.

44. Chuang YY, Huang YC. Molecular epidemiology of community-associated meticillin-resistant Staphylococcus aureus in Asia. Lancet Infect Dis. 2013; 13 (8): 698-708.

45. Coombs GW, Nimmo GR, Bell JM, Huygens F, O'Brien FG, Malkowski MJ, Pearson JC, Stephens AJ, Giffard PM; Australian Group for Antimicrobial Resistance. Genetic diversity among community methicillin-resistant Staphylococcus aureus strains causing outpatient infections in Australia. J Clin Microbiol. 2004; 42 (10): 4735-43.

46. Ho PL, Tse CW, Mak GC, Chow KH, Ng TK. Community-associated methicillin-resistant Staphylococcus aureus arrives in Hong Kong. J Antimicrob Chemother. 2004; 54 (4): 845-6.

47. Hsu LY, Koh YL, Chlebicka NL, Tan TY, Krishnan P, Lin RT, Tee N, Barkham T, Koh TH. Establishment of ST30 as the predominant clonal type among community- associated methicillin resistant Staphylococcus aureus isolates in Singapore. J Clin Microbiol. 2006; 44 (3): 1090-3.

48. Tietz A, Frei R, Widmer AF. Transatlantic spread of the USA300 clone of MRSA. N Engl J Med. 2005; 353 (5): 532-3.

49. Gottlieb T, Su W, Merlino J, Cheong E. Recognition of USA300 isolates of community-acquired methicillin-resistant Staphylococcus aureus in Australia. Med J Aust. 2008; 189 (3): 179-80.

50. Lee DG, Choi SM, Park SH, Choi JH, Yoo JH, Hur JA, ShinWS. A case of perianal abscess due to Panton-Valentine leukocidin positive community-associated methicillin-resistant Staphylococcus aureus: report in Korea and literature review from the Far East. Infect Chemother. 2008; 40 (2): 121-6.

51. Sohn KM, Chung DR, Baek JY, Kim SH, Joo EJ, Ha YE, Ko KS, Kang CI, Peck KR, Song JH. Post-infl uenza pneumonia caused by the USA 300 community-associated methicillin-resistant Staphylococcus aureus in Korea. J Korean Med Sci. 2012; 27 (3): 313-6.