Acta Microbiologica et Immunologica Hungarica 64 (3), pp. 331–341 (2017)
DOI: 10.1556/030.64.2017.020
First published online August 23, 2017

HIGH FREQUENCY OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) WITH SCC*mec* TYPE III AND *spa* TYPE t030 IN KARAJ'S TEACHING HOSPITALS, IRAN

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(Received: 25 October 2016; accepted: 8 March 2017)

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been one of the most important antibiotic-resistant pathogen in many parts of the world over the past decades. This cross-sectional study was conducted to investigate MRSA isolated between July 2013 and July 2014 in Karaj, Iran. All tested isolates were collected in teaching hospitals from personnel, patients, and surfaces and each MRSA was analyzed by SCC*mec* and *spa* typing. Antibiotic susceptibility testing was accomplished by disk diffusion method. Out of 49 MRSA isolates from the Karaj's teaching hospitals, 82%, 10%, and 6% of the isolates were SCC*mec* types III, II, and I, respectively. The main *spa* type in this study was *spa* t030 with frequency as high as 75.5% from intensive care unit (ICU) of the hospitals and high rate of resistance to rifampicin (53%) was found in MRSA isolates. In conclusion, high frequency of *spa* t030 with SCC*mec* type III and MRSA phenotype illustrated circulating of one of the antibiotic-resistant strains in ICU of Karaj's teaching hospitals and emphasizes the

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need for ongoing molecular surveillance, antibiotic susceptibility monitoring, and infection control.

Keywords: MRSA, *spa* typing, SCC*mec* typing, t030, Iran

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be as one of the most important antibiotic-resistant pathogens in many parts of the world over the past decades [1]. The proportion of MRSA among *S. aureus* isolates is between 52.7% and 93.3% in different regions of Iran and recent data show the increasing rate of these strains [2–5]. Acquiring the staphylococcal cassette chromosome *mec* (SCC*mec*) element with two essential components, the *ccr* gene complex (ccr) and the mec gene complex (*mec*) harboring the *mecA* gene by bacterium causes MRSA [6]. The *mecA* and *mecC* genes encode a modified penicillin-binding protein (PBP) designated PBP 2a that confers methicillin resistance in *Staphylococci* and treating and managing of the infections caused by these organisms are more difficult [7, 8].

Molecular epidemiological methods have been used for the continuous surveillance on MRSA clones, dissemination of the strains in hospital settings, and comparing the worldwide diverse evolutionary trajectories of MRSA lineages [1, 7]. Results of many molecular epidemiological studies in different regions of the world like Iran showed that high frequency of MRSA in health-care settings is caused by a limited number of predominant clones [4, 6]. DNA sequence-based or PCR-based methods like *S. aureus* protein A (*spa*) and SCC*mec* typing methods have become more popular than pulsed field gel electrophoresis (PFGE) for genotyping of *S. aureus* over the past years. These simple and cost-effective methods are considered as molecular epidemiological methods with high discriminatory power [7]. In the case of *spa* typing, a single-locus sequence-based typing technique that have been used for detection of polymorphisms at the 24-bp variable number tandem repeat within the 3 coding regions X of the *spa* gene [9]. Sequence analysis of the polymorphic region X of the *spa* gene of *S. aureus* can be used for molecular evolution of MRSA and hospital outbreaks. In comparison to the other typing methods like PFGE, this method is less expensive, less laborious and less time-consuming [10]. For detection of nosocomial infections, SCC*mec* typing is a suitable and reliable method [7]. SCC*mec* elements have been classified into 11 major types (I–XI). Types I, II, and III are known as predominant hospital-acquired types, whereas types IV and V have been more frequently associated with community-acquired MRSA (CA-MRSA) [11].

To the best of our knowledge, there is not any report about genotypic diversity and common clones of *S. aureus* isolates from Karaj, a large suburb of Tehran, Iran. Therefore, this study was conducted to find the common types of *S. aureus* strains circulating in this part of the country by *spa* and SCC*mec* typing methods.

Materials and Methods

Collection and identification of bacterial isolates

The cross-sectional study was conducted in Karaj on three teaching hospitals from July 2013 to July 2014. Sampling from personnel and surfaces of the hospitals was done twice and monthly, respectively during the study. Specimens were collected by swabbing of both nostrils of the personnel and hospital surfaces; all samples were cultured on Brain–Heart Infusion media. Hospitals' departments included in the study were emergency, burns, intensive care unit (ICU), surgery, neonates, children, dialysis, and internal. The isolates were cultured on sheep blood agar and mannitol salt agar media and conventional biochemical tests including catalase, tube coagulase, mannitol fermentation, and DNase were used for identification of the isolates [12]. All *S. aureus* isolates isolated from patients were also included in the study. The ethical statement of the project was approved by research vice-chancellor of the Alborz University of Medical Sciences.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) guideline by standard disk diffusion method. Antibiotics tested were: cefoxitin (30 μg), tigecycline (15 μg), vancomycin (30 μg), linezolid (30 μg), synercid (quinupristin/dalfopristin) (15 μg), mupirocin (20 μg), teicoplanin (30 μg), and rifampicin (5 μg) (Mast, UK). *S. aureus* ATCC 25923 was used as control strain.

DNA extraction and identification of MRSA isolates

Boiling method was used for DNA extraction of *S. aureus* colonies [4, 13]. For confirmation of *S. aureus*, PCR amplification of *S. aureus*-specific nuclease (*nuc*A) gene was performed as described previously [14]. Moreover, all isolates

were subjected to the *mec*A- and *mupA*-specific PCR for identification of MRSA and high-level mupirocin-resistant isolates [15, 16].

SCCmec typing

SCC*mec* typing was performed using the method described previously [4]. The MRSA isolates were subjected to the SCC*mec*-PCR with final volume of 25 µl containing 12.5 µl Master mix (Ampliqon, Odense, Denmark), 20 ρmol of each primer, and 5 µl of DNA template. Amplification was carried out in a thermal cycler (Eppendorf, Hamburg, Germany) using the following conditions: 1 cycle of 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C (SCC*mec* types I, II, III, and V) or 60.5 °C (SCC*mec* subtypes IVa, IVb, IVc, and IVd) for 45 s, extension at 72 °C for 1 min and was followed by a final extension at 72 °C for 10 min. The PCR products (3 µl) were run on 1.5% agarose gel and stained with SYBR DNA Safe stain. Electrophoresis was performed in 0.5× TBE for 90 min at 110 mV.

spa typing

According to the method described before, the X region of the *spa* gene (contains variable numbers of 21- to 27-bp repeats) was amplified [17]. In brief, the PCR assay contained the following components per reaction: 12.5 µl of 2× master mix, 20 ρmol of each primer, 5 µl of template, and PCR grade water adjusted to the final volume of 25 µl PCR condition. With the exception of annealing temperature (55 °C), amplification was performed in a condition similar to that mentioned in SCC*mec* typing. The amplified products were sent to Bioneer Company (South Korea) for sequencing. Analysis of DNA sequencing results was carried out by the *spa* typing database for determination of repeat profile and the *spa* type of each isolate (http://www.spaserver.ridom.de).

Results

The results of SCC*mec* and *spa* types are shown in Tables I and II, respectively. Out of 49 MRSA isolates from the Karaj's teaching hospitals, 82%, 10%, and 6% of the isolates were SCC*mec* type III, II, and I, respectively (Table I). The main *spa* type in this study was *spa* t030 with frequency of as high as 75.5%, followed by *spa* t037 with 12.5% (Table II). As you can see in the Table II, the main *spa* types (t030 and t037) were mostly isolated from the ICU of

Table I. Number of SCC*mec* types of MRSA isolates isolated from Karaj's teaching hospitals

SCC*mec* types	Surfaces	Personnel	Patients	Total (%)
Type I	2	1	0	3 (6)
Type II	4	1	0	5 (10)
Type III	21	12	7	40 (82)
Non-typable	1	0	0	1 (2)
Total	28	14	7	49 (100)

Table II. Number of *spa* types of MRSA isolates isolated from Karaj's teaching hospitals

	Surfaces					
spa types	Hospital department	Total	Personnel	Patients	SCC*mec* type	Total (%)
t030	ICU (17), internal (2), and emergency (1)	20	11	6	T3 (33), T1 (2), T2 (2)	37 (75.5)
t037	ICU (3), neonates (1), and emergency (1)	5	0	1	T3 (5), T1 (1)	6 (12.5)
t969	ICU (2)	2	0	0	T2 (2)	2 (4)
Non-typable	Emergency	1	0	0	Non-typable	1 (2)
t297		0	1	0	T3 (1)	1 (2)
t5799		0	1	0	Non-typable	1 (2)
t7609		0	1	0	T3 (1)	1 (2)
Total		28	14	7		49 (100)

the hospitals. The phenotypic and genotypic characteristics of MRSA isolates isolated from patients, personnel, and department's surfaces of Karaj's teaching hospitals are shown in Tables III–V. Most of the MRSA isolates were isolated from hospital No. 1. Most of MRSA isolates originated from ICU department (75%) and high rate of resistance to rifampicin (53%) was found in MRSA isolates. We did not detect *mupA* gene in the 10 isolates of *S. aureus* that recognized low-level resistance to mupirocin by disk diffusion (data are not shown).

Discussion

High frequency of MRSA in Iran in nosocomial and community-acquired infections causes many concerns about increasing rate of these organisms in the country. As we expected, there was no resistance to the effective drugs such as

Table III. Phenotypic and genotypic characteristics of MRSA isolates isolated from patients of Karaj's teaching hospitals

No	Hospital	Resistance pattern	SCC*mec*	*spa* type
1	No. 3	RP	III	t030
2	No. 3	–	III	t030
3	No. 3	RP	III	t030
4	No. 3	RP	III	t030
5	No. 1	–	III	t037
6	No. 1	RP	III	t030
7	No. 1	RP, MUP	III	t030

Note: RP: rifampicin, MUP: mupirocin.

Table IV. Phenotypic and genotypic characteristics of MRSA isolates isolated from personnel's nostrils of Karaj's teaching hospitals

No	Department	Hospital	Resistance pattern	SCC*mec*	*spa* type
1	Burns	No. 1	RP	III	t030
2	Internal	No. 1	RP	III	t030
3	Internal	No. 1	RP	III	t030
4	Burns	No. 1	RP	III	t030
5	Burns	No. 1	–	III	t030
6	Surgery	No. 2	–	III	t297
7	Surgery	No. 2	–	III	t030
8	Internal	No. 1	–	I	t030
9	Burns	No. 1	RP	III	t7609
10	ICU	No. 1	RP	III	t030
11	ICU	No. 1	RP	III	t030
12	Internal	No. 1	RP	III	t030
13	Internal	No. 1	RP	II	t030
14	Internal	No. 1	–	NT	t5799

Note: RP: rifampicin, NT: non-typable.

tigecycline, linezolid, synercid (quinupristin/dalfopristin), teicoplanin, and vancomycin that are in agreement with other studies from Iran [2, 13]. In contrast with some studies [18, 19], we found high rate of resistance to rifampicin (53%) in MRSA isolates that is in agreement with other studies [2, 20]. On the other hand, we did not find any resistance to mupirocin in our MRSA isolates which is in contrast with previous study [2].

SCC*mec* types I, II, and III are mainly known as causatives of nosocomial infections, whereas SCC*mec* types IV and V are associated with community-acquired infections [21, 22]. The most prominent SCC*mec* type in this study was SCC*mec* type III (82%) followed by SCC*mec* type II (10%) and SCC*mec* type I

Table V. Phenotypic and genotypic characteristics of MRSA isolates isolated from surfaces of Karaj's teaching hospitals

No	Source	Department	Hospital	Resistance pattern	SCC*mec*	*spa* type
1	Bed	ICU	No. 1	–	III	t030
2	Suction	ICU	No. 1	RP	III	t030
3	Bed	ICU	No. 1	RP	III	t030
4	Medical trolley	ICU	No. 1	RP	III	t030
5	Medical trolley	ICU	No. 1	MUP	III	t030
6	Chair	Internal	No. 1	RP	II	t030
7	Suction	ICU	No. 1	RP	III	t030
8	Ambu bag	ICU	No. 1	RP	III	t030
9	Suction	ICU	No. 1	RP	III	t030
10	Ambu bag	ICU	No. 1	RP	III	t030
11	Suction	ICU	No. 1	RP	III	t030
12	Ambu bag	ICU	No. 1	RP	III	t030
13	Ambu bag	ICU	No. 1	RP	II	t969
14	Ambu bag	ICU	No. 1	RP	II	t969
15	Oxygen gauge	Emergency	No. 1	–	II	t030
16	Chair	Neonates	No. 2	–	I	t037
17	Suction	ICU	No. 1	–	III	t030
18	Stethoscope	Internal	No. 3	RP	III	t030
19	Door knob	Emergency	No. 2	–	NT	NT
20	Vital sign monitor	ICU	No. 1	–	III	t037
21	Computer keyboard	ICU	No. 2	RP	I	t030
22	Suction	ICU	No. 1	RP	III	t030
23	Bed	ICU	No. 3	MUP	III	t037
24	Bed	ICU	No. 1	–	III	t030
25	Computer keyboard	Internal	No. 1	–	III	t037
26	Ambu bag	ICU	No. 1	RP	III	t030
27	Computer mouse	ICU	No. 1	RP, MUP	III	t030
28	Bed	ICU	No. 1	–	III	t037

Note: RP: rifampicin, MUP: mupirocin, NT: non-typable.

(6%). These results showed that all of the isolates from patients, personnel, and surfaces had hospital origin. In agreement with other studies [11], SCC*mec* type III was the most frequent strain among MRSA strains. SCC*mec* type III has been found to be the main type in other studies from Iran with varying ranges from 45% to 76% [11, 21, 23, 24]. Although some studies from the east of Asia including Taiwan, Thailand, Vietnam, India, Sri Lanka, China, and Hong Kong reported SCC*mec* types II and IVc as the main types, SCC*mec* type III has been found to be the most isolated type in hospital-acquired infections in this area [13].

We found six different *spa* types among MRSA strains. The most dominant type was *spa* type t030 (75.5%), followed by *spa* type t037 (12.5%). According to data provided by Ridom *Spa* server (http://spa.ridom.de/spa-t037.shtml), both

t037 and t030 *spa* types are commonly recognized as MRSA. Interestingly, these genotypes were only found among MRSA isolates in this study and most of them belonged to SCC*mec* type III. This is in agreement with another study from different regions of China which reported t030 (80.1%) with SCC*mec* type III as the commonest type among MRSA isolates [1]. Although previous study from Tehran [5] reported t790 as the main *spa* type in ICU, the prominent *spa* type in this study was t030. However, the common data in the previous studies [5, 24] indicating *spa* types t030 and t037 are the most frequent strains found in the ICU of the Tehran's hospitals. Finding the above-mentioned strains in the ICU of Karaj's hospitals indicating poor hygiene and infection control strategies in the investigated hospitals. Special antibiotic-resistant strains are becoming progressively widespread in the ICU probably as a result of wide consumption of antibiotic.

Regarding the high frequency of MRSA-SCC*mec* type III-*spa* types t030 and t037 in this study, there is a growing concern about spreading of these strains in Iran. An investigation performed on MRSA strains in China [25] during the 15 years found that t037 was replaced by t030 *spa* type in the hospitals that probably is being happened in Iran. It seems both types mostly belong to ST239-CC8 and have been reported as the most frequent strains isolated from Iran and China [26–31]. ST239, one of the antibiotic-resistant clones is now widely disseminated in many Asian countries. This strain is highly associated with the staphylococcal chromosomal cassette *mec* (SCC*mec*) type III genetic element and mainly characterized with two *spa* types: t037 and t030. Since those strains were commonly isolated from personnel of the hospitals, it seems they act as victim and reservoir simultaneously. Other *spa* types that were found in this study were t297, t969, t5799, and t7609. To the best of our knowledge and according to the data provided by Ridom *Spa* server, this is the second report of t297 *spa* type in the world. Previous report was from Sweden and all isolates were methicillin-susceptible *S. aureus* (MSSA), whereas our isolate was MRSA. Moreover, t969 *spa* type was mostly reported from Iran and all of them were MRSA except for one strain.

In conclusion, high frequency of *spa* t030 with SCC*mec* type III and MRSA phenotype illustrated circulating of one of the antibiotic-resistant strains in ICU of Karaj's teaching hospitals and emphasizes the need for ongoing molecular surveillances. Moreover, antibiotic susceptibility monitoring, effective infection control measures, and regular screening of the hospitals' surfaces and personnel in terms of MRSA isolates are other important findings in this study. We highly recommend a survey of other hospitals in Karaj for finding the transmission of main MRSA strains between health-care settings in this city.

Acknowledgement

This work is a part of project 2168344 supported by Alborz University of Medical Sciences.

Conflict of Interest

The authors declare that there are no conflicts of interest financial or otherwise in the publication of this manuscript.

References

1. Chen, Y., Liu, Z., Duo, L., Xiong, J., Gong, Y., Yang, J., Wang, Z., Wu, X., Lu, Z., Meng, X., Zhao, J., Zhang, C., Wang, F., Zhang, Y., Zhang, M., Han, L.: Characterization of *Staphylococcus aureus* from distinct geographic locations in China: An increasing prevalence of *spa*-t030 and SCC*mec* type III. PLoS One **9**, e96255 (2014).
2. Abbasi-Montazeri, E., Khosravi, A. D., Feizabadi, M. M., Goodarzi, H., Khoramrooz, S. S., Mirzaii, M., Kalantar, E., Darban-Sarokhalil, D.: The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates with high-level mupirocin resistance from patients and personnel in a burn center. Burns **39**, 650–654 (2013).
3. Askari, E., Soleymani, F., Arianpoor, A., Tabatabai, S. M., Amini, A., Naderinasab, M.: Epidemiology of mecA-methicillin-resistant *Staphylococcus aureus* in Iran: A systematic review and meta-analysis. Iran J Basic Med Sci **15**, 1010–1019 (2012).
4. Parhizgari, N., Khoramrooz, S. S., Malek Hosseini, S. A., Marashifard, M., Yazdanpanah, M., Emaneini, M., Gharibpour, F., Mirzaii, M., Darban-Sarokhalil, D., Moein, M., Naraki, M.: High frequency of multidrug-resistant *Staphylococcus aureus* with SCC*mec* type III and *Spa* types t037 and t631 isolated from burn patients in southwest of Iran. APMIS **124**, 221–228 (2016).
5. Goudarzi, M., Goudarzi, H., Sá Figueiredo, A. M., Udo, E. E., Fazeli, M., Asadzadeh, M., Seyedjavadi, S. S.: Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains isolated from intensive care units in Iran: ST22-SCC*mec* IV/t790 emerges as the major clone. PLoS One **11**, e0155529 (2016).
6. Lim, K. T., Yeo, C. C., Suhaili, Z, Thong, K. L.: Comparison of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* strains isolated from a tertiary hospital in Terengganu, Malaysia. Jpn J Infect Dis **65**, 502–509 (2012).
7. Neela, V., Moghaddam, H. G., van Belkum, A., Horst-Kreft, D., Mariana, N. S., Rad, E. G.: First report on methicillin-resistant *Staphylococcus aureus* of *Spa* type t037, Sequence type 239, SCC*mec* type III/IIIA in Malaysia. Eur J Clin Microbiol Infect Dis **29**, 115–117 (2010).
8. Paterson, G. K., Harrison, E. M., Holmes, M. A.: The emergence of mecC methicillin-resistant *Staphylococcus aureus*. Trends Microbiol **22**, 42–47 (2014).
9. Frenay, H. M., Bunschoten, A. E., Schouls, L. M., van Leeuwen, W. J., Vandenbroucke-Grauls, C. M., Verhoef, J.: Molecular typing of methicillin-resistant *Staphylococcus aureus* on the basis of protein A gene polymorphism. Eur J Clin Microbiol Infect Dis **15**, 60–64 (1996).

10. Tian, S. F., Chu, Y. Z., Nian, H., Li, F. S., Sun, J. M., Wang, Y. L., Liu, L. W., Shang, H.: Genotype diversity of methicillin-resistant *Staphylococcus aureus* in Shenyang, China. Scand J Infect Dis **45**, 915–921 (2013).
11. Mohammadi, S., Sekawi, Z., Monjezi, A., Maleki, M. H., Soroush, S., Sadeghifard, N., Pakzad, I., Azizi-Jalilian, F., Emaneini, M., Asadollahi, K., Pourahmad, F., Zarrilli, R., Taherikalani, M.: Emergence of SCC*mec* type III with variable antimicrobial resistance profiles and *spa* types among methicillin-resistant *Staphylococcus aureus* isolated from healthcare- and community-acquired infections in the west of Iran. Int J Infect Dis **25**, 152–158 (2014).
12. Mahon, C., Lehman, D. C., Manuselis, G.: Textbook of Diagnostic Microbiology. Elsevier Inc., St. Louis, MO, USA, 2007.
13. Khoramrooz, S. S., Mansouri, F., Marashifard, M., Malek Hosseini, S. A., Akbarian Chenarestane-Olia, F., Ganavehei, B., Gharibpour, F., Shahbazi, A., Mirzaii, M., Darban-Sarokhalil, D.: Detection of biofilm related genes, classical enterotoxin genes and agr typing among *Staphylococcus aureus* isolated from bovine with subclinical mastitis in southwest of Iran. Microb Pathog **29**, 45–51 (2016).
14. Sahebekhtiari, N., Nochi, Z., Eslampour, M., Dabiri, H., Bolfion, M., Taherikalani, M., Khoramian, B., Zali, M. R., Emaneini, M.: Characterization of *Staphylococcus aureus* strains isolated from raw milk of bovine subclinical mastitis in Tehran and Mashhad. Acta Microbiol Immunol Hung **58**, 113–121 (2011).
15. Zhang, K., McClure, J.-A., Elsayed, S., Louie, T., Conly, J. M.: 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 **43**, 5026–5033 (2005).
16. Perez-Roth, E., Claverie-Martın, F., Villar, J., Méndez-Álvarez, S.: Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. J Clin Microbiol **39**, 4037–4041 (2001).
17. 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 **41**, 5442–5448 (2003).
18. Mirzaii, M., Emaneini, M., Maleknejad, P., Jonaid, N., Fooladi, A. A., Aligholi, M., Jabalameli, F., Halimi, S., Taherikalani, M., Kasaeian, A.: Distribution of bacterial contamination in a teaching hospital in Tehran – A special focus on *Staphylococcus aureus*. Acta Microbiol Immunol Hung **59**, 1–11 (2012).
19. Hashemian, M., Talaie, H., Akbarpour, S., Mahdavinejad, A., Mozafari, N.: Central nervous system depressants poisoning and ventilator associated pneumonia: An underrated risk factor at the toxicological intensive care unit. Iran Red Crescent Med J **18**, e30989 (2016).
20. Hassanzadeh, P., Motamedifar, M., Hadi, N.: Prevalent bacterial infections in intensive care units of Shiraz University of Medical Sciences teaching hospitals, Shiraz, Iran. Jpn J Infect Dis **62**, 249–253 (2009).
21. Szczuka, E., Grabska, K., Trawczyński, K., Bosacka, K., Kaznowski, A.: Characterization of SCCmec types, antibiotic resistance, and toxin gene profiles of *Staphylococcus aureus* strains. Acta Microbiol Immunol Hung **60**, 261–270 (2013).

22. Lepsanovic, Z., Jeremic, L. P., Lazic, S., Cirkovic, I.: High prevalence and resistance patterns of community-associated methicillin-resistant *Staphylococcus aureus* in the Pomoravlje Region, Serbia. Acta Microbiol Immunol Hung **63**, 83–92 (2016).
23. Sadeghi, J., Mansouri, S.: Molecular characterization and antibiotic resistance of clinical isolates of methicillin resistant *Staphylococcus aureus* obtained from Southeast of Iran (Kerman). APMIS **122**, 405–411 (2014).
24. Mirzaii, M., Emaneini, M., Jabalameli, F., Halimi, S., Taherikalani, M.: Molecular investigation of *Staphylococcus aureus* isolated from the patients, personnel, air and environment of an ICU in a hospital in Tehran. J Infect Public Health **8**, 202–206 (2015).
25. Chen, H., Liu, Y., Jiang, X., Chen, M., Wang, H.: Rapid change of methicillin-resistant *Staphylococcus aureus* clones in a Chinese tertiary care hospital over a 15-year period. Antimicrob Agents Chemother **54**, 1842–1847 (2010).
26. Liu, Y., Wang, H., Du, N., Shen, E., Chen, H., Niu, J., Ye, H., Chen, M.: Molecular evidence for spread of two major methicillin-resistant *Staphylococcus aureus* clones with a unique geographic distribution in Chinese hospitals. Antimicrob Agents Chemother **53**, 512–518 (2009).
27. DeLeo, F. R., Smyth, D. S., McDougal, L. K., Gran, F. W., Manoharan, A., Enright, M. C., Song, J. H., de Lencastre, H., Robinson, D. A.: Population structure of a hybrid clonal group of methicillin-resistant *Staphylococcus aureus*, ST239-MRSA-III. PLoS One **5**, e8582 (2010).
28. Tian, S. F., Chu, Y. Z., Nian, H., Li, F. S., Sun, J. M., Wang, Y. L., Liu, L. W., Shang, H.: Genotype diversity of methicillin-resistant *Staphylococcus aureus* in Shenyang, China. Scand J Infect Dis **45**, 915–921 (2013).
29. Xiao, M., Wang, H., Zhao, Y., Mao, L. L., Brown, M., Yu, Y. S., O'Sullivan, M. V., Kong, F., Xu, Y. C.: National surveillance of methicillin-resistant *Staphylococcus aureus* in China highlights a still-evolving epidemiology with 15 novel emerging multilocus sequence types. J Clin Microbiol **51**, 3638–3644 (2013).
30. He, W., Chen, H., Zhao, C., Zhang, F., Li, H., Wang, Q., Wang, X., Wang, H.: Population structure and characterization of *Staphylococcus aureus* from bacteraemia at multiple hospitals in China: Association between antimicrobial resistance, toxin genes and genotypes. Int J Antimicrob Agents **42**, 211–219 (2013).
31. Liu, Q., Han, L., Li, B., Sun, J., Ni, Y.: Virulence characteristic and MLST-agr genetic background of high-level mupirocin-resistant, MRSA isolates from Shanghai and Wenzhou, China. PLoS One **7**, e37005 (2012).