Iranian Journal of Microbiology
Volume 5 Number 3 (September 2013) 227-232

Isolation of Asian endemic and livestock associated clones of methicillin resistant *Staphylococcus aureus* from ocular samples in Northeastern Iran

Seyed Asghar Havaei[1], Amir Azimian[2*], Hosein Fazeli[1], Mahmood Naderi[3], Kiarash Ghazvini[4], Siamak Mirab Samiee[5], Masoud Soleimani[6]

[1]Department of Pathobiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. [2]Department of Pathobiology and Anatomy, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran. [3]Molecular Biology and Genetic Engineering Department, Stem Cell Technology Research Center, Tehran, Iran. [4]Department of Microbiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. [5]Food and Drug Laboratory Research Center, Ministry of Health and Medical Education, No. 408, Emam Khomeini Ave., Tehran, Iran. [6]Department of Hematology, School of Medical Science, Tarbiat Modares University, Tehran, Iran.

Received: October 2012, **Accepted:** March 2013.

ABSTRACT

Background and Objectives: Methicillin Resistant *Staphylococcus aureus* (MRSA) strains are divided into Community Associated (CA-) and Hospital Associated (HA-) MRSA. These strains vary in antimicrobial resistance and pathogenicity. *S. aureus* is one of the most common microorganisms in ocular infections. This study was aimed to determine antimicrobial resistance patterns and genetic characteristics of MRSA strains isolated from ocular infections in Iran.

Material and Methods: Out of 171 *S. aureus* strains isolated from various clinical samples during September-December 2011 at Mashhad Emam Reza Hospital, 3 were cultured from eye discharge samples. Antimicrobial resistance tests were performed with MIC and disk diffusion methods and also genetic evaluation was done with Staphylococcal Cassette Chromosome mec (*SCCmec)*, *Accessory Gene Regulator (agr*) and *Staphylococcal Protein A (spa)* typing, Multi Locus Sequence Typing (MLST) and determination of toxin gene profile.

Results: All strains were MRSA and showed resistance to tetracycline, gentamicin and clindamycin too. Vancomycin, minocyclin and trimethoprim/sulfamethoxazole were effective on all ocular isolates. All isolates belonged to *SCCmec* IV type. MRSA1 belonged to ST239, CC8, *Spa* type t7688 and *agrIII* and had *tst1* and *hla* toxin genes. MRSA2 belonged to ST239, CC8, *Spa* type t037 and *agrI* and had the *hla* toxin gene. Finally, MRSA3 belonged to ST291, CC398, *Spa* type t304, and *agrI* and had *pvl* and *hla* toxin genes.

Conclusion: phenotypic and genotypic evaluation of the isolated MRSA strains revealed that these strains belong to endemic Asian and livestock related clones that could reach from other body sites or environment to the eye of patients and developed ocular infection.

Keywords: *Staphylococcus aureus*, MRSA, MLST, *Spa* typing, *SCCmec* type, *agr*, ocular infection.

* Corresponding author: Amir Azimian Ph.D
Address: Department of Pathobiology, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran.
Tel: +98-584-2239869
Fax: +98-584-2237076
E-mail: amir_azimian2003@yahoo.com

INTRODUCTION

Staphylococcus aureus is one of the major human pathogens causing a wide variety of infections from simple superficial skin infections to dangerous systemic infections such as septicemia, endocarditis and toxic shock syndrome (1-2). *S. aureus* is the major ophthalmic bacterial pathogen isolated from

various ocular infections such as conjunctivitis, blepharitis, external and internal hordeolum, scleritis, canaliculitis, keratitis (3-4).

The eye is impermeable to external materials. In addition, some enzymes and immunomodulatory proteins such as lysozyme, lactoferrin, secretory immunoglobulins, and defensins are present in tear and reduce bacterial colonization in the ocular surfaces (5). Infections can originate from external sources or intraocular invasion of blood borne microorganisms. Bacterial infections on the outer surface of eye may spread to adjacent tissues such as the cornea, orbit, inner eye and even the brain (6). Major risk factors for bacterial ocular infections with external sources are surgical and non-surgical trauma and contact lens use. Ocular infections with progressive lesions can lead to blindness due to destructive activity of bacterial enzymes and toxins or even in some cases lead to mortality due to expansion of infection to other organs via the blood stream or the lymphatics (6-7).

Treatment of *Staphylococcus aureus* infections has become more complicated with emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) strain in 1961 (8-9). MRSA was primarily a hospital-associated pathogen, however today multiple community acquired MRSA clones emerged in human infections. Recently, human-related MRSA infections based on their source, have been categorized into four groups; Hospital Associated MRSA (HA-MRSA), Community Associated MRSA (CA-MRSA), Hospital Associated MRSA with Community onset and Livestock Associated MRSA (LA-MRSA) (10). To date, all four types of MRSA strains have been isolated from clinical samples (10-15). HA-MRSA strains have more antimicrobial resistance and also cause more serious diseases mainly in hospitalized patients while CA-MRSA strains show less antimicrobial resistance and commonly lead to skin and soft tissue (SSI) infections (1).

Despite the fact that MRSA is one of the major topics in clinical microbiology research, very little is known about the occurrence and genetic characteristics of MRSA strains isolated from ocular infections in the world especially in Asian countries such as Iran. To the best of our knowledge, there is no report concerning the genetic characteristics of MRSA strains causing ocular infections in Iran. Here, we evaluated the genetic characteristics of three MRSAs isolated from ocular discharge samples of patients admitted to the Emam Reza Hospital, Mashhad, Khorasan Razavi, Iran.

MATERIALS AND METHODS

171 Strains of *Staphylococcus aureus* were isolated from patients admitted between 23 September 2011 to 21 December 2011 at Emam Reza Hospital, a 918 bed University teaching Hospital, Mashhad, Iran. These isolates were obtained from blood, urine, sputum, wound, abscess, nose, throat, eye and respiratory tract samples. *S. aureus* isolates were identified by conventional biochemical tests including Gram staining, catalase, manitol fermentation, slide and tube coagulase test and DNase.

Screening for methicillin and vancomycin resistance. All *S. aureus* isolates were screened for oxacillin and vancomycin resistance using agar screening method. Methicillin resistance was defined as the capability of growth in agar screening media including 4% NaCl plus 6µg/ml oxacillin and MIC ≥ 4 µg/ml whereas vancomycin resistance was defined as the capability of growth in agar screening media including 6 µg/ml vancomycin and MIC ≥16 µg/ml. Strains of *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 52199 were used as control.

Antimicrobial susceptibility testing. Antibiotic susceptibility testing was carried out using disk diffusion method (MAST DISKS™) according to guidelines of Clinical Laboratory Standards Institute (CLSI)(16-17). The list of the antibiotics used in the test is presented in Table 2. Strain of *S. aureus* ATCC 25923 was used as control. E-Test method (Biomeriux Strips) was used for MIC determination for the only *S. aureus* strain capable of growing in the agar screening media.

Genomic DNA extraction. Genomic DNA of *S. aureus* isolates were extracted using QIAamp® DNA mini kit. According to manufacturer's protocol for bacterial cells, we added lysostaphin at the final concentration of 30µg/ml in lysis buffer.

PCR. PCR amplification was performed with a TAKARA Gradient PCR TP600 thermal cycler in a volume of 50 µl. We used EmeraldAmp® MAX PCR Master Mix (Takara, Japan) for all PCR reactions.

PCR identification of *mecA* gene. The primers used for amplification of *mecA* gene are listed in Table 3. PCR was performed with the following thermal setting:

Table 1. Phenotypic characteristics of VISA isolates.

	Sex/ age	Sample/ ward	Oxa	Van	Min	Lev	Cip	Tet	Cot	Gen	Cli	Rif	Oxa MIC	Van MIC	Van Agar screen
MRSA1	F/25	Eye discharge/ out patient	R	S	S	R	R	R	S	R	R	S	128	2	-
MRSA2	F/39	Eye discharge/ out patient	R	S	S	R	R	R	S	R	R	S	128	1	-
MRSA3	F/50	Eye discharge / neurology	R	S	S	S	S	R	S	R	R	R	256	1	-

Abbreviations: Oxa: Oxacillin, Van: Vancomycin, Min: Minocyclin, Lev: Levofloxacin, Cip: Ciprofloxacin, Tet: Tetracycline, Cot: trimethoprim/sulfamethoxazole, Gen: Gentamycin, Cli: Clindamycin, Rif: Rifampicin. R: Resistant, S: Susceptible.

5 min at 94°C for initial enzyme activation followed by 40 cycles of amplification (denaturation at 94°C for 30 sec; annealing at 57°C for 45 sec; extension at 72°C for 30 sec) and final extension at 72°C for 5 min.

Multiplex PCR for detection of toxin genes. The primers used for amplification of *Panton-Valentine Leukocidin (pvl), Toxic Shock Syndrome Toxin-1 (tst1), alpha Hemolysin* (*hla*) and *Enterotoxin C* (*etc*) genes are listed in Table 3**.** PCR was performed with the following thermal setting: 5 min at 94°C for initial enzyme activation followed by 40 cycles of amplification (denaturation at 94°C for 40 sec**;** annealing at 60°C for 40 sec; extension at 72°C for 1 min) and final extension at 72°C for 5 min.

Multiplex PCR for *SCCmec* and *agr* typing. *SCCmec* and *agr* typing were performed as previously described (18-19). The primers used for the PCR are listed in Table 3.

MLST. Multi Locus Sequence Typing was carried out by PCR and sequencing of the internal fragments of carbamate kinase (*arc*)*,* shikimate dehydrogenase (*aro*), glycerol kinase (*glp*), guanylate kinase (*gmk*), phosphate acetyl transferase (*pta*), trios phosphate isomerase (*tpi*) and acetyl coenzyme A acetyl transferase (*yqi*) genes of *S. aureus* as previously described (20).

***Spa* typing.** s*pa* typing was performed by PCR and sequencing of polymorphic X region of *spa* gene was done as previously described, http://*spa*server.ridom.de/(21).

Nucleotide Sequencing. Amplified PCR products were purified with QIAquick® Gel Extraction Kit. The purified PCR products were sequenced with an ABI 3730XL DNA analyzer (Applied Biosystems) in both directions. The sequences were used for both confirmation and sequence based typing methods (MLST and *spa* typing).

RESULTS

Of 171 *S. aureus* isolates 3 were isolated from eye discharge. These 3 isolates were resistant to oxacillin, positive for *mecA* gene and showed *SCCmec* type IV. They belonged to Clonal Complexes 8 and 398 (CC8 and CC398). Genetic analysis of the isolated MRSA strains revealed that these strains were endemic Asian and livestock related clones. All three isolates were sensitive to vancomycin, minocycline and trimethoprim/sulfamethoxazole but resistant to oxacillin, tetracycline, gentamycin and clindamycin. The demographic data and antibiogram results of these strains are listed in Table 1. Genetic evaluation results in details including, *mecA* gene PCR, SCC*mec* types, *agr* groups, toxin profiles, *spa* types and Sequence Types (STs) are presented in Table 2.

DISCUSSION

This is the first report of the molecular analysis of

Table 2. Genetic characteristics of VISA isolates.

	agr	Scc *mec*	*pvl*	*hla*	*etc*	*tst1*	*mecA*	*vanA*	*spa* type	*spa* repeat profile	ST	CC	MLST allelic profile
MRSA1	III	IV	-	+	-	+	+	-	t7688	11-148-21-17-34-24-34-22-25	ST-239	8	2-3-1-1-4-4-3
MRSA2	I	IV	-	+	-	-	+	-	t037	15-12-16-02-25-17-24	ST-239	8	2-3-1-1-4-4-3
MRSA3	I	IV	+	+	-	-	+	-	t304	11-10-21-17-34-24-34-22-25	ST-291	398	3-37-19-2-20-26-32

Abbreviations: *pvl*: *Panton-ValentinLeukocidin, sec: Enterotoxin C, tst1: Toxic Shock Syndrome Toxin1, hla: alpha Hemolysin*, *agr: accessory gene regulator, SCCmec: Staphylococcal Cassette Chromosome,spa*: *Staphylococcal protein A*

Table 3. Primers used in this study.

Target	primer	Sequence (5'-3')	Product size (bp)	reference
mecA	F	AGAAGATGGTATGTGGAAGTTAG	583	(24)
	R	ATGTATGTGCGATTGTATTGC		
pvl	F	GGAAACATTTATTCTGGCTATAC	502	
	R	CTGGATTGAAGTTACCTCTGG		
hla	F	CGGTACTACAGATATTGGAAGC	744	
	R	TGGTAATCATCACGAACTCG		
sec	F	GGGAATGTTGGATGAAGG	900	
	R	AGGCAAGCACCGAAGTAC		
tst1	F	TTATCGTAAGCCCTTTGTTG	398	
	R	TAAAGGTAGTTCTATTGGAGTAGG		
agr	Pan F	ATGCACATGGTGCACATGC		(27)
	R I	GTCACAAGTACTATAAGCTGCGAT	439	
	R II	GTATTACTAATTGAAAAGTGCCATAGC	572	
	R III	CTGTTGAAAAAGTCAACTAAAAGCTC	406	
	R IV	CGATAATGCCGTAATACCCG	657	
SCCmec	F β	ATTGCCTTGATAATAGCCYTCT	937	(19)
	R α3	TAAAGGCATCAATGCACAAACACT		
	F ccrC	CGTCTATTACAAGATGTTAAGGATAAT	518	
	R ccrC	CCTTTATAGACTGGATTATTCAAAATAT		
	F 1272	GCCACTCATAACATATGGAA	415	
	R 1272	CATCCGAGTGAAACCCAAA		
	F 5R*mecA*	TATACCAAACCCGACAACTAC	359	
	R 5R431	CGGCTACAGTGATAACATCC		

three *S. aureus* strains isolated from ocular infections with different molecular typing methods in Iran. In addition, *SCCmec* types of both isolated MRSA were type IV. With regard to these data, isolated strains can be categorized to CA-MRSA. The third MRSA was isolated from a hospitalized patient who had a history of hospitalization. Given this information, this infection could be categorized into the Hospital associated group, however, the isolate showed *SCCmec* type IV and belonged to ST291 (CC398) and therefore can be placed in the LA-MRSA group. We concluded that this isolate was a livestock associated strain that either entered the hospital environment or colonized the patient before hospitalization. We don't know about the patient contact with livestock or animals.

Nadig *et al.* (2012) reported that most of the eye infections in two important Eye Specialist Hospital in India were caused by community-associated *S. aureus*. They also reported that major Sequence Type in their isolates was ST722 (belonged to Clonal Complex 1) whereas in our study isolated strains were belonged to endemic Asian and also livestock associated clones (10). The livestock associated isolate in our study epidemiologically was HA-MRSA. Currently, MRSA isolates belonging to CC398, such as ST398 or ST291 (Double Locus Variant of ST398) are newly categorized to LA-MRSA (10). There are some reports relating human infections, caused by Livestock associated Methicillin Sensitive *Staphylococcus aureus* (MSSA), to LA-MRSA (13-15). To our knowledge, this is the first report of human ocular infection with LA-MRSA. Mediavilla *et al.* reported that most of CC398 strains isolated from Clinical samples in the USA, mainly belonged to *Spa* type t2313 which is endemic to Europe, while our LA-MRSA isolate had *Spa* type t304 that has been isolated from different parts of the world (13, 22).

The MRSA1 strain belonged to *Spa* type t7688 and MRSA2 to *Spa* type t037. To date, *Spa* type t7688 has been reported for the first time from Iran (http://spa.ridom.de/spa-t7688.shtml) and t037 is one of the common *Spa* types in Asian countries (23-24). Hesje *et al*. reported that the most observed *Spa* types in their study in the USA were *Spa* types t008 and t002 that belonged to USA300 and USA100 clones,

respectively (25).

Other important genetic characteristic of these isolates is *agr* specific group. The *agr* locus of *S. aureus* is a quorum-sensing gene cluster that up-regulates the production of secreted virulence factors and down-regulates the production of cell-associated virulence factors. The relation between *agr* group, type of infection, geographical area and resistance to some antibiotics, particularly vancomycin, is highlighted in some articles (18, 26). MRSA1 belonged to *agrIII* and MRSA2 & 3 belonged to *agrI*, that is the most common *agr* group among worldwide MRSA clones (27).

Some reports have been published concerning the antimicrobial susceptibility patterns of MRSA strains isolated from clinical specimens including ocular samples (5-7, 28-32). Shanmuganathan *et al.* reported that all MRSA strains, isolated from external ocular infections, were sensitive to chloramphenicol and those isolated from patients more than 50 years old, all were resistant to ofloxacin (28). Hsiao *et al.* reported that CA-MRSA and HA-MRSA isolates were resistant to clindamycin and erythromycin and CA-MRSA isolates were more susceptible to trimethoprim/sulfamethoxazole (30). All MRSA strains in this study were resistant to tetracycline, gentamicin and clindamycin. In contrast, vancomycin, minocyclin and sulfamethoxazole/trimethoprim showed activity against all of the isolated MRSA strains (Table 2).

Epidemiological studies show that combination of *pvl* and other toxins such as entero-toxins, could increase the severity of diseases caused by *S. aureus* (6). We evaluated *pvl*, *tst1*, *etc* and *hla* genes amongst the MRSA isolate (Table1). All isolates were positive for *hla* gene and negative for *etc* gene. *hla* gene commonly found in most isolated *S. aureus* strains (33). MRSA1 was also positive for *tst1* in PCR and MRSA3 isolate was positive for *pvl* gene, but unfortunately we do not have any data about the outcome of these infections in our patients, because they were not available for more questioning.

A major limitation in this study was the small number of isolates. It was better that we simultaneously evaluated the nasal swab and eye samples. This approach helped us to find probable source of ocular infection in the patients. An interesting finding of the ocular specimens in this study was the gender of patients: all samples were obtained from female patients. However, due to small sample size it is not possible to make conclusion about this finding.

Funding. This work was supported by the Isfahan University of Medical Sciences [Grant number 390256].

ACKNOWLEDGEMENTS

We specifically thank Dr. Jean B. Patel from Division of Healthcare Quality Promotion, Centers for Disease Control for her scientific advice. Also, we thank Stem Cell Research Center (Bone Yakhteh) for supporting technical assistance. We deeply thank Prof. Richard P. Novick and Prof. K. Hiramatsu for providing standard strains. We also thank Zahra Masoumi for editing the paper.

REFERENCES

1. Chambers HF, DeLeo FR. Waves of Resistance: *Staphylococcus aureus* in the Antibiotic Era. *Nat Rev Microbiol* 2009; 7: 629-641.
2. Feng Y, Chen C-J, Su L-H, Hu S, Yu J, Chiu C-H. Evolutionand pathogenesis of *Staphylococcus aureus*: lessons learned from genotyping and comparative genomics. *FEMS Microbiol Rev* 2007; 32: 23-37.
3. Ramesh S, Ramakrishnan R, Bharathi MJ, Amuthan M, Viswanathan S. Prevalence of bacterial pathogens causing ocular infections in South India. *Indian J Pathol Microbiol* 2010; 53: 281-286.
4. Behlau I, Gilmore MS. Microbial Biofilms in Ophthalmology and Infectious Disease. *Arch Ophthalmol* 2008; 126: 1572-1581.
5. Khosravi AD, Mehdinejad M, Heidari M. Bacteriological findings in patients with ocular infection and antibiotic susceptibility patterns of isolated pathogens. *Singapore Med J* 2007; 48: 741-743.
6. Nadig S, Velusamy N, Lalitha P, Kar S, Sharma S, Arakere G. *Staphylococcus aureus* eye infections in two Indian hospitals: emergence of ST772 as a major clone. *Clin Ophthalmol* 2012; 6: 165-173.
7. Chuang C-C, Hsiao C-H, Tan H-Y, Ma DH-K, Lin K-K, Chang C-J, et al. *Staphylococcus aureus* Ocular Infection: Methicillin- Resistance, Clinical Features, and Antibiotic Susceptibilities. *PLoS ONE* 2012; 8: e42437.
8. Rehm SJ, Tice A. *Staphylococcus aureus*: Methicillin-Susceptible *S. aureus* to Methicillin-Resistant *S. aureus* and Vancomycin-Resistant *S. aureus*. *Clin Infect Dis* 2010; 51: S176-S82.
9. Sievert DM, Rudrik JT, Patel JB, McDonald LC, Wilkins MJ, Hageman JC. Vancomycin-Resistant *Staphylococcus aureus* in the United States, 2002–2006. *Clin Infect Dis* 2008; 46: 668-674.
10. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *Mbio* 2012; 3: 1-6.

11. Herold B, Immergluck L, Maranan M, Lauderdale D, Gaskin R, Boyle-Vavra S, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA* 1998; 279: 593-598.
12. Fridkin SK, Hageman JC, Morrison M, Sanza LT, Como-Sabetti K, Jernigan JA, et al. Methicillin-Resistant *Staphylococcus aureus* Disease in Three Communities. *N Engl J Med* 2005; 352: 1436-1444.
13. Mediavilla JR, Chen L, Uhlemann A-C, Hanson BM, Rosenthal M, Stanak K, et al. Methicillin Susceptible *Staphylococcus aureus* ST398, New York and New Jersey, USA. *Emerg Infect Dis* 2012;18:700-702.
14. Graveland H, Wagenaar J. A, Bergs K, Heesterbeek H, Heederik D. Persistence of livestock associated MRSA CC398 in humans is dependent on Intensity of animal contact. PLoS ONE. 2011; 6: 1-7.
15. Cleef BAGLv, Monnet DL, Voss A, Krziwanek K, Allerberger F, Struelens M, et al. Livestock associated Methicillin- Resistant *Staphylococcus aureus* in Humans, Europe. *Emerg Infect Dis* 2011; 17: 502-505.
16. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests. Standard - 11th ed CLSI document M02-A11. CLSI, 950 West Valley Rd., Suite 25002010.
17. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standard - 11th ed 2010.
18. Peerayeh SN, Azimian A, Nejad QB, Kashi M. Prevalence of *agr* specificity groups among *Staphylococcus aureus* isolates from university hospitals in Tehran. *Lab Med* 2009; 40: 27-29.
19. Boye K, Bartels M, Andersen I, Møller J, Westh H. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCCmec types I-V. *Clin Microbiol Infect* 2007; 13: 725-727.
20. 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: 1008-1015.
21. Harmsen D, Claus H, Witte W, Rothga¨nger Jr, 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: 5442-5448.
22. Emaneini M, Khoramrooz SS, Taherikalani M, Jabalameli F, Aligholi M. Molecular characterization of *Staphylococcus aureus* isolated from children with adenoid hypertrophy: Emergence of new spa types t7685 and t7692. *Int J Ped Otorhinolaryng* 2011; 75: 1446-1449.
23. Ghaznavi-Rad E, Shamsudin MN, Sekawi Z, Khoon 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: 867-872.
24. Azimian A, Havaei SA, Fazeli H, Naderi M, Ghazvini K, Samiee SM, et al. Genetic characterization of a Vancomycin-Resistant *Staphylococcus aureus* isolated from respiratory tract of a hospitalized patient in a university hospital in Northeastern Iran. *J Clin Microbiol* 2012; 50: 3581-3585.
25. Hesje CK, Sanfilippo CM, Haas W, Morris TW. Molecular Epidemiology of Methicillin- Resistant and Methicillin-Susceptible *Staphylococcus aureus* Isolated from the Eye. *Cur Eye Res* 2011; 36: 94-102.
26. Sakoulas G, Eliopoulos GM, Robert C. Moellering J, Wennersten C, Venkataraman L, Novick RP, et al. Accessory gene regulator (*agr*) locus in geographically diverse *Staphylococcus aureus* isolates with reduced susceptibility to vancomycin. *Antimicrob Agent Chemother* 2002; 46: 1492-1502.
27. Leeuwen Wv, Nieuwenhuizen Wv, Gijzen C, Verbrugh H, Belkum AV. Population studies of methicillin-resistant and -sensitive *Staphylococcus aureus* strains reveal a lack of variability in the agrD gene, encoding a staphylococcal autoinducer peptide. *J Bacteriol* 2000; 182: 5721-5729.
28. Shanmuganathan V, Armstrong M, Buller A, Tullo A. External ocular infections due to methicillin-resistant *Staphylococcus aureus* (MRSA). *Eye* 2005; 19: 284-291.
29. Schubert TL, Hume EB, Willcox MD. *Staphylococcus aureus* ocular isolates from symptomatic adverse events: antibiotic resistance and similarity of bacteria causing adverse events. *Clin Exp Optom* 2008;91:148-155.
30. 30. Hsiao CH, Chuang CC, Tan HY, Ma DHK, Lin KK, Chang CJ, et al. Methicillin-resistant *Staphylococcus aureus* ocular infection: a 10-year hospital-based study. *Ophthalmology* 2012;119(8).
31. 31. Hasani A, Sheikhalizadeh V, Hasani A, Naghili B, Valizadeh V, Nikoonijad AR. Methicillin resistant and susceptible *Staphylococcus aureus*: Appraising therapeutic approaches in the Northwest of Iran. *Iran J Microbiol* 2013;5:56-62.
32. 32. Kaleem F, Usman J, Hassan A, Omair M, Khalid A, Uddin R. Sensitivity pattern of methicillin resistant *Staphylococcus aureus* isolated from patients admitted in a tertiary care hospital of Pakistan. *Iran J Microbiol* 2010;2:143-146.
33. 33. Havaei S, Vidovic S, Narimani T, Kazemi M, Karbalaei M, Starnino S, et al. Epidemic Methicillin-Susceptible *Staphylococcus aureus* Lineages Are the Main Cause of Infections at an Iranian University Hospital. *J Clin Microbiol* 2011;49:3990-3993.