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

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**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.

**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:
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 1 min; 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. spa typing was performed by PCR and sequencing of polymorphic X region of spa gene was done as previously described, http://spaserver.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, SCCmec 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

| Sex/ age | Sample/ ward | Oxa | Van | Min | Lev | Cip | Tet | Cot | Gen | Cli | Rif | Oxa MIC | Van MIC | Van Agar screen |
|----------|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----------|----------|-----------------|
| MRSA1    | F/25         |     |     |     |     |     |     |     |     |     |     | 128      | 2        | -               |
| MRSA2    | F/39         |     |     |     |     |     |     |     |     |     |     | 5        | 1        | -               |
| MRSA3    | F/50         |     |     |     |     |     |     |     |     |     |     | 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.

Table 1. Phenotypic characteristics of VISA isolates.

Table 2. Genetic characteristics of VISA isolates.

| agr | Sec 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     | 11-10-21-17-34-24-34-23-25      | ST-291 | 398| 3-37-19-2-20-26-32 |

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

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**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 Ophthalmo* 2008; 126: 1572-1581.

5. Khosravi AD, Mehminejad 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 ST1772 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. Peeralyeh SN, Azimian A, Nejad OB, 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, Moller 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, Rothgäger 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, Taherikalamani 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, Samiec 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. Heasje 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, Niewenhuizen 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. Shannuganathan 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. 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. Hasani A, Sheikhalizadeh V, Hasani A, Naghili B, Valizadeh V, Nikoonjam AR. Methicillin resistant and susceptible *Staphylococcus aureus*: Appraising therapeutic approaches in the Northwest of Iran. *Iran J Microbiol* 2013;5:56-62.

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. 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.