Prevalence of *mecA* Gene of Methicillin Resistant *Staphylococcus spp.* Isolated from Nosocomial Infections and Environmental Specimens in Sanandaj Hospitals, Kurdistan, Iran

Rashid Ramazanzadeh [1], Himen Salimizand [1, 2], Babak Shahbazi [3], Masoumeh khonshah [3], Hanar Narenji [3*]

[1] Cellular and Molecular Research Center and Microbiology Department, Kurdistan University of Medical Sciences, Sanandaj, Iran.
[2] Department of Microbiology and Virology, Mashhad University of Medical Sciences, Mashhad, Iran.
[3] Microbiology Department, Member of Student Research committee, Kurdistan University of Medical Sciences, Sanandaj, Iran.

Abstract

Background: Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the major agents for increasing number of serious hospital and community acquired infections. The aim of this study was to investigate the occurrence of the MRSA and *mecA* gene among nosocomial and environmental specimens in Kurdistan hospitals and determining the antibiotic resistance of the isolates.

Materials and Methods: A total of 264 clinical and environmental Staphylococcus was isolated from Kurdistan medical University Hospitals, in February 2011 to June 2012 Iran, and their susceptibility patterns to different antibiotics were determined. Furthermore, agar screen method was used to determine oxacillin resistant isolates. Finally, using PCR, the oxacillin resistant isolates were tested for the presence of *mecA* gene.

Results: In this study, from 88 (93.18%) *Staphylococcus aureus* isolates, 82 were found resistant to oxacillin using agar screen method and *mecA* gene was detected in 66 strains (75%). Our results showed that the agar screen method is more reliable in determination of MRSA strains compared to PCR.

Conclusion: In this research the studied MRSA were found with high prevalence and *mecA* was widespread in *S. aureus* isolates in Sanandaj.

Keywords: Nosocomial infections; Methicillin resistance; MRSA; *mecA*

Introduction

Staphylococcus aureus (*S. aureus*) is one of the most frequently isolated bacteria from both hospital and the environment (1, 2). Clinical infections with this bacterium are most common in care units, nursing homes, and other chronic care facilities. Also, methicillin-resistant *S. aureus* (MRSA) as an emerging and important community-acquired pathogen due to resistance to all of the available β-lactam antibiotics (3, 4). Infections with MRSA are rapidly expanding throughout the world (5) soon after the first isolation of MRSA in the United Kingdom in 1961 (2). MRSA transmission in healthcare usually occurs via contaminated hands, garments, or equipment of healthcare laborer (7, 8).This kind of transmission has been a major factor

accounting for the raise in the incidence and prevalence of MRSA in acute care facilities (8). Barrier precautions are often included in recommended control measures for MRSA. For example, contact isolation in a single room has been recommended by the Centers for Disease Control (CDC) and prevention since 1983 for patients colonized or infected with MRSA (9). *mecA*, a particular penicillin-binding protein (PBP) gene called PBP2A with low affinity for methicillin and most other β-lactam drugs, is reported as the main cause of non-susceptibility to methicillin (10). However, *mecA* is also present in methicillin-resistant coagolase negative staphylococcus (MRCoNS) isolates, which could be detected both,

simultaneously, to rapidly distinguish MRSA from MRCoNS (11). Recent studies have revealed an increase in the worldwide prevalence of MRSA. European countries have maintained low rates of MRSA (12). Although, there are many reports from different cities of Iran, however, the average rate of MRSA in Iran hospitals is still unknown (6).

In this study we investigated the occurrence of MRSA and *mecA* gene among isolates from nosocomial infections and environmental specimens in Kurdistan hospitals and determined the antibiotic resistance of the isolates.

Materials and methods

Bacterial collection
A total of 264 isolates was collected from patients and healthy individuals. Of which, 156 staphylococci strains were isolated from clinical samples, 49 from the environment and 59 from the staff in wards of hospitals in Sanandaj. Characterization of isolates was confirmed by biochemical tests (catalase, coagulase, mannitol fermentation and DNase activity).

Antibiotic susceptibility testing
Sensitivity to antibiotics was determined by Kirby-Bauer methods with gentamicin (10μg), vancomycin (30μg), ciprofloxacin (5μg), and erythromycin (15μg) according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (13). Then, agar screening containing 6 μgr/mL oxacillin was used

to find MRSA isolates (14).

Molecular assay of mecA gene
DNA from isolates was extracted by commercial kit (Cinnapure-DNA, CinnaGen, Iran). Uniplex PCR assays for *mecA* gene were performed in a reaction volume of 25 μl. Primers were 3'-AAA CTA CGG TAA CAT TGA TCG CAA C-5' as forward and 3'-CTT GTA CCC AAT TTT GAT CCA TTT-5' as reverse. To make the 25 μl reaction mixture we mixed 2X-PCR Master mix (CinnaGen, Iran) 12.5μl, DW 8.5μl, 1 μL of each primer and 2 μl of the DNA. The cycling profile for *mecA* was as follows: initial denaturation at 95 °C for 5 min, and 35 cycles of denaturation at 95 °C for 45s, annealing at 57.5 °C for 45s, extension at 72 °C for 1 min and final extension at 72 °C for 10 min. The expected amplicon were 321 bp.

Ethics Statement

Since bacteria were under experiments, there was no need to consider research ethics.

Results

A total of 264 strains from patients and healthy individuals (101 men, 104 women; 49.27% and 50.73%, respectively) were isolated. Sources and types of Staphylococcus isolates are mentioned in Table 1.

Table 1. Prevalence of Staphylococcus strains isolated from the samples.

Organism	Staff	Environmental	Clinical	Total (%)
S. *aureus* –n (%)	18 (36.73)	20 (33.9)	50 (32.05)	88 (33.3)
S. *epidermidis* – n (%)	31 (63.27)	38 (64.41)	101 (64.74)	170 (64.39)
S. *saprophyticus* –n (%)	0	1 (1.69)	5 (3.21)	6 (2.27)
Total - n (%)	49 (18.56)	59 (22.35)	156 (59.09)	264 (100)

Among the 264 isolates tested by disk diffusion method, vancomycin was the most effective antibiotic against *staphylococcus* species (69.7%). The frequency of resistance to ciprofloxacin was 43.94%, gentamicin 28.3% and erythromycin 9.09%. The data analysis showed that most of the strains were resistant to at least more than one antibiotic.

Screen agar for detection of MRSA strains
After culturing Staphylococcus strains on Muller Hinton agar (Merck, Germany) containing 4% NaCl and 6 mg / L oxacillin (Sigma, Germany), the isolates were incubated for 24 hours and the results were evaluated. In total, 6 (6.82%) isolates were

methicillin susceptible and 82 (93.18%) of the isolates were resistant to methicillin (Table2).

mecA amplification
PCR for mecA gene was performed (Figure1). Results revealed that 66 (75%) out of 88 *S. aureus* isolates harbored *mecA* gene.

Discussion

S. aureus, as the most frequently isolated bacteria in both hospital and environment, has reported as a causative agent of clinical infections especially in care units, nursing homes, and other chronic care facilities (1, 2). MRSA, as an emerging pathogen, due

to resistance to all of the available β-lactam antibiotics has become a barrier in treatment of staphylococcal infection (3, 4). Data of prevalence and the rate of infections occurred by *S. aureus* isolates can help in reducing the rate of mortality and morbidity related to this pathogen.

Table 2. Frequency of methicillin resistance in *S. aureus* and coagulase negative staphylococcus groups based on agar screen method.

Organisms	Frequency (%)
MRSA	82 (93.18)
MSSA	6 (6.82)
MRCONS	151 (85.79)
MSCONS	25 (14.21)

MRSA, Methicillin-resistant *S. aureus;* MSSA, Methicillin Sensitive *S.* aureus; MRCONS, Methicillin-resistant Coagulase-negative staphylococcus; MSCONS, *Methicillin Sensitive* Coagulase-negative staphylococcus.

Azimian et al. in 235 *S. aurous* isolated from clinical samples in Iran showed that 127 strains (54%) were methicillin susceptible and 108 strains (46%) showed resistance, also PCR for *mec*A gene showed that 110 strains (47%) had *mecA* gene (15). In another study, Japoni et al. that isolated 109 *S. aureus* from nosocomial infections and environmental specimens in Iran found vancomycin as the most effective antibiotic against all of the MRSA strains (16). A survey carried out in 300 U.S.A laboratories, between 1998 and 2005, about three million microbial isolates were recovered. In which, S. aureus was the most prevalent in inpatients (18.7%) and the second in outpatients (14.7%). Also, the frequency of MRSA was 59% in ICU patients, 55% in non-ICU patients and 48% in outpatients (17). These studies in concert with others showed that MRSA has become an important problem in hospitals worldwide and MRSA strains as the major nosocomial pathogens.
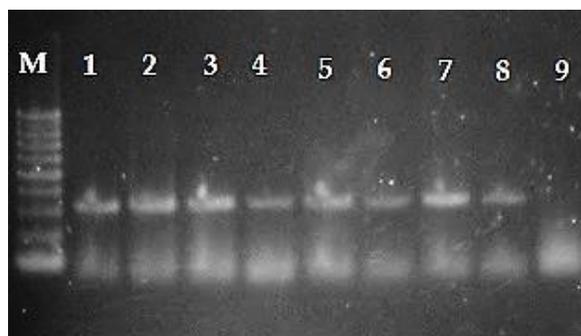
Figure 1. Product of PCR : Lane 1, 100 bp DNA marker; Lane 2 to 8 *mecA* gene 321 *bp*; Lane 9, Negative control.

Serious infections can be created by MRSA such as

bacteremia, osteomelitis, and sepsis (14, 18). *S. aureus* is the major pathogen in both community and hospital acquired infections (19). Moreover, It has been a causative of outbreaks in hospitals especially those which are resistant to beta-lactamases and erythromycin (10, 15). In the present study, 93.18 % of *S. aureus* isolates were found methicillin resistant in screen agar method and 75% *mecA* carrier by PCR. It can be concluded that screen agar is more reliable than PCR to detect methicillin resistant isolates; however, for epidemiology studies *mecA* PCR followed by sequencing of this gene is required.

Various studies reported the prevalence of MRSA in different countries. For instance, Eftekhar et al. from Iran reported MRSA in 90.9% of isolates in screen agar method, although, 75% of isolates were MRSA in PCR method (20). Also, Najar piraye et al. in 174 *S. aurous* that isolated from clinical samples in Iran showed that 47.7% of isolates were MRSA in screen agar method. moreover, PCR for *mec*A gene showed that 48.2% had *mecA* gene (21) which is in contrast with our findings. Perez et al. from USA reported 99.4% and 41.4% of isolates were MRSA in screen agar and PCR method, respectively (22). Our results are compatible with Eftekhar and Perez studies that indicate a wide distribution of *mecA* gene in the world, which has the potential of the risk of MRSA infection occurrence. In another study by Kumer et al. from India 45% of strains were resistant to methicillin (23). Nafisi et al. from Iran found 44% of the strains resistant to methicillin (24). Askaryan et al. showed that in 186 *S. aureus* isolated from clinical samples in Iran 17.2 % were MRSA in screen agar method, and PCR showed that 17.2% had *mecA* gene (25). The rise of methicillin resistance may be due to antibiotic-resistant genes spread in the community, hospitals and healthy staff (26). But the common thread among all of these studies, illustrate the variety of *mec*A gene in the risk of occurrence of resistant staph infections, Thus, health plans and control infection measures should be taken to prevent this problem.

Authors' contributions
RR and NH designed the project. ShB, KhM and NH did the experiments. NH wrote paper manuscript and SH revised the paper.

Kurdistan University of Medical Sciences.

Conflict of interest
The authors declare that there are no conflicts of interest.

References
1. Lowy FD. Staphylococcus aureus infections. New Eng J Med. 1998; 339(8):520-32. PMID: 9709046

2. Kim HB, Jang H-C, Nam HJ, Lee YS, Kim BS, Park WB, et al. In vitro activities of 28 antimicrobial agents against Staphylococcus aureus isolates from tertiary-care hospitals in Korea: a nationwide survey. Anti Agent Chem. 2004; 48(4):1124-7. PMID: 15047511

3. Muto CA, Jernigan JA, Ostrowsky BE, Richet HM, Jarvis WR, Boyce JM, et al. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of Staphylococcus aureus and enterococcus. Infect Cont Hosp Epidemiol. 2003; 24(5):362-86. PMID: 12785411

4. Hidron AI, Kourbatova EV, Halvosa JS, Terrell BJ, McDougal LK, Tenover FC, et al. Risk factors for colonization with methicillin-resistant Staphylococcus aureus (MRSA) in patients admitted to an urban hospital: emergence of community-associated MRSA nasal carriage. Clin Infect Dis. 2005; 41(2):159-66. PMID: 15983910

5. Salgado CD, Calfee DP, Farr BM. Interventions to prevent methicillin-resistant Staphylococcus aureus transmission in health care facilities: What works? Clin Mic New. 2003; 25(18):137-44. PMID: 24027796

6. Taherikalani M, Mohammadzad MR, Soroush S, Maleki MH, Azizi-Jalilian F, Pakzad I, et. al. Determining the prevalence of SCCmec polymorphism, virulence and antibiotic resistance genes among methicillin-resistant Staphylococcus aureus (MRSA) isolates collected from selected hospitals in west of Iran. J Chemother. 2015;1973947815Y0000000018. PMID: 25976554

7. Blok HE, Troelstra A, Kamp-Hopmans TE, Gigengack-Baars AC, Vandenbroucke-Grauls CM, Weersink AJ, et al. Role of healthcare workers in outbreaks of methicillin-resistant Staphylococcus aureus: a 10-year evaluation from a Dutch university hospital. Infect Control Hosp Epidemiol. 2003; 24(9):679-85. PMID: 14510251

8. Tammelin A, Klötz F, Hambræus A, Ståhle E, Ransjö U. Nasal and hand carriage of Staphylococcus aureus in staff at a Department for Thoracic and Cardiovascular Surgery: endogenous or exogenous source? Infect Control Hosp Epidemiol. 2003; 24(9):686-9. PMID: 14510252

9. Boyce JM, Pittet D. Guideline for Hand Hygiene in Health-Care Settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC /IDSA Hand Hygiene Task Force. Infect Control Hosp Epidemiol. 2002; 23(12):S3-40. PMID: 12515399

10. Stapleton PD1, Taylor PW. Methicillin resistance in Staphylococcus aureus: mechanisms and modulation. Sci Prog. 2002; 85(1):57-72. PMID: 11969119

11. Huletsky A, Lebel P, Picard FJ, et al. Identification of methicillin resistant Staphylococcus aureus carriage in less than 1 hour during a hospital surveillance program. Clin Infect Dis. 2005; 40(7):976-981. PMID: 15824989

12. Askari E, Soleymani F, Arianpoor A, Tabatabai SM, Amini A, NaderiNasab M. Epidemiology of mecA-methicillin resistant Staphylococcus aureus (MRSA) in Iran: A systematic review and meta-analysis. Iran J Bas Med Sci. 2012; 15(5):1010-9. PMID: 23493646

13. Wayne, P. A. "Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Second informational Supplement." Clinical and Laboratory Standards Institute. M100-S22. USA 2012.

14. Coban AY, Bozdogan B, Cihan CC, Cetinkaya E, Bilgin K, Darka O, et al. Two new colorimetric methods for early detection of vancomycin and oxacillin resistance in Staphylococcus aureus. J Clin Mic. 2006; 44(2):580-2. PMID: 16455915

15. 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 Mic. 2012; 43(2):779-85. PMID: 24031890

16. Japoni A, Ziyaeyan M, Jmalidoust M, Farshad S, Alborzi A, Rafaatpour N, et al. Antibacterial susceptibility patterns and cross-resistance of methicillin resistant and sensitive staphyloccus aureus isolated from the hospitalized patients in shiraz, Iran. Braz J Microbiol. 2010; 41(3):567-73. PMID: 24031530

17. Styers D, Sheehan DJ, Hogan P, Sahm DF. Laboratory-based surveillance of current antimicrobial resistance patterns and trends among Staphylococcus aureus: 2005 status in the United States. Ann Clin Microbiol Antimicrob. 2006; 5:2. PMID: 16469106

18. Lina G, Gillet Y, Vandenesch F, Jones ME, Floret D, Etienne J. Toxin involvement in staphylococcal scalded skin syndrome. Clin Infect Dis. 1997; 25(6):1369-73. PMID: 9431380

19. Cookson B. Five decades of MRSA: controversy and uncertainty continues. Lancet. 2011; 378(9799):1291-2. PMID: 21991590

20. Eftekhar F, Raei F. Correlation of Minimum Inhibitory Concentration Breakpoints and Methicillin Resistance Gene Carriage in Clinical Isolates of Staphylococcus epidermidis. Iran J Med Sci. 2011; 36(3):213-6. PMID: 23359643

21. Najar Pirayeh S, Azimian A, Mostafaee M, Siadat SD. Identification of methicillin-resistant Staphylococcus aureus by disk diffusion method, determination of MIC and PCR for mecA gene. Modares J Med Sci: Pathobiol. 2009; 12(3):61-9.

22. Perez LRR, Dias C, d'Azevedo PA. Agar dilution and agar screen with cefoxitin and oxacillin: what is known and what is unknown in detection of meticillin-resistant Staphylococcus aureus. J Med Mic. 2008; 57(8):954-6. PMID: 18628494

23. Kumar P, Shukla I, Varshney S. Nasal screening of healthcare workers for nasal carriage of coagulase positive MRSA and prevalence of nasal colonization with Staphylococcus aureus. Biol Med. 2011; 3(2):182-6.

24. Nafisi M KH, Zamanzad B, Karimi A, Farokhi E, Validi M. Comparison of agar screen and duplex-PCR in determination of methicillin resistant Staphylococcus aureus (MRSA) strains isolated from nose of personnel in Hajar hospital of Shahre-kord, 2007. Arak Uni Med Sci J. 2008; 11(2): 94 -101.

25. Askariyan M, Zeinalzadeh AH, Japoni A, Alborzi A. Risk Factors of Nasal Carriage of Methicillin-Resistant Staphylococcus Aureus and its Antibiotic Susceptibility Pattern in Namazi Hospital

Healthcare Workers in Shiraz, Iran. J Kerman Uni Med Sci. 2009; 16(1):65-77.

26. Lederer SR1, Riedelsdorf G, Schiffl H. Nasal carriage of meticillin resistant Staphylococcus aureus: the prevalence, patients at risk and the effect of elimination on outcomes among outclinic haemodialysis patients. Eur J Med Res. 2007; 12(7):284-8. PMID: 17933699