Iranian Journal of Microbiology
Volume 6 Number 5 (October 2014) 341-344

ORIGINAL ARTICLE

Comparison of four diagnostic methods for detection of methicillin resistant *Staphylococcus aureus*

Mohammad Reza Pourmand[1*], Sepideh Hassanzadeh[2], Rahil Mashhadi[3], Emran Askari[4]

[1]Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
[2]Biotechnology Research Center, Tehran University of Medical Sciences, Tehran, Iran.
[3]Urology Research Center, Tehran University of Medical Sciences, Tehran, Iran.
[4]Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Received: May 2014, **Accepted:** September 2014

ABSTRACT

Background and Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-known pathogen with a worldwide distribution. Given the increasing rate of MRSA infections, implementing of reliable, accurate and rapid testing for diagnosis of MRSA is necessary. The aim of this study was to compare four diagnostic methods for detection of MRSA isolates.

Materials and Methods: From December 2012 to April 2014, 120 *S. aureus* isolates were collected from three hospitals affiliated with Tehran University of Medical Sciences. MRSA isolates were detected by four different methods including cefoxitin disc diffusion test, oxacillin disc diffusion test, minimum inhibitory concentration (MIC) of oxacillin as determined by MIC test strip, and *mecA* detection by PCR.

Results: Out of 120 *S. aureus* isolates, cefoxitin disc diffusion test, oxacillin disc diffusion test and MIC test strip identified 60 (50%), 48 (40%), 55 (45.83%) isolates as MRSA, respectively. The sensitivity and specificity for oxacillin disc diffusion, cefoxitin disc diffusion and MIC of oxacillin were 80% and 100%, 100% and 100%, and 91.6% and 100%, respectively.

Conclusion: Cefoxitin disc diffusion test is reliable substitute for detection of MRSA in clinical laboratory where MIC detection and molecular methods are not accessible.

Keywords: *Staphylococcus aureus*, Methicillin resistance, Microbial sensitivity tests

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered as a major pathogen both in hospita land community settings (1, 2). MRSA is endemic in many hospitals causing excess mortality and economic burden compared to methicillin-susceptible isolates (3). MRSA strains are resistant to nearly all of the beta-lactam antibiotics by producing an alternative penicillin-binding protein known as PBP_{2a}. This protein is encoded by the *mecA* gene and has a low affinity to many beta-lactam antibiotics (4). MRSA strains are not only resistant to beta-lactams and cephalosporins, but also often show resistance to a wide range of antibiotics (5).

Due to high prevalence of MRSA infections among hospitalized patients, rapid and accurate identification of MRSA is needed to initiate appropriate antimicrobial therapy and prevent the spread of MRSA infections. Usually, molecular methods such as detection of the *mecA* gene are preferred for this task because of high sensitivity and specificity. The results of molecular methods are also usually available faster than that of phenotypic

*Corresponding Author: Mohammad Reza Pourmand, PhD. Department of Pathobiology, School of Public Health, and Biotechnology Research Center, Tehran University of Medical Sciences, Tehran, IR Iran.
Tel: +98-912-5168520
Email: mpourmand@tums.ac.ir

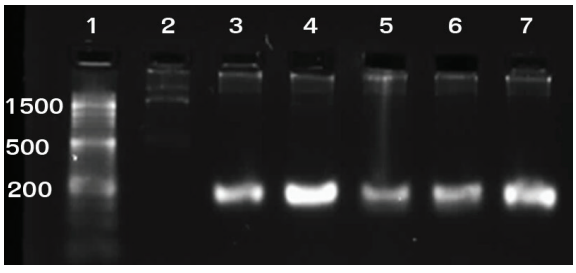

Fig. 1. 1%Agarose gel electrophoresis of the PCR-amplified *mecA* methicillin resistance gene. Lanes: 1: 50-bp ladder; 2: Negative control (*S. aureus* ATCC 8325-4); 3: Positive control (*S. aureus* strain COL); 4-7: *S. aureus* isolates showing 162 bp*mecA*amplicon.

methods (7).

Different phenotypic methods are available in clinical laboratories such as oxacillin and cefoxitin disc diffusion test, oxacillin agar screening test, and determination of minimum inhibitory concentration (MIC) for oxacillin and cefoxitin. However, the expression of resistance is affected in variant conditions such as difference in temperature, medium, inoculum size and NaCl concentration in the medium (8). In this study, we aimed to compare PCR of the *mecA* gene with three phenotypic methods including cefoxitin disc diffusion test, oxacillin disc diffusion test and MIC of oxacillin for detection of MRSA.

MATERIALS AND METHODS

Isolation and identification of *S. aureus.* From December 2012 to April 2014, we collected 120 *S. aureus* isolates from three hospitals affiliated with Tehran University of Medical Sciences. Samples were identified and confirmed by conventional biochemical tests (9). Control strains for methicillin-resistant and –susceptible *S. aureus* were COL and ATCC 8325-4, respectively.

Phenotype identification of MRSA. We performed phenotypic methods for detection of MRSA strains according to clinical and laboratory standards Institute (CLSI) guideline as follows:

Cefoxitin and oxacillindisc diffusion tests were done using cefoxitin (30µg) and oxacillin (1µg) discs purchased from MAST Company (UK). Müeller-Hinton agar (MHA) plates containing 2% NaCl were inoculated with broth suspension equivalent to 0.5McFarland. Discs were applied on to the plates and incubated at 35°C for 24h. The zone inhibitions were measured and interpreted according to CLSI guideline (10).

For oxacillin MIC strip test, isolates were cultured on MHA containing 2% NaCl and oxacillin strip was placed on the medium and incubated for 24h at 35°C. After incubation, inhibitory concentration studied and interpreted according to the CLSI criteria (10).

Genotype identification of MRSA by PCR. We extracted genomic DNA using Viogene kit (UK) based on manufacturer's instructions. We used the extracted genomic DNA as template for PCR of the *mecA* gene. Forward (5'-TCC AGA TTA CAA CTT CAC CAG G-3') and reverse (5'-CCA CTT CAT ATC TTG TAA CG-3') primers were used to amplify the 162 bp *mecA* gene of MRSA as described previously (Fig. 1) (11). Each PCR mixture was composed of 2 µl DNA template, 0.5 µl of each primer (10 µM), 12.5 µl master mix (SinaClon, Iran), and 9.5 µl sterile distilled water. PCR program began with an initial denaturation step at 97°C for 6 min followed by 30 cycles of 92°C for 30 seconds, 55°C for 30 seconds, and 72°C for 45 second, and ended with a final extension step at 72°C for 10 min. The *mecA*-positive strain COL and the *mecA*-negative ATCC8325-4 were included as positive and negative controls, respectively. The amplified PCR products were electrophoresed in 1% agarose gel at 120 V for 1h, stained with KBC (0.5 µg/ml) (Kawsar,Iran), and photographed under UV light.

Table1. Comparison of various laboratory methods for detection of methicillin-resistant *Staphylococcus aureus* isolates in this study

Method	Specificity	Sensitivity	PPV	NPV
mecA gene	100%	100%	100%	100%
Cefoxitin disc	100%	100%	100%	100%
Oxacillin disc	100%	80%	100%	83%
Oxacillin strip	100%	91.6%	100%	92%

PPV= Positive Predictive Value; NPV= Negative Predictive Value

Table2. Discordant results of minimum inhibitory concentration of oxacillin and PCR of *mecA*

Isolate number	MIC (µg/dL) of Oxacillin	*mecA*
TTH-3-25	0.5	+
TTH-1-17	0.25	+
TTH-3-11	0.25	+
TTH-3-8	0.25	+
TTH-3-3	0.125	+

TTH: Tehran Teaching Hospital; MIC: Minimum Inhibitory Concentration

RESULTS

All *S. aureus* isolates were subjected to MRSA detection by four phenotypic methods. The PCR assay targeting *mecA* gene and cefoxitin disc diffusion identified 60 (50%) isolates as MRSA. Forty-eight MRSA (40%) were identified by oxacillin disc diffusion method, including the intermediate zones (Table 1). MIC test strip found 55 (45.83%) MRSA phenotype with MIC between 8-16 µg/ml. The remaining five MRSA strains were only identified by cefoxitin disc diffusion and had an MIC of oxacillin between 0.125-0.5 µg/ml (Table 2).

DISCUSSION

In recent years, detection of *mecA* by PCR is considered as the gold standard for identification of MRSA. In this study, we evaluated other methods as alternatives to PCR (12). Cefoxitin disc diffusion test was perceived to be the most sensitive method for detection of *mecA*-mediated resistance. CLSI has also recently substituted the oxacillin disc with cefoxitin disc for detection of MRSA (13). Numerous studies including the current one have informed that the results of the cefoxitin disc diffusion test correlates better with the presence of *mecA* compared with those of the oxacillin disc diffusion test (14-16).

Our results about cefoxitin disc diffusion method are consistent with previous report (15). However, Broekeme *et al*., reported the sensitivity and specificity of this method 97.3% and 100%, respectively among 1,611 *S. aureus* isolates (16).

In current study, MIC strip test showed the sensitivity and specificity about 91.6% and 100%, respectively. In the study of Rahbar*et al.,* sensitivity and specificity were both 100% (17). Five isolates in our study showed discordant results for MIC of oxacillin and PCR. This can be probably explained by the fact that not all *S. aureus* isolates express their *mecA* gene (18). In our study, the sensitivity and specificity of oxacillin disc diffusion test were 80% and 100%, respectively. In the study of Farahani *et al*., the sensitivity and specificity of the oxacillin disc diffusion method was 100 and 73.6%, respectively (19). In previous study that performed by Pillai*et al.,* the sensitivity and specificity were reported 93.5% and 83.5%, respectively (20).

In conclusion, the present study showed that cefoxitin disc diffusion has both high sensitivity and specificity as compared with *mecA* PCR. Therefore,itcan be a good alternative to molecular methods due to its low cost for clinical laboratories.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to research council of Tehran University of Medical Sciences, Iran, for financial support (Grant number 26590).

REFERENCES

1. Mediavilla JR, Chen L, Mathema B, Kreiswirth BN. Global epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). *Curr Oppin Microbial* 2012; 15:588-595.
2. Stafani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AW, Westh H, *et al.*Meticillin-Resistant *Staphylococcus aureus* (MRSA): Global epidemiology and harmonisation of typing methods. *Int J Antimicrobial Agent*2012; 39:273-283.
3. Cosqrove SE, Qi Y, Kays KS, Herbarth S, Karchmar AW, Carmeli Y. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. *Infect ontrol Hosp Epidemiol* 2005; 56:166-174.
4. Deurenberg R, Vink C, Kalanic S, Fariedrich AW, Bruggeman CA, Stobberingh EE. The molecular evolution of methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 2007; 13:222-235.

5. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus*as a public-health threat. *Lancet* 2006;368:874-85.
6. Kaier K, Hagist C, Frank U, Conrad A, Meyer E. Two time-series analyses of the impact of antibiotic consumption and alcohol-based hand disinfection on the incidences of nosocomial methicillin-resistant *Staphylococcus aureus* infection and *Clostridium difficile* infection. *Infect Control Hosp Epidemiol* 2009; 30:346-53.
7. Barski P, Piechowicz L, Galinski J, Kur J. Rapid assay for detection of methicillin-resistant *Staphylococcus aureus* using multiplex PCR. *Mol Cell Probes* 1996; 10:471-475.
8. Afrough P, Pourmand MR, Zeinalinia N, Yousefi M, Abdossamadi Z, Yazdchi S. Molecular typing of clinical and nasal carriage isolates of *staphylococcus aureus* by *spa* gene patterns. *J Mazand Univ Med* 2012; 22:28-34.
9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 23rd informational supplement M100-S23, CLSI, Wayne, PA, 2013.
10. Pourmand MR, Abdossamadi Z, Salari M.H, Hosseini M. Slime layer formation and the prevalence of mecA and aap genes in *Staphylococcus epidermidis* isolates. *J Infect Dev Ctries*2011;5:34-40.
11. Vogelaers D. MRSA: total war or tolerance?.*Nephrol Dial Transplant* 2006;21:837-8.
12. Bhutia KO, Singh TS, Biswas S, Adhikari L. Evaluation of phenotypic with genotypic methods for species identification and detection of methicillin resistant in *Staphylococcus aureus*. *Int J Appl Basic Med Res* 2012; 2:84-91.
13. Adaleti R, Nakipoglu Y, Karahan ZC, Tasdemir C, Kaya F. Comparison of polymerase chain reaction and conventional methods in detecting methicillin-resistant *Staphylococcus aureus. J Infect Dev Ctries* 2008; 2:46-50.
14. Cauwelier B, Gordts B, Descheemaecker P, Landuyt H. Evaluation of a disk diffusion method with cefoxitin (30 µg) for detection of methicillin-resistant *Staphylococcus aureus*. *Eur J Clin Microbiol Infect Dis* 2004; 23:389-92.
15. Ananl K, Agrawal P, Kumar S, Kapila K. Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for mecA gene for detection of MRSA. *Indian J Med Microbiol* 2009; 27:27-29.
16. Broekema N.M, Van TT, Monson TA, Marshal SA, Warshauer DM. Comparison of cefoxitin and oxacillin disk diffusion methods for detection of mecA-mediated resistance in *Staphylococcus aureus* in a large-scale study. *J Clin Microbiol* 2009;47:217-219.
17. Rahbar M, Yaghoobi M, Fattahi A. Comparison of different laboratory methods for detection of methicillin resistant *Staphylococcus aureus*. *Pak J Med Sci* 2006;22:442-445.
18. Ryffel C, Kayser FH, Berger Bachi B. Correlation between regulation of *mecA* transcription and expression of methicillin resistance in staphylococci. *Antimicrobial Agent Chemother* 1992; 36:25-31.
19. Farahani A, Mohajeri P,G holamine B, Rezaei M, Abbasi H. Comparison of different phenotypic and genotypic methods for the detection of methicillin-resistant *Staphylococcus aureus*. *N Am J Med Sci*2013; 5:637-640.
20. Pillai MM, Latha R, Sarkar G. Detection of methicillin resistance in *Staphylococcus aureus* by polymerase chain reaction and conventional methods: A comparative study. *J Lab Physicians*2012; 4:83-88.