Comparison of four diagnostic methods for detection of methicillin resistant \textit{Staphylococcus aureus}

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\textbf{ABSTRACT}

\textbf{Background and Objectives:} Methicillin-resistant \textit{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.

\textbf{Materials and Methods:} From December 2012 to April 2014, 120 \textit{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 \textit{mecA} detection by PCR.

\textbf{Results:} Out of 120 \textit{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.

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

\textbf{Keywords:} \textit{Staphylococcus aureus}, Methicillin resistance, Microbial sensitivity tests
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.

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.

![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.](http://ijm.tums.ac.ir)

| 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

Table1. Comparison of various laboratory methods for detection of methicillin-resistant *Staphylococcus aureus* isolates in this study
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 Rahbataet *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 Pillaiet *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, it can be a good alternative to molecular methods due to its low cost for clinical laboratories.

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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. Cosgrove 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 control 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 meticillin-resistant Staphylococcus aureus infection and Clostridium difficile infection. Infect Control Hosp Epidemiol 2009; 30:346-53.

7. Barski P, Piechowicz L, Galinski J, Kuj J. Rapid assay for detection of meticillin-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 meticillin 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 meticillin-resistant Staphylococcus aureus. J Infect Dev Ctries 2008; 2:46-50.

14. Cauwelier B, Gordts B, Descheemaeker P, Landuyt H. Evaluation of a disk diffusion method with cefoxitin (30 μg) for detection of meticillin-resistant Staphylococcus aureus. Eur J Clin Microbiol Infect Dis 2004; 23:389-92.

15. Anani 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 meticillin 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 meticillin resistance in staphylococi. Antimicrobial Agent Chemother 1992; 36:25-31.

19. Farahani A, Mohajeri P, Hosome B, Rezaei M, Abbasi H. Comparison of different phenotypic and genotypic methods for the detection of meticillin-resistant Staphylococcus aureus. N Am J Med Sci 2013; 5:637-640.

20. Pillai MM, Latha R, Sarkar G. Detection of meticillin resistance in Staphylococcus aureus by polymerase chain reaction and conventional methods: A comparative study. J Lab Physicians 2012; 4:83-88.