Original Article

Comparison of polymerase chain reaction and conventional methods in detecting methicillin-resistant *Staphylococcus aureus*

Riza Adaleti,[1] Yasar Nakipoglu,[2] Zeynep Ceren Karahan,[3] Cihan Tasdemir,[1] Fatma Kaya.[1]

[1]*Clinical Microbiology Laboratory, Haydarpasa Numune Education and Research Hospital, Istanbul, Turkey.*
[2]*Department of Microbiology and Clinical Microbiology, Faculty of Medicine, University of Istanbul, Istanbul, Turkey.*
[3]*Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Ankara University, Ankara, Turkey.*

Abstract

Background: Accurate and rapid detection of methicillin-resistant *Staphylococcus aureus* is very important in a clinical laboratory setting to avoid treatment failure. Conventional methods were compared against the gold standard polymerase chain reaction (PCR) technique to determine the best combination of the routine procedures.

Methodology: Methicillin resistance was investigated in 416 clinical *Staphylococcus aureus* isolates by PCR, oxacillin agar screening (OAS), oxacillin disk diffusion (ODD) and cefoxitin disk diffusion (CDD) methods.

Results: Two hundred and ten (51%) out of 416 *S. aureus* strains were found to be mecA-positive by PCR. Sensitivity and specificity of the ODD, CDD and OAS methods were detected as follows: 100% and 89%, 99.50% and 100%, and 99.50% and 100%, respectively.

Conclusion: Combining the ODD and CDD methods could be a good choice for detecting methicillin resistance in *S. aureus* strains where mecA PCR cannot be performed.

Key Words: Methicillin-resistant *Staphylococcus aureus* (MRSA), polymerase chain reaction (PCR), oxacillin disk diffusion, cefoxitin disk diffusion, oxacillin agar screening.

J Infect Developing Countries 2008; 2(1):46-50.

Received 15 November 2007 - Accepted 23 December 2007.

Introduction

Since first reported in 1961, methicillin-resistant *Staphylococcus aureus* (MRSA) have become a major nosocomial pathogen worldwide [1]. Rapid and accurate identification of MRSA is required for therapeutic and epidemiological reasons: to immediately start the appropriate antimicrobial therapy and to avoid the spread of these strains [1,2].

Methicillin resistance in *S. aureus* is associated with the production of an altered penicillin-binding protein, PBP2a, encoded by the mec gene complex [3,4]. Genotypic tests involving detection of mecA gene by polymerase chain reaction (PCR) are the preferred methods [5,6], but they are not practical for routine use in many clinical laboratories.

Accepted phenotypic methods used for detecting MRSA strains include oxacillin disk diffusion (ODD), oxacillin agar screening (OAS) methods, and determination of minimal inhibition concentration (MIC) of oxacillin by broth dilution or E-test method.

Recently, oxacillin was replaced by cefoxitin for detection of methicillin resistance in *S. aureus* and all studies indicate that these tests are more reliable than those with oxacillin [7-10]. In phenotypic tests, *in vitro* conditions such as the test agent, incubation temperature, medium inoculated, inoculum size and NaCl concentration of the medium are known to affect the expression of resistance [1].

There are also commercial methods based on detection of PBP2a alone or in combination with clumping factor with high sensitivity and specificity. Disc diffusion methods remain the most widely used methods in routine clinical laboratories. Laboratory methods used to detect MRSA should have high sensitivity and specificity.

Therefore, the present study aimed to compare the performance of ODD, OAS, and cefoxitin disk diffusion (CDD) methods for the detection of

MRSA when compared to mecA-PCR which is accepted as the gold standard.

Materials and Methods

Study Strains

Non-repetitive 416 *S. aureus* strains isolated from patients hospitalized in different clinics of the Haydarpasa Numune Teaching and Research Hospital, Istanbul, Turkey, in 2004 and 2005 were included in the study. The isolates were identified as *S. aureus* by conventional methods (Gram stain morphology, catalase and DNase production), and were confirmed by the production of clumping factor and polyclonal IgG antibodies against protein A and capsular polysaccharide (Staphytect test; Oxoid Ltd, Basingstoke,Hampshire, England). *S. aureus* ATCC 25923 and *S. aureus* ATCC 700699 (Mu50) strains were used as methicillin susceptible and resistant standard controls, respectively.

DNA extraction

DNA was extracted by the method previously described by Ida *et al.* [11]. Briefly, colonies obtained from overnight *S. aureus* cultures from sheep blood agar were harvested and suspended in 100 µl of lysis solution (20 mM Tris HCl, 140 mM NaCl, 5 mM EDTA [pH 8.0]). Three units of lysostaphine were added and the suspension was incubated at 37ºC for 3 hours. 200 µl of distilled water was added and incubated at 95ºC for five minutes. Phenol-chloroform extraction and ethanol precipitation steps were then performed for DNA extraction.

PCR was performed to amplify a 310 bp portion of the mecA gene by using the primers mecA1 (5'-GTA GAA ATG ACT GAA CGT CCG ATA A) and mecA2 (5'-CCA ATT CCA CAT TGT TTC GGT CTA A). The reaction mix contained 200 µM dNTP, 2.5 mM MgCl2, 10X reaction buffer (160 mM (NH4)2SO4, 670 mM Tris HCl pH8.8, %0.1 Tween 80), 2U Taq DNA polymerase (Bioron, Germany), 30 ng DNA and 50 pmol of each primer in a total volume of 50µl [12]. PCR conditions were as follows: Initial denaturation at 94^{o}C for five minutes, followed by 30 cycles of denaturation at 94^{o}C for 30 seconds, annealing at 55^{o}C for 30 seconds, and extension for 72^{o}C for 30 seconds. A final extension was applied at 72^{o}C for 10 minutes and the products were stored at 10^{o}C.

PCR products were separated by 2% agarose gel electrophoresis and visualized under UV after staining with ethidium bromide.

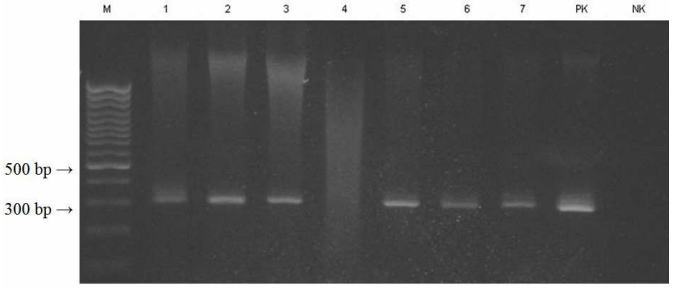
Susceptibility testing

Clinical and Laboratory Standards Institute (CLSI) criteria [13] were used for determination of susceptibility of the isolates to methicillin. Overnight *S. aureus* cultures were adjusted to turbidity of 0.5 McFarland standard. The bacterial suspension was spread on Mueller-Hinton agar (MHA) plates containing 2% NaCl for ODD and without NaCl for CDD. One µg oxacillin (Oxoid Ltd, Basingstoke,Hampshire, England)) and 30 µg cefoxitin (Oxoid Ltd, Basingstoke, Hampshire, England) disks were placed on inoculated plates. In OAS method, the bacterial suspension was streaked on MHA plates containing 4% NaCl and 6 mg/L oxacillin. All plates were incubated at 35ºC for 24 hours before reading the results. Isolates were considered as MRSA when the inhibition zone diameter was ≤ 10 mm for oxacillin, ≤19 mm for cefoxitin, and any growth on MHA plates in case of OAS.

Results

Of the 416 *S. aureus* strains, 210 (51%) were found to be methicillin-resistant by mecA PCR, which is now accepted to be the gold standard in detecting methicillin resistance (Figure 1).

Figure 1. mecA PCR results. M: Molecular size marker (GeneRuler 50bp DNA ladder plus, Fermentas, Lithuania). 1-3, 5-7: mecA-positive strains, 4: mecA-negative strain, PK: Positive control, NK: Negative control. 50-100-150-200-250-300-400-500-600-700-800-900-1031).



The three conventional methods (ODD, CDD, and OAS) reliably detected methicillin resistance in 389 (93.5%) of these 416 strains: 209 strains were found to be MRSA and 180 strains MSSA by these

Table 1. Methicillin susceptibilities of 416 *S. aureus* strains determined by PCR and three conventional methods.

mecA-PCR	Oxacillin disk diffusion (ODD)			Cefoxitin disk diffusion (CDD)			Oxacillin agar screening (OAS)	
	GIZD * (mm)	Methicillin Susceptibility	No of strains	GIZD (mm)	Methicillin Susceptibility	No of strains	Methicillin Susceptibility	No of strains
Positive (n:210)	6	R	210	6	R	204	R	204
				11	R	2	R	2
				13	R	1	R	1
				14	R	1	R	1
				16	R	1	R	1
				26	S	1	S	1
Negative (n:206)	11	S	4	28-32	S	4	S	4
	6	R	16	28-34	S	3	S	3
	6-8	R	10	27-36	S	13	S	13
	13	S	11	25-34	S	21	S	21
	15	S	16	21-34	S	16	S	16
	16	S	17	28-34	S	17	S	17
	17	S	24	27-38	S	24	S	24
	18	S	15	25-40	S	15	S	15
	19	S	13	25-35	S	13	S	13
	20	S	11	29-36	S	11	S	11
	21	S	12	26-35	S	12	S	12
	22	S	7	27-32	S	7	S	7
	23	S	4	27-35	S	4	S	4
	24	S	1	29	S	1	S	1
	25	S	8	30-32	S	8	S	8
	26	S	8	28-35	S	8	S	8
	27	S	7	26-35	S	7	S	7
	28	S	5	29-33	S	5	S	5
	29	S	3	28-31	S	3	S	3
	30	S	4	31-34	S	4	S	4
	31	S	1	31	S	1	S	1
	34	S	2	34-40	S	2	S	2
	35	S	1	40	S	1	S	1
	16	S	6	27-35	S	6	S	6

*Abbreviations: GIZD, Growth inhibition zone diameters, R, Resistant, S, Susceptible.

methods. Discrepancies between molecular methods and traditional ones were found in the results of 27 strains (one MRSA and 26 MSSA). One mecA-positive strain was found to be methicillin resistant by ODD method, but both CDD and OAS methods failed to detect methicillin resistance in this strain in triplicate studies. The inhibition zone diameter of cefoxitin for this strain was 26 mm. Twenty-six mecA-negative strains were determined as MRSA by ODD method. Growth inhibition zone diameters in ODD were 6 mm for 210 mecA-positive *S. aureus* strains and 6-8 mm for 26 mecA-negative *S. aureus* strains. In CDD method, the zone diameters varied between 6 to 16 mm for 209 mecA-positive *S. aureus* strains. One mecA-positive *S. aureus* strain was found to be sensitive (zone diameter 26 mm) to methicillin by this method (Table 1).

Sensitivity of the ODD was found to be 100%, followed with 99.50% for CDD and OAS methods. The specificities of ODD, CDD and OAS methods were found to be 89%, 100%, and 100% respectively. Sensitivities of the three methods

were 100%, 99.5%, and 99.5% for ODD, CDD and OAS methods, respectively (Table 2).

Table 2 Sensitivity and specificities of the conventional methods compared to mecA-PCR.

Strains	CDD	ODD	OAS
MRSA (n:210)	209	210	209
MSSA (n:206)	206	180	206
False negative	1	0	1
False positive	0	26	0
Sensitivity (%)	99.52	100	99.52
Specificity (%)	100	88.80	100

*Abbreviations: CDD, Cefoxitin disk diffusion, ODD, Oxacillin disk diffusion, OAS, Oxacillin agar screening.

Discussion

Methicillin resistance in *S. aureus* strains in our department is routinely demonstrated by ODD method for clinical *S. aureus* isolates and OAS method is used in case of screening for nasal carriers of MRSA.

Recently, CLSI outperformed oxacillin with cefoxitin [13] in obtaining more appropriate results. Accurate and early determination of methicillin resistance is of key importance in the prognosis of infections caused by *S. aureus*. The sensitivity and specificity values of the phenotypic methods used for determination of MRSA are known to vary depending on the media used for inoculation, the NaCl concentration, the incubation temperature and time, and the experience of the personnel examining the plates. Today, detection of mecA gene by PCR is considered to be the gold standard test but not practical for a routine clinical laboratory.

In this study we evaluated the presence of the mecA gene in 416 *S. aureus* strains by PCR and compared the results with phenotypic methods used in our department. mecA gene was detected in 210 (50.5%) *S. aureus* strains by PCR. These strains were also characterized as MRSA by ODD, the method formerly advised by NCCLS. This MRSA strain was falsely identified as MSSA by both CDD and OAS methods. We applied MRSA latex agglutination test (bioMérieux, France) to this strain, which also identified it as MRSA. MIIC value of this strain was found to be 256 mg/L by microdilution method (data not shown).

Twenty-six mecA-negative strains were falsely identified as MRSA by phenotypic methods. The growth inhibition zone diameters of these strains were as narrow as 6 to 8 mm by ODD. The low specificity of ODD (89%) was found to be mainly due to the usage of oxacillin disks; when the oxacillin disk was replaced by cefoxitin, the specificity rose to 100%. On the other hand, four strains which were considered as MSSA in ODD method had inhibition zone diameters of 11 mm which should have been interpreted as intermediate resistance to methicillin. If these strains were considered as false-positives, the specificity of the ODD method would have decreased to 87%.

Simor *et al*. [14] have reported results obtained by using OAS for the detection of MRSA from clinical specimens and they have correctly detected 102 of 104 (98%) isolates. Becker *et al.* [15] have selected 130 (99%) out of 131 of MRSA strains obtained from unselected clinical specimens by OAS. Boubaker *et al*. [1] compared two ODD (1 and 5 µg oxacillin disks) methods with CDD method for detection of MRSA, by using mecA PCR as the reference method. They found the CDD method (specificity 100%, sensitivity 96.5%) superior to the ODD methods (specificity 99%, sensitivity 90.4%). They concluded that combining the results of tests with both cefoxitin and oxacillin would give a sensitivity of 100% and a specificity of 99.1%.

Krishnan *et al*. [16] reported that the specificity of routine laboratory tests for MRSA detection was variable and it was difficult to perform PCR in routine diagnostic laboratories. They suggested the use of the Mastalex™ kit for the detection of PBP2a as an alternative method for the detection of MRSA. Smyth and Kahlmeter [17] proposed that agar containing cefoxitin supported the growth of 96.6% of the mecA-positive strains in the collection and inhibited the growth of 100% of the mecA-negative strains. They concluded that selective media containing cefoxitin was superior to those containing oxacillin for the detection of MRSA.

Velasco *et al.* [18] studied 102 clinical *S. aureus* isolates, including the following: 51 MRSA isolates, by PCR, and various phenotypic methods including oxacillin (1 µg), cefazolin, cefoxitin, cefotaxime and imipenem (all 30 µg) discs; E test for oxacillin; microdilution with oxacillin; agar screening tests (ORSAB medium) with 2 mg/L or 6 mg/L of oxacillin; and PBP2 agglutination with two different kits. They found that the cefoxitin disc, ORSAB medium and PBP2 detection had the

highest sensitivity (100%) while cefoxitin, cefazolin and imipenem discs, Etest for oxacillin, microdilution and agar screening method with 6 mg/L at 24 hours showed the highest specificity (100%). They concluded that the cefoxitin disc, which showed negative and positive predictive values of 100% and 98%, was the best method for detecting MRSA isolates. They also concluded that the cefoxitin disc was the best predictor of methicillin resistance in *S. aureus* strains among the techniques tested. Our study has shown that the ODD method had higher (100%) sensitivity but lower specificity (89%) when compared with the CDD and OAS methods (sensitivity 99.50% and specificity 100%).

In conclusion, combining CDD and ODD methods would improve the sensitivity of the cefoxitin and specificity of the oxacillin disk diffusion methods and this approach could be used in clinical laboratories for the detection of MRSA.

Acknowledgments

The authors thank Dr. Hiramatsu Keichii of Juntendo University, Tokyo-Japan, for kindly providing the *S. aureus* ATCC 700699 (Mu50) reference strain used in this study.

References

1. Boutiba-Ben Boubaker I *et al.* (2004) Evaluation of a cefoxitin disk diffusion test for the routine detection of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 10: 762–765.
2. Diederen B, Duijn IV, Belkum AV, Willemse P, Keulen PV, Kluytmans J (2005) Performance of CHROMagar MRSA Medium for Detection of Methicillin-Resistant *Staphylococcus aureus*, J Clin Microbiol 43: 1925–1927.
3. Cavassini M, Wenger A, Jaton K, Blanc DS, Bille J (1999) Evaluation of MRSA screen, a simple anti-PBP-2a slide latex agglutination kit, for rapid detection of methicillin resistance in *Staphylococcus aureus*. J Clin Microbiol 37:1591–1594.
4. Gerberding JL, Miick C, Liu HH, Chambers HF (1991) Comparison of conventional susceptibility tests with direct detection of penicillin binding protein 2a in borderline oxacillin-resistant strains of *Staphylococcus aureus*. Antimicrob Agents Chemother 35: 2574–2579.
5. Thean YT (2002) A comparison of PCR detection of mecA with two standard methods of oxacillin disk susceptibility testing for coagulase negative staphylococci. J Med Microbiol 51: 83–85.
6. Tokue Y, Shoji S, Satoh K, Watanabe A, Motomiya M (1992) Comparison of a polymerase chain reaction assay and a conventional microbiologic method for detection of methicillin resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 36: 6–9.
7. Mougeot C, Guillaumat-Tailliet J, Libert JM (2005) *Staphylococcus aureus*: new detection of intrinsic resistance using the diffusion method.Pathol Biol (Paris) 49: 199–204.
8. Felten A, Grandry B, Lagrange PH *et al.* (2002) Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disc diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. J Clin Microbiol 40: 2766–71.
9. Skov R, Smyth R, Clausen M *et al*. (2003) Evaluation of a cefoxitin 30 m g disc on Iso Sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 52: 204–207.
10. Cauwelier B *et al.* (2004) Evaluation of a disk diffusion method with cefoxitin (30 m g) for detection of methicillin-resistant *Staphylococcus aureus*. Eur J Clin Microbiol Infect Dis 23: 389–392.
11. Ida T, Okamoto R, Shimauchi C, Okubo T, Kuga A, Inoue M (2001) Identification of aminoglycoside-modifying enzymes by susceptibility testing: Epidemiology of methicillin-resistant *Staphylococcus aureus* in Japan. J Clin Microbiol 39: 3115-3121.
12. Araj GF, Talhouk RS, Siman CJ, Maasad MJ (1999) Discrepancies between mecA PZR and conventional tests used for detection of methicillin resistant *Staphylococcus aureus*. Int J Antimicrob Agents 11: 47-52.
13. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 15th informational supplement.CLSI/NCCLS. 2005; M100-S15. Clinical and Laboratory Standards Institute, Wayne, Pa.
14. Simor A E, Goodfellow J, Louie L, Louie M (2001) Evaluation of new medium, oxacillin resistance screening agar base, for the detection of methicillin-resistant *Staphylococcus aureus* from clinical specimens. J Clin Microbiol. 39: 3422.
15. Becker A, Forster DH, Kniehl E (2002) Oxacillin resistance screening agar base for detection of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 40: 4400-4401.
16. 16 Krishnan PU, Miles K, Shetty N (2002) Detection of methicillin and mupirocin resistance in Staphylococcus *aureus* isolates using conventional and molecular methods: a descriptive study from a burns unit with high prevalence of MRSA. J Clin Pathol. 55: 745-748.
17. Smyth RW and Kahlmeter G (2005) Mannitol Salt Agar-Cefoxitin Combination as a Screening Medium for Methicillin-Resistant *Staphylococcus aureus*. J Clin Microbiol 43: 3797–3799.
18. Velasco D, Mar Tomas MD, Cartelle M, Beceiro A, Perez A, Molina F, Moure R, Villanueva R, Bou G (2005) Evaluation of different methods for detecting methicillin (oxacillin) resistance in *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy 55: 379–382.

Corresponding Author: Yasar Nakipoglu, Department of Microbiology and Clinical Microbiology, Faculty of Medicine, University of Istanbul, Istanbul, Turkey, Tel: +90-212-2122000/31975, Fax: +90-212-5335888, e-mail: yasarnakip@yahoo.com

Conflict of interest: No conflict of interest is declared.