COMPARISON OF DIFFERENT LABORATORY METHODS FOR DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Objective: The aim of this study was to compare four different laboratory methods for detection of methicillin resistant *Staphylococcus aureus* (MRSA).

Design: The clinical specimens including urine, blood, wound and tracheal tube aspirates were processed for isolation of S .aureus.

Setting: The samples were obtained from patients admitted in Milad hospital in Tehran,Iran.

Subjects: 95 strains of S.aureus were tested with four different methods i.e. disk diffusion, oxacillin screen agar E-test and latex agglutination for methicillin resistantance.

Results: Of 95 tested S.aureus; E-test revealed that 51 isolates were MRSA. Oxacillin screen agar showed two false positive MRSA. The sensitivity and specificity of oxacillin screen agar method was 96% and 95% respectively. The MRSA –Screen latex agglutination showed 54(three false positive) MRSA. The sensitivity and specificity for this method was 94% and 93% respectively. Results of susceptibility testing by disk diffusion methods in comparison with other methods were conflicting.

Conclusions: Oxacillin screen agar test is a reliable alternative for detection of MRSA in clinical laboratory where MIC detection or molecular methods are not available.Also MRSA latex agglutination kit offers an interesting new approach to early detection. of MRSA.

KEY WORDS: Staphyloccus aureus, MRSA, Oxacillin.

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INTRODUCTION

The first case of methicillin –resistant *Staphylococcus aureus* (MRSA) was reported in 1961.[1,2] The importance of MRSA as a nosocomial pathogen is well documented .Because MRSA is often resistant to many antibacterial agents, infections caused by this organism are difficult to treat.[3,4]

Accurate detection of methicillin resistance in S.aureus by routine methods is difficult due to the presence of two subpopulation of S.aureus (i.e. one susceptible and other resistant) which may coexist within a culture. All cells in culture may carry the genetic information for resistance but a small numbers can express this kind of resistance in routine susceptibility testing performed in the laboratory. This phenomenon is termed heterogeneous resistance and occurs in staphylococci resistant to penicillinase-stable penicillin such as oxacillin.[5] The basis of most methicillin resistance is the production of an additional penicillin-binding protein,PBP2'or PBP2a, mediated by the mecA gene. mecA is an additional gene found in methicillin –resistant staphylococci and with no allelic equivalent in methicillin –susceptible staphylococci. There are several additional genes that affect the expression of methicillin

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resistance in *S.aureus,* but these are found in susceptible as well as resistant strains.[1,6,7]

There are many laboratory methods for detection of methicillin resistance in *S.aureus.* Most laboratories use disk diffusion method for routine tests. The gold standard, for antimicrobial susceptibility testing has been the MIC determined by a dilution or E-test method. In recent years MIC methods has been replaced by molecular methods that detect mecA gene. However the use of these assay is largely restricted to reference centers, and they are not currently available in most routine diagnostic laboratories.[1,7-10] The aim of this study was to determine the reliability of four different laboratory methods for detection of methicillin resistance in a collection of 96 isolates of *S.aureus.*

MATERIALS AND METHODS

Ninety six clinical isolates of *S.aureus* were obtained from Milad Hospital, Tehran, Iran. Milad Hospital is one the largest hospitals in Tehran with more than 1000 beds. The E-test method for antimicrobial susceptibility is performed as follows. A Mueller-Hinton agar medium is inoculated with a broth suspension equivalent to 0.5MacFarland standards prepared by directly inoculating organism from 24-hr-old agar medium and then using a cotton swab to apply the suspension .The E-test strips are applied onto the plate and incubated at 35^0C for 24hours. After incubation inhibitory concentration are seen as a formation of an elliptical zone of inhibition growth, whose intersection between the value printed on the strip edge and the zone of inhibition is the MIC. MICd″2µ/ml consider as susceptible and e″4µg/ml resistant.[11,12]

The mec A product (PBP2a) was detected by using the Mastalex ™MRSA kit. This is a commercial kit that detects the PBP-2a present in MRSA. The mastlax MRSA method was used according to the manufacture's instruction. Sufficient colonies of *S.aureus* were suspended in 200µl extraction reagent1'and heated in boiling water for 3 minutes. Tubes were cooled and 50µl 'extraction reagent 2'was added .Tubes were centrifuged at 1500g (300rpm) for five minute. Fifty microliter of suspension was mixed with 50µl sensitized latex suspension and rotated manually for 3 minute while looking for agglutination .The supernatant was tested simultaneously with a negative control latex suspension. The time at which agglutination was visible by eye was recorded.[8]

Oxacillin screen agar was performed by direct colony suspension method and adjusted to match 0.5 MacFarland turbidity standard. The suspension inoculated on Muller-Hinton agar containing 4% NaC and with 6µg/ml Oxacillin. Plates were incubated 24 hours at 35°C. Any growth on the plate containing Oxacillin considered as resistant to methicillin.[11,12]

Oxacillin disk susceptibility testing was performed according to National Clinical Laboratory Standards.[11,12] Briefly a bacterial suspension adjusted to 0.5 MacFarland was inoculated onto Muller-Hinton agar. A filter paper disk containing 1µg oxacillin from four different commercial companies, two local products (A, B) and two imported (C, D) was placed on the inoculated Muller Hinton agar. All plates were incubated in 35°C for 24 hours. The diameter of zone of inhibition was measured and following criteria were chosen for interpretation of results.[11,12] Susceptible (e″ 13mm) Intermediate (11-12mm) Resistant (d″13mm)

RESULTS

By using E-test method, of 96 isolates 51 isolates were MRSA and 45 isolates susceptible to methicillin. Oxacillin screen agar showed only two false positive MRSA in comparison with E-test .The sensitivity and specificity of oxacillin screen agar methods was 96% and 95% respectively in comparison with E-test. The MRSA –Screen latex agglutination showed 54 (three false positive) MRSA isolates and 42 isolate were susceptible to methicillin. The sensitivity and specificity for this method was 94% and 93% respectively. Table-I

The results of susceptibility testing by disk diffusion method was very conflicting. Of 96 tested strains of *S. aureus* by using local prod-

Table-I: Comparison of different laboratory methods for detection of MRSA

Method	*R*	*I*	*S*	*sensitivity*	*Specificity*
E-test	51	-	45	100	100
Oxacillin screen agar	53	-	43	96	95
Latex agglutination	54	-	41	94	93
Disk diffusion (D)	52	8	36	77	97

R= resistant I=intermediate S= Susceptible

uct oxacillin disks from Company A(Iran Daru, Iran), 94 (98%) isolates were MRSA. There were only two strains intermediate. By using the other local oxacillin disks from company B (Pad Tan, Iran) 60 strains were MRSA, 21 strains intermediate and 15 strains susceptible to methicillin. .Results of disk diffusion method by using imported product disks; in comparison with local product oxacillin disks were better. For example by using imported oxaacillin disk company C (Hi Media India) 52 strains were MRSA, 22 strains, intermediate and 22 strains susceptible to methicillin. Finally results of disk diffusion method by using other imported oxacillin disks company D (Becton, Dickinson and Company USA) were similar like other conventional methods such as oxacillin screen agar. By using these oxacillin disks 52 strains were MRSA, 8 strains intermediate and 36 strains susceptible to methicillin. Table-II summarizes results of disk diffusion methods by using oxacillin disks from four different companies for detection of MRSA.

DISCUSSION

The accurate diagnosis of MRSA in microbiology laboratories is vital for patients management. It is also essential for meaningful interpretation of surveillance data. Currently surveillance data for MRSA are difficult to interpret, because there is no uniform testing method for detection of MRSA, and laboratories vary in their standard operating procedure and interpretation of breakpoint values.[13] There are many different methods for detection of MRSA phenotypically. The oxacillin disk diffusion test, oxacillin screen agar test, rapid latex agglutination and E-test are four important methods. Amplification tests like those based on the polymerase chain reactions (PCR) for detecting mecA gene are gold standard methods.[14]

Table-II: Results of disk diffusion method for detection of MRSA by using oxacillin disks from different home and foreign made companies

Source of disks	*Resistant*	*Intermediate*	*Susceptible*
Local produc (A)	94	2	1
Local product(B)	60	21	15
Imported (C)	52	22	22
Imported (D)	52	8	36
E-test	51	-	45

Disk diffusion method is an easy method for performance in microbiology laboratories for detection of MRSA. As already reported, the oxacillin disk diffusion test was the least reliable test for detection of MRSA.[15] In our study also disk diffusing method raveled the least reliability especially with home made oxacillin susceptibility testing disks. Table-II

Among the four methods tested, E-test gives MIC result and affected by test conditions in a similar way to MIC and diffusion methods. The E-test has an advantages over other MIC methods in that it is easy to set up as a disk diffusion method.[10] The oxacillin screen agar test has been evaluated thoroughly, In studies performed since 1990 that used the presence of the mec A gene the gold standard, the sensitivity of agar screen test for detection of MRSA was excellent. However two reports noted that when very heteroresistant strains were tested, the sensitivity decreased. Conversely, the specificity among susceptible strains tested was very good and strains with borderline MICs was included.[14] Our study revealed that oxacillin screen agar is an alternative method with high sensitivity and specificity for detection MRSA. There have been many recent evaluations of the MRSA– screen latex agglutination test. Most studies reported the sensitivity of this method for detection of MRSA as more than 97%.[14,16, 17]

As shown in this as well as other studies, disk diffusion method is not reliable enough for detection of MRSA especially in our country which quality of home made oxacillin testing

antibiotic disks are poor. It is recommended to supply oxacillin disks from approved companies and performance of quality control for all oxacillin disks as recommended by NCCLS. It is also recommended performance of other confirmatory tests such oxacillin screen agar or PCR for detection of mec A. However it is difficult to perform detection of mecA gene in routine diagnostic laboratories and test costs are relatively high.[3,18] The latex agglutination kit for detection of PBP2a is an alternative that could be used in most laboratories. This method would be practically useful for urgent confirmation of resistance

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REFERENCES

1. Brown DFJ. Detection of methicillin/oxacillin resistance in *staphylococci*. J Antimicrob Chemother 2001; 48:65-70.
2. Merlino J. Watson J, Funnel G, Gottlieb T. New screen medium for detection and identification of methicillin/oxacillin-resistant. *Staphylococcus aureus* for nosocomial surveillance. Eur J Clin Microbiol Infect Dis 2002; 21: 414-6.
3. Tiemersma EW, Bronzwaer SLAM, Lyytikainen O, Degener JE, Schrijnemakers P. Methicillin-resistant *Staphylococcus aureus* in Europe,1999-2002. Em Infect Dis 2004; 10:1627-34.
4. Novak SM, Hindler J, Bruckner DA. Reliability of two novel methods Alamar and E-Test, for detection of methicillin – resistant *Staphylococcus aureus*. J Clin Microbiolol 1993; 31:3056-7.
5. Cavassini M, Wenger A, Jaton K, Blanc DS, Bille J. Evaluation of MRSA-screen, a simple anti-PBP2a slide latex agglutination kit for rapid detection of methicillin resistance in *Staphylococcus aureus*. J Clin Microbiol 1999; 37: 1591-4.
6. Tokue Y, Shoji SSK, Watanabe A, Motomiya. Comparison of polymerase chain reaction assay and a conventional microbiologic method for detection of methicillin–resistant *Staphylococcus aureus*. Anti Agent Chemother 1992; 36:6-9.
7. Louie L, Matsumura SO, Choi E, Louie M, Simor AE. Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus*. J Clin Microbiol 2000; 38: 2170-3.
8. Walpole BD. Evaluation of the Mastalex latex agglutination test for methicillin resistance in Staphylococcus aureus grown on different screening media. J Antimicrob Chemother 2001; 187-9.
9. Cavassini M, Wenger A, Jaton K, Blanc DS, Bille J. Evaluation of MRSA-screen, a simple anti-PBP2a slide latex agglutination kit for rapid detection of methicillin resistance in *Staphylococcus aureus*. J Clin Microbiol 1999:37: 1591-4.
10. Brown D, Edddwards DI, Hawkey PM, Morrison D, Ridgway GL. Guidelines for the laboratory diagnosis of and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA) J Antimicrob Chemothe 2005; 56:1000-18.
11. National Committee for Clinical Laboratories Standards. Performance standard for antimicrobial disk susceptibility tests. Eight edition: Approved standards M2-A8.NCCLS, Wayen, PA, USA, 2003.
12. National Committee for Clinical Laboratories Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard M7-A6. NCCLS. Wayen. P A. USA, 2003.
13. Krishanan UMK, Shetty N. 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 Pathology 2002; 55:745-8.
14. Swenson JM, Williams PP, Killgor G, Mohr C. Performance of eight methods including two new rapid methods ,for detection of oxacillin resistance in a challenge set of *Staphylococcus aureus* organisms. J Clin Microbiol 2001; 39:3785-8.
15. Fleming DW, Hegerson SD, Mellery BL, Foster LR, White MC. Methicillin –resistant *staphylococcus aureus*: How reliable is laboratory reporting. Infect Control 1986; 7: 164-7.
16. Humphreys H, Glynn G, Rossney A, McDonald P, Johnson H, McDoneel R et al Methicillin-resistant *Staphylococcus aureus*: Laboratory detection methods in use in the Republic of Ireland and Northern Ireland. Br J Biomed Sci 2002; 59: 7-10.
17. Smyth RW, Kahlmeter, Liljequist BO, Hoffman B. Methods for identifying methicillin resistance in *Staphylococcus aureus, J* Hosp Infect 2001;48:103-7.
18. Cavassini M, Wenger A, Jaton K, Blanc DS, Bille J. Evaluation of MRSA-screen, a simple anti-PBP2a slide latex agglutination kit for rapid detection of methicillin resistance in *Staphylococcus aureus*. J Clin Microbiol 1999:37: 1591-4.