

Original Article

Evaluation of Five Phenotypic Methods for Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA)

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ABSTRACT

Background and Objectives : Rapid and accurate detection of methicillin resistant *Staphylococcus aureus* (MRSA) is an important role of clinical microbiology laboratories to avoid treatment failure. The aim of this study was to compare conventional methods against the E-test minimum inhibitory concentration (MIC) method to determine the best phenotypic method.

Materials and Methods: Methicillin resistance was studied among clinical isolates of *S. aureus* from April to October 2009 in Milad Hospital of Tehran. These methods included E-test MIC, oxacillin screen agar, oacillin disk diffusion, cefoxitin disk diffusion, and CHROMagar- MRSA methods.

Results: Out of 294 isolates of *S. aureus*, one hundred and six (36%) strains of MRSA were isolated from clinical specimen. Oxacillin screen agar and CHROMagar-MRSA showed both 110 MRSA isolates. The sensitivity and specificity for these two methods were 100% and 97.9%, respectively. The sensitivity and specificity of oxacillin disk diffusion method was similar to those of oxacillin screen and CHROMagar-MRSA. One hundred and eight strains of *S. aureus* were MRSA by cefoxitin disk diffusion method. The sensitivity and specificity of cefoxitin disk diffusion method was 100% and 98.1% respectively. All isolates including MRSA were susceptible to vancomycin. Nearly al MRSA isolates were resistant to erythromycin, clindamycin, chloramphenicol, tetracycline, ceftriaxone and ciprofloxacin.

Conclusion: All phenotypic methods had high sensitivity and specificity for detection of MRSA. However, cefoxitin disk diffusion method in comparison to other methods had higher specificity.

Keywords: Methicillin-Resistant *Staphylococcus aureus*, Phenotypes

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Introduction

Staphylococcus aureus is an important etiological agent of hospital and community acquired infections (1,2). The organism has a differential ability to spread and cause outbreaks especially in hospitals (3). Currently one of the most serious aspects concerning treatment of *S. aureus* infections is resistance of this organism to methicillin(4). The first case of methicillin resistant *S. aureus* (MRSA) was reported in 1961. The importance of MRSA as a nosocomial as well as community acquired pathogen is well documented (5,6). Emerging of MRSA worldwide has led to the overuse of glycopeptides antibiotics, and to the emergence of vancomycin-resistant *S. aureus* (7). Methicillin resistance in *S. aureus* is based on the production of an additional penicillin binding protein, PBP2 or PBP2a, which is mediated by the *mecA* gene (8). MRSA strains are frequently resistant to many different classes antibiotics, second -and third-line antimicrobial resistance is a growing concern(9).

Considering the increasing rate of infections caused by MRSA, performance of reliable, accurate and rapid testing for detection MRSA is essential for both antibiotic therapy and infection control measures(10). There are many traditional and commercial systems for detection of MRSA in clinical microbiology laboratories. Most laboratories use disk diffusion methods for routine test. The gold standard method for antimicrobial susceptibility testing has been the MIC determined by dilution methods. In the recent years, MIC methods have been replaced by molecular methods which detect *mecA* gene as a gold standard for determining classical methicillin resistance in *S. aureus*. However, the use of molecular methods for detection MRSA is largely restricted to reference laboratories and is not utilized in many microbiology laboratories as a routine test (5, 6, 11-13).

The aim of this study was to determine the incidence of methicillin resistance in an Iranian large hospital located in Tehran. We also compared five standard methods for detection of MRSA. E-test MIC were used as a gold standard method in this evaluation.

Material and Methods

Clinical isolates of *S. aureus* from different specimens including tracheal aspirates, wound and soft tissue, urine, blood and other specimens between April 2009 and October 2009 in Milad Hospital of Tehran were studied. Milad Hospital is 1000-bed non-teaching tertiary care hospital. In total, 106 strains of *S. aureus* isolated from patients admitted to our hospital were included. The majority of patients were hospitalized patients. Briefly, the samples were cultured aerobically in blood and MacConky agar. The plates were incubated overnight at 35°C. All isolates were identified using gram stain, biochemical tests including catalase, coagulase and DNase.

All isolates were tested with oxacillin (1 µg) and cefoxitin (30 µg) disks, using Mueller Hinton agar with a suspension equivalent to 0.5 McFarland standards of the *S. aureus* isolates. All plates were incubated at 35°C for 24h. Zone of inhibition were measured and interpreted as guideline recommended by CLSI(14).

The E-test method (AB Biodisk Solna Sweden) was used to determine the minimum inhibitory concentration (MIC) as recommended by manufacture. Briefly, using Muller-Hinton agar supplemented with 2% NaCl and an inoculum density equivalent to 0.5 McFarland standards. We used also oxacillin screening agar which was performed by inoculating a direct colony suspension (0.5 McFarland standard) with a swab spotting an area 10 to 15mm in diameter on Mueller-Hinton agar supplemented with 4% NaCl and 6 µg/ml oxacillin. After incubation at 35°C for 24 hours any growth was interpreted as positive for MRSA (14). Briefly, for detection of MRSA on MRSA CHROMagar, a suspension of 0.5 Mac Farland was prepared and 10µL of bacterial suspension was streaked on above mentioned medium. All plates were incubated at 35°C for 24h. Strains growing on CHROMagar-MRSA and yielding colonies with rose to mauve were considered MRSA as recommended by manufacture (15). *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as a control strains for quality control of susceptibility testing antibiotics disks.

We also used *S. aureus* ATCC 29213 as a positive control for MRSA. Statistical calculations were performed with SPSS statistical software (version 16.0; SPSS, Chicago, IL, USA). E-test MIC was our gold standard method. Sensitivity and specificity of other methods were compared with it.

Results

From April to October 2009, totally, 294 strains of *S. aureus* were isolated from clinical specimens of patient admitted to the hospital. Of 294 isolates, 106(36%) were MRSA by E-test MIC method. Of 106 MRSA isolates, 51 (48%) strains isolated from tracheal aspirate, 26 (24.5%) strains from wound, 10 (9.4%) strains from blood cultures, and 19 isolates (17.9%) from other specimen. Of 106 patients, 62 were male and 38 were female.

By using E-test method, of 294 isolates 106(36%) were MRSA, Oxacillin screen agar and

CHROMagar-MRSA showed both 110 MRSA isolates. The sensitivity and specificity for these two methods were 100% and 97.9%, respectively. The sensitivity and specificity of oxacillin disk diffusion method were similar to those of oxacillin screen and CHROMagar-MRSA methods. One hundred and eight strains of *S. aureus* were MRSA by cefoxitin disk diffusion method. The sensitivity and specificity for cefoxitin disk diffusion method were 100% and 98.1%, respectively. Performance characteristics of the all-phenotypic methods are shown in Table-1. All methods were considered satisfactory in detecting MRSA and showed similar sensitivity. Although the specificity and positive predictive value of Oxacillin screen agar, CHROMagar-MRSA and oxacillin screen agar seemed to be lower than the other methods. Differences in sensitivities and specificities were not statistically significant. The results of susceptibility testing of MRSA isolates to other antibiotics are shown in Table 2

Table. 1: Sensitivity and specificity of phenotypic methods for detecton of MRSA.

Methods	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
E-MIC	100	100	100	100
Oxacillin disk diffusion	100	97.9	96.36	100
Cefoxitin disk diffusion	100	98.9	98.1	100
Oxacillin screen agar	100	97.9	96.3	100
CHROMagar MRSA	100	97.9	96.3	100

MIC=Minimum Inhibitory Concentration ,PPV=Positive Predictive Value ,NPV= Negative Predictive Value

Table. 2: Drug resistance pattern of MRSA isolated from clinical specimens

Antibiotic	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Erythromycin	2(1.88)	1(0.94)	103(97.16)
Co-trimoxazole	75(70.75)	0(0.0)	31(29.25)
Vancomycin	106(100)	0(0.00)	0(0.00)
Gentamycin	5(4.71)	0(0.00)	101(95.29)
Clindamycin	3(2.83)	0(0.00)	103(97.16)
Chloramphenicol	3(2.83)	0(0.00)	103(97.16)
Tetracycline	0(0.00)	1(0.94)	105(99.05)
Ceftriaxone	1(0.90)	0(0.00)	105 (99.09)
Rifampin	31(29.24)	0(0.00)	75(70.76)
Ciprofloxacin	4(3.77)	0(0.00)	102(96.22)

Discussion

Multiple antibiotics resistant especially MRSA constitute a major health care problem (16). Accurate detection of MRSA by using high sensitivity and specificity methods provide a major guideline for treatment of infections caused by this organism. There are several methods for detection MRSA including routine methods such as disk diffusion, MIC determination (broth dilution-test) oxacillin screening agar, and recently developed methods like disk diffusion using cefoxitin instead of oxacillin, latex agglutination and CHROMagar-MRSA (5). Methicillin resistance in *S. aureus* strains in our laboratory is routinely detected by oxacillin disk diffusion method for clinical isolates of *S. aureus*, and oxacillin screen agar method is used as a confirmatory test for detection of MRSA.

Testing of oxacillin (methicillin) resistance in *S. aureus*, has been a challenge for clinical laboratories in recent years. Several studies have been showed that detection of *mecA* gene is a gold standard method for diagnosis of MRSA in clinical microbiology laboratories (11). However, most laboratories especially in developing countries are not in position to perform molecular methods. In the present study, we evaluated different phenotypic methods for the detection of MRSA.

We used E-test MIC as a gold standard method for detection of MRSA. The E-test method has the advantages of being easy to perform as a disk diffusion test and approaches the accuracy of PCR for *mecA*. There are many studies comparing E test MIC with broth dilution and PCR methods with generally has been yielded satisfactory results (17). The presence of resistance in *S. aureus* isolate on an oxacillin screen agar plate generally means that the isolates *mecA* positive. Occasionally, however heteroresistant *mecA*-positive strains is not detected due to low expression of resistance. Oxacillin agar screen generally dose not detect borderline resistant strains, when studies have included strains whose resistance is heterogeneous the test has been shown to perform less well (18).

CHROMagar-MRSA is another phenotypic method, which is being used for detection of MRSA. Recent studies have shown a high sensitivity and specificity for MRSA CHROMagar. Our recent study showed 100 sensitivity and specificity for this method (16).

Recently CLSI has replaced oxacillin with cefoxitin for detection of MRSA (11). Regarding cefoxitin disk diffusion, many studied reported that the results of cefoxitin disk diffusion tests correlate better with the presence of *mecA* than do the results of disk diffusion tests using oxacillin (19). In a study by Anand *et al.* results of cefoxitin disk diffusion method for detection of MRSA was in concordance with the PCR for *mecA* gene (18). Another study by showed a high correlation between MICs of cefoxitin and presence of *mecA* in staphylococcus spp. (20) Recently it is showed that cefoxitin disk diffusion method was reliable as oxacillin disk diffusion method for detection of MRSA (21, 22).

The sensitivity and specificity value of phenotypic methods used for identification of MRSA are known to vary depending on the media used for incubation, the concentration of NaCl used in medium, the incubation time and temperature and the experience of personnel's which carry out the tests. (11)

Conclusions

Our study reveled that cefoxitin disk diffusion method had a high sensitivity and specificity comparative to other routinely used methods for detection MRSA. This method can be preferred in clinical microbiology laboratories because it is easy to perform, do not require special technique, media preparation and finally more cost-effective in comparison to other methods.

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