Original Article

Comparison of Different Phenotypic and Genotypic Methods for the Detection of Methicillin-Resistant *Staphylococcus Aureus*

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Abstract

Background: *Staphylococcus aureus* is known as a powerful pathogen that causes various infections. Emergence of methicillin-resistant *S. aureus* (MSRA) is responsible for nosocomial and community-acquired infections worldwide. **Aims:** The present study aimed to evaluate the performance and ability of eight different phenotypic and genotypic methods for the detection of MSRA. **Materials and Methods:** A total of 186 *S. aureus* isolates were defined as methicillin-susceptible *S. aureus* (MSSA; 95) and MSRA (91) using polymerase chain reaction (PCR) as the gold standard. Susceptibility to methicillin was investigated using oxacillin, methicillin, cefotetan, cefoxitin, and cefmetazole disks, by oxacillin Adata Tab and strips. For all *S. aureus* isolates minimal inhibitory concentrations of oxacillin were determined using the broth microdilution method according to Clinical and Laboratory Standards Institute guidelines. **Results:** Among the diagnostic methods studied, broth microdilution and the cefoxitin disk had the highest specificity (98.9 and 94.7%), sensitivity (100 and 98.9%), and concordance with PCR results (98.9 and 93.6%). The cefotetan and cefmetazole disks had the lowest concordance with PCR results. **Conclusion:** Our results suggest that microdilution and cefoxitin disk methods have high sensitivities compared with other methods for detection of MSRA. The cefoxitin disk method may be preferred in clinical laboratories because it is easy to perform and does not require special equipment.

Keywords: Cefoxitin, Detection method, Genotypic, Methicillin, Methicillin-resistant *S. aureus,* Phenotypic, *Staphylococcus aureus*

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Introduction

Staphylococcus aureus is known as one of the most important human pathogens frequently implicated in nosocomial infections. *S. aureus* has the ability to grow in different environmental conditions and as a part of the normal human flora, it can colonize areas such as the anterior nares, perineum, armpit, and groin.[1,2] In addition, *S. aureus* is capable of causing a wide range of infections including skin infections (folliculitis boils, furuncles, and carbuncles), abscesses, toxic shock and scalded skin syndrome, food poisoning, bacteremia, endocarditis, septicemia, osteomyelitis, and pyoarthritis.[3,4]

β-Lactams are considered as the first-choice antibiotics to treat Staphylococcal infections. Currently, the increasing resistance against antibacterial drugs is a major public health concern and one of the biggest challenges faced by physicians. In *S. aureus*, resistance to methicillin occurs because of variations in the alteration of constitutive penicillin-binding proteins (PBPs) or expression of the *mecA*.[5,6] Because of an increasing prevalence of methicillin-resistant *S. aureus* (MRSA) infections, hospital infection control units, and health authorities should investigate the local MRSA prevalence to provide comprehensive and practical programs to prevent the spread of this organism. Therefore, providing fast and

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reliable methods for the detection of MRSA isolates is considered a prerequisite to ensure optimal treatment for patients with MRSA infections.

Detection of MRSA is based on phenotypic and genotypic characterization of bacterial isolates. In most cases, phenotypic methods are faster and easier than genotypic methods, while genotypic methods may have better accuracy and precision.[7] Phenotypic methods include broth microdilution, agar dilution, the agar screening method, disk diffusion, and latex agglutination methods; while genotypic methods comprises polymerase chain reaction (PCR)-based detection techniques.[8] This study was performed to determine the relative importance of these diagnostic techniques.

Materials and Methods

This work is approved (# 0089024) by the research ethic committee of Kermanshah University of Medical Sciences (KUMS). In this way, written informed consent was obtained from all participating patients after careful explanation of the study.

Bacterial isolates

One hundred and eighty-six nonduplicate *S. aureus* isolates were collected from the anterior nares of patients hospitalized in different wards of the Kermanshah Hospital in the west of Iran to be screened for MRSA colonization.

Cotton swabs soaked in sterile saline were entered into the patient's anterior nostrils and rotated five times and subsequently transferred to the mannitol salt agar medium. *S. aureus* isolates were identified by conventional methods, that is, colonial morphology, gram staining characteristics, production of catalase, coagulase, DNase, and mannitol salt agar fermentation.

Genotypic identification of MRSA

According to the method of Anand *et al*., *S. aureus* isolates were examined using PCR for the presence of *mecA*.[9] MRSA ATCC 43300 (oxacillin resistant) and methicillin susceptible *S. aureus* (MSSA) ATCC 25923 (oxacillin sensitive) were used as positive and negative controls, respectively.

Determination of antimicrobial susceptibility profiles

Susceptibility to oxacillin (1 μg), methicillin (5 μg), cefoxitin (30 μg), cefotetan (30 μg), and cefmetazole (30 μg) was determined by disk diffusion testing using the Kirby-Bauer method. Antibiogram results were interpreted according to the Clinical and Laboratory Standards Institutes (CLSI) 2007 standard tables.[10]

Oxacillin broth microdilution

For all isolates, the broth microdilution method (microsterile plate) with oxacillin powder (Sigma-Aldrich, Germany) was used to determine the minimum inhibitory concentration (MIC).[11]

Adata Tab oxacillin test

According to the manufacturer's protocol, each oxacillin tablet was dissolved in 100 mL of nutrient broth medium containing 5% sodium chloride. Bacteria were subsequently cultured on this medium and incubated for 24 h. Resistance was indicated by bacterial growth after 24 h.

Oxacillin strip test

Bacteria were cultured on nutrient agar medium containing 5% sodium chloride. Subsequently, an oxacillin strip was placed on this medium and incubated for 24 h at 37°C. Observed bacterial growth around the strip indicated resistance.

Statistical analysis

Data were described by two-dimensional tables using the k_2 test for association and kappa concordance measures for levels of concordance. Sensitivity of a certain method was calculated as the number of resistant *S. aureus* isolates determined using this method, divided by the sum of *mecA*-positive strains. Specificity was calculated as the number of MSSA strains determined by this method, divided by the sum of *mecA*-negative strains. To calculate the positive predictive value (PPV), the number of true positives (*mecA* positive) was divided by the number of positive results by the other tests; and to calculate negative predictive value (NPV), the number of true negatives (*mecA* negative) was divided by the number of negative results by the other tests.

Results

Of the 186 *S. aureus* isolates, 95 and 91 were confirmed using PCR as MSSA and MRSA, respectively. All *mecA*-positive isolates had a MIC of ³ 4 mg/mL for oxacillin. The broth microdilution and A data Tab methods indicated 100% sensitivity, and the cefmetazole and oxacillin disk methods had 100% specificity [Table 1]. According to our results, the broth microdilution and cefoxitin disk methods had the highest concordance (98.9 and 93.6%, respectively) and the cefmetazole disk method had the lowest concordance (47.8%). As displayed in [Table 2], 42.8% MRSA had an MIC > 2,048 mg/mL for oxacillin.

Table 1: Comparison of various laboratory methods for detecting resistant *Staphylococcus aureus* isolates

Methods	Specificity (%)	Sensitivity (%)	NPV (%)	PPV (%)	Concordance with PCR (%)
Oxacillin strip	93.4	92.6	92.4	93.6	86
Microdilution	100	98.9	98.9	100	98.9
Adata Tab	100	88.4	89.2	100	88.2
Methicillin disk	87.9	92.6	92	88.9	80.6
Cefoxitin disk	98.9	94.7	94.7	98.9	93.6
Cefotetan disk	98.5	91.4	91.5	94.6	84
Cefemtazol disk	47.3	100	100	66.4	47.8
Oxacillin disk	73.6	100	100	79.8	74

PPV = Positive predictive value, NPV = Negative predictive value, PCR = Polymerase chain reaction

Table 2: MIC distribution of *Staphylococcus aureus* isolates determined by oxacillin microbroth dilution

Number	Percent	MIC
39	42.8	≥2,048
11	12.1	1,024
8	8.8	512
8	8.8	256
5	5.5	128
12	13.2	64
3	3.3	32
3	3.3	16
1	1.1	8
1	1.1	4
95	51.1	<4

M IC = Minimum inhibition concentration.

Discussion

MRSA is a major nosocomial pathogen causing significant morbidity and mortality.[12] Because of the necessity and importance of identifying MRSA among *S. aureus*, it is imperative to apply the appropriate and precise laboratory methods to identify these isolates. Because the *mecA* cannot be observed in MSSA strains, molecular methods such as PCR and hybridization that can detect the *mecA* are considered as the gold standard methods for the identification of MRSA.[8] Therefore, the presence and absence of the *mecA* indicates MRSA and MSSA, respectively.[13]

In the present study, among the phenotypic methods, the microdilution method was observed to be the most sensitive (100%) for the detection of *mecA*-mediated resistance. However, since this method requires oxacillin powder and skilled laboratory staff for its implementation, and as it is time consuming, it cannot easily be implemented in routine laboratories. The cefoxitin disk test, which by contrast is easy to perform and does not require special equipment, also demonstrated high sensitivity (98.9%) and specificity (94.7%) for MRSA detection. These findings are consistent with those of Broekeme *et al*., where the sensitivity and specificity of the cefoxitin disk method were reported as 97.3 and 100%, respectively among 10,611 *S. aureus* isolates examined.[14] Accordingly, CLSI has replaced the oxacillin disk with a cefoxitin disk for the detection of MRSA.[15] Several studies including the current one have reported that the results of the cefoxitin disk diffusion test correlate better with the presence of *mecA* compared with those of the oxacillin disk diffusion test.[16] Cefoxitin is a better inducer of *mecA* expression; this could explain why heterogeneous MRSA populations variably expressing the *mecA* are better detected by disk diffusion with cefoxitin than with oxacillin, which is a weak inducer of PBP2a production. This is considered to be the underlying mechanism for the higher sensitivity of cefoxitin than oxacillin. Anand *et al*. reported the sensitivity and specificity of the cefoxitin disk method to be 100%, which was slightly better than that observed in our results. In addition, in that study, the sensitivity and specificity of the oxacillin disk was determined to be 87.5% and 100%, respectively. The lower sensitivity in the present study (73.6%) could be because of differences in the manufacturer's disk.[9] In the study of Sakoulas *et al*., the sensitivity and specificity of the oxacillin MIC method was 99 and 98.1%, respectively, and the specificity finding was consistent with the results of the present study (100 and 98.9%, respectively).[17] Wallet *et al*., compared the MIC method with PCR and the sensitivity was 96%, which was slightly lower than results in this study (100%).[18]

In the Adata Tab method, the medium can be easily made and interpreted, but this test is expensive to perform and not available in all laboratories. Because an appropriate and reliable test must have both high sensitivity and specificity, PCR was used as the gold standard to calculate concordance.

One feature of the present study was the simultaneous evaluation of the three antibiotics; cefoxitin, cefmetazole, and cefotetan. These antibiotics are a category of cephamycins and are classified as second generation

cephalosporins because of their similarity with cephalosporins.[19] We hypothesized that these three antibiotics would show the same sensitivity and specificity; however, the results did not support this hypothesis.

Conclusion

The present study revealed that the microdilution and cefoxitin disk methods have high sensitivities compared with other methods for detection of MRSA. The cefoxitin disk method may be preferred in clinical laboratories because it is easy to perform and does not require special equipment. However, use of the other investigated methods may not be appropriate because of the relatively lower levels of concordance with PCR.

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