Evaluation of phenotypic and genotypic methods for detection of methicillin-resistant Staphylococcus aureus strains

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ABSTRACT

Food-borne pathogenic is a group of micro-organisms that cause food-borne illness thus, the research for finding effective drugs against this infection is necessary. Staphylococcus aureus is the major cause of food-borne and nosocomial infection in last decade and methicillin resistant S. aureus (MRSA) has emerged as a major clinical problem. The aim of the present study was to compare different phenotypic methods with genotypic method by polymerase chain reaction (PCR) for the detection of MRSA strains. Sensitivity to methicillin was also investigated by oxacillin, methicillin, cefotetan, cefoxitin, cefmetazole disks, the data tab, and disk diffusion method and oxacillin strip. Minimum inhibitory concentration (MIC) of these isolates was determined by using micro-broth dilution. A total of 186 isolates of S. aureus with PCR method (gold standard) were detected. About 95 isolates were methicillin-sensitive S. aureus, and 91 isolates were MRSA. Among the diagnostic methods studied, micro-broth dilution and cefoxitin disk had the most specificity with 98.9% and 94.7%, respectively. The sensitivity of them was 100.0% and 98.9%, respectively. Furthermore, the concordance with PCR was 98.9% and 93.6%, respectively. The cefotetan and cefmetazole disks had the lowest concordance with the results of PCR. Due to the necessity of using simple, reliable and low-cost methods in routine diagnostic laboratories it seems use of cefoxitin disk still be considered as one of recommended methods for detecting MRSA isolates.

1. Introduction

Food-borne pathogenic is a group of micro-organisms that cause food-borne illness thus, the research for finding effective drugs against this infection is necessary. Staphylococcus aureus is the major cause of food-borne and nosocomial infection in last decade and methicillin resistant S. aureus (MRSA) has emerged as a major clinical problem (1).

MRSA strains were reported in a British study in 1961 and then methicillin became available for clinical use. S. aureus is the major cause of nosocomial infections in last decade and MRSA has emerged as a major clinical problem. Therefore, rapid and accurate detection of these nosocomial infections is essential in order to choose appropriate treatment, to avoid unnecessary use of antibiotics and to take necessary measures for infection control (2-7) Antibiotics that contain a beta-lactam ring are considered as the choice antibiotics to treatment infections caused by these nosocomial infections.

The increasing resistance against anti-bacterial drugs has remained as a serious problem of public health and threat to the health of many. Methicillin is the first semi-synthetic penicillin resistant to β-lactamase, but since its introduction to market, resistance to this antibiotic has increased (8-10) Resistance to methicillin is considered resistance to all penicillinase-resistant penicillins and cephalosporins (11).

Due to increasing prevalence of infections caused by MRSA, the infection control unit in hospitals and health authorities should investigate prevalence of
MRSA and provide comprehensive and practical programs to prevent the spread of this organism.

Thus providing fast and reliable methods for detecting MRSA isolates is considered as a prerequisite to ensure optimal treatment in patients with infections caused by this organism. These diagnostic methods are based on phenotypic and genotypic characteristics of bacterial isolates. In most cases, phenotypic methods are faster and easier than genotypic methods, while genotypic methods have more accuracy and precision. Phenotypic methods still preferred for detection, but for the reliable detection of MRSA should include a combination of tests and apply a genotypic method (12,13). Phenotypic methods are including: micro-dilution broth, macro-dilution broth, agar dilution, agar screening method, disc diffusion with methicillin, oxacillin, cefoxitin, and latex agglutination methods and genotypic methods are polymerase chain reaction (PCR), multiplex PCR and real-time PCR (14). This study was performed to determine the relative importance of these diagnostic techniques.

2. Materials and methods

2.1 Sample collection

Samples were collected from the patient admitted to Emam Reza Teaching Hospital of Kermanshah city (Iran) after obtaining informed consent and assigning special code to each patient confidentially.

For sampling, cotton swabs soaked in sterile saline entered into the patient’s anterior nostrils and rotated 5 times, transferred on mannitol salt agar medium in the place of sampling then was cultured. Up to 2 h transferred to medical school and was incubated at 35° C for 24-48 h. Yellow colonies (fermentor of mannitol) suspected to S. aureus was cultured on nutrients agar medium for subsequent testing. Colony morphology, gram stain, hemolysis test, catalase test, slide coagulase test, tube coagulase test and DNase testing was performed to detect bacteria.

2.2 Determination of resistant isolates by PCR

S. aureus isolates were examined by PCR as the gold standard method, and the strains of MRSA ATCC 43300 and MSSA ATCC 25923 were used for positive and negative controls by PCR amplification of the mecA gene (15).

2.3 Measuring the sensitivity of bacteria to antibiotics

Sensitivity to oxacillin (1 µg), methicillin (5 µg), cefoxitin (30 µg), cefotetan (30 µg), and cefmetazole (30 µg) were determined by disk diffusion testing with Kirby-Bauer Method. Antibiogram results were interpreted using of Clinical and Laboratory Standards Institute (CLSI) 2008 standard tables (16).

2.4 Sensitivity determination of MRSA for oxacillin broth micro-dilution

The preparation of oxacillin (Sigma-Aldrich, St. Louis, USA) stock solution and testing conditions for oxacillin micro-broth dilution was done as per CLSI guidelines (17).

2.4.1. ADATA TAB oxacillin test

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

2.4.2. Oxacillin strip test

Bacteria were cultured on nutrients agar medium containing sodium chloride 5%. Then, oxacillin strip placed on a medium and incubated for 24 h at 37° C. After this time growth around of strip indicated resistance and growth absence indicated sensitivity.

2.5 Statistical analysis

Data described by two-dimensional tables. For their association $k^2$ test and for levels of concordance kappa concordance measure was used. For calculate the sensitivity, cases which were resistant divided into mecA-positive sum of results and to calculate the specificity, cases that were susceptible divided into mecA-negative sum of results. To calculate the positive predictive value, MecA-positive cases were divided into total resistance and for calculating the negative predictive value, MecA-negative cases were divided into total sensitive cases.

3. Results

In this study, 186 samples were examined, and the numbers of 91 samples were resistant and 95 were susceptible to methicillin. As shown in table 1, micro-dilution and ADATA TAB methods (both 100%) showed the most sensitivity and cefmetazole and oxacillin disk (both 100%) had the most specificity. Since an appropriate and reliable test must have both high sensitivity and specificity so cefoxitin disk and micro-dilution methods look better. PCR was used as the gold standard so concordance of each method was measured according to PCR. According to our results micro-dilution and cefoxitin disk had the most rate of concordance (98.9% and 93.6% respectively), and cefmetazole disk had the least rate of concordance (47.8%). MRSA isolates were studied different MIC, as can be seen in table 2, the most MIC frequency was more than 2048 µg/mL concentration that was 42.8% of total MRSA.
One feature of our study was evaluating three antibiotics (cefoxitin, cefmetazole, and cefotetan) simultaneously. These antibiotics are among the category of cefomycins and because of the similarity with cephalosporins are classified as the second-generation cephalosporins (20). Our prediction was that these three antibiotics show the same sensitivity and specificity, but the results proved otherwise.

Optimal methods to detect MRSA should possess high sensitivity and specificity. On the other hand, higher concordance of any applied test with gold standard method (PCR) is desired.

In the present study, micro-broth dilution method had the maximum level of concordance (98.9%).

Then cefotetan disk showed a considerable concordance (93.6%). Concordance of other used methods in this study was ranged from 74.0% to 88.2%. Cefmetazole disk had the lowest concordance (47.8%). Each of studied methods has specific advantages and disadvantages. For example, disk diffusion method is quick and inexpensive that requires no special experience and in some cases expected that some disks can be replaced by others. Of course, one should always consider the quality of disks and their storage conditions.

In the ADATA TAB method, the medium can be easily made and interpreted easily, but this test is expensive to perform and not available in all laboratories. Micro-broth dilution is an accurate method that can even determine the amount of MIC, but needs oxacillin powder and skilled laboratory staff and also is time-consuming.

### Table 1. Comparison of various laboratory methods for detecting susceptible Staphylococcus aureus isolates

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
<th>Concordance with PCR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin strip</td>
<td>93.4</td>
<td>92.6</td>
<td>92.4</td>
<td>93.6</td>
<td>86.0</td>
</tr>
<tr>
<td>Micro-dilution</td>
<td>100.0</td>
<td>98.9</td>
<td>98.9</td>
<td>100.0</td>
<td>98.9</td>
</tr>
<tr>
<td>ADA TAB</td>
<td>100.0</td>
<td>88.4</td>
<td>89.2</td>
<td>100.0</td>
<td>88.2</td>
</tr>
<tr>
<td>Methicillin disk</td>
<td>87.9</td>
<td>92.6</td>
<td>92.0</td>
<td>88.9</td>
<td>80.6</td>
</tr>
<tr>
<td>Cefoxitin disk</td>
<td>98.9</td>
<td>94.7</td>
<td>94.7</td>
<td>98.9</td>
<td>93.6</td>
</tr>
<tr>
<td>Cefotetan disk</td>
<td>98.5</td>
<td>91.4</td>
<td>91.5</td>
<td>94.6</td>
<td>84.0</td>
</tr>
<tr>
<td>Cefmetazol disk</td>
<td>47.3</td>
<td>100.0</td>
<td>100.0</td>
<td>66.4</td>
<td>47.8</td>
</tr>
<tr>
<td>Oxacillin disk</td>
<td>73.6</td>
<td>100.0</td>
<td>100.0</td>
<td>79.8</td>
<td>74.0</td>
</tr>
</tbody>
</table>

4. Discussion

In the present study, due to necessity and importance of identifying antibiotic-resistant isolates of MRSA, it seems imperative to apply the appropriate and precise laboratory methods to identify these isolates. With study of methods identifying resistant isolates by phenotypic and genotypic characterization can achieve efficient and useful results. Since mecA gene cannot be found in methicillin-sensitive Staphylococcus strains, molecular methods such as PCR and hybridization that can detect MecA gene are considered as the gold standard methods (14).

Anand et al. have reported that sensitivity and specificity of cefoxitin disk was 100.0% that was slightly more than our results. Furthermore in that study, sensitivity and specificity of oxacillin disk was determined 87.5% and 100.0% respectively that had equal specificity, but the sensitivity of our study (73.6%) was lower, which could be due to differences in manufacturer’s disk (17).

In the study of Broekema et al. by examining 10,611 S. aureus isolates, the sensitivity and specificity of cefoxitin disk were reported 97.3% and 100.0% respectively that was consistent with our results (98.9% sensitivity and 94.7% specificity) (18).

In the study of Sakoulas et al. MIC sensitivity and specificity was 99.0% and 98.1% respectively that was consistent with sensitivity and specificity of our study results (100.0%, and 98.9%) (13).

Wallet et al. compared the MIC method with PCR and sensitivity was equal to 96.0% that slightly was lower than our results (100.0%) (19).

### Table 2. Minimum inhibitory concentration frequency of methicillin resistant Staphylococcus aureus isolates by micro-broth dilution

<table>
<thead>
<tr>
<th>MIC</th>
<th>Percent</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤22048</td>
<td>42.8</td>
<td>39</td>
</tr>
<tr>
<td>1024</td>
<td>12.1</td>
<td>11</td>
</tr>
<tr>
<td>512</td>
<td>8.8</td>
<td>8</td>
</tr>
<tr>
<td>256</td>
<td>8.8</td>
<td>8</td>
</tr>
<tr>
<td>128</td>
<td>5.5</td>
<td>5</td>
</tr>
<tr>
<td>64</td>
<td>13.2</td>
<td>12</td>
</tr>
<tr>
<td>32</td>
<td>3.3</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>3.3</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>1.1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1.1</td>
<td>1</td>
</tr>
</tbody>
</table>
alternative method. Due to relatively lower levels concordance with PCR, using other methods may not be appropriate.

**Conflict of Interests**
Authors have no conflict of interest.

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**References**