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Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) by CHROMagar Versus Cefoxitin Disk Diffusion Method

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ABSTRACT

Background and Aim: MRSA, or methicillin-resistant *Staphylococcus aureus*, has arisen as a nosocomial and community-acquired infection throughout the world. MRSA identification in the laboratory is difficult for a variety of reasons. The aim of this study was to investigate several phenotypic methods for the detection of MRSA compared with the PCR-based method as the gold standard.

Materials and Methods: A total of 220 clinical isolates of *S. aureus* were recovered from diverse clinical specimens between August 1, 2019 and June 30, 2020 at Milad Hospital in Tehran, Iran. Cefoxitin discs, CHROMagar™ MRSA medium, and identification of the *mecA* gene by Polymerase Chain Reaction (PCR) as the gold standard method were used to assess methicillin resistance.

Results and Conclusion: PCR testing revealed that 105 (47.72%) of 220 *S. aureus* isolates were positive for the *mecA* gene. The results of the Cefoxitin disc diffusion method showed that it has similar sensitivity and specificity to PCR method. The sensitivity and specificity of CHROMagar™ MRSA medium were both 100%. The Cefoxitin disc diffusion method had the same sensitivity and specificity as the PCR method for detecting the *mecA* gene. For MRSA detection, the Cefoxitin disc diffusion method can be employed as an alternative to PCR.

Keywords: Methicillin Resistant; *mecA* gene; *Staphylococcus aureus*; CHROMagar; MRSA

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1. Introduction

Staphylococcus aureus is a major bacterial human pathogen that causes serious nosocomial and community-acquired infections **(1)**. Infections caused by methicillin resistant *Staphylococcus aureus* (MRSA) are a major health problematic issue especially in intensive care units (ICU) in Iran and worldwide **(2)**. Increasing prevalence of MRSA is one of the therapeutic challenges in health setting **(3)**. In Europe about 20% of *S. aureus* isolates are MRSA; this rate in USA is higher than %50 **(1)**. With the exception of Iraq, the frequency of MRSA in the Islamic Republic of Iran is higher than in neighbouring Middle Eastern nations

(4). It is estimated that 52 % of *S. aureus* isolates in our country are MRSA **(5)**.

MRSA detection in microbiology laboratories is critical for racial treatment and drug resistance prevention **(6)**. Microbiology laboratories have a critical role for detection of MRSA **(7)**. There are many laboratory methods including phenotypic and genotypic methods for detection of MRSA **(8)**. Some of these methods such as molecular techniques including PCR has a higher sensitivity and gives rapid results but unfortunately these methods are expensive and are not available in many microbiology laboratories especially in developing countries **(9)**. Therefore, reliable screening method that is inexpensive and simple to use is needed. Culture based methods using selective media such as oxacillin screening agar, are used widely for detection of MRSA, because of their simplicity and cost effectiveness **(10)**. In recent decades, other culture-based methods such as use of chromogenic media are introduced for detection of MRSA. CHROMagar ™ MRSA cause reduction of detection time and workload and do not require expensive equipment. So, it can be a safe, reliable and low-cost method for screening of MRSA in medical microbiology laboratory **(9)**. For this reason, in this study, we will compare different routine laboratory methods including Cefoxitin disk diffusion and CHROM agar to show that CHROMagar ™ is a reliable and cost effectiveness method.

2. Materials and Methods

2.1. Samples Collection

During an 11-month period (1 August 2019 to 30 June 2020), a total of 220 *Staphylococcus aureus* strains were isolated from different clinical specimens including urine, blood, wound and respiratory tract specimens of patients who were admitted at Milad hospital of Tehran. Milad hospital is a 1000-beds tertiary care hospital affiliated by Iranian social security. All isolates of *S. aureus* were identified by routine laboratory methods such as colony morphology, Gram staining, catalase, coagulase, DNase test and other biochemical reactions.

2.2. Preparation of CHROM agar™ Medium

The CHROM agar Company provided CHROM agar™ for MRSA identification (Paris, France). The product composed of this medium included agar (15g/l), peptone (40g/l), NaCl (25 g/l) and proprietary chromogenic mix (3g/l), totally 82.5g/l. The culture media was prepared as guideline that recommended by manufacture. Briefly 82g of CHROM agar™ MRSA was dispersed in 1 L of purified water and stirred completely. The mixture was autoclaved at 110ºC for 5 minutes and then cooled in water bath (45-50ºC).

In step two, aseptically, CHROM agar MRSA supplement (ref. SU620) was rehydrated in 20 ml of sterile water and mixed slowly. 1 ml of CHROM agar supplement was add to prepared CHROM agar medium and dispensed in sterile plates. After solidification, all plates were stored up to one month under refrigeration (2-8°C) until use. Quality control was performed by using microorganisms as recommended by manufacture.

2.3. Inoculation of Strains on CHROM Agar

For testing of *S. aureus* one fine 24-hour colony isolates processed by direct streaking on CHROM agar MRSA. Plates were incubated for 12-18 hour. Growth of intense colonies with mauve color considered as MRSA according to manufacture instruction.

2.4. Susceptibility Test

For detection of MRSA isolates, disk diffusion test using Cefoxitin (30µg) was used according to CLSI instructions. Bacterial suspension in 0.9% NaCl solution at a density equivalent to 0.5 McFarland standards was prepared with 4-5 fresh colonies of *S. aureus* on blood agar and inoculated to Mueller-Hinton agar plates. Cefoxitin disk (ROSCO. Co Denmark) aseptically placed on Mueller-Hinton agars. Plates were incubated at 35ºC for 24 hours. Inhibition zone diameters were measured in millimeter and interpreted according to instructions.

2.5. Detection of the *mecA* Gene by Polymerase Chain Reaction

The DNA extraction process was performed by the rapid method **(11)**. An overnight culture on blood agar plates was prepared. One colony of each sample was re-suspended in 25 µl of sterile distilled water. The suspension was then placed in a heating block (100ºC) in water for 15 min. Ten-microliter volumes from this suspension was directly used as a template for PCR amplification. The 162 bp fragment of the Methicillin-resistant gene (*mecA*) was amplified using the primers *mecA-F* (5'-TCCAGATTACAACTTCACCAGG-3') and the *mecA*-R (5'-CCACTTCATATCTTGTAACG-3') **(6)**. An aliquot of 10 µl of extracted DNA was added to 40 µl of PCR mixture consisting of PCR buffer (1.5X), Taq DNA polymerase (0.1 U/µlit), dNTP mix (0.25mM of each), $MgCl_2$ (1.5mM), the primer (0.3 pmol) and with the following thermal cycling profile: denaturation step at 94°C for 5 min, followed by annealing at 58 °C for 50 sec, extension at 72 °C for 50 sec and the final extension step at 72 °C for 10 min. Under a UV Transilluminator, the PCR products were visualized on a 1.5% agarose gel with ethidium bromide dye.

Amplicons of 162 bp were found to be compatible with the amplification of the *mecA* gene (Fig. 1).

Methicillin resistant *S aureus* (MRSA ATCC 43300) and methicillin sensitive *S. aureus* (MSSA ATCC 25293) were used as positive and negative controls, respectively. These strains were provided from Iranian reference health laboratory.

3. Results & Discussion

S. aureus was found in 220 of the 74572 surveillance specimens sent to Milad Hospital's microbiology lab. Out of 220 patients, 56.4% were male and 43.6% female. PCR testing revealed that 105 (47.72%) of the 220 *S. aureus* isolates were positive for the *mecA* gene. Figure 1 illustrates the electrophoresis of PCR product produced from *S. aureus mecA* genes on an agarose gel. The Cefoxitin disc diffusion method showed the highest sensitivity and specificity among phenotypic methods. Table 1 compares the sensitivity and specificity of various phenotypic approaches to the PCR method as a gold standard process for MRSA detection.

Various laboratory methods including phenotypic and genotypic have been introduced for detection of MRSA **(5, 6, 12)**. Although the molecular methods are rapid and have a high sensitivity, but these methods are not achievable in many microbiology laboratories. In contrast phenotypic methods are time consuming but give a comparable sensitivity and lower price. There are different phenotypic methods for detection of MRSA, which are widely used in microbiology laboratories. The main phenotypic methods are based on disk diffusion methods such as using oxacillin and Cefoxitin disks. Other methods such oxacillin screen agar, latex agglutination (Penicillin binding protein 2a) and chromogenic media are also available in some laboratories **(13-15)**.

The oxacillin disc diffusion method is one of the oldest methods for detecting MRSA in clinical microbiology laboratories. Recently studies have shown a lower reliability of oxacillin disk for detection of MRSA and therefore Cefoxitin disk is substituted for oxacilln to detection of MRSA. Cefoxitin disk diffusion method is highly recommended by CLSI and this method is very reliable method with high sensitivities and specificity.

There are several culture-based media for isolation and identification of MRSA. With the advent of chromogenic media, diagnosis of MRSA has undergone a revaluation. Chromogenic medium has a number of advantages for the enumeration, detection, and identification of *S. aureus.* CHROMagar was the first medium which introduced for detection of MRSA. Using of CHROMagar™ leads to significant reductions in detection time and workload, which it necessitated a wide-scale patient screening to determine its clinical utility **(9)**. Many studies have been shown a higher level of sensitivity and specificity in comparison oxacillin containing media. Their results of CHROMagar and cefoxitin disk diffusion method are comparable. In this study both methods have 100% sensitivity and specificity **(9, 10, 16, 17)**. In Brennan *et al.*, study, that was performed in Ireland, the sensitivity of chromogenic agar ranging from 98% to 100% **(10)** which is similar to our study. In another study has been conducted in university of Manitoba by Manickam *et al.*, reported a sensitivity of 98% and a specificity of 100% **(17)**. Because of the changing epidemiology of MRSA, rapid detection of MRSA from clinical specimen is very important issue for infection control and effective patent manage.

Table 1. Comparison of PCR and phenotypic methods for detection of MRSA.

Method	No of MRSA	Sensitivity %	Specificity %
PCR for detection of *mecA* gene	105	100	100
Cefoxitin Disk (30 µg)	105	100	100
CHROMagar™ MRSA	105	100	100

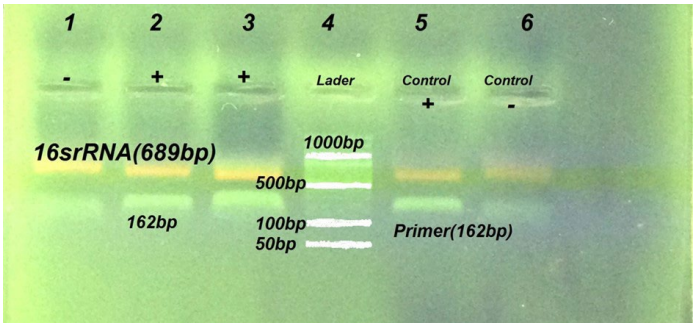

Figure 1. Detection of *mecA* gene by PCR method. MRSA strains isolated from clinical specimens showing in lane 2 and 3 while lane 4 contain 100bp ladder, lane 5 is having positive control (*S. aureus* ATCC 33591) and lane 6 is negative control (S. *aureus* ATCC 29213).

5. Conclusion

Oure study revealed a high sensitivity and specificity for identification of MRSA by CHROMagar which comparable with result of Cefoxitin disk diffusion method. Both methods have 100% sensitivity and specificity in comparison with PCR as a gold standard method for detection of *mecA*.

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Conflict of Interest

The authors have no conflict of interest.

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