

EVALUATION OF MRSA CHROME AGAR FOR THE DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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Abstract

The aim of this study was to evaluate the efficacy of MRSA Chrome agar to detect methicillin resistant *Staphylococcus aureus* (MRSA) and compare it with 1µg oxacillin disc diffusion tests and detection of *mecA* gene by PCR. A total 116 *Staphylococcus aureus* (*S. aureus*), isolated from various clinical samples, were obtained from three tertiary care hospitals of Dhaka city. *S. aureus* was identified by colony characters, Gram stain and standard biochemical procedures. MRSA was detected by susceptibility to 1µg oxacillin disc, growth of denim blue color colonies of *S. aureus* on the Brilliance MRSA Chrome agar at 24 and 48 hours of incubation. PCR was performed for amplification of *mecA* gene as a gold standard method. Out of 116 isolated *S. aureus*, 33 (28.44%) were MRSA by oxacillin disc diffusion test where *mecA* gene was detected in 28 strains. On MRSA Chrome agar, 29 (25.0%) *S. aureus* produced denim blue colonies at 24 hours, of which 28 isolates possessed *mecA* gene. At 48 hours incubation, an additional 4 isolates yielded denim blue colonies from which *mecA* gene could not be identified. All the strains of *S. aureus* that produced denim blue colonies at 24 and 48 hours were resistant to oxacillin. The sensitivity, specificity and accuracy of oxacillin disc diffusion test were 100%, 94.31% and 95.68% and Chrome agar at 24 hours were 100%, 98.86% and 99.13% respectively. Thus MRSA Chrome agar could be good choice in clinical microbiology laboratory for rapid and accurate identification of MRSA.

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**Key words:** MRSA chrome agar, media, detection, *Staph Aureus*

Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections worldwide. Methicillin resistance in *Staphylococcus aureus* (*S. aureus*) is mediated by the presence of *mecA* gene that is found on a characteristics piece of flanking DNA, which is termed as staphylococcal chromosomal cassette *mec*.<sup>1</sup> The *mecA* gene confers methicillin resistance by encoding penicillin binding protein 2a (PBP2a), an enzyme with decreased affinity for β-lactam antibiotics. The detection of methicillin resistance in *S. aureus* is complicated by the fact that phenotypic expression in many strains is heterogeneous.<sup>2</sup> In the routine laboratories, different types of phenotypic methods are used in detecting

methicillin resistance of which 1 µg oxacillin disc diffusion test is a commonly employed one. Although oxacillin disc diffusion test in Mueller-Hinton agar (MHA) has a high sensitivity (90-100%) and specificity (85-100%) for detection of methicillin resistance in *S. aureus* isolates, it is time consuming and the results may be affected by various components of MHA, temperature and duration of incubation.<sup>3</sup>

The methods currently used for screening of MRSA include standard culture and susceptibility to oxacillin, chromogenic media and molecular based testing. FDA approved PCR assays have been proven to be an excellent method for a rapid detection of MRSA. But the cost is very high as it requires expensive equipments

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and highly trained personnel. In recent years, the chromogenic media have been introduced for the rapid detection of *S. aureus* in clinical samples.<sup>4</sup> This media detects key enzyme as diagnostic marker for MRSA by the use of “chromogenic” substrates incorporated into a solid agar based matrix and antibiotics for selective growth of MRSA.<sup>4</sup> In contrast to conventional culture media, chromogenic media allow direct colony color-based identification of the MRSA from the primary culture. Therefore, the total turnaround time for the detection of MRSA is reduced to 1.4-1.7 days by eliminating the need for further biochemical testing.

Therefore, the present study was undertaken to evaluate the efficacy of chromogenic agar in detecting MRSA compared to 1 $\mu$ g oxacillin disc diffusion test.

## Materials and Methods

### Samples

A total 116 *S. aureus* isolates were obtained from Mitford, BIRDEM and National Medical College hospitals of Dhaka city during the period between January to December 2011. The organisms were isolated from different clinical samples namely pus, wound swab, blood, urine and throat swab. *S. aureus* was identified by colony characters, Gram stain and standard biochemical procedures. For detection of MRSA all isolated *S. aureus* were subjected to 1  $\mu$ g oxacillin disc diffusion test, culture in MRSA Chrome agar and detection of *mecA* gene by PCR. Reference strain of *S. aureus* ‘MR-108’ was used as a positive control in all the above tests and was obtained from Sapporo Medical University, Japan.

### Oxacillin Disc Diffusion Method

A 0.5 McFarland standard suspension of the isolated *S. aureus* was prepared. Mueller Hinton agar (supplemented with 4% NaCl) was inoculated with 0.5 McFarland suspension of the isolate which was spread evenly by rotating the plate approximately 60° for three times to get a uniform distribution of inoculums. Oxacillin (1 $\mu$ g) disc was placed and incubated at 35°C for 24 hours. The diameter of the inhibition zones was measured as per recommendations of the National Committee for Clinical Laboratory Standards (NCCLS).<sup>5</sup> An inhibition zone diameter of  $\leq$ 13 mm was considered as resistant.

### Culture on to Brilliance MRSA Chrome Agar (Oxoid, UK)

Brilliance MRSA Chrome agar (Oxoid, UK) was inoculated with 0.5 McFarland suspension of the isolate and incubated at 35°C. The plates were read after 24 and 48 h of incubation. The growth of denim blue colored colonies indicates presence of MRSA.

### Polymerase Chain Reaction (PCR) for identification of *mecA* gene

This method was done according to Ercis *et al.*<sup>6</sup> For extraction of DNA, rapid lysis procedure was done, colonies were obtained from fresh subculture mixed with 300  $\mu$ l of distilled water and bacterial suspension was heated at 95°C for 10 minutes and then quickly placed on ice. This suspension was then microcentrifuged at 14000 rpm for 10 minutes. Supernatant was used as template DNA. Primer Sequences used in this study were:

Mec-A1 (+) 5' AAAATCGATGGTAAAGGTTGGC-3' and

Mec-A2 (-) 5' AGTTCTGCAGTACCGGATTTGC-3'

PCR were performed in a thermal cycler (Eppendorf) by using supermix (Promega, USA) following the manufacturer's recommendations. 12.5  $\mu$ l super mix and 2 $\mu$ l template DNA were taken to each PCR reaction tube. 8.5  $\mu$ l PCR water and 1.0  $\mu$ l *mecA*1, 1.0  $\mu$ l *mecA*2 primer was added to each tube. So the final volume in each tube was 25  $\mu$ l. The following amplification protocol was used: initial denaturation for 3 minutes at 94°C, then final denaturation continued for 40 cycles of 30s at 94°C, 45s at 60°C, annealing at 60°C for 1.5 minutes. Extension of primer was done at 72°C for 3.5 minutes with a total of 40 cycles.

The amplified PCR products were detected by 0.8% agarose gel with ethidium bromide dye under UV transilluminator. A 533-bp DNA fragment indicated presence of target *mecA* gene and photographed under UV illumination.

### Determination of sensitivity and specificity

The sensitivity and specificity of Chrom Agar and Oxacillin disc diffusion test were determined by the standard formula as described elsewhere.<sup>7</sup>

## Results

Among 116 *S. aureus* isolates, 33 (28.44%) were oxacillin resistant in which *mecA* gene was found in

**Table-1:** Detection of MRSA by oxacillin disc diffusion test and MRSA Chrome agar compared to presence of *mecA* gene by PCR (N=116)

| Method                         | Number (%) | <i>mecA</i> gene |              |
|--------------------------------|------------|------------------|--------------|
|                                |            | Present n (%)    | Absent n (%) |
| Oxacillin disc diffusion test* |            |                  |              |
| Resistant                      | 33(28.44)  | 28(84.84)        | 5(15.16)     |
| Sensitive                      | 83(71.56)  | 0                | 83 (100)     |
| MRSA Chrome agar:              |            |                  |              |
| Growth at 24 hrs               |            |                  |              |
| Present                        | 29(25.00)  | 28(96.56)        | 1(3.44)      |
| Absent                         | 87(75.00)  | 0                | 87(100)      |
| Growth at 48 hrs               |            |                  |              |
| Present                        | 33(28.34)  | 28(84.85)        | 5(15.15)     |
| Absent                         | 83(71.66)  | 0                | 83(100)      |

Note: \* After 24 hours of incubation

28 (84.84%) strains while in 5 isolates *mecA* gene could not be detected. The *mecA* gene was not detected in 83 oxacillin sensitive strains of *S. aureus*. In Chrome agar media, 29 (25%) isolates were positive for MRSA by yielding denim blue colonies at 24 hours, of which 28 (96.56%) isolates carried *mecA* gene. At 48 hours, an additional 4 isolates became Chrome agar positive. But all of them were for *mecA* gene by PCR. The detailed result is shown in Table-1. In this study, MRSA Chrome agar showed the highest sensitivity (100%), specificity (98.86%) and accuracy (99.13%) when incubated for 24 hrs. But the specificity reduced to 94.31 when the culture plates were incubated further up to 48 hours. Compared to MRSA Chrome agar the

**Table-2:** Sensitivity and specificity of MRSA Chrome Agar and oxacillin disc diffusion test for the detection of MRSA

| Method                    | Sensitivity (%) | Specificity (%) | Accuracy (%) |
|---------------------------|-----------------|-----------------|--------------|
| MRSA Chrome agar          |                 |                 |              |
| a. after 24 h incubation  | 100             | 98.86           | 99.13        |
| b. 48 h after incubation  | 100             | 94.31           | 95.68        |
| Oxacillin disc diffusion* | 100             | 94.31           | 95.68        |

Note: \* After 24 hrs incubation

specificity and accuracy of oxacillin disc diffusion test were 94.31% and 95.68% respectively (Table-2).

### Discussion

Methicillin resistant *S. aureus* was first identified in 1961, since then MRSA has been increasingly reported in both hospital and community settings.<sup>8</sup> Rapid and accurate identification of MRSA is important for effective patient care and prevention of its further spread. The present study evaluated the MRSA Chrome agar for rapid detection of MRSA. In this study, detection of MRSA was done by oxacillin disc diffusion test, culture on MRSA Chrome agar (Brilliance MRSA agar, Oxoid, UK) and detection of *mecA* gene by PCR. Detection of *mecA* gene by PCR was considered as a gold standard method for MRSA confirmation.<sup>9</sup> MRSA Chrome agar is a selective chromogenic medium, which is more reliable and faster for screening of MRSA than oxacillin disc diffusion method and also less costly compared to the PCR.<sup>10</sup> Different studies have reported the sensitivity and specificity of MRSA Chrome agar as 90% to 100%.<sup>4</sup> It is easy to perform and the interpretation of results does not need any expertise.

In the present study, out of 116 isolated *S. aureus*, 33 (28.44%) were oxacillin resistant, of which 28 were detected as MRSA by polymerase chain reaction (PCR). In five oxacillin resistant isolates, *mecA* gene was not detected. It might be possible that these strains were hyper beta-lactamase producer thereby they were accounting for non-*mecA* mediated oxacillin resistance or they had borderline resistance to methicillin.<sup>11</sup> On the other hand, these five isolates could represent false positive resistance to oxacillin. Therefore, the specificity of oxacillin disc diffusion test became 94.31%. This result is in agreement with Mimica *et al* (2007) who found the specificity of oxacillin disc method as 97.7%.<sup>12</sup>

On MRSA Chrome agar, 29 (25.00%) strains of *S. aureus* produced denim blue colonies at 24 hours indicating MRSA. Additional four strains produced denim blue colonies at 48 hours. So at 48 hours, a total of 33 (28.34%) isolates were MRSA Chrome agar positive. All of these strains were resistant to oxacillin. Out of 29 positive strains at 24 hour, *mecA* genes were detected in 28 (96.56%) strains. No *mecA* gene was found in the additional 4 isolates that produced denim blue colonies at 48 hours. The sensitivity,

specificity and accuracy of MRSA Chrome agar at 24 hours were 100%, 98.86% and 99.13% respectively, which was better than the sensitivity (100%) and specificity (94.31%) at 48 hours (Table-2). This finding was similar to the results of Peterson *et al.* (2010) who reported the sensitivity and specificity at 24 hours 96% and 99.6% and at 48 hours 96% and 95.2% respectively.<sup>13</sup> Almost similar result was reported by Kristien *et al.* (2010), in which the sensitivity and specificity were 95% and 97.4% at 24 hours and 98.9% and 89.4% at 48 hours respectively.<sup>14</sup> The remaining false positive results could be due to an inherent limitation of any subjective test. The result of MRSA Chrome agar permitted sensitive and specific detection of MRSA at 24 hours which was more specific than that achieved at 48 hours and of oxacillin disc diffusion test. Methicillin sensitive *S. aureus* was not grown on the chromogenic media as it contained combination of antibacterial compounds.<sup>14</sup> MRSA can be detected within 24 hour when specimens are inoculated directly on to it, which is a one day improvement in turnaround time compared to oxacillin disc diffusion tests which usually needs 2 to 3 days to detect MRSA and the specificity is also less. Gene detection by conventional PCR requires labor, time, costs and special set up.

Thus, rapid and accurate detection of MRSA by Chrome agar is an effective method than that of oxacillin disc diffusion test and can be an alternative to PCR.

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