Evaluation of Oxacillin Resistant Screening Agar for Rapid Conventional Screening of Methicillin Resistant Staphylococcus Aureus in Infection Control Unit

Abdalla A. El-Bialy, and Ayman A. Allam
Microbiology and Immunology Department, Zagazig Faculty of Medicine

ABSTRACT

Rapid assessment of clinical specimens for the presence of methicillin resistant Staphylococcus aureus (MRSA) is an important part of the infection control measures taken to control the spread of MRSA and thus, to decrease hospitalization costs. A novel medium, Oxacillin resistant screening Agar (ORSA) medium, was evaluated for the screening of specimens for MRSA in our infection control unit. Swabs of the nose, throat, perineum, and other infected sites (1527) were inoculated onto the ORSA medium and into an enrichment broth (Muller- Hinton broth supplemented with Na Cl and oxacillin{OX-MH broth}). After 24h of incubation, the enrichment broth was sub-cultured onto one ORSA plate and one lipovitellin chapman salt agar plate. All MRSA isolates were confirmed by PCR amplifying MecA gene. Out of 1527 specimens, 242 (16%) were positive for MRSA. A sensitivity of (70%) was obtained when ORSA medium was used alone as a primary culture, whereas the sensitivity was 88% when a single selective enrichment was used before subculture on ORSA. Among the 414 blue colonies observed on ORSA plates, only 48% were found to be MRSA, 42% were coagulase – negative staphylococci, 4.4% were Enterococcus species, and 3.3% were methicillin sensitive S. aureus. Among 43 MRSA colonies, 21 (49%) were visible only after 48 h of incubation. However, when ORSA plate was used for subculture, 88 % of blue colonies were detected after only 24 h of incubation. In conclusion, the ORSA is suitable for screening of MRSA in a simple laboratory of infection control unit with the advantage of the ease of recognition of mannitol fermenting bacteria. An enrichment broth is still needed to ensure a good sensitivity for the recovery of MRSA, and incubation time of 48 h is required for primary culture on ORSA medium.

INTRODUCTION

Since the first report of methicillin resistant Staphylococcus aureus as a major nosocomial pathogen in 1960s, the incidence of infections caused by this organism continues to increase worldwide (14). Rapid assessment of clinical specimens for the presence of MRSA is an important part of the infection control measures taken to control the spread of MRSA and, thus, to decrease hospitalization costs. In hospitals with low rates of MRSA, it is probably important to detect each patient colonized or infected with MRSA(15). Thus, the negative predictive value of the screening test should be high. PCR- based methods for the direct detection of MRSA in screening specimens have been developed (4, 8&10) but these methods remain expensive and cannot be done in simple laboratories. Evaluation of results of these molecular techniques, as well as their costs and benefits should be undertaken (14). Thus, new screening media for the improved detection of MRSA have been developed (3,11,16 &18). A medium with aniline blue to detect mannitol fermentation in Staphylococci, the Oxacillin Resistant Screening Agar (ORSA), was recently used for the screening of specimens for MRSA in a hospital setting(19). Therefore, this work was carried out to evaluate (i) the sensitivity of the procedure for the recovery of MRSA from patient specimens by using ORSA base medium alone as a primary culture medium or as an enrichment broth medium for subculture, (ii) the proportion of samples that do not contain MRSA but that show the growth of blue colonies on ORSA medium and (iii) the optimal incubation time.

MATERIAL & METHODS

This work was conducted in the infection control unit, Zagazig Faculty of Medicine, during the period from Mars 2008 to December 2009.

Specimens:

Screening samples were obtained from the following individuals: (i) rehospitalized patients known to have been positive for MRSA during a previous hospitalization in Zagazig University Hospitals, (ii) patients transferred from a foreign hospital, (iii) the contact of a patient known to be infected or colonized with MRSA infection, (iv) staff members and/or patients from a ward
with a suspected cluster of cases of MRSA infection, and (v) patients screened after treatment for MRSA eradication. The screening samples obtained from each patient included swabs (sterile transport swab; Copan, Brescia, Italy) of the nose, throat, perineum, and all infected sites. The nose, throat and perineal swabs from all patients with a negative or unknown history of MRSA infection or colonization were pooled for each analysis.

**Bacteriological Methods:**

Each specimen was inoculated onto two primary culture media: an ORSA base medium plate composed of (per liter) peptone (11.8 g), yeast extract (9.0 g), mannitol (10.0 g), NaCl (55.0 g), lithium chloride (5.0 g), aniline blue (0.2 g) and supplemented with 100,000 IU of polymyxin B and 2.0 mg of oxacillin (Oxoid limited) and Muller Hinton broth composed of (per liter) beef extract (3.0 g), acid hydrolysate of casein (17.5 g), and starch (1.5 g) (BBL Becton Dickinson, Heidelberg, Germany) supplemented with 6 mg of oxacillin per liter and 45 g of Na Cl per liter (OX-MH broth). After 24 h incubation, the OX-MH broth was sub-cultured on one ORSA plate and one lipovitellin salt agar (lipovitellin salt mannitol [LSM]) plate composed of (per liter) pancreatic digest of casein (5.0 g), peptic digest of animal tissue (5.0 g), beef extract (1.0 g), NaCl (75.0 g), D-mannitol (10.0 g), phenol red (25 mg), and agar (15.0 g) (BBL Becton Dickinson) supplemented with 20 g of egg yolk per liter (12). All agar plates were incubated aerobically for 48 h at 35 °C and were read every day.

Each morphotype of mannitol-fermenting colonies was sub-cultured onto a blood agar plate and identified by conventional biochemical tests, including Gram staining, and catalase test, DNase and latex agglutination (Postrex Staph Plus; Bio-Rad, Redmond, Wash) tests. Resistance to methicillin was determined by the disc diffusion method and the oxacillin agar screen test according to the guidelines of the National Committee for Clinical Laboratory Standards (9). The MRSA screen (Denka-Seiken, Tokyo, Japan) was also used on several occasions to confirm the resistance. Species identification of isolates other than S. aureus was performed with ID 32 Strep test (Biomerieux).

**PCR**

All MRSA strains isolated were confirmed by PCR amplification of mecA gene.

**DNA extraction:**

Genomic DNA was extracted after a 10-min treatment at 37°C in TE containing 100 µg/ml of lysostaphin (Ambicin; Applied Microbiology, Tarzeytown, N.Y.). DNA concentration and purity were assessed by spectrophotometry (15).

**Amplification:**

Primers (Pharmacia Biotech) used for detection of the mecA gene were MecA1 (5’ GTA GAA ATG ACT GAA CGT CCG ATA A‘3) and MecA2 (5’ CCA ATT CCA CAT TGT TTC GGT CTA A‘3), yielding a 310-bp amplicon (6). PCR was performed in a 25-µl volume with 1 µl PCR buffer (Roche Molecular Systems), 3 mM MgCl2, a 200 µM concentration of each deoxynucleoside triphosphate, 2.5 U of Taq polymerase (Roche), 0.5 µM mecA primers with 1 µl of template DNA.

The PCR cycling conditions were as follows: initial denaturation at 94°C for 4 min, followed by 30 cycles of 45 s at 94°C, 45 s at 50°C, and 60 s at 72°C, with a final extension step at 72°C for 2 min. Ten-microliter aliquots were loaded onto agarose gel electrophoresis gels (1% agarose, Tris-buffered EDTA; 90 V for 90 min) and stained with 10 µg of ethidium bromide/ml after electrophoresis.

**RESULTS**

In order to optimize the procedure for the detection of MRSA, the sensitivity of each medium alone or in combination was analyzed. From Mars 2008 to December 2009, 1527 specimens were analyzed. These specimens were obtained from 466 patients or staff members. Among these specimens, 242 (16%) were positive for MRSA confirmed by PCR. This served as reference value to define a sensitivity of 100%. The sensitivity for the detection of MRSA was calculated for each medium alone or the two media in combination (Table 1). A low sensitivity (70%) was obtained when ORSA medium was used alone as a primary culture. The sensitivity increased with the number of media included in the procedure. Primary plating on ORSA and OX-MH broth enrichment followed by subculture on ORSA gave sensitivity of 96% and the sensitivity increased to 100% when subculture was done on ORSA and LSM plates.
Table (1): Sensitivities for the detection of MRSA in each medium alone or in combination

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. with MRSA*</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORSA plate</td>
<td>169</td>
<td>70</td>
</tr>
<tr>
<td>OX-MH broth + ORSA plate</td>
<td>201</td>
<td>83</td>
</tr>
<tr>
<td>OX-MH broth + LSM plate</td>
<td>211</td>
<td>87</td>
</tr>
<tr>
<td>OX-MH broth + ORSA and LSM plates</td>
<td>218</td>
<td>90</td>
</tr>
<tr>
<td>ORSA and OX-MH broth + ORSA plate</td>
<td>232</td>
<td>96</td>
</tr>
<tr>
<td>ORSA and OX-MH broth + ORSA and LSM plates</td>
<td>242</td>
<td>100</td>
</tr>
</tbody>
</table>

*The number of specimens found to be positive for MRSA among the 242 MRSA-positive specimens.

All MRSA strains isolated were confirmed by PCR amplification of mec A gene. The amplified product was detected at 310 bp (Fig. 1).

In order to evaluate the proportion of the blue colonies growing on ORSA plates that were not MRSA, we analyzed 311 specimens for which complete data were available after 24 h and 48 h of incubation. They were obtained from 117 patients or staff members. Blue colonies grew from 88 of these specimens. Among the 91 blue colonies, 48.4% were found to be MRSA, 41.8 were coagulase negative staphylococci, 3.3% were methicillin sensitive S. aureus and 4.4% were Enterococcus species (Table 2).

![Fig.(1): Lane 1,3 &4 show 310-bp band (Positive). Lane 2 shows no band (negative)](image)

Table (2): Distribution of the blue colonies on ORSA plate at 48 hr of incubation (primary culture and subcultures) by species and sampling site

<table>
<thead>
<tr>
<th>Sample(S)</th>
<th>No.(1%) of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Nose, Throat and perineal swabs</td>
<td>124</td>
</tr>
<tr>
<td>Nose swab</td>
<td>37</td>
</tr>
<tr>
<td>Throat swab</td>
<td>37</td>
</tr>
<tr>
<td>Perineal swab</td>
<td>36</td>
</tr>
<tr>
<td>Wound Swab</td>
<td>25</td>
</tr>
<tr>
<td>Urine</td>
<td>42</td>
</tr>
<tr>
<td>Others</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>311</td>
</tr>
</tbody>
</table>

MSSA = Methicillin susciptable S. aureus, CoNS = Coagulase negative staphylococci.
The optimal incubation time for the ORSA plates was evaluated with the data for the 43 MRSA colonies. Among these colonies, 21 (49%) were visible only after 48 h of incubation (no blue colonies were observed after 24 h of incubation). However, when ORSA plate was used for subculture, 88% of blue colonies were detected after only 24 h of incubation.

DISCUSSION

Although many different microbiologic media and techniques have been studied for the recovery of MRSA from clinical specimens, no consensus exists as to the most sensitive and accurate method (14).

MRSA is a multi-resistant organism so the use of a laboratory procedure that is sensitive for the detection of MRSA from patient specimens is crucial to limit its spread. The use of an enrichment and selective broth for MRSA detection is known to increase sensitivity but requires an extra day of incubation (14&17). So the main drawback of this method, which has a high sensitivity, is prolonged delay between the time of specimen collection and the time that results are available (a minimum of 3 days).

Our results showed that a low sensitivity (70%) was obtained when ORSA medium was used alone as a primary culture. A sensitivity of 74% was reported in the study of Blanc et al in Switzerland (1) while higher sensitivities (90% & 91.5%) was reported in studies done in India (11) and Japan respectively (7). These differences can be attributed to differences in the type of specimens used.

Our study showed that the sensitivity increased with the number of media included in the procedures so one can not avoid the use of an enrichment broth without a substantial loss of sensitivity. Thus, ORSA plates and enrichment should be used for primary culture. Prior studies have reported enhanced sensitivity and additional yield of 14 to 25% for detection of MRSA with pre-enrichment of the specimen in salt containing media (5&14).

An advantage of the ORSA is the easy detection of mannitol fermenting colonies (The appearance of a blue colour). In our study, 48% of the blue colonies were MRSA and additional methods for identification should be used to confirm MRSA identification. This proportion agrees with other study done in Switzerland (3) but disagrees than that reported in another study (74%) (10). This difference can be attributed to different number and nature of specimens investigated (from colonized sites {nose and perineum} or not). Colonization with other staphylococcal species might lead to false positive results (4).

In this study, 21 (49%) out of 43 MRSA colonies were visible only after 48 h of incubation. This result agrees with previous studies (15&1) which reported that very few cultures showed MRSA positivity at 24 h (5, 1). However when ORSA plate was used for subculture, 88% of blue colonies were detected after only 24 h of incubation, probably because the bacteria are already in the growth phase in the broth (9).

These results allowed us to optimize our procedure for the detection of MRSA in screening specimens. The major advantage of this procedure is the reduced amount of time for the detection of MRSA (2 days instead of 3 days) because the ORSA subculture is incubated for only 24 h. The ORSA medium also has the advantage of easy interpretation, especially for specimens other than perineal swabs as most of the non-MRSA blue colonies were observed in these specimens.

In conclusion, the main advantage of the ORSA medium is the ease of recognition of mannitol fermenting bacteria. However, further identification tests are needed to confirm the identification of S. aureus since half of the blue colonies were not S. aureus. Moreover, an enrichment broth is still needed to ensure a good sensitivity of method for the recovery of MRSA, and incubation time of 48 h is required for the primary culture. Thus, the procedure still requires a minimum of 2 days before final results can be obtained.

REFERENCES


تقييم استخدام الأجار الماسح المقاوم للأوكساسيلين للمسح التقليدي السريع للميكروبات العنقودية الذهبية المقاومة للميسيلين في وحدة مكافحة العدوى

عبد الله البيلي و أيمن علام
قسم الميكروبيولوجي و المناعة - كلية الطب البشري - جامعة الزقاق.

لا شك أن التشخيص السريع لوجود الميكروبات العنقودية الذهبية المقاومة للميسيلين في العينات الأوليكبانية هو جزء مهم من إجراءات مكافحة العدوى المتبعة لمقاومة الميكروبات العنقودية الذهبية المقاومة للميسيلين، و ذلك نظراً لتقليل تأخير العلاج المناسب. أجريت هذه الدراسة بغرض تقييم الأجار الماسح المقاوم للأوكساسيلين (ORSA) فيما يتعلق بوجود الميكروبات العنقودية الذهبية المقاومة للميسيلين في وحدة مكافحة العدوى. تم زراعة 252 عينة من الأنف والحلق ومنطقة ما حول المريض وعنواح أخرى على الأجار الماسح المقاوم (enrichment broth) للأوكساسيلين، وسط المرقة المحمصة (OXMH broth) و بعد 48 ساعة من الحضانة تم زراعة وسط المرقة المحمصة على طبق الأجار الماسح المقاوم للأوكساسيلين و طبق أجار ليويفتالي شامان المغذي وقد تم التأكد من كل معاوزات الميكروبات العنقودية الذهبية المقاومة للميسيلين بعمل اختبار تفاعل البلمرة المتمثلي.

وقد بينت نتائج الدراسة أن نسبة الميكروبات العنقودية الذهبية المزودة المقاومة للميسيلين كانت 16%. وكانت حساسية الأجار الماسح المقاوم للأوكساسيلين 79% عند استخدامه كوسط مسح وسيلة الحصانة إلى 88% عند زراعة العينات مباشرة على وسط المرقة المحمصة. وأوضحت الدراسة أيضاً أن 44% من المستعمرات الزائدة في مستعمرات الميكروبات العنقودية الذهبية المقاومة (MRSA) كانت 2% من مستعمرات الميكروبات العنقودية الذهبية سالبة كاوجيلوز، و 64% كانت من سلالة Enterococcus. و 3% كانت ميكروبات عنقودية ذهبية حساسية للميسيلين. وقد تم تأسيس 49% من مستعمرات الميكروبات العنقودية الذهبية المقاومة للميسيلين على طبق الأجار الماسح المقاوم للأوكساسيلين بعد 48 ساعة عند استخدامه كوسط مسح وسيلة الحصانة. بينما بينت نتائج الدراسة أن نسبة الميكروبات العنقودية الذهبية المقاومة للميسيلين على طبق الأجار الماسح المقاوم للأوكساسيلين عند زراعة العينات مباشرة على وسط المرقة المحمصة 88%.

وانتهت الدراسة إلى أن الأجار الماسح المقاوم للأوكساسيلين (ORSA) حالياً كوسط مسح مسحي للعينات وجدته نتائج مثالية وعملية لتفحص العينات المختارة. الميكروبات العنقودية الذهبية المقاومة للميسيلين (MRSA) في وحدة مكافحة العدوى وتسمح للعازب المتضرر على البيكربيا المخبرة (enrichment broth) للعلاج، ولا زال هناك احتياج للزمن لاجتماع الميكروبات العنقودية الذهبية المقاومة للميسيلين لتطابق الأجار الماسح المقاوم للأوكساسيلين (ORSA) عند استخدامه كوسط مسح مسحي لعينات المرضى.