

Evaluation of a New CHROMagar Medium for Detection of Methicillin-Resistant *Staphylococcus aureus*

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Abstract: Four laboratory methods including, E-test MIC, Oxacillin screen agar, manitol salt agar plus oxacillin and CHROMagar MRSA were compared for detection of methicillin resistant *Staphylococcus aureus* (MRSA). CHROMagar MRSA had a high sensitivity and specificity comparing to conventional E-test method. Sensitivity and specificity by manitol salt agar was 95 and 100%, respectively, while oxacillin screening agar method had 100% sensitivity and 95% specificity.

Key words: Methicillin resistant *Staphylococcus aureus*, CHROMagar MRSA

INTRODUCTION

Methicillin-Resistant *Staphylococcus aureus* (MRSA) has emerged worldwide as a nosocomial pathogen of major importance and the incidence of infections caused by MRSA continues to increase (Archer, 1998; Diedem *et al.*, 2006; Voss and Doebbelin, 1995). According to the latest report from the National Nosocomial Infection Control Surveillance system approximately 60% of all *S. aureus* nosocomial infections in intensive care units were methicillin resistant in 2003, representing an 11% increase in resistance compared to the preceding 5 years period (Flayhart *et al.*, 2005). Infections caused by MRSA strains are associated with longer hospital stay, days of antibiotic administration and higher costs than infections caused by Methicillin-Susceptible *Staphylococcus aureus* (MSSA) strains. More importantly, several studies and one large meta-analysis have shown that patients who develop MRSA bacteremia have a higher mortality than patients with MSSA infections after adjusting for underlying severity of illness. (Flayhart *et al.*, 2005). There are many different laboratory methods for detection of MRSA (Stoakes *et al.*, 2006). Methods used to detect MRSA in clinical samples ideally should have high sensitivity and specificity and should report the results within a short time.

The purpose of this study was to evaluate the *in vitro* sensitivity and specificity of recently developed medium called CHROMagar, which we used a well-defined collection of strains to identify MRSA.

MATERIALS AND METHODS

A collection of 97 well defined isolates of *S. aureus* including 58 methicillin resistant and 39 susceptible strains has been tested in present study. All isolates were from Milad hospital of Tehran having unique typing patterns. Susceptibility testing was performed by E-test MIC method as recommended by the manufacturer (AB Bio Disk Sweden). Two other methods included Oxacillin screen agar (Muller-Hinton agar supplemented with 4% NaCl and 6 $\mu\text{g mL}^{-1}$ oxacillin (Anonymous, 1997) and Mannitol salt agar supplemented with 6 $\mu\text{g mL}^{-1}$ oxacillin and CHROMagarTM MRSA. All isolates were first inoculated on Sheep blood agar and incubated for 24 h at 35°C. A suspension was prepared at 0.5 MacFarland of which 10 μL was streaked on each above mentioned media. All plates were incubated at 35°C for 24 h. Strains yielding colonies on Oxacillin screen agar and Mannitol salt agar were considered as MRSA since they had been previously also characterized as *S. aureus* by complementary tests. Strains growing on CHROMagarTM MRSA with rose to mauve colour colonies were considered as MRSA as recommend by manufacture Fig. 1.

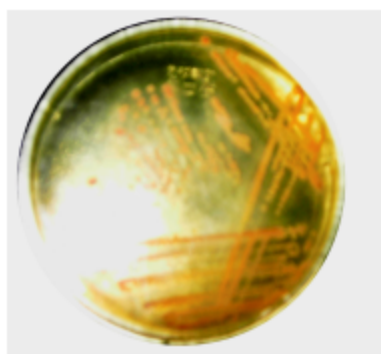


Fig. 1: Colonies of MRSA on CHROMagar

RESULTS AND DISCUSSION

In our evaluation by E-test MIC method, 57 isolates were MRSA and 39 isolates were MSSA while, in Oxacillin screen agar method we had 2 false positive MRSA. By using Mannitol salt agar method 2 strains of MRSA could not be identified. The results of this method were equivalent to E-test with our strains previously characterized as *S. aureus*. We found excellent results by using CHROMagar™ MRSA: sensitivity and specificity for this method was 100%. Sensitivity and specificity by mannitol salt agar was 95 and 100%, respectively, while oxacillin screening agar method had 100% sensitivity and 95% specificity.

A wide range of techniques has been used to detect and identify MRSA from clinical specimens. Selective and differential culture media, especially Mannitol Salt Agar (MSA) supplemented with oxacillin, are most widely employed (Table 1). However all MSA media are not the same. They differ in their salt and antibiotic contents and their performance may not be comparable (Stoakes *et al.*, 2006).

Other studies have shown the clinical performance of chromogenic agar for detection of MRSA. Merlino *et al.* (1997) studied a sample of 136 staphylococcus strains including 114 *S. aureus* and 22 coagulase-negative staphylococci. They compared chromogenic media with conventional mannitol salt agar by using DNase agar plates and tube coagulase test. The CHROMagar Staph aureus medium was supplemented with methicillin at a concentration of 4 mgL⁻¹. None of MSSA strains grew on the plates and all 36 highly resistant MRSA strains grew on the plates and all 36 highly resistant MRSA strains grew on the plates. They also tested 12 community-acquired MRSA strains. Only four of these 12 strains (33.3%) grew on methicillin-supplemented CHROMagar Staph aureus (Simor *et al.*, 2001), compared Oxacillin Resistance Screening Agar Base (ORSAB), supplemented

Table 1: Comparison of different methods for identification of MRSA

Methods	MRSA	MSSA
E-test	58	39
Chromagar MRSA	58	39
Mannitol salt agar plus oxacillin	55	42
Oxacillin screening agar	60	37

with oxacillin, with a conventional mannitol salt agar plate (MSA), supplemented with 2.0 mg of oxacillin per liter, for the detection of MRSA in clinical specimens. When specimens from patients at high risk for MRSA colonization were screened with ORSAB, 102 of 104 MRSA-positive clinical specimens (98%) were correctly identified. In total, 138 clinical specimens yielded blue, mannitol-fermenting colony types; therefore, the positive predictive value of ORSAB-positive specimens for MRSA was only 74%. In a study by Perry *et al.* (2004), CHROMagar MRSA appeared to be inferior to all other media for the isolation of MRSA from perineal swabs, possibly due to the impact of the competing flora. In a study by Taguchi *et al.* (2004) they compared three different media including MSA with oxacillin, ORSAB and CHROMagar MRSA for detection of MRSA. In their studies CHROMagar MRSA achieved 100% specificity and sensitivity. They examined 57 MRSA isolates holding the DNA of *mecA* gene and 43 isolates were found to be MSSA. CHROMagar MRSA was successful in detecting all 57 isolates of MRSA. On the contrary, with MSA and ORSAB, 4 isolates from different patients were not detected in both MSA and ORSAB media.

The present evaluation is different from the local studies using consecutive clinical MRSA isolates in an area of endemicity, where only a limited number of clonal types are tested. This circumstance leads to over- or underestimation of the true value of test under evaluation. In the present study, a specific type was included only once and many different strains were tested. This study provides results that represent a broad array of strains.

However, this study has some important limitations. First, a high inoculum of MSSA isolates in pure culture was used, in contrast to the low concentration usually present in clinical samples. Second, the presence of coagulase-negative staphylococci or the influence of other species was not evaluated. This may influence both sensitivity and specificity when CHROMagar MRSA medium is used on clinical samples.

CONCLUSION

Present study revealed that CHROMagar MRSA is highly sensitive and specific for detection of MRSA. It is recommended to use this culture media routinely in microbiology laboratories as an alternative method for detection of MRSA.

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