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Research Article

PERFORMANCE OF CHROM AGAR MEDIUM AND CONVENTIONAL METHODS FOR DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for hospital and community acquired infections. There are many laboratory methods for detection of MRSA. Chromogenic media have been used for the last few years for the quick detection of MRSA. The aim of this study was to compare the performance of conventional methods and chromogenic media for the detection of MRSA in a tertiary care hospital.

Methods: A total of 200 consecutive isolates of *S. aureus* confirmed by conventional methods, collected in a tertiary care hospital, were used for this study. Cefoxitin and oxacillin disc diffusion test used as conventional methods and chromogenic media, i.e., oxacillin resistant screen agar base (ORSAB) was used for the detection of MRSA. All confirmed MRSA were checked by gold standard *mecA* base polymerase chain reaction (PCR) method.

Results: Out of 200 isolates of *S. aureus,* 50, 52 and 47 strains were MRSA by cefoxitin disc diffusion method, oxacillin disc diffusion method and ORSAB method, respectively. Specificity was 100%, 98.66%, 98.66% by cefoxitin disc diffusion, oxacillin disc diffusion, and ORSAB method, respectively.

Conclusion: In conclusion, cefoxitin disc diffusion was the best for the phenotypic detection of MRSA because their sensitivity and specificity were better than oxacillin and ORSAB.

Keywords: Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus, mecA, Chromogenic media, Oxacillin resistant screen agar base, Specificity.

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INTRODUCTION

Staphylococcus aureus is the most common causes of nosocomial or community-based infections, leading to serious illnesses with high rates of morbidity and mortality. Methicillin-resistant *S. aureus* (MRSA) is a major pathogen causing bacteremia, pneumonia, and soft-tissue infections that result in significant morbidity, mortality, and longer hospital stays [1]. MRSA has become a serious clinical and epidemiological problem not only because this antibiotic is considered as the first option in the treatment of staphylococci infections but also resistance to this antibiotic implies resistance to all β-lactam antibiotics [2,3]. Rapid identification of MRSA from clinical specimens and screening of high-risk patients for MRSA colonization have been found to be cost-effective measures for limiting the spread of the organism in hospitals [4,5]. This global spread of MRSA constitutes one of the most serious contemporary challenges to the treatment of hospital-acquired infections [6].

There are many laboratory methods for the detection of MRSA. Chromogenic media have been used for the last few years for the quick detection of micro-organisms from clinical specimens [7]. These media contain chromogenic substrate which is integrated into a solid agar-based medium. This substrate detects specific enzymes produced by the micro-organisms which are the identification markers for micro-organisms [8]. Therefore, in contrast to other conventional methods, chromogenic media identify the pathogen by direct colony color from the first culture. This is a time-saving method which minimizes further sub-culturing for further biochemical testing until a result is obtained [9].

In recent years, the use of chromogenic media has become a key method for the rapid identification of micro-organisms in clinical samples. Optimal surveillance methods need diagnostic testing that is sensitive, specific, and rapid with a high negative predictive value so that MRSA colonized patients can be identified quickly and placed into isolation from other patients. Published reports on methods suggest a variety of approaches and confound the determination of which technique is the most effective. Currently available chromogenic media for MRSA detection incorporate chromogens to differentiate *S. aureus* from other pathogens and antibiotics for selective growth of MRSA. These all media differ in their chromogenic substrates, antibiotic formulations, and concentrations, factors that impact their sensitivity and specificity for MRSA detection [10].

An among different methods, oxacillin resistance screening agar base is a modification of a mannitol salt agar supplemented with oxacillin, in which mannitol-positive isolates turn blue due to an acid-dependent chromogenic component, i.e., aniline blue.

The purpose of our study was to compare the performance of conventional methods and chromogenic media for the screening of MRSA. We evaluated oxacillin-resistance screening agar base on 200 consecutive isolates of *S. aureus* collected in a tertiary care hospital.

METHODS

A prospective study was conducted at Bharati Vidyapeeth Deemed University a tertiary care hospital, Sangli. A total 200 clinical isolates of *S. aureus* from various clinical specimens were included in the study. Isolates were identified as *S. aureus* based on conventional methods as per the standard protocol. Antibiotic sensitivity testing of isolates of *S. aureus* to various antimicrobial discs was carried out by using Kirby-Bauer disc diffusion method. All antimicrobial discs were obtained from Hi-media Laboratories Pvt. Ltd., Mumbai, India. Zone diameters were measured by following Clinical and Laboratory Standards Institute (CLSI) guidelines [11].

Isolates of *S. aureus* were identified as MRSA using cefoxitin disc as surrogate marker. *S. aureus* ATCC 25923 and ATCC 43300 strains were used as negative and positive controls, respectively, for standardization of procedure and quality control.

In this study, all testing was done according to the CLSI as well as the manufacturer's recommendations. Conformed strains of *S. aureus* were identified as MRSA using cefoxitin disc as surrogate marker and oxacillin disc diffusion method as per CLSI guidelines. *S. aureus* ATCC 25923 and ATCC 43300 strains were used as negative and positive controls, respectively, for standardization of procedure and quality control. *mecA* detection by polymerase chain reaction (PCR) method was used as the gold standard method for MRSA.

Cefoxitin and oxacillin disc diffusion test

Cefoxitin disc diffusion test was carried out using a 30 µg disc of cefoxitin on Muller-Hinton agar plate, and oxacillin disc diffusion test was carried out using a 1 µg disc on Muller-Hinton agar plate containing 2% Nacl on all isolates of *S. aureus*. Lawn culture of the bacterial suspension standardized to 0.5 McFarland standards was done on the agar plates. The plates were incubated at 37°C for cefoxitin and 35°C for oxacillin disc for 18 to 24 hrs. Zone diameters were measured. Zone diameters ≤19 mm was reported as methicillin-resistant, and zone diameters ≥22 mm was considered as methicillin sensitive for cefoxitin disc. When zone diameters ≤10 mm was reported as methicillin-resistant and zone diameters ≥13 mm was considered as methicillin sensitive for oxacillin disc.

Oxacillin resistant screen agar base (ORSAB)

ORSAB is a selective media developed to detect MRSA in clinical specimens. The medium uses aniline blue to detect mannitol fermentation in *S. arueus.* The antibiotic supplements (oxacillin, 2.0 µl; polymixin B, 50,000 IU/I) of 5.5% NaCl reduce the growth of non-staphylococcal organisms and helps in the selection of MRSA. The test was carried out as per the manufacturer's instructions. 51.75 g of ORSAB was suspended in 500 ml of distilled water and boiled to dissolve the contents. The medium was sterilized by autoclaving at 121°C for 15 minutes, cooled to 50°C and aseptically added the contents of one vial of the antibiotic supplement after reconstituting it in 2 ml of sterile water. 20 ml of the medium was poured in sterile Petri plates and cooled to solidify. Colonies form each culture was taken in a loop and mixed in peptone water to bring it to 0.5 McFarland standards. The plates were subsequently inoculated by spot inoculation method and incubated at 37°C for 48 hrs. When blue colored colonies are seen within 24-48 hrs, it was considered for MRSA strains and no growth even after 48 hrs of incubation, considered as MSSA strain.

Detection of *mecA* by PCR method

Molecular detection of *mecA* gene by PCR was done using the standard procedures on MRSA isolates as per result of different phenotypic methods. PCR for the detection of *mecA* gene is done. Bacterial DNA was extracted from overnight cultures of *S. aureus* by CTAB-NaCl method [12]. The quality and quantity of isolated DNA was determined using nano-drop 1000 spectrophotometer (JH Biosciences, USA. Model: ND1000) at 260/280 nm, as well as visually by horizontal gel electrophoresis in 1% agarose. PCR for the detection of *mecA* was carried out following the method of Unal *et al.* [13]. Primer sequences used for *mecA* detection are *mecA* (F): 5'- GTA GAA ATG ACT GAA CGT CCG ATA A-3' and *mecA* R 5' CCA ATT CCA CAT TGT TTC GGT CTA A 3'.

Briefly, 1 µl of 60 ng of the extracted DNA was added to 24 µl of PCR amplification mix consisting of 16 µl of doubled distilled autoclaved water, 2.5 µl of ×10 Taq buffer, 1 µl of 2.5 mM dNTP mix (Merck, India), 0.5 µl of 3 U/µl Taq polymerase (Merck, India), and 0.5 mM of each primer. The *mecA* gene was amplified using the primers (Sigma, India) as described by Jonas *et al*., 1999. [14] Amplifications were carried out in a thermal cycler (iCycler, BioRad Inc., USA) with conditions that consisted of 30 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 1 minute with a final extension at 72°C for 2 minutes. Amplicons of 310 bp were consistent with *mecA* gene amplification. The PCR products were subjected to agarose gel electrophoresis using gel red dye and images were acquired using alpha imager gel documentation system.

RESULT

A total of 200 clinical isolates of *S. aureus* were evaluated. Out of these, total 50 strains were MRSA and 150 strains were MSSA by cefoxitin disc diffusion method, 52 and 148 strains were MRSA and MSSA, respectively, by oxacillin disc diffusion method, and 47 strains were identified as MRSA and 153 strains were identified as MSSA by ORSAB method.

The results for ORSAB were recorded after 24 hrs and 48 hrs. Among these 47 isolates, 37 strains showed growth with fermentation after 24 hrs and 10 strains showed growth with fermentation after 48 hrs. 153 strains were not grown even after 48 hrs Fig. 1.

All these 52 strains were taken for confirmation of *mecA* gene by PCR method which included all strains which were detected as MRSA by cefoxitin disc diffusion method, oxacillin disc diffusion method, and ORSAB media. In the 50 strains which were MRSA by cefoxitin disc diffusion method and oxacillin disc diffusion method had *mecA* gene. *mecA* was present in the 3 strains, which were detected as MSSA, only by ORSAB media which were actually false negative and same strains were MRSA by cefoxitin disc diffusion method. In 2 strains which were MRSA by oxacillin disc diffusion test, had not *mecA* gene. It is suggested that oxacillin disc diffusion method showed the false positive result.

DISCUSSION

MRSA has become a major infection control challenge not only within the hospitals and community also. The rapid and reliable identification of MRSA appears, nowadays, to be essential for proper patient care, control of spreading of such strain and use of antimicrobial guidelines. Active surveillance for MRSA revealed an efficient and recommended strategy to control hospital and community-associated MRSA infections but requires rapid identification. Recent some molecular methods are used in routine laboratories for reliable MRSA identification remains based on cultures using selective agar media [15]. Numerous reports in the literature have described screening media for MRSA identification showing variable performance. Detection of the *mecA* gene is considered as the reference and gold standard method for determining resistance to methicillin. However, many laboratories worldwide do not have the capacity or the experienced staff required to develop molecular techniques for detecting MRSA and it is therefore most essential and useful, screening methods are required in the routine clinical practice.

The accurate rapid diagnosis of MRSA in microbiology laboratories is very important for patients' management. It is also essential for meaningful interpretation of surveillance data. Currently, surveillance data for MRSA is difficult to interpret because there is no uniform testing method for the detection of MRSA, and laboratories vary in their standard operating procedures and interpretation of breakpoint values [16]. Phenotypic methods based on oxacillin containing medium are not satisfactory in terms of sensitivity and specificity. These methods are sensitive to incubation temperature time and inoculums density therefore; MRSA identification using cefoxitin containing media appears better and is now recommended by the CLSI [17,18].

The main objective of this study was to evaluate cefoxitin disc diffusion test, oxacillin disc diffusion test, and ORSAB. Timely detection of MRSA is still problematic with the majority of techniques taking longer than 48-72 hrs [19].

In this study, cefoxitin disc diffusion test, oxacillin disc diffusion test, ORSAB showed 100% sensitivities and specificities of 100%, 98.66%, 98.66%, respectively. The low specificity of oxacillin disc diffusion test and ORSAB medium prevent the use of each alone to predict methicillin-resistance in staphylococci (Table 1).

Chromogenic media have many advantages like rapid detection, high sensitivity, highly specific, needles to further biochemical test in micro-organism identification [20]. The low specificity of the ORSAB

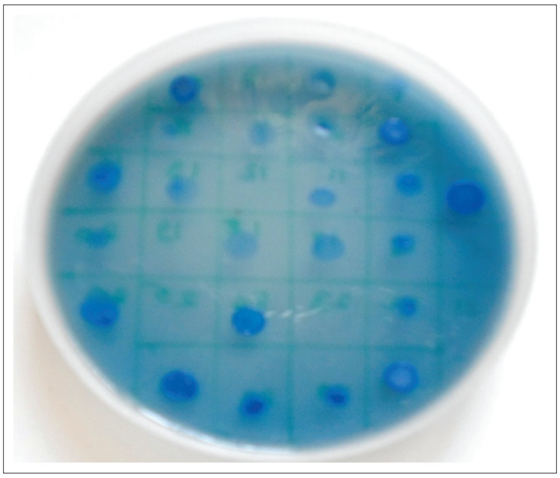

Fig. 1: Methicillin resistant *Staphylococcus aureus* on chromogenic (oxacillin resistant screen agar base) media

Table 1: Sensitivity and specificity of conventional and chromogenic media for detection of MRSA

Result of tests	Methods		
	Conventional method		Chromogenic media
	Cefoxitin disc diffusion (%)	Oxacillin disc diffusion (%)	ORSAB (%)
Sensitivity	100	100	100
Specificity	100	98.66	98.66
PPV	100	96.15	96
NPV	100	98.66	98.66

ORSAB: Oxacillin resistant screen agar base, MRSA: Methicillin resistant *Staphylococcus aureus*

and oxacillin disc diffusion medium prevents its use, at least alone, in predicting MRSA. This medium has previously been reported to show good sensitivity, although in a study in which the agar base was supplemented with antibiotics [21].

However, conventional screening methods used in the present study require prolonged incubation and confirmatory testing up to 48 hrs and separate incubation temperature (30°C) required for oxacillin disc diffusion method. During this time, MRSA negative patients may be held in unnecessary isolation, whereas unidentified MRSA positive individuals remain a hidden reservoir for cross infection.

Krishnan *et al*. reported that the specificity of routine laboratory tests for MRSA detection was variable and it was difficult to perform PCR in routine diagnostic laboratories [16].

Several chromogenic media and other deferential MRSA selective agars have been used to identify MRSA within 18-24 hrs [22-24]. In this study, we used ORSAB for the detection of MRSA. ORSAB agar is a good, cost-effective medium for the detection of MRSA because cost of screening on conventional culture medium is similar to the cost of chromogenic medium. The specificity and sensitivity were 98.66% and 100%, respectively.

Our result correlates with Velasco *et al*., he studied 102 isolates of *S. aureus* by PCR and various phenotypic methods including oxacillin (1 µg), cefoxitin, cefazolin, cefotaxime, and imipenem (all 30 µg) discs, E-test for oxacillin, microdilution with oxacillin, ORSAB medium and PBP2 agglutination with two different kits. They found that the cefoxitin disc, ORSAB medium and PBP2 detection had the highest sensitivity (100%). They concluded that the cefoxitin disc was the best method for detecting MRSA isolates [25].

These 2 false positive results by oxacillin disc diffusion test could be attributed to the presence of alternate resistance mechanisms either moderate oxacillin-resistant *S. aureus* (MODSA) or borderline oxacillin-resistant *S. aureus* (BORSA) in these isolates.

Simor *et al*. compared ORSAB, supplemented with oxacillin, with a conventional mannitol salt agar plate, supplemented with 2.0 mg of oxacillin per liter, for the detection of MRSA in clinical specimens [26]. Becker *et al*. suggested that ORSAB medium has limitations for surveillance applications not only due to lower sensitivity but also because some coagulase-negative staphylococci (mainly *Staphylococcus* hemolyticus) appear blue. Thus, utilization of this plate warrants confirmatory tests for MRSA identification and should rather be considered in high prevalence settings [27]. This change in the specificity may be due to the difference in the collection of samples, clones circulating in different parts of the world and prevalence of MRSA [28].

CONCLUSION

In conclusion, cefoxitin disc diffusion was the best for the phenotypic detection of MRSA because their sensitivity and specificity were better than oxacillin and ORSAB. The low specificity of oxacillin disc diffusion test and ORSAB medium prevent the use of each alone to predict methicillin-resistance in staphylococci. In most of the laboratories, oxacillin disc is used for the detection of MRSA. It is, therefore, recommended that oxacillin discs should be replaced by the cefoxitin discs test method because of its effectiveness. ORSAB is also effective and could be used as the second option for the direct inoculation of specimens in the laboratories.

REFERENCES

1. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, *et al.* Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 2007;298(15):1763-71.
2. Archer GL. *Staphylococcus aureus*: A well-armed pathogen. Clin Infect Dis 1998;26(5):1179-81.
3. Voss A, Doebbeling BN. The worldwide prevalence of methicillin-resistant *Staphylococcus aureus*. Int J Antimicrob Agents 1995;5(2):101-6.
4. Jernigan JA, Clemence MA, Stott GA, Titus MG, Alexander CH, Palumbo CM, *et al.* Control of methicillin-resistant *Staphylococcus aureus* at a university hospital: One decade later. Infect Control Hosp Epidemiol 1995;16(12):686-96.
5. Papia G, Louie M, Tralla A, Johnson C, Collins V, Simor AE. Screening high-risk patients for methicillin-resistant *Staphylococcus aureus* on admission to the hospital: Is it cost-effective? Infect Control Hosp Epidemiol 1999;20(7):473-7.
6. Szczepanik A, Koziol-Montewka M, Al-Doori Z, Morrison D, Kaczor D. Spread of a single multiresistant methicillin-resistant *Staphylococcus aureus* clone carring a variant of staphylococcal cassette chromosome mec Type III isolated in a university hospital. Eur J Clin Microbiol Infect Dis 2007;26(1):29-35.
7. Felten A, Grandry B, Lagrange PH, Casin I. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): A disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. J Clin Microbiol 2002;40(8):2766-71.
8. Perry JD, Freydière AM. The application of chromogenic media in clinical microbiology. J Appl Microbiol 2007;103(6):2046-55.
9. Malhotra-Kumar S, Abrahantes JC, Sabiiti W, Lammens C, Vercauteren G, Ieven M, *et al.* Evaluation of chromogenic media for detection of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2010;48(4):1040-6.
10. Malhotra-Kumar S, Haccuria K, Michiels M, Ieven M, Poyart C, Hryniewicz W, *et al*. Current trends in rapid diagnostics for methicillin-resistant *Staphylococcus aureus* and glycopeptide-resistant *Enterococcus* species. J Clin Microbiol 2008;46(5):1577-87.
11. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, 15th Informational Supplement, M100–S15. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
12. Ausubel FM, Brent R, Kingston RE, Moore AD, Seidman JG, Smith JA,

et al. Protocols in Molecular Biology. 4th ed. New York: John Wiley and Sons Inc.; 1999.
13. Unal S, Werner K, DeGirolami P, Barsanti F, Eliopoulos G. Comparison of tests for detection of methicillin-resistant *Staphylococcus aureus* in a clinical microbiology laboratory. Antimicrob Agents Chemother 1994;38(2):345-7.
14. Jonas D, Grundmann H, Hartung D, Daschner FD, Towner KJ. Evaluation of the *mecA* femB duplex polymerase chain reaction for detection of methicillin resistant *Staphylococcus aureus*. Eur J Clin Microbiol Infect Dis 1999;18(9):643-47.
15. Arnold MS, Dempsey JM, Fishman M, McAuley PJ, Tibert C, Vallande NC. The best hospital practices for controlling methicillin-resistant *Staphylococcus aureus*: On the cutting edge. Infect Control Hosp Epidemiol 2002;23(2):69-76.
16. Krishnan PU, Miles K, Shetty N. Detection of methicillin and mupirocin resistance in *Staphylococcus aureus* isolates using conventional and molecular methods: A descriptive study from a burns unit with high prevalence of MRSA. J Clin Pathol 2002;55(10):745-8.
17. Skov R, Smyth R, Larsen AR, Bolmstrôm A, Karlsson A, Mills K, *et al.* Phenotypic detection of methicillin resistance in *Staphylococcus aureus* by disk diffusion testing and Etest on Mueller-Hinton agar. J Clin Microbiol 2006;44(12):4395-9.
18. Blanc DS, Wenger A, Bille J. Evaluation of a novel medium for screening specimens from hospitalized patients to detect methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2003;41(8):3499-502.
19. Hardy KJ, Hawkey PM, Gao F, Oppenheim BA. Methicillin resistant *Staphylococcus aureus* in the critically ill. Br J Anaesth 2004;92(1):121-30.
20. Tavakoli H, Bayat M, Kousha A, Panahi P. The application of chromogenic culture media for rapid detection of food and water borne pathogen. Am-Euras J Agric Environ Sci 2008;4(6):693-8.
21. Kluytmans J, Van Griethuysen A, Willemse P, Van Keulen P. Performance of CHROM agar selective medium and oxacillin resistance screening agar base for identifying *Staphylococcus aureus* and detecting methicillin resistance. J Clin Microbiol 2002;40(7):2480-2.
22. Flayhart D, Hindler JF, Bruckner DA, Hall G, Shrestha RK, Vogel SA, *et al.* Multicenter evaluation of BBL CHROMagar MRSA medium for direct detection of methicillin-resistant *Staphylococcus aureus* from surveillance cultures of the anterior nares. J Clin Microbiol 2005;43(11):5536-40.
23. Nahimana I, Francioli P, Blanc DS. Evaluation of three chromogenic media (MRSA-ID, MRSA-select and CHROMagar MRSA) and ORSAB for surveillance cultures of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 2006;12(12):1168-74.
24. Stoakes L, Reyes R, Daniel J, Lennox G, John MA, Lannigan R, *et al.* Prospective comparison of a new chromogenic medium, MRSASelect, to CHROMagar MRSA and mannitol-salt medium supplemented with oxacillin or cefoxitin for detection of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2006;44(2):637-9.
25. Velasco D, del Mar Tomas M, Cartelle M, Beceiro A, Perez A, Molina F, *et al.* Evaluation of different methods for detecting methicillin (oxacillin) resistance in *Staphylococcus aureus*. J Antimicrob Chemother 2005;55(3):379-82.
26. Simor AE, Goodfellow J, Louie L, Louie M. Evaluation of a new medium, oxacillin resistance screening agar base, for the detection of methicillin resistant *Staphylococcus aureus* from clinical specimens. J Clin Microbiol 2001;39(9):3422.
27. Becker A, Forster DH, Kniehl E. Oxacillin resistance screening agar base for detection of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2002;40(11):4400-1.
28. van Loo IH, van Dijk S, Verbakel-Schelle I, Buiting AG. Evaluation of a chromogenic agar (MRSASelect) for the detection of meticillin-resistant *Staphylococcus aureus* with clinical samples in the Netherlands. J Med Microbiol 2007;56(4):491-4.