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Evaluation of Chromogenic AGAR Medium in The Rapid Phenotypic Detection of MRSA Strains from Clinical Isolates

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Abstract

Introduction: Methicillin-resistant Staphylococcus aureus (MRSA) has been recognized as one of the major pathogens in hospitals as well as community settings. The prevalence of MRSA is 30 to 70% and many studies have suggested an alarming rate of infections caused by this organism. In spite of modern diagnostic procedures and technological advancement, infections caused by Methicillin-resistant Staphylococcus aureus still remain difficult to diagnose in developing countries like India. The aim of this study is to evaluate the efficacy of chromogenic agar medium in the identification of Methicillin-resistant Staphylococcus aureus strains isolated from clinical specimen.

Materials & Methods: A total of 166 S. aureus isolates from various samples were identified by standard protocol. Out of which

44 were methicillin resistant S. aureus. Methicillin resistance was evaluated by Mannitol salt agar (MSA), Oxacillin screen agar (OSA) & Hicrome MeReSa agar.

Results: Among the 44 MRSA strains tested by Mannitol salt agar, Oxacillin screen agar & Hicrome MeReSa Staph agar medium; the sensitivity, specificity, positive predictive value (PPV) & negative predictive value (NPV) were 100%, 100%, 100% and 100% respectively for HiCrome MeReSa Staphy agar.

Conclusion: The early detection of Methicillin-resistant Staphylococcus aureus is very important in the prognosis of S. aureus infections. An integrated awareness program, good hand wash technique, epidemiological

studies and effective control measures are the goals for elimination of Methicillin-resistant Staphylococcus aureus in hospitals.

Keywords: Methicillin-resistant Staphylococcus aureus, Oxacillin screen agar, HiCrome MeReSa agar medium

Introduction

Staphylococcus aureus is one of the pathogens most frequently isolated within clinical specimens and is currently the most common cause of nosocomial infections leading to serious manifestations with high mortality and morbidity. Methicilln resistance in Staphylococcus aureus is based on the production of an altered Penicillin binding protein 2a (PBP2a) which is mediated by mecA gene. The Penicillin binding protein 2' (PBP2a) which encodes the mecA gene is carried on the mobile genetic element Deoxtriboneuclic acid (DNA) called the Staphylococcal cassette chromosome mec (SCCmec) and confers an intrinsic resistance to all beta-lactams and their derivatives. Resistance levels in methicillin-resistant Staphylococcus aureus (MRSA) depend on efficient PBP2' (PBP2a) production and are modulated by chromosomal factors. Depending on the genetic background of the strain that acquired mecA, resistance levels range from phenotypically susceptible to highly resistant.

Staphylococcus aureus has been recognised as an important pathogen responsible for a wide spectrum of infections from mild pyogenic skin infections to serious bacteremia, septicaemia and important system related manifestations.

The detection of Methicillin-resistant Staphylococcus aureus is difficult and has become complicated due to many factors especially the genetic and environmental. The resistance to methicillin in Staphylococcus aureus is predominamtly heterogenous where a subset of a microbial population is resistant to certain antibiotics, while the majority of the population is susceptible.

It is associated with various deleterious health outcomes like increased treatment failure, chronic infections, long-term hospitalization, increased patient mortality, increased treatment costs and prolonged blood stream infections.

Approaches to rapid detection of Methicillin-resistant Staphylococcus aureus includes rapid culture & molecular methods. A number of studies have evaluated the role of rapid detection methods as a component of Methicillin-resistant Staphylococcus aureus control strategies. [1, 2]

Addition of NaCl or sucrose to culture medium, incubation at 30^0C or subculture in the presence of beta lactum antibiotics enhances the expression of resistance.

Molecular diagnostic methods can reduce the "turnaround time" for detection of Methicillin-resistant Staphylococcus aureus colonization, leading to earlier isolation of colonized patients and lower the rates of its transmission. Treatment of infections caused by Staphylococcus aureus has become more problematic due to the development of antimicrobial resistance. Currently the most important problem is methicillin resistant Staphylococcus aureus. Since Methicillin-resistant Staphylococcus aureus strains are resistant to all beta-lactam antibiotics, the therapeutic options are limited significantly. In addition, most Methicillin-resistant Staphylococcus aureus strains are resistant to other groups of antimicrobials.

Therefore, control and management of Methicillin-resistant Staphylococcus aureus infection is a global challenge. In this regard, rapid and reliable detection of Methicillin-resistant Staphylococcus aureus is crucial for both effective control and optimal therapeutic outcome.

The incidence of infections caused by Methicillin-resistant Staphylococcus aureus continues to increase worldwide. Screening for Methicillin-resistant Staphylococcus aureus is important for therapeutic and epidemiological reasons. [2]

Rapid identification of Methicillin-resistant Staphylococcus aureus from clinical specimen and screening of high risk patients for Methicillin-resistant Staphylococcus aureus colonization have been to be cost effective measures for limiting the spread of organisms in hospitals and healthcare settings. The use of rapid and sensitive screening assays for Methicillin-resistant Staphylococcus aureus detection could help to further improve the infection control and also decrease the hospitalization costs. The currently available chromogenic media differ in their chromogenic substrates, antibiotic formulation and concentration which are the factors that impact their sensitivity and specificity for detection of Methicillin resistance Staphylococcus aureus.

Methods used to detect Methicillin-resistant Staphylococcus aureus in clinical samples ideally should have high sensitivity and specificity and should report the results within a short time. To identify Staphylococcus aureus from contaminated samples more easily and reliably, selective media have been developed. Ideally, selective media achieve isolation of Staphylococcus aureus and detection of methicillin resistance in one step.

Phenotypic methods are used by most clinical laboratories for Methicillin-resistant Staphylococcus aureus detection because it is easy to perform and interpret. Various methods have evolved for rapid detection of Methicillin-resistant Staphylococcus aureus, but the optimum method for its detection remains controversial. [3, 4]

Conventional methods for identification of Methicillin-resistant Staphylococcus aureus, take more time and are influenced by environmental conditions like temperature, pH, salt concentration and duration of incubation. These factors need a sensitive, rapid, simple & accurate method for Methicillin-resistant Staphylococcus aureus detection in routine diagnostic laboratories. The conventional methods practiced for the detection of Methicillin-resistant Staphylococcus aureus in the clinical laboratories are oxacillin agar screen test (OSA), oxacillin disc diffusion (ODD) and oxacillin MIC by agar, or broth dilution. [5]

Cefoxitin disc diffusion is recommended by CLSI for detection of methicillin resistance. Cefoxitin is considered as a better inducer of mec-A gene expression than oxacillin or methicillin, and can be used to screen heterogeneous Methicillin-resistant Staphylococcus aureus populations. The advantage of using cefoxitin is that the test conditions are similar to those used for other antibiotics. [6]

Apart from these, a latex agglutination kit has been developed by Denka Seiken Co., Japan which uses specific moAbs directed towards the PBP2a antigen for the detection of Methicillin-resistant Staphylococcus aureus. [7]

In addition, use of chromogenic substances in the medium is another method for the identification of Methicillin-resistant Staphylococcus aureus. In the present days, the use of chromogenic media has become a key method for the rapid identification of microrganisms from the clinical samples. These media through the use of chromogenic substrates incorporated into a solid agar based medium, detects the key

microbial emzymes which are diagnostic markers of the pathogen. The chromogenic media allows the colour based identification of the pathogen from the direct colonies on the primary culture medium. This reduces the need for subculture and further identification by biochemical tests in turn speeding up the reporting process. [8,11]

In the present study an attempt was made to evaluate three diffeerent chromogenic media mannitol salt agar, oxacillin screen agar and HiCrome MeReSa agar medium for the screening of Methicillin Resistant Staphylococcus aureus from clinical isolates.

Aims and Objectives

The aim and objective of the present study is to evaluate the usefulness and efficacy of a chromogenic medium (Mannitol salt agar, Oxacillin screen agar and HiCrome MeReSa agar) in the rapid identification of Methicillin-resistant Staphylococcus aureus with the aim of selecting a rapid, sensitive, specific, cost effective and easy method for the phenotypic detection of Methicillin-resistant Staphylococcus aureus in routine diagnostics, in comparison to mannitol salt agar (MSA) and oxacillin screen agar (OSA), keeping cefoxitin disc diffusion (CxDD) as the gold standard which is equivalent to mecA gene detection by Polymerase Chain Reaction (PCR).

Materials and Methods

The present one-year prospective study from February 2019 – January 2020 was undertaken in our teaching hospital after getting clearance from the Institutional ethics committee. A total of 166 consecutive non-duplicated clinically significant Staphylococcus aureus strains from various specimen were collected at our microbiology laboratory. All the Staphylococcus aureus strains were subjected for the following chromogenic phenotypic tests based on the conventional methods as per standard protocol for the identification of Methicillin-resistant Staphylococcus aureus.

Quality control: ATCC 25923 for Methicillin sensitive Staphylococcus aureus and ATCC 43300 for Methicillin resistant Staphylococcus aureus were used as positive and negative control strains.

Detection of Methicillin-resistant Staphylococcus aureus by Chromogenic medium

Rapid culture makesw use of chromogenic agar, which contains media substrates that change colour in the presence of Staphylococcus aureus; Use of such agar allows identification of Methicillin-resistant Staphylococcus aureus from primary isolation plates within 24 to 48 hours, without the need for additional subcultures or biochemical tests.

Mannitol Salt AGAR is prepared as per manufacturer's instructions. It is used for the selective isolation of pathogenic Staphylococci. It is recommended for identification of Coagulase positive Staphylococci from clinical specimen.

The medium contains beef extract and protease which makes it very nutricioiu as they provide essential growth factors trace nutrients. Bacteria other than Staphylococci are inhibited by 7.5 NaCl. The differential action of the agar is attributed to D-mannitol. Staphylococcus aureus ferments mannitol to produce yellow coloured colonies which is due to the indicator phenyl red indicator, which changes the colour of the medium. This indicator at alkaline pH is red and at acidic pH is yellow in colour.

HiCrome MeReSa Agar Base M1674 (Hi-Media, India) was used for detection of the Methicillin-resistant Staphylococcus aureus among clinical isolates of Staphylococcus aureus. The medium was prepared by suspending 41.65 g of the medium into 500 ml of boiled

distilled water. The medium was cooled to around 45-50°C, MeReSa selective supplement (FD229) reconstituted with 5 ml sterile distilled water into methicillin vial having 2.0 mg of methicillin as per manufacturer instructions (HiMedia-India). It was added and mixed very well, poured into Petri plates, cooled and sterility check of the prepared plates done. [8] The growth of Staphylococcus aureus from Mannitol salt agar was sub cultured onto HiCrome MeReSa chromogenic agar and any bluish green colony was considered to be positive, indicating Methicillin-resistant Staphylococcus aureus, while all others were recorded as Methiciliin sensitive Staphylococcus aureus (MSSA) strains[9].

Oxacillin screen agar test: A bacterial inoculum of each strain was made and turbidity was adjusted to 0.5 McFarland. The suspension was inoculated on Mueller–Hinton agar (MHA) containing 4% NaCl and 6µg/ml oxacillin (Hi-Media)[8]. Plates were incubated at 35°C for 24 hours. Any strains showing growth on the plate containing oxacillin were considered to be resistant to methicillin[10].

Results:

Figure 1, 2 & 3

Growth of Methicillin Resistant Staphylococcus aureus on various chromogenic media - Mannitol salt agar, Oxacillin screen agar & HiCrome Staph MeReSa agar respectively.

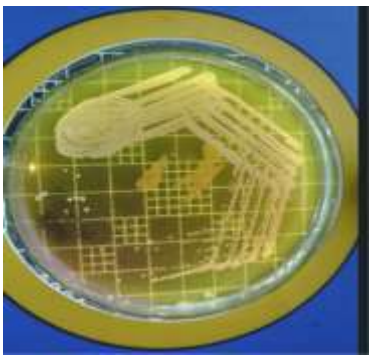

Figure 1: MSA with growth of MRSA

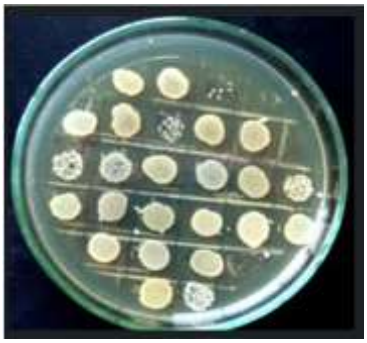

Figure 2: OSA with growth of MRSA

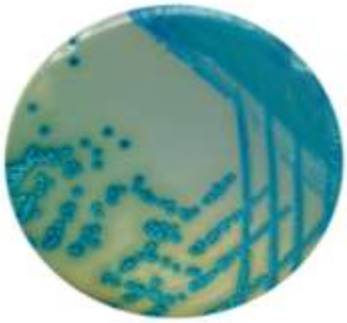

Figure 3: HiCrome Staph with growth of MRSA

Table 1: Statistics of total Staphylococcus aureus isolated:

MRSA	MSSA	CONS	MRSA & MSSA	Total
44	122	511	166	677

Table 2: Statistics of MRSA isolated on Hi crome Staph medium:

Staphylococcus aureus strains	No of strains isolated / Total no. of strains at:	
	24 hrs Incubation	48 hrs Incubation
MSSA	0/122 (0%)	0/122 (0%)
MRSA	42/44 (95.4%)	44/44 (100%)

Table 3: Comparative statistics of MRSA isolates on various Chromogenic screening media:

Chromogenic medium	MRSA (44)	%	Sensitivity	Specificity	False positives	False negatives	NPV	PPV
Mannitol salt agar	41	93.2	93.18%	100%	0	3	97.60	100
Oxacillin screen agar	43	97.7	97.72%	100%	0	1	99.18	100
MeReSa (HiCrome Staph aureus)	44	100	100%	100%	0	0	100	100

Discussion

Rapid and accurate diagnosis of Methicillin-resistant Staphylococcus aureus is very important in the management of infections caused by Staphylococcus aureus. Chromogenic agar containing substrates, which changes colour in the presence of Methicillin-resistant Staphylococcus aureus was used in the present study.

Many phenotypic methods to detect Methicillin-resistant Staphylococcus aureus have been developed but they vary in sensitivity and specificity. Selectivity for Methicillin-resistant Staphylococcus aureus is achieved by incorporation of antibiotics into the agar. Use of such agar allows identification of Methicillin-resistant Staphylococcus aureus from primary isolation plates within 24 or 48 h after enrichment, obviating the need for additional biochemical tests which may save time & money and provide overall results that are equivalent to the results of the Polymerase chain reaction (PCR) method.[9]

In the present study, the prevalence of Methicillin-resistant Staphylococcus aureus is 26.5% (44/166)

The oxacillin screen agar test showed 97.72% sensitivity and 100% specificity for Methicillin-resistant Staphylococcus aureus detection in our study. This sensitivity could be increased to 100% by increasing the incubation period of HiCrome MeReSa agar from 24 to 48 hrs. [10]

HiCrome MeReSa agar showed 100% sensitivity and 100% specificity. The subculture of Staphylococcus aureus isolates from Manitol salt agar and Oxacillin screen agar on HiCrome MeReSa agar medium increased the detection rate of Methicillin-resistant Staphylococcus aureus (from 93.2% to 100% and 97.2% to 100% of sensitivity respectively).

Conclusion

The current gold standard for MRSA detection is identification of the mecA gene. However, the use of molecular methods for routine clinical practice may not be feasible in the resource-constraint settings. Therefore, it is desirable to identify an accurate, rapid and cost-effective phenotypic method for the detection of MRSA from clinical isolates.

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