Section: Microbiology

Original Research Article

EVALUATION OF CHROME AGAR METHOD TO DETECT METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN CLINICAL SAMPLES: A COMPARATIVE STUDY IN A TERTIARY CARE HOSPTIAL , BANGALORE

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ABSTRACT

Background: Methicillin resistant Staphylococcus.aureus (MRSA) strains emerged soon after the introduction of Methicillin into clinical practice. In addition to being a nosocomial pathogen, MRSA has become a community pathogen. MRSA strains harbour the mecA gene which encodes the modified penicillin binding protein (PBP2a) having low affinity for methicillin and all β-lactum antibiotics. Resistant to this antibiotic implies resistance to all β-lactum antibiotics leaving few therapeutic options to treat such severe infections. The treatment of infections caused by these organisms has become problematic. So rapid and accurate identification of MRSA is required to immediately start the appropriate antimicrobial therapy and to avoid the spread of these strains. Aim of the study is evaluate the chrome agar in screening of MRSA detection and to estimate the percentage of MRSA isolates among the clinical samples received at tertiary care hospital. **Materials and Methods:** The study was conducted in department of Microbiology in a tertiary care hospital. All Staphylococcus aureus(S.aureus) isolates from clinical samples were included in this study and performed as per the standard operating procedures. MRSA was detected by using cefoxitin (30µg) disk diffusion method, chrome agar, and standard PCR. **Result:** In our study, among 100 staphylococcal species 70 were S.aureus isolates, 30 were coagulase negative staphylococcal species, 60 were MRSA isolates, Most of the S. aureus were isolated from pus samples 35% (n= 35) followed by Blood samples 15% (n=15), sputum samples 5%(n=5) and urine samples 5%(n=5). **Conclusion:** MRSA plays a significant role to cause severe infections both in-patients and out patients. Phenotypic methods like use of chrome agar (can be considered as rapid screening method for the detection of methicillin resistance in S. aureus, as it can be done in low cost and less time, whereas conventional culture method or molecular method will take more time and technical expertise.

INTRODUCTION

MRSA strains surfaced soon after the introduction of Methicillin into clinical practice. In addition to being a nosocomial pathogen, MRSA has gained popularity as a community pathogen. MRSA strains harbour the mecA gene which encodes the modified penicillin binding protein (PBP2a) having low affinity for methicillin and all β-lactum antibiotics. Resistant to this antibiotic implies resistance to all β-lactum antibiotics leaving few therapeutic options to treat such severe infections. The treatment of infections caused by these organisms has become problematic.[1] So rapid and accurate identification of MRSA is required to immediately start the appropriate antimicrobial therapy and to avoid the spread of these infections.[2] Several studies have reported a number of phenotypic methods for identification of MRSA. However, these methods are inadequate as the expression of resistance is subject to environmental and conditional expression of PBP2a antigen. Discrepancies in interpretation are leading to an adverse effect on patient management. So there is a need of highlighting the importance of accuracy in detection within a short period of time.[2,3] Hence the current study was conducted to

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find out the accuracy of the rapid chrome agar method to detect MRSA in health care facility centers, and we need to estimate the percentage of MRSA strains among the clinical isolates of Staphylococcal species.[4-10]

Objectives of the study

1. To evaluate the accuracy of chrome agar for MRSA detection, by comparing with Polymerase chain reaction (PCR)
2. To estimate the percentage of MRSA isolates among clinical samples received at tertiary care teaching hospital.

MATERIALS AND METHODS

The study was conducted in the tertiary care hospital, Bangalore. Material & data which is required for the study were collected from the central laboratory of the Dr. B. R. Ambedkar Medical college & Hospital. Around 100 number of staphylococcal species isolates were collected from the clinical samples which are positive for routine culture methods. Identification and isolation of Staph.aureus has been done by standard bacteriological procedures. This includes both inpatient samples and outpatient's samples. The study was initiated by collecting the clinical samples from the patients like pus, blood, sputum, and urine. These samples were immediately inoculated on blood agar, Maconkey agar nutrient agar and after overnight incubation, developed colonies were observed macroscopically for characteristic features like β-hemolysis, opaque, smooth, round, convex and golden yellow pigmentation. and examined microscopically by Gram's staining for Gram positive cocci in clusters. suspected colonies were performed for glucose O/F test, catalase test, tube coagulase test.[4] The pure cultures of S.aureus were performed for cefoxitin disk diffusion by using McFarland 0.5 standard tube. By observing zone of inhibition 22 mm [5]. Antibiotic sensitivity testing was done according CLSI-2024 guidelines. Simultaneusly pure cultures of staph.aureus were inoculated on chrome agar plate (Hicrome Rapid MRSA Agar base with a selective suppliment) to detect MRSA colonies after 18 to 24 hrs of incubation by observing greenish yellow coloured colonies [6]. Media plates preparation was performed according to literature manual.

Standard Polymerase Chain Reaction (PCR) method to detect MRSA.[7-10]

PCR method to detect the mecA gene, considered as Gold standard method. The positive control was Staphylococcus aureus ATCC 43300 (MRSA) while the negative control was ATCC 25923 (MSSA). The chromosomal DNA of the isolates was extracted by a simple lysis method. single colony of the isolate was inoculated in 1 ml of Luria Bertani Broth (LB Broth Difco) in small Eppendorf tubes and incubated overnight at 37◦C. Then turbid tubes were centrifuged at 6000 rpm for 5 minutes. The supernatant was discarded, and the cell pellet was collected and resuspended in 400 µl of sterile nuclease-free water. Then heated at 85º C for 15 minutes in a water bath and immediately transferred to -20º C, which causes cell lysis. Master Mix of a single reaction: A 20µL PCR reaction consisted of PCR buffer (2µL), MgCl2 (2µL), dNTPs (1.5µL), MecA F primer (0.5µL), MecA R primer (0.5µL), Taq polymerase (0.3µL), Template DNA (1µL), and MilliQ H2O (12.2µL). The mecA F primer (5'-AAAATCGATGGTAAAGGTTGGC-3'), which corresponds to nucleotides 1282 to 1303, and the mecA R primer (5'-AGTTCTGCAGTACCGGATTTGC-3'), which is complementary to nucleotides 1581 to 1598 within the coding frames, were used for the amplification of the 533-base pair (bp) fragment of the mecA gene. The PCR technique included a 5-minute denaturation stage at 95ºC, followed by 30 amplification cycles. Each cycle consisted of a 60-second denaturation at 94ºC, a 60-second annealing at 55ºC, and a 90-second extension at 72ºC. The programme ended with a 10-minute extension at 72ºC. The PCR products are observed on a 1% agarose gel containing ethidium bromide dye under a UV transilluminator (Gel Doc, Bio-Rad US). A 100-base-pair DNA ladder serves as the molecular-weight size marker.

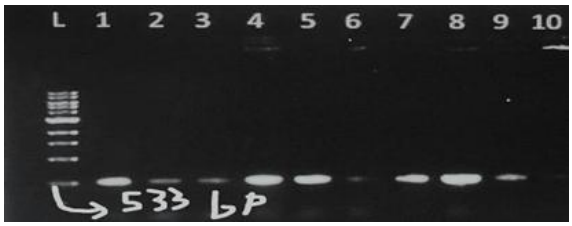

Figure 1: Gel pic of mecA gene

Lane 1: positive for mecA gene showing 533bp
Lane 2-9: samples
Lane 10: negative control

RESULTS

Table 1: Distribution of MRSA isolates among the clinical samples.

Sample name	Pus	Blood	sputum	Urine
No. of MRSA isolates	35	15	5	5

Table 2: Detection of MRSA isolates by various methods.

Employed method	Conventional Culture method	Standard PCR	Chrome agar method
No. of MRSA isolates detected	60	60	54

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Table 3: Evaluvation of chrome agar to detect MRSA

Sensitivity	93%
Specificity	100%
Positive predictive value	100%
Negative predictive value	44%
Accuracy	93%

Statistical Analysis: Chrome agar method was considered as test for evaluation and PCR test was considered as Gold standard method to detect MRSA among clinical isolates, and 2x2 contingency table was used for accuracy calculation.

DISCUSSION

MRSA pathogens have become increasingly resistant to antimicrobial therapies, hindering effective treatment and increasing patient morbidity. The prevalence of MRSA among S.aureus isolates ranges from 40% to 70% in India.[11] V.kodi et al(2024) from south India reported 60% MRSA in clinical isolates of staph.aureus, whereas Sudeshna Das, kirti nirmal et al (2024) reported 73% of MRSA from north India.[11,12] In our study we found 60% of MRSA isolates among 100 isolates of staphylococcal species isolated from various clinical samples. Our study results of MRSA percentage are positively correlating with the results of V.kodi et al's study. However To curb the emergence and transmission of this pathogen, surveillance of individuals at high, regular monitoring can limit the unnecessary misuse of antibiotics and enable timely implementation of infection control measures. In this study, we explored a practical MRSA detection method that can be easy to perform in laboratories with limited resources and expertise. The reference and gold standard method for MRSA identification is detecting the mecA gene by PCR. Our study aimed to find an easy to perform, rapid and accurate method for MRSA detection for microbiological laboratories where molecular assays are unavailable, and the prevalence of MRSA is high. The main objective of this study was to evaluate the chromogenic agar in screening of MRSA detection and to estimate the percentage of MRSA isolates among the clinical samples. Some of the previous studies have evaluated the accuracy of commercially available chromogenic agars to detect MRSA. A study done by Al-Zaidi and Al-Sulami et al [13] In the clinical samples screening for MRSA their results reveals that the sensitivity, specificity, positive predictive value and negative predictive value of HiCrome Agar medium test was 94%, 100%, 100 % and 95.6%, respectively. Another study done by Sharma S et al [14] for MRSA detection by chrome agar (Himedia) they reported that 98% and 97% of sensitivity and specificity respectively. In our study we used chromogenic agar (Hichrome rapid MRSA agar base) to detect MRSA and found the values of 93%,100%,100%,44% and 93% for sensitivity, specificity, positive predictive value, negative predictive value and accuracy Respectively. The results of our study for evaluation of Chrome agar to detect MRSA in clinical samples is positively correlating the studies of Al-zaidi and Sulami et al, so we can suggest that in hospital setups we can use this kind of phenotypic chrome agar method (Hichrome rapid MRSA agar base) is good for screening, but not 100% accurate while comparing with PCR method.

CONCLUSION

MRSA is an emerging pathogen playing a significant role to cause high rate (60%) of severe infections in both in-patients and out-patients. Phenotypic method like chromogenic agar can be considered as a kind of rapid screening method for the detection of MRSA as it is highly sensitive and specific to detect MRSA and can be done in low cost and less time, whereas conventional culture method or molecular method will take more time, expensive and need of technical expertise.

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