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Original Article

Utility of Chromogenic Medium for Early Detection of Nasal Carriage of Methicillin Resistant *Staphylococcus Aureus* (MRSA) in Healthcare Professionals

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ABSTRACT
Introduction: *Resistance to methicillin in staphylococci is mediated by an altered penicillin-binding protein (PBP2a), which is encoded by the mecA gene and confers resistance to most of the current β-lactam antimicrobial agents. Methicillin-resistant S. aureus (MRSA) infections account for 40-60% of all nosocomial (hospital acquired) S. aureus infections in many centers across the world. HCWs are likely to play a large role in MRSA transmission. Screening is a useful technique to identify the reservoir, initiate contact precautions and eradication measures. The conventional screening method is a multistep process. The chrome agar method is not only a single step process but also easier one. By this we save time and decrease the workload.* This Cross-sectional Analytical study was carried out to *look for the utility of chromogenic media as screening tool for early detection of MRSA strains from the nasal carriage of healthcare workers in tertiary care hospital in central India.*
Methods: *Thirty non repetitive samples each from four groups of health care provider i.e. Consultants, Residents, Nursing staff & Cleaning Staff were collected after informed consent and ethical clearance. Samples from anterior nares were processed for isolation of S. aureus and detection of MRSA by conventional and by Chromagar. Data maintained in Microsoft office Excel was analyzed with statistical tools like tests of proportion & Chi Square test for significance.*
Results: *By conventional method, out of 120 samples 68 were S. aureus of which 26(21.66%) were MRSA. Chrome agar detected 27(22.5%) MRSA. Time required for MRSA detection conventionally was 48 hours while by Chrome agar detection was in 24 hours for 24 (88.88%) isolates and 48 hours for 03 isolates.*
Conclusion: *Chrom agar MRSA is highly specific and sensitive to detect MRSA. In majority of cases MRSA detection was within 24 hours.*
Keywords: *Chrom agar, Nasal colonization, MRSA, PBP2a, Health care provider, Hospital Acquired Infections*
Key Messages: *The control and prevention of the infection ascribed to MRSA can only be achieved when there is a regular screening of carriers among healthcare providers thus preventing the spread of MRSA in hospital settings as well as community. Chrome agar can be a better option to conventional method as it is highly sensitive and specific and time saving.*

INTRODUCTION

Staphylococci are widespread in nature, their normal habitats being the skin and mucous membranes of human beings. Human skin is densely colonized with several of the coagulase-negative species, and to a lesser extent with *Staphylococccus aureus* (*S. aureus*).[1] The anterior nares of nose are the most frequent carriage site for *S. aureus*. Extra nasal carriage sites that typically harbour *S. aureus* include the skin, perineum, pharynx and less frequent carriage sites include the gastrointestinal tract, vagina, and axilllae and can be responsible for transmission of mild to life threatening infections. [2,3,4,5]

Methicillin resistant *Staphylococcus aureus* (MRSA), a specific strain of the S. aureus bacterium, which is intrinsically resistant to methicillin and all β-lactams[6]. Resistance to methicillin in staphylococci is mediated by an altered penicillin-binding protein (PBP2a), which is encoded by the mecA gene and confers resistance to most of the current β-lactam antimicrobial agents[7]. Methicillin-resistant *S. aureus* (MRSA) infections account for 40-60% of all nosocomial (hospital acquired) S. *aureus* infections in many centers across the world. MRSA is a problem in hospitals worldwide and an important cause of health-care-associated infect-ions since 1970's.[8] According to meta-analysis, the average rate of MRSA colonization among healthcare workers (HCWs) is approxim-ately 4.6% worldwide, and evidence suggests that HCWs are likely to play a large role in MRSA transmission.[9] The majority of infections result in asymptomatic carriage. People especially medical staffs carry Staphylococcus bacteria on their skin or inside their nose or throat.[10]

MRSA can spread through direct skin to skin contact or contact with towels, sheets, clothes, dressings or other objects that have been used by person infected or colonized with MRSA.[11] Hand hygiene practice, environmental cleaning and disinfection, timely identification of MRSA-colonized or infected patients and their contacts is included in preventive measures. This can be implemented on a broader scale to prevent the horizontal transmission of MRSA.[12] Isolation of infected patients is also among a major preventive strategy as it reduces the direct and airborne transmission.

Screening is a useful technique to identify the reservoir, initiate contact precautions and eradication measures.[13] In screening, a nasal swab is collected from the external nares of a person which is then conventionally cultured, *S. aureus* is identified and then checked for susceptibility to cefoxitin. In the chromagar screening method, instead of blood agar, chrome agar which directly detects the presence of Methicillin Resistant *S. aureus* is used.[14] The conventional method is a multistep process and time consuming. The chrome agar method is not only a single step process but also easier one. By this we can save time and decrease the workload.

Thus, this project was undertaken to look for the utility of chromogenic media as screening tool for early detection of MRSA strains from the nasal carriage of healthcare workers in tertiary care hospital in central India.

REVIEW OF LITERATURE

In a study by Sharon Rainy Rongpharpi, et al. *Staphylococcus aureus* was isolated in 70 cases (22.22%). Methicillin resistance was seen in11.43% of the *S.aureus* isolates, both by the disc diffusion test and by the Oxacillin Resistance Screen Agar (ORSA) test.[15]

The number of strains *of S. aureus* which were isolated by Radhakrishna M, et al. from 200 participants was 35, with a rate of 17.5% of the 35 isolates of *S. aureus*, 5 (14.3%) were MRSA[16]

In another study carried out by Rudrakshi Singh, et al, thirty five S. aureus strains were isolated from120 samples collected of which 15 were MRSA.[17]

AA Poojary, et al. isolated 40 (16.26 %) MRSA out of 246 specimens. The earliest turnaround time (TAT) for MRSA identification with the conventional methods was 48 hours. 36 isolates (90%) of the MRSA isolates were identified at 24

hours using the BBL CHROM agar. The remaining 4 isolates (10%) were identified at 48hours .[18]

F.E. Hardic, et al. found out of 79 cultures positive for MRSA, BAP/MSO detected 61 (77.2%) while C-MRSA detected 69 (87.3%).[19]

Bram Diederen, et al. collected MRSA strains from Neatherland between 1989 and 1998 in which CHROMagar MRSA was evaluated for its ability to identify methicillin-resistant *Staphylococcus aureus* (MRSA). The sensitivity of CHROMagar MRSA after 24 h of incubation was 95.4%, increasing to 100% after 48 h. The specificity was 100% after 24 hr.[20]

AIM

To assess the utility of chromogenic medium as screening tool for early detection of MRSA as compared to conventional method.

OBJECTIVES

- To screen the health care workers for presence of MRSA strains in anterior nares using chromogenic medium.
- To isolate & identify *Staphylococcus aureus* form anterior nares of Health care providers.
- To determine the burden of nasal carriage of MRSA in Health care providers by using phenotypic confirmatory test.
- Comparative evaluation of chromogenic medium against conventional gold standard test for early detection of MRSA.

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, associated with tertiary care hospital at Bhopal during a time period of 2 months.

Study Population: Health Care Providers of tertiary care hospital, Bhopal

Study Variables: All ages and any sexes.

Inclusion Criteria: 1) All Health Care Providers (HCP) consenting for study.

Exclusion Criteria: 1) All Health Care Providers (HCP) not consenting for study.

Study Type: Cross-sectional Analytical study.

Sample Size: One hundred and twenty (120) Non-Repetitive samples from Health Care Providers (HCP) of tertiary care hospital.

Study duration: 1st August to 1st October 2016

Procedure for Sampling:

After obtaining the Ethical Clearance from the Institutional Ethics Committee, Informed Written Consent was taken from the Heath Care Providers and nasal swabs were collected. Nasal swabs were taken with the help of sterile cotton swab moistened with sterile distilled water from Health Care Providers (HCP) of Tertiary Care Hospital, Bhopal. The samples were transported immediately to Microbiology Laboratory.

Health Care Providers were divided into 4 groups –

Group A (30 Participants) : Consultants

Group B (30 Participants): Junior & Senior Residents

Group C (30 Participants) : Nursing Staff

Group D (30 Participants) : Cleaning Staff/ Ward-boys/ Mausibai etc.

Preparation Chrom Agar:

Chromagar was prepared by using HiCrome™ MeReSa Agar Base (M1674-100G) of HiMedia Laboratories Pvt. Ltd. as per the manufactures instructions. Sterile rehydrated contents of 1 vial of MeReSa Selective Supplement (FD229) and Cefoxitine Supplement (FD259) for selectivity were added as per the manufactures instructions and stored as per the manufactures instructions.

Processing of samples

The samples were inoculated immediately on dried Chromagar & Blood agar plates with preparation of smear thereafter on a clean grease free glass slide

Screening on Chromagar Plates:

Inoculated and streaked Chromagar plates were incubated for 18-24 Hrs at 37°C. MRSA strains were visualized as green colored colonies. If green colonies were not found, then plates further

incubated for next 24 Hrs. After 48 Hrs of incubation, formation of green colored colonies indicated the presence of MRSA strain but if green colored colonies were not found so MRSA strains were absent in the collected specimen.

Culture & Identification on Blood Agar:

The samples were inoculated immediately on Blood Agar and smear was prepared for Direct Examination by Gram staining. Inoculated Blood agar plates were incubated for 18-24 Hrs. at $37^{O}C$. The Beta hemolytic colonies on blood agar were further identified as Staphylococci by Gram Staining which shows Gram positive cocci in clusters. All these colonies were subjected to Slide coagulase test. If slide coagulase test is negative then Tube Coagulase test was performed to confirm it to be *Staphylococcus aureus*.[21]

The identified strains of *Staphylococcus aureus* were further tested for Methicillin Resistance by using disk diffusion by Cefoxitin Disk which is a phenotypic confirmatory test at par with PCR for detection of mecA gene for PCR and is considered a gold standard. [24] MRSA detection was done as per CLSI 2014 guidelines. [22]

All data was maintained in Microsoft office Excel. All statistical analysis was carried out using Excel and test of proportion and test of significance were applied for interpretation of data.

RESULTS

Out of a total of 120 nasal samples collected (30 in each group) and cultured on Blood agar, all samples showed growth. Small round beta hemolytic colonies were seen in 68/120 (56.6%), of which 26 (21.67%) were found to be MRSA by gold standard i.e. Cefoxitin disk diffusin test and rest to be MSSA. A total of 36/120 (30 %) non hemolytic colonies colonies turned out to be Coagulase Negative Staphylococcus and 16/120 (13.3%) were identified as Gram negative bacilli on gram staining which were not processed further keeping in view the objectives of our study. Table 1 shows the distribution of the isolated organisms in different groups of Healthcare workers. [Table 1]

Chromagar as scrrening methods detected a total of 27/120 (22.5%) MRSA strains whereas 26/120 (21.66 %) were detected by Phenotypic confirmatory test (PCT). The distribution of samples yielding MRSA stains in different groups by both the methods shown in table 2 and time taken for detection of the total number of MRSA strains is as shown in Table 3, (Table 2 & 3)

Detection of MRSA strains directly from the samples as screening tool was compared to the gold standard test i.e PCT and was found to be highly significant when tested with Pearsons Chi Square test for significance with Chi Square value of 56.02 and p value of <0.0001 with 1 degree of freedom. Table 4 shows the sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) (Table 4)

Table 1: Nasal samples showing distribution of organisms identified by conventional method

Health Care Workers Group	MRSA	MSSA	CONS	GNB
GROUP A (30)	5	9	11	5
GROUP B (30)	5	13	9	3
GROUP C (30)	7	12	6	5
GROUP D (30)	9	8	10	3
TOTAL (120)	26 (21.66%)	42 (35%)	36 (30%)	16 (13.33%)

Table 2: MRSA isolates by Chromagar and conventional methods in different groups of health care workers

Health Care Workers Groups		Number of MRSA strains detected by phenotypic confirmatory test	Number of MRSA strains detected by by Chromagar
GROUP A (n=30)	**Consultants**	5	4
GROUP B (n=30)	**Junior & Senior Residents**	5	5
GROUP C (n=30)	**Nursing Staff**	7	8
GROUP D (n=30)	**Cleaning Staff/ Ward-boys/ Mausibai etc**	9	10
TOTAL (n=120)		26	27

Table 3: Comparison of time required for MRSA detection

	MRSA by Chromagar (n = 27)		MRSA by Phenotypic confirmatory Test (n = 26)
Time of Reading	24 Hours	48 Hours	48 Hours
Number of MRSA strains	24 (88.88 %)	3 (11.11 %)	26 (100 %)

Table 4: Comparative evaluation of MRSA detection by Chromagar against phenotypic confirmatory test of Cefoxitin Disk Diffusion

	Cefoxitin Resistant (MRSA)	Cefoxitin Sensitive (MSSA)	Total	Chi Square value & p value
Chrom agar positive	25	2	27	56.02
Chrom agar negative	1	40	41	
Total	26	42	68	p value <0.0001
Sensitivity : 96.15%				
Specificity : 95.23%				
PPV : 92.59%				
NPV : 97.56%				

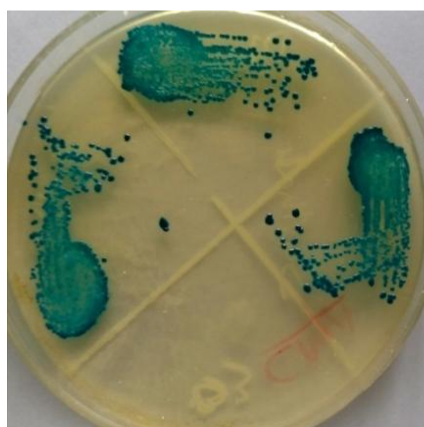

Figure 1: Green coloured colonies on Chromagar

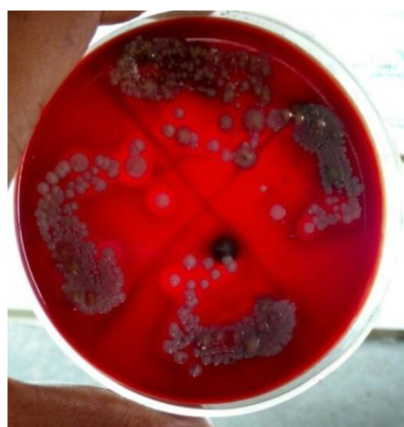

Figure 2: Beta haemolytic colonies on blood agar

Figure 3: Phenotypic confirmatory test (PCT)

DISCUSSION

Out of 120 samples processed, 104 (86.66%) showed growth of Staphylococcus. 68 of 104 (65.38%) strains turns out to be *Staphylococcus aureus* and rest 36 (34.61%) were Coagulase Negative Staphylococcus (CONS). Overall isolation percentage of *Staphylococcus aureus* from 120 nasal samples turned out to be 68/120 i.e 56.66% which is well in accordance with the isolation rates in different part of the world.

In a study carried out in Iran by Moghadam SO et al isolated 39 (14.44%) *Staphylococcus aureus* from nasal carriage in Health Care workers. Whereas in a study conducted by Lakshmi S. Kakhandki and B.V. Peerapur in Bijapur, Karnataka 33(23.6%) strains were isolated form the nasal carriage.[23,24]

26 out of 68 (38.23%) were found to be Methicillin Resistant (MRSA) whereas 42 out of 68 strains (61.76%) strains were found to be Methicilline Sensitive (MSSA) in our study. In a similar study conducted by Kaur DC & Narayan PA out of 140 HCWs, *S. aureus* was isolated in 38 (27.14%) out of which MRSA and methicillin sensitive *S. aureus* (MSSA) were 20 (14.28%) and 18 (12.86%) respectively. [25]

27 out of 68 (39.71%) were detected Methicillin Resistant (MRSA) by Chrome agar. 24 out of 27 (88.88%) were detected in 24 hrs while the remaining 3 out of 27 (11.11%) were detected in 48 hrs. This is in accordance with the study carried out by Department of Pathology and Microbiology, Breach Candy Hospital Trust, Mumbai, India in which 36 isolates (90%) of the MRSA were identified at 24 hours using the BBL CHROM agar. The remaining 4 isolates (10%) were identified at 48hours .[18]

Around 88.88 % strains of MRSA were detected by Chromagar within 24 hrs directly. When compared with the gold standard PCT, the chromagar results were highly significant with Perasons Chi square value of 56.02 and p value of <0.0001 and sensitivity, specificity, PPV and NPV of 96.15% , 95.23 %, 92.59 & 97.56 % respectively.

9 out of 26 (34.61%) which is maximum is found in group D i.e. cleaning staff. 7 out of 26(26.92%) is detected in group C i.e. nursing staff. Minimum but still alarming, 5 out of 26 (19.23%) MRSA was detected in group A i.e. Consultant group as well as in group B i.e. junior and senior residents group. In a similar study carried out in a tertiary health care center of central India by Rudrakshi Singh, also found similar trends with higher number of MRSA Carriage in cleaning staff/ward boys/mausi bais; followed by nursing staff, residents and consultants. This may be because of poor hand hygiene practice in low socio-economic group. In our study, chrome agar has detected 27 MRSA out of which 2 isolates were identified as MSSA by PCT and one isolate which was MRSA by PCT was not detected by chrome agar.

The principal mode of MRSA transmission within an institution is from patient to patient through the already colonised hands of hospital personnel who acquire the organism after direct patient contact or after handling the contaminated materials.

All these groups are the people who are in regular contact with patients for a longer period of time, more so with nursing, ward boys & cleaning staff, therefore surveillance through regular screening for Nasal carriage & treatment of the carriers should be obligatory for prevention of HAI. By this study we come to know that there is the need for a periodic screening of all the healthcare personnel and required measures should be taken to treat the carriers. This study also indicates that instead of using conventional method for detection of MRSA which is multi step process and more time consuming, it can be replaced by chrom agar so that there is early detection of carriers. Early detection leads to early treatment, if required and prevention of transmission of MRSA strains to other patients.

CONCLUSION

Chrom agar MRSA is highly specific and sensitive to differentiate between MRSA and MSSA directly from the clinical samples. Chromagar helps in rapid screening of the clinical samples within 24 hours and helps decrease the workload of the overburdened laboratories obviating the need for phenotypic confirmatory tests. So chromogenic agar can be used routinely for detection of MRSA in clinical samples as well as screening.

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