

Diagnostic Accuracy of CHROMagar MRSA for Detection of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) from Screening Swab Specimens

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ABSTRACT

Objective: To determine the diagnostic accuracy of CHROMagar MRSA for detecting MRSA from screening swab specimens keeping the Cefoxitin disk diffusion test as the reference method.

Study Design: A cross-sectional validation study.

Place and duration of study: Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi Pakistan, from Mar to Aug 2019.

Methodology: A total of 243 screening swab specimens, e.g., axillary, nasal and web swabs, each of hospitalized patients and healthcare workers (HCW) submitted for MRSA screening were included in the study and were processed simultaneously on blood agar, MacConkey agar and CHROMagar MRSA. The agar plates were incubated at 35°C ± 2°C for 18-24 hours in ambient air. The cefoxitin disk diffusion test followed the isolation and identification of MRSA according to the latest Clinical and Laboratory Standards Institute (CLSI) guidelines. In CHROMagar MRSA screening, after incubation, plates were examined for the presence of mauve colonies (MRSA detected), and the results were obtained and validated against the reference method of Cefoxitin disk diffusion.

Results: Overall, the diagnostic accuracy of CHROMagar MRSA for detecting MRSA was 97.53%. Diagnostic accuracy of CHROMagar MRSA was 95.1%, 97.5% and 100% in axillary, nasal and web specimens, respectively. The rate of MRSA detection was maximum in axillary swabs, i.e., 40.7%, followed by 29.6% and 9.8% in nasal and web swabs, respectively.

Conclusion: CHROMagar MRSA is found to be accurate for the detection of MRSA. It is reliable, easy to perform, less time-consuming, and cost-effective. It is an affordable alternative to the conventional method of detection of MRSA in resource-poor settings.

Keywords: Cefoxitin disk diffusion, CHROMagar MRSA, Methicillin-resistant *Staphylococcus aureus* (MRSA).

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global menace to patient management and infection control.¹ The ability of *Staphylococcus aureus* (*S. aureus*) to colonize the host safely contributes to its success as a pathogen. However, its prevalence is alarmingly on the rise while showing resistance to a large panel of antibiotic groups, limiting therapeutic options and responsible for more extended hospital stays with high morbidity and mortality rates.² A study in Karachi reported a 52% prevalence rate of MRSA strains among *S. aureus*.³ The global prevalence of MRSA is continuously growing. For example, a European study documented 65% MRSA in ICU clinical samples.⁴ The increasing rate of MRSA, its changing epidemiology, and the imminent threat of Vancomycin-resistant strains call for the global control of

multidrug-resistant staphylococci.

Rapid and accurate identification of MRSA is necessary for curbing the spread and initiating early antimicrobial therapy in patients, and healthcare workers (HCWs) colonized with MRSA. Conventional phenotypic detection methods of MRSA, such as cefoxitin disk diffusion, require two to three days of processing. There are a plethora of PCR methods that have been developed to detect methicillin resistance in *S. aureus*. Polymerase chain reaction (PCR) methods are regarded as the gold standard test for MRSA, and the target comprises *mecA* gene detection. It is restricted by its high cost, availability and shortage of qualified staff.⁵

Chromogenic media are evolving as a vital tool for rapidly identifying microorganisms in clinical specimens. MRSA screening methods require diagnostic testing that is rapid and accurate for the identification of MRSA carriers and their isolation from other patients. Chromogenic media contain various chromogens

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and antibiotics for selective growth of MRSA. Various chromogenic media vary in their compositions, influencing their sensitivity and specificity for MRSA detection.⁶ Therefore, compared to alternative conventional approaches, chromogenic media save time as they isolate the pathogen directly from the sample and minimize further sub-culturing/biochemical testing. CHROMagar MRSA claims an accurate detection of MRSA with better sensitivity than media containing oxacillin and provides fast and simple interpretation, i.e., intense pale purple colour colony in 18-24 hours. CHROMagar MRSA significantly reduces detection time and workload and enables a mass-level screening of patients.⁷ According to an international study, CHROMagar's sensitivity to detect MRSA is 98.07%, and specificity is 97.80% after 24h of incubation.⁸ This study was planned to determine the diagnostic accuracy of CHROMagar MRSA for MRSA detection from screening swab specimens keeping cefoxitin disk diffusion as a reference method which will help in the timely detection of MRSA but will also help in the early treatment of disease and transmission will be prevented.

METHODOLOGY

This cross-sectional validation study was done at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi Pakistan (Reference Laboratory) from March to August 2019. Permission was obtained from Institutional Review Board, IRB ID 573. The sample size was calculated by sensitivity-specificity using CHROMagar MRSA media sensitivity 91.9% (assuming 91% for sample size calculation) and specificity 99.5% (assuming 99% for sample size calculation),⁹ MRSA prevalence of 52%,⁹ confidence level at 95% and margin of error at 5%. The estimated sample size was 243. Consent was taken from all the patients fulfilling the inclusion and exclusion criteria. Demographic information like name, age, gender and address was obtained. Non-probability consecutive sampling was done.

Inclusion Criteria: All the screening swab specimens, e.g. axillary, nasal and web swabs of hospitalized patients and healthcare workers (HCW) submitted for MRSA screening at the Department of Microbiology, AFIP, were included in the study.

Exclusion Criteria: Duplicate samples were excluded.

Samples were directly streaked on blood agar (BA), MacConkey agar and CHROMagar MRSA

simultaneously and incubated at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 24 hours.

When isolating a staphylococcus from clinical or screening specimens, it is of the utmost importance to ensure that it is, in fact, *S.aureus* rather than coagulase-negative staphylococcus, as the latter can be an opportunistic pathogen. Round, smooth, creamy white and distinctive yellow colonies on BA were deemed presumptive for *S.aureus*. Gram stain was performed on presumptive isolates, and Gram-positive cocci were dealt with for further biochemical tests, including Catalase, Coagulase (slide/tube) and DNase tests. The disc diffusion susceptibility testing using cefoxitin impregnated discs (30 μg) was performed according to the latest Clinical and Laboratory Standards Institute (CLSI 2019) guidelines. The inoculum was prepared, and density was adjusted to obtain semi-confluent growth after incubation. Sterile swabs were used to uniformly inoculate the bacterial suspension on Mueller Hinton agar plates. The cefoxitin impregnated discs (30 μg) were dispensed onto the same agar plates and incubated at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 24 hr. The interpretation of measured diameters of zones of inhibition was made using interpretative criteria of *S.aureus* given in the latest CLSI 2019, as shown in Figure-1.

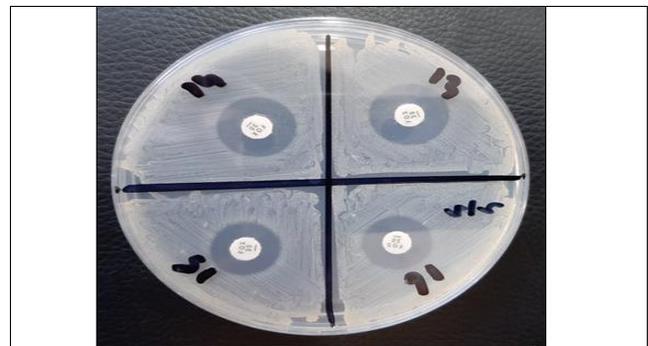


Figure-1: Cefoxitin disk diffusion test.

CHROMagar MRSA screening agar plates were allowed to attain room temperature before inoculation. Screening swab specimens were processed by direct streaking on the agar. The agar plates were incubated at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 18-24 hours in aerobic conditions. After incubation, plates were examined for the presence of mauve colonies, and the results were obtained. The presence of mauve colonies was indicative of a positive result (MRSA), and colonies with any other colour or no growth were considered negative (no MRSA), as shown in Figure-2.

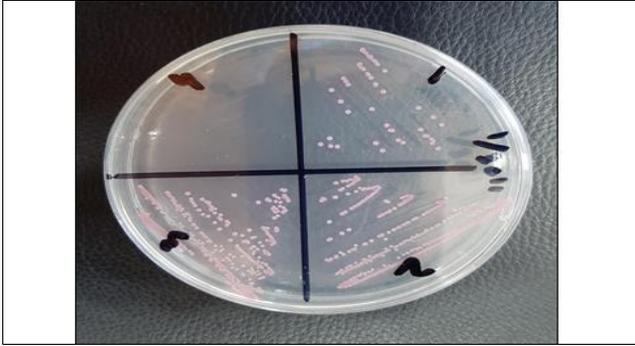


Figure-2: Chromagar MRSA screening agar plate.

The data obtained was entered in SPSS (version 25) software for statistical data analysis. Both qualitative and quantitative variables were calculated using descriptive statistics. Among quantitative variables like age, mean and standard deviation (SD) were calculated and gender frequency, and percentage were calculated for qualitative variables. A 2x2 was made to calculate sensitivity, specificity, positive predicted value (PPV), negative predictive value (NPV) and diagnostic accuracy of CHROMagar MRSA against cefoxitin disk diffusion test (Reference Method) for screening swab specimens. The likelihood ratio and ROC curve were also measured. The *p*-value was calculated by analysis in SPSS (version 25) using covariation and Pearson equation for two tailed studies. The *p*-value of ≤ 0.05 was considered significant, and results of CHROMagar MRSA screening were validated against the Cefoxitin disk diffusion test as the reference method.

RESULTS

A total of 243 patients and HCWs specimens were considered in the study. The average age of the patients and HCWs in this study was 23.56 ± 9.67 years. Most of the patients and HCW, 81 (33.33%), were between 26 to 30 years of age. Of 243 patients, 171 (70.37%) were males, and 72 (29.63%) were females.

In axillary swab specimens, the rate of detection of MRSA was 40.7%, and sensitivity, specificity, PPV, NPV, and diagnostic accuracy was 90.9%, 97.9%, 96.8%, 94% and 95.1%, respectively. Similarly, as for nasal screening swabs, cefoxitin disk diffusion confirmed 29.6% of cases as MRSA and sensitivity, specificity, PPV, NPV, and diagnostic accuracy were 95.8%, 98.2%, 95.8%, 98.2% and 97.5% respectively. By web screening, cefoxitin disk diffusion confirmed 9.8% of cases as MRSA and sensitivity, specificity, positive predictive value, negative predictive value and diagnostic

accuracy were 100% each. Overall diagnostic accuracy of CHROMagar MRSA for MRSA detection was 97.53%, as shown in Tables-I, II.

Table-I: diagnostic parameters of chromagar mrsa for screening swab specimens taking cefoxitin disk diffusion as reference method (n=243).

Screening Swab Specimens	Chromagar MRSA	Cefoxitin Disk Diffusion	
		Positive	Negative
Axillary	Positive	90	3
	Negative	9	141
Nasal	Positive	69	3
	Negative	3	168
Web	Positive	24	0
	Negative	0	219

On ROC curve, AUC of axillary, nasal and web swab specimens were 0.944, 0.970 and 1.000 respectively as shown in Figure-3.

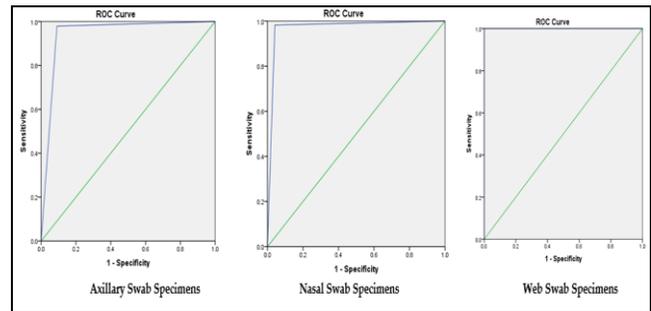


Figure-3: ROC-AUC analysis of axillary, nasal and web swab specimens.

DISCUSSION

MRSA hinders the daily management of patients by causing treatment failure and prolonged morbidity. Surveillance of MRSA of high-risk patients' samples taken from different body sites is pivotal in patient care to limit the emergence and spread of such strains and avoid unnecessary antimicrobials.¹⁰ MRSA screening appears to be a cost-effective method of controlling nosocomial MRSA transmission. Clinical microbiology laboratories must be proficient in the detection of MRSA. Our study aimed to find a method for MRSA detection that is easy to perform, rapid and accurate, especially in microbiological laboratories where molecular assays are unavailable, and the prevalence of MRSA is high. The reference and gold standard method for MRSA identification is detecting the *mecA* gene by PCR. Although PCR yields results quickly, it is expensive, requires experienced staff and is not applicable in every laboratory.¹¹ Some chromogenic screening media are used for reliable MRSA detection

CHROMagar MRSA

based on cultures showing variable performance in various laboratories.¹² was 84.8%, 87.9%, 80.8%, and 83.8% after 18 hours; 91.9%, 94.9%, 90.9% and 92.9% after 24 hours

Table-II: Diagnostic parameters of chromagar mrsa for screening swab specimens taking cefoxitin disk diffusion as reference method (n=243).

Screening Swab Specimens	Diagnostic Parameters	CHROMagar MRSA % values
Axillary	Sensitivity = $90/90+9 \times 100$	90.9%
	Specificity = $141/141+3 \times 100$	97.9%
	Positive Predictive Value= $90/90+3 \times 100$	96.8%
	Negative Predictive Value= $141/141+9 \times 100$	94%
	Diagnostic Accuracy= $90+141/90+141+3+9$	95.1%
Nasal	Sensitivity = $69/69+3 \times 100$	95.8%
	Specificity = $168/168+3 \times 100$	98.2%
	Positive Predictive Value= $69/69+3 \times 100$	95.8%
	Negative Predictive Value= $168/168+3 \times 100$	98.2%
	Diagnostic Accuracy= $69+168/69+168+3+3 \times 100$	97.5%
Web	Sensitivity = $24/24+0 \times 100$	100%
	Specificity = $219/219+0 \times 100$	100%
	Positive Predictive Value= $24/24+0 \times 100$	100%
	Negative Predictive Value= $219/219+0 \times 100$	100%
	Diagnostic Accuracy= $24+219/24+219+0+0 \times 100$	100%

Many previous studies have assessed the efficiency of different commercial chromogenic media for MRSA detection. For example, Cherkaoui *et al.*¹³ reported that primary plating on MRSA ID and MRSA Select was more sensitive, i.e., 90 and 91%, than screening done with ORSAB and Chromogen oxacillin *S.aureus* (Axon Lab). Nahimana *et al.*¹⁴ stated that MRSA Select displayed 65% sensitivity and 100% specificity after inoculation with direct specimen and incubation for 16-18 hours. Further, the sensitivity was increased for all media by prolonging the incubation time to 42 hours. In a study by Van *et al.*¹⁵ CHRO Magar MRSA, MRSASelect and MRSA ID from nasal swabs reflected sensitivities of 75%, 68%, and 72% after 24 hours of incubation and 79%, 77%, and 82% after 48 hours of incubation. In contrast to our study, lower sensitivity was detected from a non-nasal site, in which higher sensitivity (%) was observed in non-nasal swabs.

In this study, the rate of MRSA detection was maximum in axillary swab specimens, i.e., 40.7%, followed by 29.6% and 9.8% in nasal and web swab specimens, respectively. Diagnostic accuracy of CHROMagar MRSA was 95.1%, 97.5% and 100% in axillary, nasal and web swab specimens, respectively. Previous studies reported the sensitivity and specificity of CHRO Magar MRSA ranging from 95.4 % to 100 % and 95 % to 100 %, respectively.¹⁶⁻¹⁸

In Rahbar *et al.* study,⁹ the sensitivity of CHRO Magar MRSA, MRSA Select, MRSA ID and MSA-FX

respectively. The specificity of CHROMagar MRSA, MRSA Select, MRSA ID and MSA-FX was 99.8%, 99%, 98.7% and 97.7% after 18 hours and 99.5%, 98.5%, 98.1% and 97.1% after 24 hours respectively. These results are concordant with our study.

The significant benefit of CHROMagar MRSA is that the agar is available in the market as agar base form and already prepared agar plates, enabling it to be conveniently transported to the testing lab without sufficient cooling.¹⁹ The agar base could be stored for up to 2 years at 2°C to 8°C.^{19,20} This study's limitations included several procedure variables, including the type of specimen, incubation period, and broth enrichment phase that affect the output of chromogenic media.²¹ Possible exposure to light before and during incubation may destroy chromogens and gives false results. Few strains of coagulase-negative *staphylococci* may produce mauve colonies. Prolonged incubation, i.e., more than 24 hours, may increase the number of false-positive results. The presence of coagulase-negative *staphylococci* was not evaluated in this study. This may affect CHROMagar MRSA's utility in clinical samples regarding sensitivity and specificity. Initial inoculation restricted to only one MRSA screening agar was considered one of the drawbacks of this study.

CONCLUSION

CHROMagar MRSA for detecting MRSA is reliable, easy to perform, less time-consuming and cost-effective. It showed acceptable sensitivity and specificity and has not only dramatically improved our ability of rapid but also

accurate detection of MRSA from screening swab specimens. It is a suitable alternative to conventional methods of MRSA detection, i.e. cefoxitin disk diffusion. It can be an affordable alternative to the molecular detection method of MRSA in a resource-poor setting. CHROMagar MRSA is recommended for routine use to rapidly detect MRSA, which will help in infection prevention and control measures. Further studies are needed and recommended for the use of CHROMagar MRSA for MRSA detection in direct clinical specimens.

Conflict of Interest: None.

Author's Contribution

FA: Data collection, data analysis, result and discussion of literature, WH: Review of article and correction, IAM: Data analysis and result, SA: Data analysis, data interpretation, discussion of literature and review, UK: Discussion and review of article and correction, MS: Discussion and Literature review.

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