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ORIGINAL ARTICLE

Diagnostic accuracy of MRSA (Methicillin-resistant Staphylococcus aureus) chrom agar comparing it with cefoxitin disc diffusion method.

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ABSTRACT… Objective: To evaluate the sensitivity and specificity of MeReSa Chrom agar to detect methicillin-resistant Staphylococcus aureus in clinical specimens. **Study Design:** Cross sectional. **Setting:** Department of Microbiology, Allama Iqbal Medical College, Jinnah Hospital Lahore. **Period:** January 2024 to January 2025. **Methods:** The 525 iolates of Staphylococcus aureus was isolated from various clinical samples. Cefoxitin disc diffusion method was used to isolate MRSA. All isolates were inoculated on Chrom agar, and growth was noted after 24 and 48 hours of incubation. All isolates were later analyzed for the presence of the mec A gene through PCR. Sensitivity, specificity, positive predictive value and negative predictive value were calculated through SPSS 27.0. **Results:** From total 525 Staphylococcus aureus isolates, 180 were resistant to methicillin (MRSA), and the remaining 345 were sensitive to methicillin (MSSA). All 525 isolates were cultured on Chrom agar, and sensitivity and specificity were 95.5% and 97.96%, respectively, with 96.1% positive predictive value (PPV) and 97.7% negative predictive value (NPV) noted against the cefoxitin disc diffusion method. **Conclusion:** HiCHROM™ MeReSa Chrom agar can be used to detect MRSA from clinical specimens due to its high sensitivity and specificity. It can operate as selective and confirmatory media for MRSA detection.

Key words: Chrom Agar, Cefoxitin Disc Diffusion Method, Methicillin-resistant Staphylococcus Aureus, MRSA, Mec-A gene.

INTRODUCTION

Staphylococcus aureus is a common pathogen for humans that can cause broad range of infections.[1] Staphylococcus aureus associated infections may range from minor folliculitis[2] to severe meningitis[3] and toxic shock syndrome.[4] Humans are the natural reservoir for this highly minacious gram-positive pathogen. After escaping from the human immune response by forming an antiphagocytic capsule, it can cause nosocomial, community-acquired, and prosthetic device infections.[5] Staphylococcus aureus has an incredible ability to acquire resistance against many antibiotics. Methicillin-resistant Staphylococcus aureus (MRSA) shows resistant to the almost all β-lactam antibiotics.[6] And due to this broad resistant pattern, detection of MRSA and the drug of choice to treat it have great importance. Vancomycin and Linezolid are helpful for the treatment of MRSA.[7]

Various methods are currently available to detect MRSA. In molecular biology, detection of the mecA gene by PCR reaction is considered a gold standard due to its enormous diagnostic accuracy.[8] Detection of MRSA from cefoxitin disc diffusion method[9], PBP2a latex agglutination test[10], and the use of selective media Chrom agar are three good phenotypic methods for the detection of MRSA with high sensitivity and specificity. To limit the spread of MRSA, early detection has supreme importance. To overcome this issue, selective media for MRSA identification can be used. So, Chrom agar has unique formation, as it shows specific color in the presenc of MRSA.[11]

HiCHROM™ MeReSa Chrom agar (M1674) is a selective media for identifying MRSA.[12] This chromogenic media has a unique chromogenic mixture, cefoxitin supplement (FD259) and MeReSa selective supplements (FD229).

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Only MRSA strains grow and cleave the chromogenic variety and get bluish-green colored colonies.[13] This study observed the diagnostic accuracy of MeReSa Chrom agar by comparing it with the cefoxitin disc diffusion method and PCR results for the mec-A gene.

METHODS

This study was approved from the ethical review board (ERB 145/21/04-01-2024/SIERB) of Allama Iqbal Medical College/Jinnah Hospital Lahore. This crossectional study was conducted in the Microbiology laboratory of pathaology department of Allama Iqbal Medical College from January 2024 to January 2025. Non random probability samplimg technique was used to collect the samples from both indoor and outdoor patient departments. All samples with the growth of Staphylococcus aureus were included in this study and all other and repeat sample from same patient were excluded.

Staphylocccus aureus was isolated from various clinical specimens on blood agar and from urine on CLED agar. It confirmed by gram stain, catalase test, coagulase test, and yellow-colored growth on mannitol salt agar. After confirmation of specie, cefoxitin disc (30μg) was applied on all isolates using the modified Kirby Bauer disc diffusion antimicrobial susceptibility method to differentiate between MRSA and methicillin sensitive Staphylococcus aureus (MSSA).[14] Culture plates were incubated for 24 hours at 37°C. Zone sizes were measured according to CLSI 2024. Isolates that were resistant to cefoxitin were considered MRSA and susceptible to methicillin concluded as MSSA.[15]

Genotypic analysis for the presence of the mec-A gene was performed on all isolates. DNA was isolated using an illustra extraction kit according to the guidelines of the manufacturing company.[16] Amplification was performed in an Eppendorf thermocycler. DNA was isolated by using an illustra extraction kit according to the guideline of the manufacturing company. Amplification conditions were: 92 °C for 3 min, followed by 30 cycles of DNA denaturation at 92 °C for 1 min, annealing at 56 °C for 1 min, and extension at 72 °C for 3 min. The final reaction volume was 35 μL containing 10.2 μL autoclaved Milli-Q water, 3.5 μL deoxyribonucleotide triphosphates (dNTP), 0.8 μL Taq DNA polymerase, 2.5 μL $MgCl_2$-free buffer, 2.5 μL $MgCl_2$, 0.14 μM of each 16S primer, 0.86 μM of each MRS primer, 0.35 μM of each SAU primer, 0.57 μM of each COA primer, and 3.5 μL of bacterial DNA. The amplification products were analyzed by electrophoresis on 3% agarose gel at 70 V.[17]

All isolates were cultured on HiCHROM™ (M1674) MeReSa Chrom agar, and growth was noted after 48 hours of incubation at 37°C. Staphylococcus aureus grown with round, small and bluish-green color colonies (Figure-1) were concluded as MRSA. Staphylococcus aureus whose growth was inhibited on Chrom agar, was considered MSSA. Sensitivity and specificity of HiCHROM™ (M1674) MeReSa Chrom agar was calculated in comparison with cefroxitin disk diffusion method and PCR for mecA using SPSS 27.0.

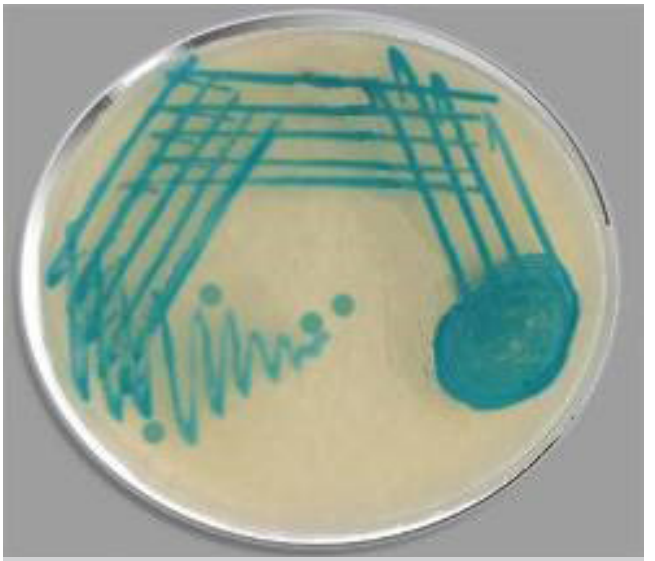

Figure-1. Growth of methicillin-resistant Staphylococcus aureus on HiChrom ™ MeReSa agar

RESULTS

In this study, a total of 525 samples were collected from patients with different ages. The mean age (+ standard deviation) was 36.99±19.49. Patients were divided into five age groups 0-15, 16-30, 31-45, 46-60, and >60 years. Highest percentage 36.76% were from age group 16-30 years. From all the patients, 53.14% were female and 46.86%

were males. Among all the samples, the frequency of Staphylococcus aureus from pus samples was the highest which was 77.4% (Table-I).

Variables	Frequency (%)
Age group (Years)	
0-15	30 (5.71%)
16-30	193 (36.76%)
31-45	170 (32.40%)
46-60	108 (20.56%)
>60	24 (4.57%)
Gender	
Female	279 (53.14%)
Male	246 (46.86%)
Clinical sample type	
Pus	407 (77.5%)
Fluids	25 (4.8%)
Blood culture	20 (3.8%)
Sputum	17 (3.2%)
Urine	15 (2.9%)
CVP line tip	13 (2.5%)
High vaginal swab	10 (1.9%)
Tissue culture	8 (1.5%)
Nasal swabs	6 (1.1%)
Tracheal secretions	4 (0.76%)

Table-I. Frequency and percentage of different variables

After susceptibility testing using cefoxitin (30μg), it was concluded that among 525 Staphylococcus aureus isolates, 180 were resistant, and all 345 isolates were sensitive to cefoxitin. Sensitivity, Specificty, PPV and NPV of cefoxitin disc diffusion method was first calculated comparing it with PCR results of mecA gene detection and shown in Table-II which was 99.4% and 100% respectively. After this sensitivity, specificity, positive predictive value, & negative predictive values of chrom agar were calculated by comparing with cefoxitin disc diffusion method and it was 94.1%, 98.23%, 96.66%, & 96.81% respectively as shown in Table-III.

DISCUSSION

In this cross sectional study, total 525 samples were tested and 53.14% were female and 46.86% were male. Highest 53.14% patients were from age group 16-30 years and mean age was 36.99±19.49. Staphylococcus aureus were isolated from pus, body fluids, blood cultures, sputum, urine, CVP line tips, HVS, tissue culture, nasal Swab, and tracheal secretions. 77.5% were pus samples, which was the the highest count among all. Out of 525 samples, 180 were resistant to methicillin (MRSA) and remaining 345 were sensitive to methicilline (MSSA). A similar study was conducted in Iran by (Koupahi et al. 2023), in which male patients were 56.4% and female were 43.6% and it was opposite to our study. Iranian study also showed the sensitivity and specificity of chrom agar and both were 100%, and results of our study are very close to Irnanin study.

			mec A Gene Detection	
			Detected	**Not Detected**
Cefoxitin Disc Diffusion Test	MRSA	Count	180	0
		% within mec A Gene	99.4%*	0.0%
		% within Cefoxitin DD Test	100%*	0.0%
	MSSA	Count	1	344
		% within mec A Gene	0.6%	100.0%*
		% within Cefoxitin DD Test	0.3%	99.7%*

Table-II. Sensitivity, specificty, PPV, NPV of cefoxitin disc diffusion method
***Sensitivity of Cefoxitin DD Test: 99.4%, Sepecificty of Cefoxitin DD Test: 100%, PPV: 100%, NPV: 99.7%**

			Susceptibility to Cefoxitin	
			MRSA	**MSSA**
Growth on HiChrom ™ MeReSa agar	Growth with Blue-Green Colonies	Count	174	06
		% within Cefoxitin Disc Diffusion Test	94.05%*	1.76%
		% within Growth on HiChrom ™ MeReSa agar	96.66%*	3.34%
	No Growth	Count	11	334
		% within Cefoxitin Disc Diffusion Test	5.94%	98.23%*
		Growth on HiChrom ™ MeReSa agar	3.19%	96.81%*

Table-III. Growth on HiChrom ™ MeReSa agar & susceptibility to cefoxitin
***Sensitivity of HiChrom ™ MeReSa: 94.05%, Sepecificty of HiChrom ™ MeReSa: 98.23%, PPV: 96.66%, NPV: 96.81%**

As, sensitivity and specificity of our study was 94.1% and 98.23% respectively.[18]

Another study was conducted by (Bhoi, Swain & Otta 2021) and their results are comparable to our study, MRSA were 38.4% and in our study it was 34.28%, both are very close. In contrast to our study, they compared cefoxitin disc diffusion method with Vitek 2 MIC system. While we did it with the comparison of PCR for mec A gene. But, if we compare sensitivity and specificity of chrom agar then results are very close which were 93.75% and 97.36% respectively. They additionally reported antimicrobial susceptibility pattern which is lacking in our study.[19]

Another prospective cross sectional study was conducted by (Madhavan et al. 2021) and results were much similar to our study, but the study was different slightly because they compared with Vitek 2 system and found that sensitivity and specificity of cefoxitin disc diffusion method were 97.2% and 100% respectively. Senstivity of chrom agar was 100% which is comaparable to ours but there was an anough gap between specificity of (Madhavan et al. 2021) study and our study. Specificity in study by (Madhavan et al. 2021) was only 78.6% but in our study it was 98.23%. They also reported positive and negative predictive values which are much close to positive and negative predictive values of our study. They reported 92.3% positive predictive value while we found 96.66% and negative predictive value was 96.81% in our study and they reported it 100%. This difference may be due to low sample count in study by (Madhavan et al. 2021). As they worked on 100 samples and only 72 were MRSA and in our study total sample count was 525 and MRSA were 180. Another important point is that, they reported more MRSA as compare to MSSA.[20]

Another study was conducted in tertiary care hospital of central India in which 38.6% MRSA count was very close to our study and they reported that 95.1% MRSA isolated after 24 hours while remaining 4.9% after 48 hours incubation on chrom agar. Both contrast and similarty exsits if we compare sample type. Most of the Staphyloccus aureus were isolated from Pus samples in both studies. But, they reported high MRSA from blood samples which was 42.1% and in our study overall Staphylococcus aureus isolated from blood samples was 3.8% only.[21]

CONCLUSION

Chrom agar has high sensitivity and specificity to isolate MRSA. There is a need for rapid identification of MRSA to minimize the spread. So, Chrom agar can be used for routine screening of MRSA from any clinical specimens. Cefoxitin disc diffusion testing can also be used as another cheap and rapid method for MRSA detection and can replace expensive PCR-based methods.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORSHIP AND CONTRIBUTION DECLARATION	
1	**Muhammad Touqeer Hanif:** Actual concept writing of paper.
2	**Farhan Rasheed:** Reviewing.
3	**Zainab Yousaf:** Statistical analysis.
4	**Dania Niaz:** Data collection.
5	**Sara Arif:** Data collection.
6	**Afshan Zia:** Drafting.