Comparison of cefoxitin disc diffusion test, HiCrome rapid MRSA agar and PCR for *mec*A, PVL and *fem*B genes for detection of methicillin-resistant *Staphylococcus aureus* (MRSA)

Sineth Jeewantha Wijekoon[1], Harshi Abeygoonawardena[1], Ridmy Amaya Karawgoda[2], Nambage Shirani Chandrasiri[3], Nilanthi Priyadarshika Senanayake[2]

(Key words: MRSA, HiCrome rapid MRSA agar, cefoxitin disk diffusion, *mec*A, PVL, *fem*B)

Abstract

This study evaluates the effectiveness of cefoxitin disk diffusion as a method to detect methicillin-resistant *Staphylococcus aureus* (MRSA), comparing with HiCrome MRSA screening agar and PCR-based *mec*A gene detection. Both PCR and the cefoxitin disk diffusion test exhibited 100% sensitivity and specificity, while HiCrome agar showed slightly lower sensitivity (90%) and specificity (95.24%) out of 72 *Staphylococcus aureus* isolates. The MRSA detection rate was found to be 41.66%, with *fem*B and PVL genes present in 86.6% and 13.3% of MRSA isolates, respectively. Cefoxitin could serve as a reliable substitute marker for MRSA detection, particularly in settings with limited resources.

Introduction

Staphylococcus aureus, a Gram-positive bacterium, is known for causing various infections. Its resistance to antibiotics has been a growing concern since the introduction of penicillin in the 1940s [1]. This led to the development of methicillin, but methicillin-resistant *S. aureus* (MRSA) emerged shortly after its introduction in 1959 [2]. MRSA's resistance is mainly due to the acquisition of a mobile element called Staphylococcal cassette chromosome encoding methicillin resistance (SCCmec), which carries crucial genes including *mec*A. These genes alter the bacterium's response to antibiotics [3,4].

The traditional gold standard for identifying MRSA involves PCR detection of the *mec*A gene, but this method can be expensive and technically demanding [5,6]. Alternative methods like oxacillin disc diffusion have limitations. Cefoxitin, a potent inducer of the mec complex, has shown promise as a cost-effective and reliable alternative, often producing clear distinctions between MRSA and methicillin-sensitive *S. aureus* (MSSA). Furthermore, cefoxitin-resistant *S. aureus* tends to be resistant to various β-lactam antibiotics, reinforcing its value as an MRSA marker [7].

HiCrome rapid MRSA agar is another method that utilizes selective growth conditions and specific chromogenic reactions to distinguish MRSA colonies [8]. The current study aims to assess the effectiveness of cefoxitin disc diffusion and HiCrome MRSA screening agar in comparison to PCR for detecting MRSA. The goal is to identify the most accurate phenotypic method for MRSA detection, which is crucial for effective antibiotic treatment and infection management.

Moreover, the study aims to determine the prevalence of the *fem*B and Panton-Valentine Leukocidin (PVL) genes in MRSA isolates. This genetic information is relevant for understanding MRSA strains and their potential virulence factors. Additionally, the study considers practical aspects such as turnaround time and cost-effectiveness, crucial factors in choosing routine MRSA detection methods for diagnostic labs.

Methods

A total of 107 clinical isolates taken from wound swabs, blood, tracheal secretions and urine received from the Department of Microbiology, Colombo South Teaching Hospital, Sri Lanka, from 1st February 2018 to

Ceylon Medical Journal 2023; **68:** 81-84

DOI: https://doi.org/10.4038/cmj.v68i2.9765

[1]*Faculty of Medicine, General Sir John Kotelawala Defense University, Sri Lanka.* [2]*Faculty of Medicine, University of Colombo, Sri Lanka.* [3]*Department of Microbiology, Colombo South Teaching Hospital, Sri Lanka.*

Correspondence: NPS, *e-mail: <nilanthi@micro.cmb.ac.lk>.* *Received 12 March 2023 and revised version 11 June 2023 accepted 22 June 2023*

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

1st April 2018. All isolates were cultured on blood agar, chocolate agar, and MacConkey agar and incubated at 35°C for 24 hours. *S. aureus* isolates were identified by Gram staining and positive results for the catalase test, slide coagulase test, tube coagulase test, mannitol salt agar (MSA) test and DNase agar test methods. Methicillin-sensitive *S. aureus* (MSSA) ATCC 25923 and methicillin-resistant *S. aureus* (MRSA) ATCC 43300 standard strains were used as negative and positive controls, respectively, throughout the study.

CEYGEN Bacto-Spin D™ Bacterial DNA extraction kit (column based) was used for DNA extraction. Multiplex PCR was carried out for the detection of *mec*A, *fem*B and PVL genes. The genes were amplified using the primer sequences and PCR was performed described by Jonas *et al.* (2002) and Lina *et al.* (1999), and given in Table 1. Cefoxitin disc diffusion and HiCrome™ rapid MRSA test were performed according to the CLSI guidelines.

Statistical analysis

Sensitivity, specificity, positive and negative predictive values were calculated manually using PCR identification of strains as the standard and values were computed. IBM SPSS version 25 (SPSS Inc. Chicago, USA) was used for other statistical tests such as Friedman test was used to calculate the p-value to identify which method(s) is/are statistically more accurate in identifying MRSA. Significance level (α) was considered as 0.05. Accuracy of the three sets being identical was considered as the null hypothesis.

Results

Of the 107 clinical isolates 72 were identified as *S. aureus* by initial tests (growth on MacConkey agar, catalase test, slide coagulase test, tube coagulase test, mannitol salt agar (MSA) test and DNase agar test). Of the 72 samples, 30 were positive for the *mec*A by PCR. PCR results further indicated that 22 samples carried both *mec*A and *fem*B genes while only 4 samples carried all three tested genes. Gene distribution of these 30 samples is depicted in Figure 1. PCR, which is considered the golden standard for MRSA identification, revealed that the overall percentage of MRSA was 41.66% (30 out of 72 samples) while the remaining 42 isolates were regarded as MSSA.

Percentage of MRSA by cefoxitin disc diffusion and HiCrome rapid MRSA agar was 41.66% (30/72) and 37.5% (27/72), respectively. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the methods are given in Table 2. Overall accuracy of cefoxitin disc diffusion was 100%, whereas it was 93.06% for the Hicrome rapid MRSA agar test. An overview of the test results between PCR, cefoxitin disc diffusion and HiCrome rapid MRSA agar tests relative to identifying MRSA are given in Figure 2.

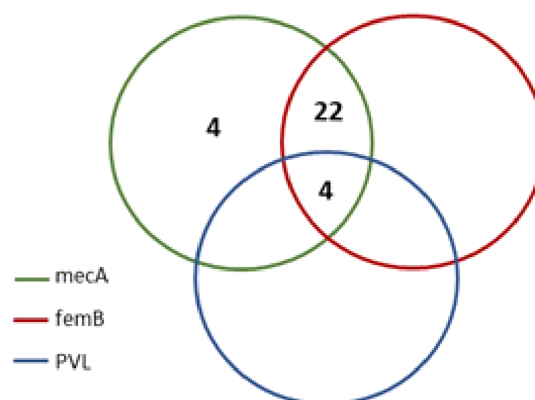

Figure 1. **Distribution of *mec*A, *fem*B and PVL genes in MRSA samples (n=30) as observed from multiplex PCR results.**

Table 1. Primer sequences used for the single-tube multiplex-PCR analysis of *mec*A, *fem*B and PVL gene amplifications [9,10]

Gene	*Primer sequence*	*Amplicon*
*Mec*A1	5'- GTA GAA ATG ACT GAA CGT CCG ATA A -3'	310 bp
*Mec*A2	5'- CCA ATT CCA CAT TGT TTC GGT CTA A -3'	
*Fem*B1	5'- TTA CAG AGT TAA CTG TTA CC -3'	651 bp
*Fem*B2	5'- ATA CAA ATC CAG CAC GCT CT -3'	
Luk-PV-1	5'- ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A -3'	433 bp
Luk-PV-2	5'- GCA TCA AST GTA TTG GAT AGC AAA AGC -3'	

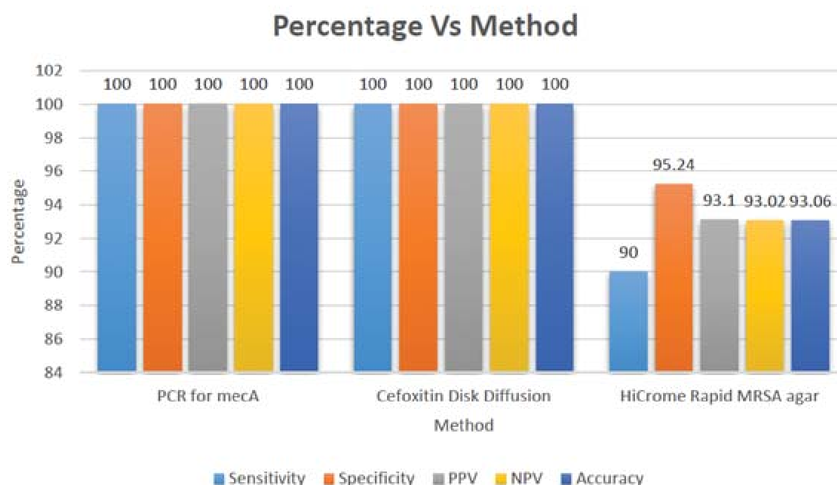

Figure 2. **Comparison of sensitivity, specificity, PPV, NPV and accuracy results between PCR, cefoxitin disc diffusion and HiCrome rapid MRSA agar tests.**

Table 2. Comparison of phenotypic methods for MRSA detection

Method	*Sensitivity (at 95% CI)*	*Specificity (at 95% CI)*	*PPV (at 95% CI)*	*NPV (at 95% CI)*
Cefoxitin disc diffusion	100.00% (88.43-100.00)	100.00% (91.59-100.00)	100.00 -	100.00% -
HiCrome rapid MRSA agar	90.00% (73.47-97.89)	95.24% (83.84-99.24)	93.10% (77.64-98.13)	93.02% (81.97-97.51)

CI – confidence interval

Friedman test was used to calculate whether there was a significant difference between the accuracy of used PCR, cefoxitin disc diffusion and HiCrome rapid MRSA agar tests. Significance value of .000 was observed indicating a significant difference between the accuracy of tested methods. The cost analysis was done to compare the two methods. Cost-effectiveness involves evaluating the efficiency of a method in relation to its cost. In the study, the cost per sample for each method was calculated. The cost per sample for PCR was Rs. 3915/=, and the cefoxitin disc diffusion test was Rs. 89/=. This suggests that the cefoxitin disc diffusion test is significantly cheaper per sample compared to PCR.

Cefoxitin disc diffusion method	*Cost per sample*	*PCR method*	*Cost per sample*
Muller hinton agar 500g (plates 364) = Rs. 8473.20	Rs. 23.27	CEYGEN Bacto-Spin D™ Bacterial DNA extraction kit (column based) Rs. 36,000/100	Rs. 360
Cefoxitin 30 mcg (50 dsc) 1 vial Rs. 1392	Rs. 27.84	PCR master mix Rs.173,985 = 50 Nos.	Rs. 3479.70
Culture swabs 500 = Rs. 11,000	Rs. 22.00	Primers 1343 × 6 = 8058	Rs. 75.30
Cation adjusted muller hinton broth 250g = Rs. 10,764	Rs. 15.89		
Total	Rs. 89.00		Rs. 3915.00

Discussion

In the present study, sensitivity and specificity of cefoxitin disc diffusion testing was found to be 100%, which was in complete concordance with the PCR results. Detection of *mec*A gene by PCR is the gold standard for MRSA confirmation. Considering these cost and time, cefoxitin disc diffusion testing is a cost-effective, reproducible, practical method for resource-constrained, developing countries such as Sri Lanka and can be used as a replacement for PCR for MRSA screening in a large population or during an outbreak. The cost per PCR sample was Rs. 3,915/= and cost per cefoxitin disc diffusion per sample was Rs. 89/= at the time of this study. Therefore, cefoxitin can be used as an accurate surrogate marker for the detection of MRSA. Hence cefoxitin disc diffusion can be used as an alternative for the technically demanding PCR, in resource-constrained settings.

Author contributions

All authors are equally contributed to the study.

Competing interests

Authors declare no conflicts of interest.

Acknowledgements

We are grateful to the patients and staff of Department of Microbiology, Colombo South Teaching Hospital for their support of sample collection.

Ethics approval

Ethical clearance was obtained from the Ethical Review Committee of the Faculty of Medicine, General Sir John Kotelawala Defence University, Ratmalana (Reference number RP/BCAS/2018/03).

Sources of funding

We are grateful to the General Sir John Kotelawala Defence University for providing funding for the purchasing kits and reagents.

References

1. Hartman BJ, Tomasz A. Low-affinity penicillin-binding protein associated with β-lactam resistance in *Staphylococcus aureus*. *Journal of Bacteriology* 1984; **158**(2): 513-6.
2. Eriksen KR, Erichsen I. Resistance to methicillin, isoxazolyl penicillins, and cephalothin in *Staphylococcus aureus*. *Acta Pathol Microbiol Scand* 1964; **62**: 255-75.
3. Chen FJ, Huang IW, Wang CH, *et al. mec*A-positive *Staphylococcus aureus* with low-level oxacillin MIC in Taiwan. *Journal of Clinical Microbiology* 2012; **50**(5): 1679-83.
4. Gunawardena N, Thevanesam V, Kanakaratne N, Abeysekera D, Ekanayake A, Perera N. Molecular identification of methicillin resistance and virulence marker in *Staphylococcus aureus*. *Sri Lankan Journal of Infectious Diseases* 2012; **2**(2): 18-29.
5. Madhavan A, Sachu A, Balakrishnan A, Vasudevan A, Balakrishnan S, Vasudevapanicker J. Comparison of pcr and phenotypic methods for the detection of methicillin resistant *staphylococcus aureus*. *Iranian Journal of Microbiology* 2021; **13**(1): 31-6.
6. Alharthi AA, Gaber A, Hassan MM. Molecular characterization of *mec*A and SCCmec Genes in pathogenic Staphylococcus spp. collected from Hospitals in taif Region, KSA. *Biotechnology* 2016; **15**(1-2): 26-34.
7. Pourmand MR, Hassanzadeh S, Mashhadi R, Askari E. Comparison of four diagnostic methods for detection of methicillin resistant *Staphylococcus aureus*. *Iranian Journal of Microbiology* 2014; **6**(5): 341-4.
8. Himedia Laboratories, India. Technical data sheet for HiCrome™ Rapid MRSA Agar Base, 2020. https://himedialabs.com/TD/M1974.pdf (accessed on Oct 18, 2022)
9. Jonas D, Speck M, Daschner FD, Grundmann H. Rapid PCR-Based Identification of Methicillin-Resistant *Staphylococcus aureus* from Screening Swabs. *Journal of Clinical Microbiology* 2002; **40**(5): 1821-3.
10. Lina G, Pie Y, Godail-gamot F, Peter M. Involvement of Panton-Valentine Leukocidin - Producing *Staphylococcus aureus* in Primary Skin Infections and Pneumonia. *Clinical Infectious Diseases* 1999; **29**(5): 1128-32.