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Original Research Article

Evaluation of cefoxitin disc diffusion test as a rapid phenotypic method for detection of methicillin resistance *Staphylococcus aureus*

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ABSTRACT

Background and Objectives: Accuracy and promptness in the detection of methicillin resistance are of key importance in ensuring correct antibiotic treatment in infected patients and control of MR staphylococci in the hospital environment. The aim of this study was to detect MRSA phenotypically by oxacillin screen agar and Oxacillin MIC method and to evaluate cefoxitin disc diffusion test as a screening tool for MRSA detection.

Materials and Methods: In the present study, a total of 50 isolates of *Staphylococcus aureus* from various clinical samples collected were used for the detection of Methicillin resistant *Staphylococcus aureus* (MRSA). Methicillin resistance was determined by oxacillin disc diffusion, cefoxitin disc diffusion the oxacillin screen agar test and MIC.

Result: Out of 50 *Staphylococcus aureus* isolates 21 (42%) isolates were detected as MRSA based on MIC method, which is considered as gold standard method for the detection of MRSA. All the isolates of MRSA were 100% susceptible to vancomycin and linezolid. \r\nIn the present study, cefoxitin diffusion method has given 100% sensitivity and specificity in concordance with MIC method. However, the oxacillin screen agar method showed 95.24% sensitivity and 96.55% specificity.

Conclusion: As per our study and previous reports elsewhere on phenotypic detection of MRSA, cefoxitin is more potent inducer of the *macA* regulatory system and an accurate surrogate marker for the detection of MRSA in the routine susceptibility testing.

1. Introduction

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.[1]

Accuracy and promptness in the detection of methicillin resistance are of key importance in ensuring correct antibiotic treatment in infected patients and control of MR staphylococci in the hospital environment.[2]

In the recent past, there have been multiple reports on the use of cefoxitin as a surrogate marker for detection of *mec A* gene-mediated methicillin resistance.[3] Cefoxitin is a potent inducer of the *mecA* regulatory system.[4]

Cefoxitin, a cephamycin, is a more potent inducer of the *mecA* regulatory system than are the penicillins.[5] Several groups of investigators have reported that the results of cefoxitin disk diffusion (DD) tests correlate better with the presence of *mecA* than do the results of disk diffusion tests using oxacillin.[6]

Susceptibility to oxacillin by disc diffusion has been used for the detection of MR staphylococcal strains in routine testing; however, some recent studies have reported low sensitivity and low specificity of oxacillin compared with cefoxitin for the detection of MR isolates.[7]

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2. Need for the Study

1. Accuracy and promptness in the detection of methicillin resistance are of key importance in ensuring correct antibiotic treatment in infected patients and control of MR staphylococci in the hospital environment.
2. The *mecA* gene is highly conserved in staphylococcal strains and thus is a useful marker of methicillin/oxacillin resistance. Its detection is considered the gold standard for detection of MR isolates. However, many laboratories throughout the world do not have the capacity to use molecular techniques to detect MR staphylococci in routine clinical practice.
3. There are several phenotypic methods, such as MIC determination [by agar dilution (AD), broth dilution and E-test], the oxacillin screen agar (OSA) method and disc diffusion (DD) testing, for detection of MR staphylococci. Phenotypic expression of resistance can vary depending on the growth conditions (e.g. temperature, osmolarity and culture medium supplements such as NaCl or sucrose), making susceptibility testing by standard microbiological methods potentially problematic.

3. Objective

1. To determine antibiograms to guide clinicians in prescribing proper antibiotic and controlling hospital infection.
2. To screen for Resistance pattern of isolates with reference to Cefoxitin as per CLSI guidelines.
3. To detect MRSA phenotypically by oxacillin screen agar and Oxacillin MIC method and to evaluate cefoxitin disc diffusion test as a screening tool for MRSA detection.

3.1. Study design

This laboratory based prospective cross section study will be carried out in the Department of Microbiology, Navodaya Medical College, Hospital and Research Center, Raichur, Karnataka.

3.2. Sample size

In the present study, a total of 50 *Staphylococcus aureus* isolates will be evaluated phenotypically for methicillin resistance.

3.3. Inclusion criteria

Consecutive non-duplicate isolates of Staphylococcus aureus from various clinical samples received in the microbiology laboratory for bacterial culture and sensitivity from patients admitted in medical and surgical wards and ICUs were included in this study.

3.4. Exclusion criteria

1. Patients from outpatient departments were excluded.
2. Patients with culture positive for other organisms.

3.5. Identification of Staphylococcus aureus

A total of 50 strains of *S. aureus* isolated from clinical samples were used in the study. Confirmation of the strains was done as per standard laboratory procedures using standard tests like catalase, slide and tube coagulase, and growth on Mannitol salt agar.

3.5.1. Antibiotic susceptibility testing

It was performed by Kirby Bauer disc diffusion method[8] for the following antibiotics: ampicillin (10 g), amoxicillin/clavulanic acid (20/10 g), ciprofloxacin (5 g), erythromycin (15 g), clindamycin (2 g), gentamicin (10 g), oxacillin (1 g), and vancomycin (30 g).

3.5.2. Oxacillin screening agar (OSA) test

All plates were prepared with Mueller–Hinton agar supplemented with 4% (w/v) NaCl containing oxacillin. Two types of plate were prepared, one containing oxacillin at a concentration of 4 μg/ml(OSA 4 μg/ ml) and another containing oxacillin at a concentration of 6 μg/ml(OSA 6 μg/ml). All plates were spot inoculated with a cotton swab dipped into a 0.5 McFarland standard suspension of each isolate, according to the procedures outlined by the CLSI (2005). Oxacillin resistance was confirmed by bacterial growth after 24 h incubation at 35 ^{o}C.[9,10]

3.5.3. Agar dilution test

The MIC for oxacillin was determined by an AD method, following CLSI guidelines. Briefly, for each isolate, a minimum of four to five colonies isolated from an overnight growth were transferred to sterile saline. The suspension was adjusted to a 0.5 McFarland standard (10^{8}CFU/ ml) and spot inoculated on Mueller–Hinton agar plates supplemented with 2% NaCl and containing 256–0.125 μg oxacillin/ ml in serial doubling dilutions. The oxacillin Mueller–Hinton plates were incubated at 35^{o}C for 24 h. The oxacillin susceptibility breakpoint.[11]

3.6. Statistical analysis

All findings were entered in an MS Excel data sheet and on completion of the study data will be analyzed with a descriptive statistics wherever appropriate. The *chi*-square and Fisher's exact test will be used to evaluate the statistical significance of differences in the results. The p-value of <0.05 is considered statistically significant. Statistical analysis will be performed by software package used for statistical analysis (SPSS) version 16.0.

Table 1: Comparison of four Laboratory methods for detection of MRSA

Methods	Total No. of MRSA (21)	False Negative	False Positive	Sensitivity	Specificity	PPV	NPV
MIC	21	0	0	100%	100%	100%	100%
OXA. Screen Agar	20	1	1	95.24%	96.55%	95.24%	96.55%
Cefoxitin Disc Diffusion	21	0	0	100%	100%	100%	100%
Oxacillin Disc Diffusion	17	3	1	90.00%	96.67%	94.74%	93.55%

PPV – Positive Predictive Value, NPV – Negative Predictive Value

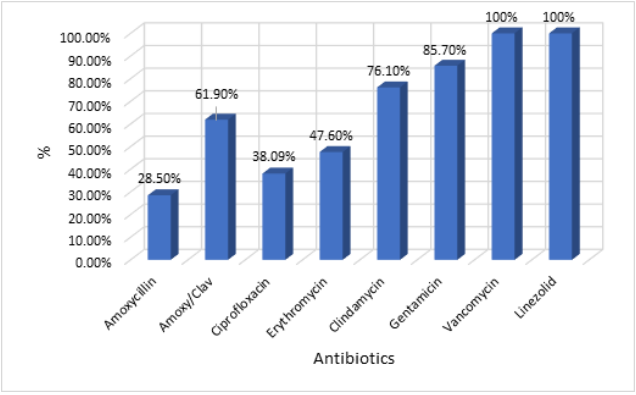

Fig. 1: Susceptibility of Methicillin Resistant *Staphylococcus aureus* isolates

3.7. Implications

The knowledge of antibiotic susceptibility pattern and drug resistance in a geographical area might help in the formulation of an appropriate hospital antibiotic policy which is a paramount aspect in the control of hospital acquired infections.

Because of the possible association of MRSA with multiple drug resistance and relatively difficult and higher cost of treatment, the accurate and rapid identification of MRSA is crucial in clinical world for timely management of the infections caused by this superbug.

4. Results

In the present study, a total of 50 isolates of *Staphylococcus aureus* from various clinical samples collected between November 2019 and February 2020 were used for the detection of MRSA. The study was conducted in the Microbiology department, Navodaya Medical College Hospital & Research Centre, Raichur, Karnataka. The isolates were identified by routine microbiological procedures like Gram stain, Colony Morphology, Catalase test, tube & slide Coagulase test and mannitol fermentation reaction. Antibiotic susceptibility testing was done as per CLSI Guidelines.

Out of 50 *Staphylococcus aureus* isolates 21 (42%) isolates were detected as MRSA based on MIC method, which is considered as gold standard method for the detection of MRSA.

The results of susceptibility testing of MRSA isolates to different antibiotics is shown in Figure 1.

All the isolates of MRSA were 100% susceptible to vancomycin and linezolid. However, amoxycillin (28.5%), ciprofloxacin (38.09%) and erythromycin (47.6%) lesser susceptibility to MRSA isolates.

The comparison of four phenotypic laboratory methods for the detection of MRSA are shown in Table 1.

In the present study, cefoxitin diffusion method has given 100% sensitivity and specificity in concordance with MIC method. However, the oxacillin screen agar method showed 95.24% sensitivity and 96.55% specificity. Similarly, Oxacillin disc diffusion method showed 90% sensitivity and 96.69% specificity.

5. Discussion

The genotypic method for detection of MRSA isolates involving *mecA* gene by PCR considered to be the reference, are not practical for routine use in microbiology laboratory and also in resource limited setups of developing countries. There are several phenotypic methods for detection of MRSA are available but optimal method of detection remains controversial.[4–12]

The Oxacillin resistance in *S. aureus* isolate by Oxacillin screen agar plate method generally means that the isolate is *mecA* -positive. Occasionally, heteroresistant *mecA*-positive strain is not detected due to low expression of resistance. Hence, oxacillin screen agar test does not detect borderline resistant strains.[13] The present study recorded that the cefoxitin disc diffusion test correlates better with Oxacillin MIC method compared with Oxacillin disc diffusion method, which is in agreement with the report of Cauwelier et al.[14] The cefoxitin is better inducer of *mecA* expression; this could explain why heterogenous MRSA populations variably expressing the *mecA* are better detected by disc diffusion with cefoxitin than oxacillin, which is a weak inducer of PBP2a production.

6. Conclusion

As per our study and previous reports elsewhere on phenotypic detection of MRSA, cefoxitin is more potent inducer of the *macA* regulatory system and an accurate surrogate marker for the detection of MRSA in the routine susceptibility testing. The cefoxitin disc diffusion method can be performed routinely in clinical microbiology laboratory as the test is easy to perform, do not require special technique, equipment and expertise. The method is cost effective as well.

Our study revealed that the cefoxitin disc diffusion method has a high sensitivity, specificity and positive predictive value compared to other routinely used methods for the detection of MRSA.

7. Source of Funding

None.

8. Conflict of Interest

The authors declare no conflict of interest.

References

1. Swenson JM, Patel JB, Jorgensen JK. Special phenotypic methods for detecting antibacterial resistance. In: Manual of Clinical Microbiology. Washington DC: ASM Press; 2007. p. 1175–6.
2. Velasco D, Tomas MDM, Cartelle M, Beceiro A, Perez A, Molina F, et al. Evaluation of different methods for detecting methicillin (oxacillin) resistance in Staphylococcus aureus. *J Antimicrob Chemother*. 2005;55:379–82.
3. Swenson JM. Tenover FC; the Cefoxitin Disk Study Group. Results of disk diffusion testing with cefoxitin correlate with presence of mecA in Staphylococcus spp. *J Clin Microbiol*. 2005;43:3818–23.
4. Swenson JM, Lonsway D, Mcallister S, Thompson A, Jevitt L, Patel JB. Detection of mecA-mediated Resistance Using Cefoxitin Disk Diffusion (DD) in a Collection of Staphylococcus aureus Expressing Borderline oxacillin MICs. *Diagn Microbiol Infect Dis*. 2007;58:33–9.
5. Mckinney TK, Sharma VK, Craig WA, Archer GL. Transcription of the gene mediating methicillin resistance in Staphylococcus aureus (mecA) is corepressed but not coinduced by cognate mecA and Beta-lactamase regulators. *J Bacteriol*. 2001;183:6862–8.
6. Boubaker IBB, Abbes B, Abdallah KB, Mamlouk, Mahjoubi A, Kammoun A, et al. Evaluation of a cefoxitin disk diffusion test for the routine detection of methicillinresistant Staphylococcus aureus. *Clin Microbiol Infect*. 2004;10:762–5.
7. Palazzo IC, Darini A. Evaluation of methods for detecting oxacillin resistance in coagulase-negative staphylococci including cefoxitin disc diffusion. *FEMS Microbiol Lett*. 2006;257:299–305.
8. Clinical and Laboratory Standards Institute / NCCLS Performance standards for Antimicrobial disc diffusion tests; Approved standards. 9th ed. CLSI Document M2-M9. Wayne Pa: Clinical and Laboratory Standards Institute; 2006.
9. CLSI (2005). Performance Standards for Antimicrobial Susceptibility Testing, 15th Informational Supplement M100-S15. Wayne, PA: Clinical Laboratory Standards Institute.
10. Isenberg HD, editor. Clinical microbiology procedures hand book. 2nd ed. Washington DC: ASM Press; 2004.
11. Swenson JM, Spargo J, Tenover FC, Ferrarom J. Optimal inoculation methods and quality control for the NCCLS oxacillin agar screen test for detection of oxacillin resistance in Staphylococcus aureus. *J Clin Microbiol*. 2001;39:3781–4.
12. Stulens MJ, Denis O. Rapid molecular detection of methicillin-resistant Staphylococcus aureus: cost effective tool for infection control in critical care. *Crit Care*. 2006;10(2):128.
13. Anand KB, Agrawal P, Kumar S, Kapila K. Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for mecA gene for detection of MRSA. *Indian J Med Microbiol*. 2009;27(1):27–9.
14. Cauwelier B, Gordts B, Descheemaecker P, Landuyt H. Evaluation of a disk diffusion method with cefoxitin (30 microg) for detection of methicillin resistant Staphylococcus aureus. *Eur J Clin Microbiol Infect Dis*. 2004;23:389–92.

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