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Evaluation of different phenotypic methods for the detection of methicillin resistant Staphylococcus aureus and antimicrobial susceptibility pattern of MRSA

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ABSTRACT

Background: Rapid and accurate detection of methicillin resistant *Staphylococcus aureus* (MRSA) is an important role of clinical microbiology laboratories to avoid treatment failure. The aim of this study was to compare conventional methods against the cefoxitin disc diffusion method to determine the best phenotypic method.

Methods: Study was carried out in the Department of Microbiology, National Institute of Medical Sciences & Research, Jaipur (India), between July 2016 - December 2016. The methods included were Oxacillin E-test MIC, Oxacillin screen agar, Oxacillin disk diffusion, Cefoxitin disk diffusion and CHROMagar- MRSA methods. Antimicrobial susceptibility performed as per CLSI guidelines.

Results: Out of 142 isolates of *S. aureus*, fifty three (37.32%) strains of MRSA were isolated from clinical specimen. E-MIC test was selected as gold standard method. The sensitivity and specificity of Oxacillin screen agar and CHROMagar-MRSA were same 98.07% and 97.80%, respectively. The sensitivity and specificity of oxacillin disk diffusion were 94.23% and 98.89%. Fifty three strains of S. aureus were MRSA by cefoxitin disk diffusion method and Oxacillin Ezy MIC test. The sensitivity and specificity of cefoxitin disk diffusion method and Oxacillin Ezy MIC method was 100% and 100% respectively. All isolates including MRSA were susceptible to Vancomycin and Linezolid.

Conclusions: All phenotypic methods had high sensitivity and specificity for detection of MRSA. However, cefoxitin disk diffusion method in comparison to other methods had higher sensitivity and specificity.

Keywords: Oxacillin resistant S. aureus, Cefoxitin resistant S. aureus, Oxacillin ezy strip, Oxacillin screen agar, MeReSa chrom agar

INTRODUCTION

Staphylococcus aureus is an important etiological agent of hospital and community acquired infections. 1,2 The organism has a potential to spread and is reason of outbreaks particularly in hospitals.³ Methicillin resistant S. aureus (MRSA) was first reported in 1961. Now a day matter of concern for microbiologist and clinician is how overcome methicillin resistance problem.⁴ The importance of MRSA as a nosocomial as well as community acquired pathogen is well documented.^{5,6} Emerging of MRSA worldwide has led to the overuse of glycopeptides antibiotics, and to the emergence of Vancomycin resistant *S. aureus*. Methicillin resistance in S. aureus is mediated by the mecA gene which codes for an additional penicillin binding protein, PBP2 or PBP2a.8 MRSA strains are frequently resistant to many different classes of antibiotics, second and third-line antimicrobial

resistance is a growing concern. Considering the increasing rate of infections caused by MRSA, performance of reliable, accurate and rapid testing for detection MRSA is essential for both antibiotic therapy and infection control measures. There are many molecular and conventional phenotypic methods are available for detection of MRSA in clinical microbiology laboratories. Molecular methods are not affordable by every laboratory especially in India, so it is essential to evaluate an accurate sensitive method which can provide equivocal results with molecular methods.

The aim of this study was to determine the incidence of methicillin resistance and to compare various methods for detection of methicillin resistance with Cefoxitin disc diffusion method at National Institute of Medical Sciences and Research located in Jaipur. E-MIC test was selected as gold standard method.

METHODS

Sample processing

This is a prospective study and the clinical isolates of S. aureus from different specimens including tracheal aspirates, wound and soft issue, urine, blood and other specimens between July 2016 to December 2016 in National Institute of Medical Sciences & Research, Jaipur, 142 strains of S. aureus isolated from patients admitted to our hospital were included. The majority of patients were hospitalized patients. Briefly, the samples were cultured aerobically in blood and MacConkey agar. The plates were incubated overnight at 37°C. All isolates were identified using gram stain, biochemical tests including catalase, coagulase. Antimicrobial susceptibility testing of MRSA isolates by Modified Kirby Bauer disk diffusion method (MKBDDM) as per CLSI Guidelines 2016.¹¹

Methicillin resistance detection

1. Cefoxitin disk diffusion

All strains of *S. aureus* were tested with 30 mg cefoxitin discs (Hi-Media) on Mueller–Hinton agar plates. For each strain, a bacterial suspension adjusted to 0.5 McFarland was used. The zone of inhibition was determined after 16–18 h incubation at 37°C. Zone size was interpreted according to CLSI (2016) criteria: susceptible, >22 mm; resistant, <21 mm. ¹²

2. Oxacillin disk diffusion

All strains of *S. aureus* were tested with 1 mg oxacillin discs (Hi-Media) on Mueller–Hinton agar with an addition of 4% NaCl. For each strain, a bacterial suspension adjusted to 0.5 McFarland was used. The zone of inhibition was determined after 16-29 h incubation at 35°C. Zone size was interpreted according to CLSI

(2016) criteria: susceptible, >13 mm; intermediate, 11-12 mm; and resistant <10 mm. 12

3. E-test method (Ezy MIC strip OXA 0.016 – 256 mcg/ml Hi-media)

The conditions for testing include Muller- Hinton with 2% NaCl. The inoculum density was adjusted equivalent to 0.5 to 1.0 McFarland standards. The plates were swabbed accordingly. By the help of an applicator MIC strip on each plates was placed and were kept at 35° C for 24 hrs. The MIC less than 2 ug were considered sensitive and more than 4 ug as resistant. ¹³

4. Oxacillin screening Agar (Oxacillin resistance screening agar base Hi-media)

This method requires suspending test organism to the density of 0.5 McFarland and inoculating MH agar containing 4% NaCl and 6 mg/ml oxacillin with a spot or a streak of the organism. Plates were incubated at 35⁰ C for 24hrs. Any growth other than a single colony was considered as resistant.¹⁴

MRSA CHROMagar (Hi Crome MeReSa agar Himedia)

CHROMagar (Hi-Media) is a new chromogenic medium for the identification of MRSA. For each strain, a bacterial suspension adjusted to 0.5 McFarland was used. Subsequently, a swab was dipped in the suspension and streaked onto a CHROMagar plate. The plates were incubated at 35^o C for 18-24 hrs. The growth of any green color colony was considered to be MRSA positive. ¹⁵

S. aureus ATCC 25923 was used as a control strains for quality control.

RESULTS

Out of 142 isolates of *S. aureus*, fifty three (37.32%) strains of MRSA were isolated from clinical specimen. E-MIC test was selected as gold standard method. All isolates of MRSA were 100% susceptible to vancomycin and linezolid.

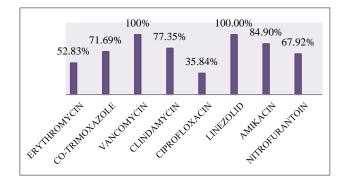


Figure 1: Susceptibility of MRSA isolates.

Methods	Total No of MRSA	False - VE	False +VE	Sensitivity (%)	Specificity (%)	PPV	NPV
E-test	53	00	00	100.00	100.00	100	100
CHROMagar	50	01	02	98.07	97.80	96.36	98.89
OXA. Screen AGAR	50	01	02	98.07	97.80	96.36	98.89
Cefoxitin disk	53	00	00	100	100.00	100	100

01

94.23

Table: 1 Sensitivity and Specificity of different methods.

Sensitivity and specificity of different methods is illustrated in Table 1. The result of susceptibility testing of MRSA isolates to other antibiotics is shown in Figure 1.

49

03

diffusion Oxacillin disk

diffusion

Table 1 show the E-test method and Cefoxitin disk diffusion method gives 100% sensitivity and specificity comparison to other tests for the MRSA detection.

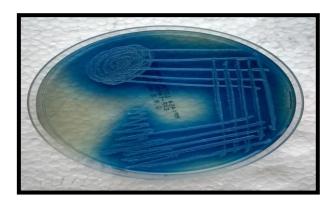
Figure 1 shows the vancomycin and linezolid drugs are higher sensitive comparison to other drugs and both are drug of choice for the treatment of MRSA patients.



Figure 2: Cefoxitin disk diffusion method for MRSA detection.



Figure 3: Oxacillin disk diffusion method for MRSA detection.



98.89

98.15

96.74

Figure 4: Oxacillin screen agar method for MRSA detection.

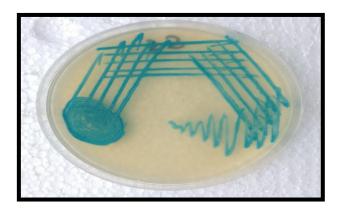


Figure 5: MeReSa Chrome agar method for MRSA detection.



Figure 6: Oxacillin E- strip (E-test) method for MRSA detection.

DISCUSSION

Testing of methicillin resistance in *S. aureus* has been a challenge for clinical laboratories for many years. Several studies have shown that detection of *mecA* gene is a gold standard method for diagnosis of MRSA in clinical microbiology laboratories, but most of the developing countries cannot afford molecular methods in routine due to its high cost, so it is essential to evaluate an easy, cost effective, accurate and sensitive method for MRSA detection that can be used in routine lab and also which can give equivocal results with molecular method.

In the present study, we evaluated five different phenotypic methods for the detection of MRSA. A total 142 *S. aureus* were isolated from different clinical samples. All of the 142 *S. aureus* isolates were processed for methicillin detection by the cefoxitin disc diffusion test as described above in material method section. 53 (37%) were observed as Methicillin Resistant *S. aureus* (MRSA) by cefoxitin disc test with 100% sensitivity and 100% specificity.

These 53 MRSA strains were then compared with oxacillin disc diffusion test, oxacillin screen agar, MRSA chrome agar and E-test strip method, we observed 49 strains as true MRSA, 3 as false negative MRSA and 1 isolate as false positive MRSA by oxacillin disc diffusion test with 94.23% sensitivity and 98.89% specificity. 50 strains detected as true MRSA, 1 false negative MRSA and 2 false positive MRSA by oxacillin screen agar with 98.07% sensitivity and 97.80% specificity. Similarly 50 strains were detected as true MRSA, 1 false negative MRSA and 2 false positive MRSA by chrome agar method with 98.07% sensitivity and 97.80% specificity. The E- test strip method gave equivocal results to cefoxitin disc diffusion test i.e. 53 strains were confirmed as true MRSA with 100% sensitivity and 100% specificity.

There is variation among the results reported regarding sensitivity of different conventional methods used for detection of MRSA by number of authors. ¹⁶ However, most of the studies reported 100% sensitivity and 100% specificity with cefoxitin disc diffusion method which is in accordance with our results. ¹⁷ Karami et al depicted 100% sensitivity and 100% specificity with E Test MIC which is also similar to our observation. ¹⁸

Our observation does not suggest oxacillin disc diffusion, oxacillin screen agar and MRSA chrome agar to be used in routine as there are chances of misinterpretation ultimately risk of treatment failure which is not acceptable and affordable at any cost. E test MIC detected all of 53 isolates as true MRSA that can also be an alternate to molecular method also it is easy to perform in routine.

Recently CLSI has replaced oxacillin with cefoxitin for detection of MRSA. Regarding cefoxitin disk diffusion,

many studies reported that the results of cefoxitin disk diffusion tests correlate better with the presence of *mecA* than do the results of disk diffusion tests using oxacillin. In a study by Anand et al, results of cefoxitin disk diffusion method for detection of MRSA were in concordance with the PCR for *mecA* gene. Another study by Anupurba et al, showed a high correlation between MICs of cefoxitin and presence of *mecA* in staphylococcus spp. Recently it is shown that cefoxitin disk diffusion method is more reliable than oxacillin disk diffusion method for detection of MRSA.

In our hospital, 37.32% of all *S. aureus* infections are caused by MRSA. Susceptibility test profiles revealed a higher level of resistance to commonly prescribed antimicrobial agents among MRSA. All isolates were sensitive to Vancomycin and Linezolid. These results were comparable to studies carried out by others (Anupurba et al). ¹⁹ In the present study the antibiotic sensitivity pattern of MRSA included Erythromycin (52.83%), Co-trimoxazole (71.69%), Clindamycin (77.35%), Ciprofloxacin (35.84%), Nitrofurantoin (67.92%) and Amikacin (84.90%). Vancomycin and Linezolid are (100%) susceptible to all MRSA isolates. In other study conducted by Datta et al, also found (100%) MRSA strains were sensitive to Vancomycin and Linezolid. ¹²

CONCLUSION

In our study we found that the cefoxitin disc is a good method for MRSA detection but it should be supplemented with some other method so that no MRSA is missed. No other method (oxacillin disc diffusion, MRSA chrome agar and oxacillin screen agar method) was as sensitive and specific as cefoxitin disc diffusion test and Oxacillin E test strip was so it is advisable to combine two methods, one with high sensitivity and the other with high specificity. According to our results, the best combination is the cefoxitin disc diffusion method and the Oxacillin E- strip test. Since the Oxacillin E- strip test is expensive it cannot be applied to all tests. Therefore, isolates that give a zone diameter of less than 20 mm can be easily reported as MRSA and only those with zone diameters of 20-22 mm need to be confirmed by Oxacillin E-strip.

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