Original Article

DOI: 10.7860/JCDR/2022/53064.16018

Comparison of Different Phenotypic Methods Including E-test, Cefoxitin and Oxacillin Disk Diffusion for Detection of Methicillin Resistant *Staphylococcus aureus*

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ABSTRACT

Introduction: Methicillin-Resistant *Staphylococcus aureus* (MRSA) has spread throughout the world as a hospital and community-acquired illness. Although, a variety of strategies have been employed, laboratory identification of MRSA remains a difficulty.

Aim: To examine several phenotypic approaches for accuracy results, with an (Epsilometer) E-test based method serving as the gold standard for MRSA identification.

Materials and Methods: A prospective observational study was conducted in the Microbiology Department of Santosh Medical College, Ghaziabad, Uttar Pradesh, India, from August 2020 to July 2021. Total of 384 isolates *S. aureus* were identified by using the required samples including pus, swab, blood, wound and urine, etc., which were collected from the Microbiology department and the comparison was done between E-test serving as the gold standard for MRSA identification with Cefoxitin Disk Diffusion (CDD)/Oxacillin Disk Diffusion method (ODD). The diagnostic kit for using E-test in collected samples was purchased from Himedia Laboratries Pvt., Ltd., Mumbai, India (EM0065). The data was calculated by using MS-Excel.

Results: A total of 113 strains were revealed to be MRSA in clinical specimens out of 384 isolated *S. aureus* according to Cefoxitin (disc diffusion method). The gold standard method was chosen to be the E-test, which had found a high sensitivity of 79.8% and a specificity of 94.2% compared to the cefoxitin/ODD method. Isolates including MRSA were highly susceptible to teicoplanin and linezolid.

Conclusion: The present study concludes that E-test (strip) method is a high sensitivity and highly specific for detecting MRSA in comparison to other disk methods used in this study. Due to less number of sample size and lesser time period more studies are needed to establish this fact.

Keywords: Linezolid, Mueller hinton agar, Oxacillin disc test, Resistant, Teicoplanin

INTRODUCTION

The most common cause of skin and soft tissue infections is *Staphylococcus aureus*. *S. aureus* carriage in the anterior nares or elsewhere, which is seen in 20-30% of all individuals [1]. *S. aureus* is contagious among patients. Hospitals put a lot of effort into preventing direct patient-to-patient transmission as well as transmission through employees and the environment [2]. Asymptomatic colonisation with MRSA has been found as a risk factor for MRSA infection in the future [3]. Methicillin is an antibiotic derived from penicillin, which has been used as a drug clinically since 1960 [4]. MRSA strains have become a severe clinical and epidemiological problem in recent years, as resistance to this antibiotic suggests resistance to beta-lactam antibiotics [5]. Because of the costs of other forms of surgery, this is a strong recommendation for cardiothoracic and orthopaedic surgery and a moderate conditional recommendation when practical [6]. According to the Centers for Disease Control and Prevention, MRSA has caused over 80,000 invasive infections and over 11,000 deaths in United States [7].

Methicillin-resistant by definition, MRSA has the mecA gene. The mecA gene produces Penicillin Binding Protein (PBP) 2a, which is distinct from *S. aureus* native PBPs. In the presence of β-lactam antibiotics, PBP 2a allows MRSA to continuously build its cell wall. Unlike HA-MRSA, CA-MRSA is sensitive to a wide range of antibiotics, with the exception of β-lactams and erythromycin [8]. Methicillin resistance developed in the hospital Infection with *Staphylococcus aureus* (HA-MRSA) is more common in hospitalised individuals. The elderly and persons with compromised immune systems are more susceptible to HA-MRSA. If a long-term hospitalised patient has a device implanted into his or her body, such as a catheter or intravenous line, the risk of infection increases. HA-MRSA infection is widespread in nursing homes due to the close person-to-person interaction between patients. According to epidemiological data, there is a higher risk of contracting Community-Acquired Methicillin *Staphylococcus aureus* (CA-MRSA) in the United States as a prevalent infection in areas with a high risk of cross-infection, such as schools, poor and homeless young adults, military personnel, and athletes are at risk of infection from close contact [9].

Recently authors have indicated high prevalence of MRSA infections among hospitalised patients [10], due to which timely and precise MRSA diagnosis is required to begin appropriate antibiotic therapy and prevent MRSA infections from spreading. In clinical laboratories, phenotypic methods such as the Oxacillin Disc Diffusion (ODD) method and Cefoxitin Disc Diffusion (CDD) method, or the E-test strip method, are available, as well as the measurement of the Minimum Inhibitory Concentration (MIC) for phenotypic methods [11]. Since, previous studies has been stated that there are different method exists to detect the MRSA so the aim of this study was to test how valuable the E-test is to detect MRSA comparing to other disc diffusion methods.

MATERIALS AND METHODS

This prospective observational study took place in the Microbiology Department at Santosh Medical College in Ghaziabad, Uttar Pradesh,

India, for a year from August 1, 2020, to July 31, 2021. This study was in accordance with the declaration of Helsinki and ethically approved from the Institutional Committee (SU/2021/2131[6]) of Santosh Medical College, Ghaziabad. Before collecting the sample informed written consent was obtained each and every participant.

Ward	Pus	Urine	Sputum	Wound swab	Blood	Vaginal swab	Pleural fluid	Throat swab	Total
Surgery	43	29	6	20	14	0	05	0	117
Medicine	20	35	07	01	33	0	03	06	105
ICU	24	30	03	06	07	0	01	01	72
OBG	08	15	0	12	03	24	0	0	62
ENT	04	02	01	10	06	0	0	05	28
Total	99	111	17	49	63	24	9	12	384

[Table/Fig-1]: Collection of different samples from different wards.

Sample Collection

Total of 384 clinical isolates as *Staphylococcus aureus* from various clinical specimens collected from patients admitted in different wards of associated hospital were obtained and included during the time period. The samples were cultured aerobically in blood and MacConkey agar. The plates were incubated overnight at 37°C.

MRSA Identification and Antimicrobial Susceptibility Testing by Various Methods

1. (Epsilometer) E-test method

These are automated systems for determining bacteria's MIC. The inoculum was plated on Mueller Hinton Agar (MHA) supplemented with 2% NaCl and was standardised to 0.5 McFarland turbidity. On the MHA surface, MIC strips for oxacillin were mounted with the MIC scale facing downwards. Plates were incubated at 37°C for 24 hours before being examined. The MIC is read from the scale at the zone-strip junction. MICs less than 2 g were deemed sensitive, whereas those over 4 g were considered resistant [12]. To perform E-test, the diagnostic kits from Himedia Laboratries Pvt., Ltd., Mumbai, India (EM0065) was purchased.

2. Cefoxitin Disk diffusion method

On MHA plates, all *S. aureus* strains were evaluated with a 30 mg cefoxitin disc. A bacterial suspension calibrated to 0.5 McFarland will be used for each strain. After 16-18 hours of incubation at 37°C, the zone of inhibition was assessed. The Clinical and Laboratory Standards Institute (CLSI) (2017) criteria were used to interpret zone size: susceptible zone greater than 22 mm and resistant zone less than 21 mm [13].

3. Oxacillin disk diffusion method

A 1 mg oxacillin disc on MHA with a 4% NaCl addition was used to test all *S. aureus* strains. Each strain was evaluated with a 0.5 McFarland-calibrated bacterial suspension. After 16-29 hours of incubation at 35-37°C, the zone of inhibition was measured. CLSI (2017) criteria were used to calculate the size of the zone: Sensitive to a depth of more than 13 mm, moderate to 11-12 mm, and resistant to a depth of less than 10 mm [13].

STATISTICAL ANALYSIS

Descriptive statistics were used to analyse the data. The data was calculated by Microsoft Excel.

RESULTS

Total 384 *staphylococcus aureus* strains were collected from 18-60 years (39.59±10.74 years) of age group, including both male (223) and female (161), patients admitted in different wards as described in [Table/Fig-1]. There were 113 (29.4%) strains resistant to MRSA among them. In this study, different phenotypic methods were used to detect MRSA the best result was found from E-test (oxacillin) 114 (29.6%). Comparison by CDD method 113 (29.4%), ODD 99 (25.7%) out of 384 isolate *S. aureus* growth [Table/Fig-2].

Methods	N=384	Susceptibility test	
		MRSA	MSSA
Cefoxitin (disc diffusion method)	Resistance	113	0
	Susceptible	0	271
Oxacillin (disc diffusion method)	Resistance	99	0
	Susceptible	0	285
E-test (oxacillin)	Resistance	114	0
	Susceptible	0	270

[Table/Fig-2]: Comparison of phenotypic methods for detection of MRSA.
MSSA: Methicillin sensitive *staphylococcus aureus*

In this study, sensitivity and specificity were detected from phenotypic comparison methods. The oxacillin (E-test) strip gold standard had high sensitivity of 79.8%, specificity of 94.2% while Positive Predictive Value (PPV) was 86.8% or Negative Predictive Value (NPV) 90.7%, followed by the cefoxitin disc diffusion method, which had sensitivity of 75.8%, specificity of 92.7% while PPV was 83.2% or NPV 88.9%. In the last ODD, sensitivity was 60.5%, specificity 90.8%, while PPV was 75.8% or NPV 82.8% [Table/Fig-3].

Methods	Sensitivity	Specificity	PPV	NPV
	N (%)	N (%)	N (%)	N (%)
Cefoxitin (disc diffusion method)	75.8	92.7	83.2	88.9
Oxacillin (disc diffusion method)	60.5	90.8	75.8	82.8
E-test (oxacillin)	79.8	94.2	86.8	90.7

[Table/Fig-3]: Sensitivity and specificity of phenotypic methods for detection of MRSA.

From the antibiotic sensitivity pattern of *S. aureus*, it was observed a high incidence of resistance to other antibiotics such as erythromycin 265 (69.0%), followed by clotrimozole 228 (59.4%), tetracycline 144 (37.5). We also observed Methicillin-Sensitive *Staphylococcus Aureus* (MSSA) by linezolid 354 (92.2%) followed by teicoplanin 325 (84.6%), gentamycin 272 (70.8%), and amoxyclave 281 (73.2%).

DISCUSSION

In recent years, MRSA has posed a challenge for clinical laboratories. As a result, determining methicillin resistance accurately and quickly is crucial in the prognosis of *S. aureus* infections. To maintain PPV or NPV, which give a primary CLSI guideline for treating infections caused by this organism, several phenotypic disc diffusion or E-test strip procedure with high accuracy, sensitivity, and specificity are required [14].

There were 384 *staphylococcus aureus* strains tested in this study, with 113 (29.4%) of them being methicillin resistant. A study conducted by Joshi S et al., in India found that 42% of cases of MRSA were found [15]. In a similar way, Choudhary D and Chakravaty P observed a slightly greater prevalence (42.96%) than the present study [16]. Different phenotypic approaches were

utilised to identify MRSA, with the E-test (oxacillin) yielding the best results 114 (29.6%), followed by the CDD method 113 (29.4%), and the ODD method (99.6%) (25.7%). In accordance of the findings of this investigation, Sharma S et al., concluded that the E-test can be used as a substitute for the molecular method and is simple to perform in routine [17]. With the E-test MIC, Rahbar M et al., reported 100% sensitivity and 100% specificity, which is identical to present findings [18].

Similar to this work, Kumar VA et al., found that the MICs of oxacillin for isolates were in the susceptible range by E-test [19]. Despite this, Rahbar M and Safadel N reported that the CDD method is a good alternative to the ODD for MRSA detection when compared to the E-test strip method [20]. The E-test, on the other hand, has the advantage of being as easy to set up as a disc diffusion test. In a study comparable to this one, Shariati L et al., showed that the phenotypic E-test oxacillin technique detected MRSA 100% of the time [21]. In the antibiotic sensitivity pattern of *S. aureus*, a significant rate of MRSA antibiotic resistance was found to cefoxitin 113 (29.4%) and oxacillin 99 (25.8%), as confirmed by Demir T et al., who concluded that oxacillin (1 g) resistance was 29% and cefoxitin (30 g) resistance was 31% out of 100 isolates of pure *S.aureus* growth followed by other antibiotics [22]. Similar results were reported by Dhuria N et al., and Anand KB et al., in terms of determining antibiotic sensitivity/resistant patterns [23,24]. MSSA in present study was found to be highly antibiotic sensitive to linezolid 354 (92.2%), tiecoplanin 325 (84.6%), gentamycin 272 (70.8%). While Shanthi M et al., identified linezolid, teicoplanin, and many other medicines to be 100 percent sensitive in their investigation [25]; the pattern is identical to the present findings. In addition to the findings of this research, a study from Iran concluded the E-test accuracy and its superiority to disk diffusion method in detecting multi drug resistance. Since, the outcomes of this study suggest the reliability of E-test over disk diffusion method in detecting drug resistance, so it can be used for routine purpose for better results.

Limitation(s)

The present study shows the result of E-test using oxacillin drug only, not with other drug i.e., cefoxitin as this study was self-financed so this might be the limitation of this study.

CONCLUSION(S)

Methicillin-resistant *S. aureus* is one of the most common causes of nosocomial infections, responsible for causing skin diseases to life-threatening infections. In this study high sensitivity and specificity for the E-test method were observed compared routinely used CDD/ODD for detection of MRSA. The results of the E-test and the Polymerase Chain Reaction (PCR) are in agreement. The molecular technique is prohibitively expensive for patients. Since, it is cheap and easy to perform compare to PCR, the E-test appears to be the best alternative for routine use in most clinical laboratories especially in developing countries. Other research may be undertaken in the future to confirm this fact.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Nov 01, 2021
- Manual Googling: Dec 31, 2021
- iThenticate Software: Jan 12, 2022 (8%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Oct 30, 2021**
Date of Peer Review: **Nov 27, 2021**
Date of Acceptance: **Jan 12, 2022**
Date of Publishing: **Feb 01, 2022**