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COMPARISON OF FOUR DIAGNOSTIC PHENOTYPIC METHODS FOR DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

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ABSTRACT

Rapid and accurate detection of Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important role of microbiology laboratories. The present study was aimed to compare various phenotypic methods for detection of MRSA.92*Staphylococcus aureus* isolates obtained from various samples were tested for methicillin resistance by cefoxitin disc diffusion, oxacillin disc diffusion and oxacillin screen agar considering E test MIC(oxacillin) as gold standard. Antibiotic susceptibility testing was done for MRSA.32 (34.78%) isolate were identified as MRSA by E test MIC. Cefoxitin disc diffusion showed 100% sensitivity and 98.30% specificity while oxacillin disc diffusion and oxacillin screen agar showed 100% sensitivity and 86.66% and 90% specificity respectively. Among MRSA high degree of resistance was seen for routinely use antibiotics but 100% sensitivity to Linezolid and Vancomycin. It is conclude that cefoxitin disc diffusion test is reliable substitute for detection of MRSA in laboratory where MIC detection and molecular methods are not accessible.

KEYWORDS: **Cefoxitin disc diffusion, E-test, Oxacillin disc diffusion, Oxacillin screen agar, MRSA**

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INTRODUCTION

Staphylococcus aureus has differential ability to spread and cause outbreak in hospital[1]. They can be cultured from dried clinical material after several months, and are relatively heat resistant. Therefore, it is not surprising that despite the availability of potent antimicrobial agents, improved public health conditions and hospital infection control measures, *Staphylococcus aureus* has remained a major human pathogen[2]. Infection caused by *Staphylococcus aureus* used to respond to beta-lactam and related group of antibiotic. However due to development of methicillin resistant *Staphylococcus aureus* (MRSA); treatment of these infection has been problematic. Presently methicillin-resistant *Staphylococcus aureus* (MRSA) is well documented cause of nosocomial as well as community acquired infection. Methicillin resistance in *Staphylococcus aureus* is based on the production of an additional penicillin binding protein, PBP2 or PBP2a, which is mediated by the mecA gene[3]. Heterogeneous resistance to methicillin also occurs due to variations in the expression of the mecA gene, or alteration of constitutive PBPs[4]. Indiscriminate use of multiple antibiotic, prolong hospital stay, intravenous drug abuse, carriage of MRSA in nose are few important risk factor for acquisition of MRSA[1]. Another major concern about MRSA is that these isolates are frequently resistant to many different classes of antibiotics. Thus, limiting the treatment options to fewer and expensive antibiotics like vancomycin, linezolid and tigecycline[5]. 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[3]. There were several phenotypic methods, such as minimum inhibitory concentration (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 MRSA. Phenotypic expression of resistance can vary depending on the growth conditions. (e.g. temperature, osmolarity and culture medium supplements such as NaCl or sucrose)[6]. Most laboratories use disc diffusion methods for routine test. In the recent years, MIC methods have been replaced by molecular methods which detect mecA gene become a gold standard for determining MRSA. However, there use is largely restricted to reference laboratories[7,8]. In the present study, we compare various phenotypic methods (cefoxitin disc diffusion, oxacillin disc diffusion, oxacillin screen agar) for detection of MRSA using E test MIC oxacillin as gold standard method. We also aimed to study the resistance pattern of MRSA isolates.

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, Dr. V M Government Medical College, Solapur, Maharashtra from June 2014 to December 2014. *Staphylococcus aureus* was isolated from 92 clinical samples taken from various sites including blood, pus, surgical site wounds, burn wounds, tracheal aspirates, central venous pressure tips and urine. Conformation of the strains was done using standard tests like catalase, slide and tube coagulase, and growth on Mannitol salt agar[9].Routine antibiotic susceptibility testing was performed by Kirby Bauer disc diffusion method for the following antibiotic: Penicillin (10ug), Gentamycin (30µg), Erythromycin(15µg), Cotrimoxazole (1.25/23.70µg), Ciprofloxacin (5µg), Tetracycline(30µg),Vancomycin (30µg), Linezolid(30µg). *Staphylococcus aureus* ATCC25923 was used as a control strain. The results were interpreted according to the guidelines of the CLSI 2014[10].

Detection of Methicillin resistance by phenotypic methods

All the *Staphylococcus aureus* isolates were tested for methicillin resistance by oxacillin disc diffusion test (1µg), cefoxitin disc diffusion test (30µg)and oxacillin screen agar test[10]. MIC for oxacillin was determined with the E test -strips (Hi-Media Mumbai), which was used as a gold standard method in the present study[3].Result of E test-strips (oxacillin MIC) were interpreted according to Clinical and Laboratory Standards guideline (CLSI) 2014[10] and sensitivity and specificity of other methods were compared with it.

RESULTS

Among the 92 *Staphylococcus aureus* isolates 32 (34.78%) were identified as MRSA by E-Test MIC method. Of 32 MRSA isolates, 15 (46.87%) strains were isolated from pus, 8 (25%) from blood, 2 (6.25%) from each surgical site wounds, burn wounds, tracheal aspirates and urine and 1 (3.12%) from central venous pressure tips. Methicillin resistance was detected by oxacillin disc diffusion, cefoxitin disc diffusion and oxacillin screen agar test in 40, 33, 38 isolates respectively. The sensitivity and specificity of various phenotypic methods in comparison to E-Test MIC (gold standard), for the detection of MRSA, are Summarized in (Table-I). The result of antibiotic susceptibility testing of MRSA isolates from various clinical sample to different antibiotics are shown in Table-II. They were 100% sensitive to vancomycin, and linezolid.

Table I
Sensitivity and Specificity of Phenotypic Methods for Detection of MRSA

Methods	No. of MRSA detected (n=92)	Sensitivity (%)	Specificity (%)
E-MIC (Oxacillin)	32	100	100
Oxacillin disc diffusion	40	100	86.66
Cefoxitin disc diffusion	33	100	98.30
Oxacillin Screen Agar	38	100	90

Table II
Resistance rates of other antimicrobials tested in MRSA (n=32)

Antibiotics	Resistant isolate (%)
Penicillin	32(100)
Erythromycin	29(90.62)
Gentamycin	24(75)
Ciprofloxacin	25(78.12)
Tetracycline	27(84.37)
Cotrimoxazole	14(43.75)
Linezolid	0 (0)
Vancomycin	0 (0)

DISCUSSION

Accuracy and promptness in the detection of methicillin resistance are of key importance in ensuring correct antibiotic treatment in infected patients and control of MRSA in the hospital and community-acquired infections[11]. Presently limited therapeutic alternatives are available to treat the MRSA isolates, so the detection of methicillin resistance should be done in the clinical microbiology laboratory with meticulous care, keeping in mind the sensitivity and the specificity of the methods which are used for detection of resistance[12].Polymerase chain reaction (PCR) for amplification of the mecA gene is presently considered as the gold standard for detecting MRSA. In spite of growing consensus in the literature for this method, it is not yet available in all clinical laboratories due to financial and technical constraints, therefore phenotypic methods, although dependent on many environmental and conditional factors still remains a method of choice in resource constraint laboratories[13].The incidence of MRSA in India ranges from 30 to 70%[14].The present study revealed 34.78% methicillin resistance among *Staphylococcus aureus* isolates. Similar result were found in studies by Sasirekha B et al[4] and Rajaduraipandi K et al[15].MRSA isolates were predominantly isolated from the pus (46.87%) in present study, similar findings were reported by Anupurba et al[16] and Vyas et al[13]. In present study E-Test MIC determination for oxacillin was used as a gold standard for MRSA detection. The advantage of E-Test method is that it is easy to perform as a disc diffusion test and approaches the accuracy of PCR for mecA gene[13, 17]. Cefoxitin disc diffusion test was perceived to be the most sensitive method for detection of mecA-mediated resistance. CLSI has also recently substituted the oxacillin disc with cefoxitin disc for detection of MRSA[18].Numerous studies have informed that the results of the cefoxitin disc diffusion test correlates better with the presence of mecA compared with those of the oxacillin disc diffusion test and Oxacillin Screen agar test.[19,20,21]Similar finding were observed in present study. In this study cefoxitin disc diffusion was found to be100%sensitive and 98.30% specific while the sensitivity of oxacillin disc diffusion and oxacillin screen agar was 100% and specificity was 86.66% and 90% respectively. Similar results were quoted by several other studies[3,4,13,14]. Detection of heteroresistant mecA-positive strains by oxacillin screen agar is not possible due to low expression of resistance. So generally it does not detect borderline resistant strains, when studies have included heteroresistant strains the test has been shown to perform less well[21].On the other hand Cefoxitin is a better inducer of the expression of the mecA gene that's why it better detect heterogeneous MRSA populations that variably express the mecA[20].The isolates which were resistant to oxacillin but sensitive to cefoxitin were also negative by E-test MIC in present study. In present study, oxacillin disc diffusion method was only 86.66% specific but 100% sensitive. The high false positivity of oxacillin disc diffusion method in this study could be due to hyper production of β- lactamases which may lead to phenotypic expression of oxacillin resistance but do not possess the usual genetic mechanism for such resistance[4]. Methicillin resistance in S. aureus restricts therapeutic options for clinical isolates and the incidence of MRSA is escalating in India. Antibiotic susceptibility testing has been found to be a good epidemiological marker for MRSA phenotyping. In present study, among MRSA isolates high degree of resistance was encountered for Penicillin (100%), Erythromycin (90.62%), Tetracycline (84.37%), Ciprofloxacin (78.12%), and Gentamycin (75%). This is similar to the finding of studies carried out by Sasirekha et al[4] and vyas et al[13] which also found a high level of resistance to Erythromycin and Ciprofloxacin. The present study revealed high percentage of resistance to Gentamycin (75%). Similar finding were also reported in a studies carried out by Quereshi et al[22] and Kandle et al[23]. In present study no strain was found resistant to vancomycin and linezolid which was similar to the finding

of other studies[15, 16, 24, 25]. Due to high cost of this drug they are less frequently use in our hospital setup. Thus, decreasing the selection pressure for drug resistance on them.

CONCLUSION

Present study revealed that the cefoxitin disc diffusion method had a high sensitivity and specificity comparative to other routinely used methods for detection of MRSA. This method can be preferred in clinical microbiology laboratories where molecular methods are not feasible as a routine because it is easy to perform, do not require special technique, media preparation and finally more cost-effective in comparison to other methods. A large proportion of these MRSA were found to be multidrug resistant, which call for urgent attention whereby strict antibiotic policy should be enforced to curtail irrational use of antibiotics. Vancomycin, linezolid are effective drugs for treatment of MRSA and should be considered as reserve drugs only.

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