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Original Article

Microbiology Section

Evaluation of Genotypic and Phenotypic Methods for Detection of Methicillin Resistant *Staphylococcus aureus* in a Tertiary Care Hospital of Eastern Odisha

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ABSTRACT

Introduction: Methicillin resistant *Staphylococcus aureus* has emerged as an important pathogen in nosocomial and community acquired infections. Accurate and rapid identification of MRSA in clinical specimens is essential for timely decision of effective antimicrobial chemotherapy.

Aim: The present study was conducted to compare efficacy of four conventional phenotypic methods, with *mec- A* based polymerase chain reaction (PCR) for MRSA identification.

Materials and Methods: Methicillin resistance was determined in 200 *S.aureus* isolates by oxacillin disc diffusion, cefoxitin disc diffusion, Oxacillin Resistance Screening Agar and E-test. The results were compared with *mec-A* based PCR.

Results: Among 200 *S.aureus* isolates 62 (31%) were positive for *mec-A* gene by PCR. Cefoxitin disc diffusion, Oxacillin Resistance Screening Agar and E-test showed 100% specificity. Oxacillin disc diffusion had lowest sensitivity (82.5%) and specificity (98.5%) among all. The conventional methods take more time than PCR for diagnosing MRSA. Linezolid, Vancomycin & Dalfopristin were the highly sensitive drugs against MRSA isolates.

Conclusion: Cefoxitin disc diffusion, is rapid, simple and cheaper, hence can be used routinely as an alternative to PCR for detection of MRSA in resource constraint laboratories.

Keywords: Cefoxitin, E-test, *mec A*, MRSA, ORSA

INTRODUCTION

Methicillin-resistant *S. aureus* (MRSA) has been a growing problem in hospital-acquired as well as community associated MRSA (CA-MRSA) infections [1]. The first case of MRSA was isolated way back in 1961 [2]. Since then, the rate of infections caused by MRSA has been increasing worldwide. In India, the prevalence of nosocomial infections caused by MRSA varies between 20 and 40% [3]. Methicillin resistance is caused by the presence of *mec- A* gene, which encodes a low affinity penicillin binding protein (PBP)-2a or PBP2' which has a low affinity for β-lactam antibiotics [4]. Therefore, presence of *mec-A* gene indicates methicillin resistance in *Staphylococci*. Detecting *mec-A* gene by polymerase chain reaction is now considered the gold standard for identifying methicillin resistance in *S. aureus* [5]. In spite of the growing consensus in the literatures for this method, it is not yet available in all clinical laboratories, therefore phenotypic methods still remain as the methods of choice in the resource constraint settings. Detection of MRSA is difficult & has become complicated because of many factors. Resistance to methicillin in *S. aureus* is heterogeneous in majority of the isolates [6]. Heterogeneous strains are composed of two populations of bacteria; one is relatively susceptible and other highly resistant population. Thus only few (one in 10^4 -10^6) of them express the phenotype. Other factors also influence the phenotypic expression of resistance. Addition of sodium chloride or sucrose to culture medium, incubation at 30^0C or passage in the presence of β-lactam antibiotics enhances the expression of resistance [3]. Further, conventional methods for identification of MRSA, take more time and are influenced by environmental conditions like temperature, pH, salt concentration and duration of incubation. These factors need a sensitive, rapid, simple & accurate method for MRSA detection in routine diagnostic laboratories. The conventional methods practiced for the detection of MRSA in the clinical laboratories are oxacillin agar screen test, oxacillin disc diffusion and oxacillin MIC by agar, or broth dilution or by E-test [7]. Recently cefoxitin disc diffusion is recommended by CLSI for detection of methicillin resistance [8]. Cefoxitin is considered as a better inducer of *mec*-A gene expression than oxacillin or methicillin, and can be used to screen heterogeneous MRSA populations. The advantage of using cefoxitin is that the test conditions are similar to those used for other antibiotics [9]. Apart from these, a latex agglutination kit have been developed by Denka Seiken Co., Japan which uses specific mAbs directed towards the PBP2a antigen for the detection of MRSA [3]. In addition, use of chromogenic substances in the medium (CHROM agar) is another method for the identification of MRSA [10].

AIM

The objective of the present study was to evaluate the usefulness of four phenotypic methods namely, the disc diffusion method using oxacillin and cefoxitin disc, oxacillin resistance screening agar & E-Test keeping *mec-A* gene detection by PCR for MRSA as the gold standard and to evaluate the antibiotic sensitivity pattern of MRSA and MSSA isolates.

MATERIALS AND METHODS

Study Type – Observational Cross Sectional Study

Study was conducted in a 1500 bedded tertiary care & teaching hospital (S.C.B Medical College, Cuttack, Odisha, India) from January 2012 to October 2014. Clinical specimens such as urine, pus, wound swab, body fluids, blood etc. from patients suffering from respective diseases & attending to different departments of S.C.B. Medical College, Cuttack were included in the study. *S.aureus* were isolated from different clinical specimens using blood and Mac-Conkey agar and identified using tests like Gram stain, catalase, coagulase, urease and DNAse as per standard procedures [11]. All the *S.aureus* isolates were subjected for the following phenotypic and genotypic tests.

Cefoxitin and Oxacillin Disc Diffusion Test [8,12]

S.aureus suspension equivalent to 0.5 Mc Farland standard were prepared for all isolates & tested with cefoxitin (30µg) and oxacilin (1µg) disc, using Muller Hinton agar. All plates were incubated at 35^{0}C for 24 hours. Zone of inhibition were measured and interpreted as guideline recommended by CLSI [13]. For cefoxitin disc, zone diameter < 19 mm was reported as MRSA and >22 mm as MSSA. For oxacillin disc, zone diameter < 10 mm was reported as MRSA and >13 mm as MSSA.

MIC by E-Test [8]

The E-Test (Hi Media Laboratories Pvt. Ltd. Mumbai, India) for determining oxacillin MICs was performed on Muller Hinton agar supplemented with 2% NaCL as per manufacturer's instruction. Plates were incubated at 35^{0}C for full 24 hours before reading results. MIC value > 4µg/ml was considered as MRSA.

Test with Oxacillin Resistant Screening Agar (ORSA) [8]

S.aureus suspensions equivalent to 0.5 Mc Farland standards were inoculated on ORSA medium (Hi Media Laboratories Pvt. Ltd., India) and incubated at 35^{0}C for complete 48 hours. ORSA contains supplements of oxacillin (2µl) and 5.5% NaCl to inhibit non-*Staphylococcal* growth and aniline blue dye to detect mannitol fermentation by *S.aureus*. Growth of any number of blue colonies indicated the presence of MRSA.

Detection of *Mec A* gene by PCR [5]

Mec-A gene detection in MRSA strains were taken as the reference gold standard to compare sensitivity, specificity and rapidity of other phenotypic methods adopted in the study. The DNA extraction of all the isolates were performed and the target gene was amplified using forward primers of sequence 5'-GTAGAAATGACTGAACGTCCGATAA-3' and reverse primer of sequence 5'-CCAATTCCACATTGTTTCGGTCTAA-3' (Enovio Bio solutions Pvt. Ltd, Bhubaneswar). Standard protocol was followed for PCR procedure & the products were visualized in gel documentation under UV Trans- illuminator using 1.5% agarose gel with Ethidium bromide. A product of 310 bp size was considered as positive for *mec-A* gene.

Antimicrobial Susceptibility Testing [8]

This was done by Kirby Bauer disc diffusion method using antibiotic discs (Hi-Media, Mumbai, India) such as cotrimoxazole (25 µgm), azithromycin (15 µgm), clindamycin (2 µgm), ciprofloxacin (5 µgm), gentamycin (10 µgm), linezolid (30 µgm), vancomycin (30 µgm) and dalfopristin/quinpristin (15 µgm) & interpreted as per CLSI (2008) guidelines.

Quality Control

ATCC 25923 for MSSA & ATCC 43300 for MRSA were used as quality control strains.

STATISTICAL ANALYSIS

The diagnostic ability of the phenotypic tests to detect MRSA was evaluated by calculating sensitivity, specificity & positive and negative predictive values of each test. K2 test and kappa concordance measures were used for evaluating association & levels of concordance of the data respectively.

RESULTS

Out of 200 *S.aureus* isolates tested for *mec-A* gene by PCR, 62(31%) were *mec-A* positive hence, designated as MRSA and rest 138 were *mec-A* negative (MSSA). Methicillin resistance was detected by cefoxitin disc diffusion, oxacillin disc diffusion, oxacillin resistant screening agar and E-Test in 53 (26.5%), 60(30 %), 59(29.5 %) and 61(30.5%) isolates respectively. The MSSA strains were 100% sensitive to vancomycin, linezolid & dalfopristin & 95 % to gentamycin & 68-89 % to rest of the drugs used. The MRSA strains were 100% sensitive to linezolid, >90% to vancomycin & dalfopristin & 50-77% sensitive to others [Table/Fig-1]. The sensitivity, specificity & positive and negative predictive values of phenotypic methods in comparison to PCR for MRSA detection is summarized in [Table/Fig-2].

DISCUSSION

S.aureus is one of the common cause of nosocomial and community acquired infections with high mortality and morbidity [1]. Increase in methicillin resistance among *Staphylococci has* posed great difficulty in managing such infections [14]. Hence, an accurate and rapid detection of methicillin resistance is essential not only to choose appropriate antibiotic but also to control the spread of MRSA [5]. Many phenotypic methods to detect MRSA have been developed but they are slow and vary in sensitivity and specificity [3]. Currently, detection of *mec-A* gene by PCR is the gold standard for MRSA identification [5]. However, use of molecular methods for routine practice is not affordable to many resource constraint laboratories. Therefore it is essential to develop a rapid, accurate and sensitive phenotypic method for detection of MRSA [13]. Our study revealed that 32% of the *S.aureus* isolates were MRSA. The results were comparable to the studies carried out by others [15,16]. In this study the PCR assay identified 62 MRSA and 138 MSSA. By the disc diffusion method for MRSA, sensitivity of cefoxitin disc was 96.7% and of oxacillin disc was 82.5%. Similarly specificity of cefoxitin and oxacillin discs were 100% & 98.5% respectively. Several workers have also reported higher sensitivity and specificity of cefoxitin than oxacillin [9,17]. Higher sensitivity of cefoxitin can be explained by the fact that it is a better inducer of mec A gene to express PBP 2' than oxacillin. MRSA detection by ORSA medium had sensitivity of 95.1% and specificity of 100% in our study but in the study conducted by Velasco et al., ORSA medium had high sensitivity and low specificity [18]. Others have noted low sensitivity of ORSA with hetero resistant strains and low specificity with strains having border line MIC [12,19]. Incubating the plate up to full 48 hours increases the sensitivity

Antibiotic	% of MSSA (n= 138)	% of MRSA (n= 62)
Cotrimoxazole	89.1	77.4
Erythromycin	78.2	56.4
Clindamycin	86.9	72.5
Ciprofloxacin	68.1	50
Gentamycin	95.6	79
Vancomycin	100	90.3
Dalfopristin/quinpristin	100	93.5
Linezolid	100	100

[Table/Fig-1]: Antibiotic susceptibility patterns of *S. aureus* (n=200).

Methods	No. of False negatives	No. of False positives	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Concordance with PCR (%)
Oxacillin disc diffusion (53)	9	2	82.5	98.5	96.3	93.7	94.5
Cefoxitin disc diffusion (60)	2	0	96.7	100	100	98.5	99
Oxacillin Resistant Screening agar (59)	3	0	95.1	100	100	97.8	98.5
E-Test(61)	1	0	98.3	100	100	99.2	99.5

[Table/Fig-2]: Comparison of various methods for MRSA detection (n=200).

but the delay in getting results reduces the efficacy of this method [20]. E-Test MIC in our study detected MRSA with high sensitivity (98.3%) and specificity (100%) and concordance with PCR was 99.5%. Satisfactory results by E-Test MIC as compared to other phenotypic and PCR methods for MRSA detection is reported as it has the advantage of being easy to perform as disc diffusion test and approaches the accuracy of PCR for *mec-A*, but results of test strictly depends upon specific test conditions [21].

LIMITATIONS

Study could have included MSSA strains in addition to the MRSA isolates as with regard to infection prevention and therapy, isolates harboring SCC*mec* element should be treated as MRSA, even though they are identified by PCR to be *mec-A* negative [22].

CONCLUSION

In this study, cefoxitin was superior to oxacillin for detection of MRSA by disc diffusion method. Results of cefoxitin disc diffusion, ORSA and E-test were in concordance with results of PCR. PCR is too costly to be routinely implemented in most of the clinical laboratories. Though ORSA is cheaper and easy to perform, but delay in getting results is the major drawback. Therefore cefoxitin can be a good surrogate marker to detect MRSA. But another test of high sensitivity and specificity like E-test should combine cefoxitin disc diffusion to confirm *S.aureus* strains showing inhibition zone diameter between 20-22 mm. Linezolid, vancomycin & dalfopristin were the highly sensitive drugs against MRSA isolates.

REFERENCES

[1] Mandell LA, Wunderink R. Methicillin-Resistant *Staphylococcus aureus* and Community-Acquired Pneumonia: An Evolving Relationship. *Clin Infect Dis*. 2012;54(8):1134-36.

[2] Jevons MP. Celbenin-Resistant *Staphylococcus*: *Br Med J.* 1961;1:124-25.

[3] Datta P, Gulati N, Singla N, Vasdeva HR, Bala K, Chander J, et al. Evaluation of various methods for the detection of meticillin resistant *Staphylococcus aureus* strains and susceptibility patterns. *Journal of Medical Microbiology*. 2011;60:1613–16.

[4] Mbah AN, Isokpehi RD. Identification of Functional Regulatory Residues of the β-Lactam Inducible Penicillin Binding Protein in Methicillin-Resistant *Staphylococcus aureus*. *Chemotherapy Research and Practice*. 2013;2013:614670. 10 pages.

[5] Pillai MM, Latha R, Sarkar G. Detection of Methicillin Resistance in *Staphylococcus aureus* by Polymerase Chain Reaction and Conventional Methods: A Comparative Study. *J Lab Physicians*. 2012;4(2):83–88.

[6] Hobul T, Bozdoğan B, Haznedaroğu T, Ozyurt M. Heterogeneous macrolide resistance in methicillin-resistant *Staphylococcus aureus* isolates: investigation of resistance mechanisms and clonality. *Mikrobiyol Bul*. 2013;47(2):211-22.

[7] Harbarth S, Hawkey PM, Tenover F, Stefani S, Pantosti A, Struelens MJ. Update on screening and clinical diagnosis of meticillin-resistant *Staphylococcus aureus* (MRSA). *International Journal of Antimicrobial Agents*. 2011;37(2):110–17.

[8] Clinical and Laboratory Standards Institute/NCCLS. Performance Standards for Antimicrobial Susceptibility Testing; 2012, M 100-S22,Vol32 N0-3, page- 70- 80.

[9] Anand K, 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;21:27–29.

[10] Wendt C, Havill NL, Chapin KC, Boyce JM, Dickenson R, Eigner U, et al. Evaluation of a New Selective Medium, BD BBL CHROMagar MRSA II, for Detection of Methicillin-Resistant *Staphylococcus aureus* in Different Specimens. *J Clin Microbiol*. 2010;48(6):2223-27.

[11] Gram Positive cocci-part-1 Staphylococci & related Gram positive cocci- In Koneman's Color Atlas & Textbook of Diagnostic Microbiology,Eds Washington WJ, Stephen A,William J,Elmer K, Gary P, Paul S & Gail W.,6th edn,2006,Lippincott Williams & Wilkins,Philadelphia:645-48.

[12] Mathews AA, Thomas M, Appalaraju B, Jayalakshmi J. Evaluation and comparison of tests to detect methicillin resistant *S. aureus*. *Ind J Pathol Microbiol.* 2010;53(1):79-82.

[13] Bhutia KO, Shantikumar Singh T, Biswas S, Adhikari L. Evaluation of phenotypic with genotypic methods for species identification and detection of methicillin resistant in *Staphylococcus aureus*. *Int J Appl Basic Med Res*. 2012;2(2):84–91.

[14] David MZ, Daum RS. Community-Associated Methicillin-Resistant *Staphylococcus aureus*: *Epidemiology and Clinical Consequences of an Emerging Epidemic Clin Microbiol Rev*. 2010;23(3):616–87.

[15] Ahmad MK, Asrar A. Prevalence of Methicillin Resistant Staphylococcus aureus in pyogenic community and hospital acquired skin and soft tissues infections. *J Pak Med Assoc.* 2014;64(8):892-95.

[16] Falagas ME, Karageorgopoulos DE, Leptidis J, Korbila IP. MRSA in Africa: Filling the Global Map of Antimicrobial Resistance. *PLOS ONE*. 2013;8(7):1-12. | www.plosone.org .

[17] Ekrami A, Samarbafzadeh A, Alavi M, Kalantar E, Hamzeloi F. Prevalence of methicillin resistant Staphylococcus species isolated from burn patients in a burn center. *Jundishapur Journal of Microbiology*. 2010;3(2):84-91.

[18] Cesur S, Yildiz E, Irmak H, Aygün Z, Karakoç E, Kinikli S, et al. Evaluation of oxacillin resistance screening agar and chromogenic MRSA agar media for the detection of methicillin resistance in Staphylococcus aureus clinical isolates. *Mikrobiyol Bul*. 2010;44(2):279-84.

[19] Buchan BW, Ledeboer NA. Identification of Two Borderline Oxacillin-Resistant Strains of *Staphylococcus aureus* From Routine Nares Swab Specimens by One of Three Chromogenic Agars Evaluated for the Detection of MRSA. *American Journal of Clinical Pathology*. 2010;134(6):921-27.

[20] Carson J, Lui B, Rosmus L, Rennick H, Fuller J. Interpretation of MRSA *Select* Screening Agar at 24 Hours of Incubation. *J Clin Microbial*. 2009;47(3): 566-68.

[21] Ercis S, Sancak B, Hascelik G. A comparison of PCR detection of mecA with oxacillin disk susceptibility testing in different media and sceptor automated system for both Staphylococcus aureus and coagulase negative staphylococci isolates. *Indian J Med Microbiol*. 2008;26(1):21-24.

[22] Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, et al. Detection of Staphylococcal Cassette Chromosome *mec* Type XI Carrying Highly Divergent *mecA*, *mecI*, *mecR*, *blaZ*, and *ccr* Genes in Human Clinical Isolates of Clonal Complex 130 Methicillin-Resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2011;55(8):3765-73.

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