Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2017; 5(4E):1610-1613 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2017.v05i04.073

Original Research Article

Detection of Methicillin Resistant *Staphylococcus aureus* in Various Clinical Samples Isolated from a Teaching Hospital

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Abstract: Methicillin resistant Staphylococcus aureus (MRSA) became a major health care problem. Early and accurate detection of MRSA is essential for implementation of infection control practices. The resistance mechanism in Staphylococcus aureus is mediated by mecA gene. This is the prospective cross sectional study conducted in Microbiology Laboratory, Mediciti Institute of Medical Sciences from November 2012 - June 2014. Detection of MRSA was done using Cefoxitin disc diffusion (CDD) method according to Clinical and Laboratory Standard Institute (CLSI) guidelines with an objective to calculate prevalence of MRSA. Other phenotypic methods of detection of MRSA like Oxacillin disc diffusion (ODD) and Oxacillin agar dilution (OAD) were also done and results of all three methods were compared. Among 1350 clinical samples collected, 120 Staphylococcus aureus were isolated and processed for detection of MRSA. Total MRSA detected were 38(31.66%), 34(28.33%), 37(30.83%) by CDD, ODD, OAD methods respectively. So the prevalence of MRSA was 31.66%.More number of MRSA was detected by CDD. But when results were tested by chi square test, the P value showed no statistical significance. The minimum Inhibitory concentration [MIC] of Oxacillin for maximum number of MRSA isolates was found to be high (32 µg/ml) indicating increase in emergence of highly resistant strains. In conclusion, CDD is good method for detection of MRSA, but it should be supplemented with another feasible phenotypic method, so that MRSA strains exhibiting heterogenous resistance will not be missed.
Keywords: MRSA, Cefoxitin disc diffusion, Oxacillin disc diffusion, Oxacillin agar dilution, minimum Inhibitory concentration of Oxacillin, prevalence of MRSA

INTRODUCTION:

Staphylococcus aureus is a silent killer pathogen and it has remarkable propensity for development of antibiotic resistance [1, 2]. The term Methicillin resistant Staphylococcus aureus (MRSA) is used for all the strains of S.aureus which exhibit resistance to several commonly used antibacterial agents such as beta-lactam antibiotics, Cephalosporins, aminoglycosides, glycopeptides etc. The incidence of MRSA has been on the rise for the past 20 years. So, rapid and accurate and cost effective diagnosis of MRSA is important for proper management, prevention of transmission and to start correct treatment [3, 4].

MATERIALS AND METHOD

A prospective cross sectional study included all clinical samples collected in the Microbiology Laboratory, Mediciti Institute of Medical Sciences from November 2012 - June 2014. Direct smears of the clinical samples were prepared and observed under microscope for cells and organisms**.** The samples were inoculated on nutrient agar**.** Colonies resembling Staphylococcus aureus in morphology were further confirmed by Grams stain from pure cultures. These isolates were taken and specific identification of Staphylococcus aureus was done by Catalase test, Coagulase test (slide & tube), and Phosphatase test, Inoculation on selective media like Milk agar, High salt agar, Mannitol salt agar, DNase agar and Mannitol fermentation test.

Detection of MRSA was done by three phenotypic methods. Cefoxitin disc diffusion test was done using 30µg cefoxitin disc and results were

interpreted according to CLSI guidelines [5]. S.aureus with zone of inhibition ≥ 22 mm was taken as Cefoxitin sensitive and ≤ 21 mm as Cefoxitin resistant. Oxacillin disc diffusion test was done with 1µg Oxacillin disc. S.aureus strains with zone of inhibition ≥13 mm – Sensitive, 11 – 12 mm – Intermediate resistant and ≤ 10 mm – Resistant according to CLSI guidelines [5]. By Oxacillin agar dilution method the minimum Inhibitory concentration (MIC) for oxacillin was determined by following CLSI guidelines [6]. Sterile antimicrobial stock solution was prepared using Oxacillin sodium salt monohydrate powder [HI MEDIA]. Dilutions were made by using sterile distilled water as diluent. Intermediate antimicrobial agent solution was prepared by making 1:2, 1:4, 1:8 dilution using dilution format / Serial two fold dilutions. Then one part of 10X antimicrobial solution was added to nine parts of molten agar and plates were prepared. For determination of agar dilution, Plates were inoculated with the isolates, allowed to stand at room temperature until moisture in the inoculums spots had been absorbed into agar that is spot dried but not for more than 30 minutes. Plates were inverted and incubated at 35 ± 2ºC for 16 to 20 hours. Reference strain MRSA –ATCC 43300 was inoculated with each batch of testing strains. Drug free plates were also prepared from base media and were used as Control plates. Plates were kept in dark, nonreflecting surface to determine the end point. Lowest concentration of antimicrobial agent that completely inhibited the growth of S.aureus was recorded as MIC. Single colony/ faint haziness caused by inoculum was disregarded. The results were reported as MIC (µg/ ml).

RESULTS

Among total 1350 clinical samples collected in the study, 120 Staphylococcus aureus were isolated and processed for detection of MRSA. Among 120 coagulase positive S.aureus, 38 isolates showed a Cefoxitin zone diameter of ≤ 21mm (MRSA) and 82 isolates (MSSA) with zone diameter of ≥22mm.So prevalence of MRSA is 31.66%.

All the strains which were detected resistant by oxacillin disc diffusion and oxacillin agar dilution methods were also resistant by cefoxitin disc diffusion method. But 4 strains and 1 strain which were detected as sensitive by oxacillin disc diffusion and oxacillin agar dilution methods respectively were detected resistant by cefoxitin disc diffusion method. Though P value calculated showed no statistical significance among three methods.

Table-1: Oxacillin-MIC of 37 MRSA detected by agar dilution method

Concentration of Oxacillin	**MIC (µg/ml)**									
	128	**64**	**32**	**16**	**8**	**4**	**2**	**1**	**0.5**	**0.25**
No. of isolates	0	1	11	10	8	7	0	0	0	0

In the present study (According to CLSI guidelines) all strains which had Oxacillin minimum inhibitory concentration(MIC) value ≥ 4µg/ml were considered MRSA and those with MIC ≤ 2µg/ml were taken as Methicillin sensitive Staphylococcus aureus (MSSA).

Table-2: Percentage of MRSA

No. *S. aureus*	Test	No of MRSA detected	% of MRSA
120	CDD	38	31.66
	ODD	34	28.33
	OAD	37	30.83

Table-3: Comparision of MRSA detection methods

MRSA DETECTION METHODS COMPARED	X^2 (CHI-SQUARED)	P VALUE	Statistically Significant [P < 0.05]
CDD - ODD	0.317	0.5731	NO
ODD - OAD	0.180	0.6714	NO
CDD - OAD	0.019	0.8892	NO

DISCUSSION

Staphylococcus aureus strains expressing MecA gene are termed as MRSA. Resistance mechanism may be either homogeneous or heterogeneous. Cefoxitin disc is considered as surrogate marker for mecA gene. CDD test require no special media or incubating temperature. Cefoxitin is preferred to Oxacillin in detection of MRSA as Cefoxitin is the potent inducer of mecA regulatory system. CDD gives clear endpoint and it is easy to read. However Oxacillin maintain its activity and prevent degradation in storage and it can be used for detection of heterogeneous population of MRSA. Recent studies indicate that CDD is far superior to the most of the currently recommended phenotypic methods like ODD and OAD methods. This is now accepted method for MRSA detection by all referral groups like CLSI [7]. So in our study also CDD was used for screening and confirmation of MRSA. The prevalence of MRSA in the present study is 31.66%.This is in accordance with Mehta *et al.;* study reporting prevalence of 32% [8],Whereas in a study by Gopalakrishnan *et al.;* in 2010 in Chennai it was as high as 40-50% [9]. In KB Anand *et al.;* study in 2009 it was found that detection of mecA gene using CDD test is best for MRSA detection[10].According to N. Kaur *et al.;* study in 2013, results obtained with CDD were comparable with Polymerase chain reaction (PCR) [11].

In present study, 34 isolates detected resistant by ODD were also detected by CDD as resistant but 4 isolates detected resistant by CDD were sensitive by ODD. This is in accordance with KB Anand *et al.;* study in which among 50 strains of S.aureus, 38 were detected MRSA by CDD and 28 were detected by ODD [10]. In a study conducted by Anila A Methew *et al.;* though higher number of MRSA were obtained using ODD than CDD, they stated that it could be due to high false positive results obtained, which might be due to hyper production of beta-lactamases which lead to phenotypic expression of Oxacillin resistance [12]. In our study, the strains which were detected as MRSA by OAD (MIC) were also resistant by CDD. But 1 strain which was detected sensitive by OAD was detected resistant by CDD. All 34 strains detected as MRSA by ODD were also detected resistant by OAD. In contrast to the present study, Kunsung O Butia *et al.;* in 2012 found that the performance of OAD was better than CDD and ODD [11]. In our study, MIC value of Oxacillin for maximum number of MRSA isolates was found to be high (32 µg/ml) indicating increase in emergence of highly resistant strains over years. However still higher MIC values were reported in S Vidhani *et al.;* study in 2001[13].

Riza Adaleti *et al.;* in 2008, stated that combining ODD and CDD could be a good choice for detection of MRSA, where mecA PCR cannot be done. According to Priya Datta *et al.;* though CDD is good method for detection of MRSA but it should be supplemented with another feasible phenotypic method, so that no MRSA is missed. However benefit of using more than one phenotypic methods have to be still evaluated and proved [14, 15].

CONCLUSION

MRSA represents a challenge for virtually all healthcare Institutions. So continuous surveillance is therefore essential. In developing countries, molecular tests for MRSA detection are expensive and all laboratories cannot afford these tests, therefore, it is essential to develop a rapid, accurate and sensitive, simple, easy to perform, inexpensive phenotypic methods for screening and confirmation of MRSA. In our study Cefoxitin disc diffusion test proved to better than other phenotypic methods tested.

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