ORIGINAL ARTICLE

Detection of MecA Mediated Methicillin Resistance in Staphylococcus aureus by Cefoxitin Disc Diffusion Method and Latex Agglutination Test

FAIQA ARSHAD, IFFET JAVED, SOHAILA MUSHTAQ, *SAEED ANWER*

ABSTRACT

Aim: To assess the accuracy of cefoxitin disk diffusion method and PBP2a latex agglutination test for detection of methicillin resistance in *Staphylococcus aureus*.
Study Design: Descriptive Study
Place and duration of study: Pathology Department, Post Graduate Medical Institute, Lahore from February 2013 to October 2013
Methods: A total of 130 *Staphylococcus aureus* isolates from different clinical specimens were tested for methicillin resistance by disk diffusion method. Disk diffusion method was performed using cefoxitin (FOX) 30µg disc on Mueller Hinton agar (MHA) and incubation was done at 35ºC for 24 hours. Results were interpreted according to CLSI recommendations. Confirmation of MRSA strains was done by PBP2a latex agglutination test.
Results: The results of the study showed that out of 130 *Staphylococcus aureus* isolates, 54 were cefoxitin resistant, but 4 isolates were *mecA* gene product (PBP2a) negative when they were confirmed with PBP2a latex agglutination kit. So, 4(7.41%) *mecA* –ve isolates were falsely identified as MRSA by cefoxitin disc Diffusion method.
Conclusion: Latex agglutination test was a rapid and accurate method and could be the best predictor to detect MRSA when the molecular methods are not available.
Keywords: Methicillin Resistant Staphylococcus aureus, Penicillin Binding Protein 2a,

INTRODUCTION

Staphylococcus aureus is an important cause of both Hospital acquired and Community acquired infections [1]. After the development of resistance of *Staphylococcus aureus* to beta lactum drugs (e.g., penicillins and cephalosporins), betalactamase stable penicillin i.e. methicillin was introduced into clinical practice in 1959[2].Methicillin is a semi synthetic penicillin but resistance against this drug developed by the acquisition of *mecA* gene found on mobile genetic element called the Staphylococcal chromosomal cassette mec (*SCCmec*)[3]. This *mecA* gene codes for altered penicillin binding protein PBP2a and resulted in the emergence of strains called methicillin resistant *Staphylococcus aureus* (MRSA). These MRSA strains are resistant to all ß-lactam agents including cephalosporins and carbapenems[4,5]. First case of MRSA was reported in United Kingdom in 1961[6]. Today, MRSA is endemic in hospitals worldwide and now it has become a great threat even for healthy persons in general community[7]. Invasive infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are difficult to treat and are associated with high morbidity and mortality[8].

Proper detection of MRSA strains is very important for both therapeutic and epidemiological purposes[9]. There are a number of phenotypic methods to detect methicillin resistance including classical methods determining the minimal inhibitory concentration (MIC) by the broth dilution or E-test, screening techniques with solid culture medium containing oxacillin, oxacillin or cefoxitin disk diffusion, automated-system methods; genotypic methods that detect the *mecA* gene or its protein product (PBP2a) i.e., PCR and Latex agglutination test respectively[10,11].

Cefoxitin is surrogate marker of *mecA* gene and disk diffusion tests using cefoxitin give clearer end points and are easier to read than tests with Oxacillin. In phenotypic tests, many factors are known to affect the expression of resistance. These include *in vitro* conditions such as the test agent, incubation temperature, medium inoculated, inoculum size and salt concentration of the medium[12,13].

Regarding accurate detection of MRSA, identification of *mecA* gene by PCR or its product

Pathology Department, Post Graduate Medical Institute, Lahore
[1]Microbiologist, PGMI, Lahore.
[2]Associate Professor, Pathology Department, PGMI, Lahore.
[3]Assistant Professor, Pathology Department, PGMI, Lahore.
[4]Professor, Pathology Department, CMH, Lahore.
Correspondence to Dr. Faiqa Arshad, 631-A Karim Block, Allama Iqbal Town, Lahore. Cell: 03054914991 E-mail: drfaiqa@hotmail.com

(PBP2a) by latex agglutination is considered as Gold standard[14, 15]. PCR and the latex agglutination tests have the major advantage over the other phenotypic methods as they are not influenced environmental conditions[16].

PCR, although it is a good confirmatory test with high sensitivity and specificity but is expensive and is not available in most of the routine laboratories. The latex agglutination is a very rapid test and can serve as a good preliminary screen with high sensitivity and specificity equivalent to PCR. It can be easily performed in most of the routine microbiology laboratories in our setup[9].

The present study was planned to detect methicillin resistance in *Staphylococcus aureus* by conventional susceptibility (cefoxitin disc diffusion) method and to evaluate latex agglutination test for the detection of PBP2a.

MATERIAL AND METHODS

Different clinical specimens were taken from hospitalized patients of Lahore General Hospital (LGH) from February 2013 to October 2013. The specimens were processed in microbiology laboratory of Pathology department, PGMI, Lahore. One hundred and thirty *Staphylococcus aureus* isolates were identified by the standard microbiological procedures. Preliminary identification included Colonial morphology, Gram staining and Catalase test. Further biochemicals like coagulase and DNase were performed for the confirmation of organism.

The phenotypic resistance to methicillin was determined using cefoxitin disk 30µg (Oxoid Ltd) on Mueller Hinton agar, inoculated with the organism suspension adjusted according to 0.5McFarland turbidity standards. The plates were incubated at 35°C for 24 hrs and the results were interpreted according to the clinical and laboratory institutes (CLSI) guideline[17]. MRSA ATCC 33591 and MSSA ATCC 25923 were used as positive and negative control respectively .All isolates that showed cefoxitin resistance were tested for *mecA* product (PBP2a) using latex agglutination kit (Slidex, Biomeurix) for confirmation of MRSA by following the manufacturer's instructions provided with the kit.

Latex Agglutination Test for Detection of Penicillin Binding Protein 2a (PBP2a): Latex particles sensitized with monoclonal antibodies directed against PBP2a, specifically react with MRSA to cause agglutination visible to the naked eye (Figure 2). Methicillin-susceptible *Staphylococcus aureus* (MSSA) do not agglutinate the latex particles. All the reagents were stored at 2-8 °C.

RESULTS

The results of the study showed majority of MRSA strains were recovered from Surgery wards (42%), followed by Medicine (20%), Gyane (10%), Neuro (8%), Ortho (8%), Paeds (8%) and Urology (4%) as shown in Table 1. Out of 130 *Staphylococcus aureus* isolates, 54 isolates showed cefoxitin resistance (Table 2) but 4 isolates were *mecA* gene product (PBP2a) negative when they were confirmed with PBP2a latex agglutination kit (Fig. 1). So, 4(7.41%) *mecA* –ve isolates were falsely identified as MRSA by Cefoxitin disc Diffusion method.

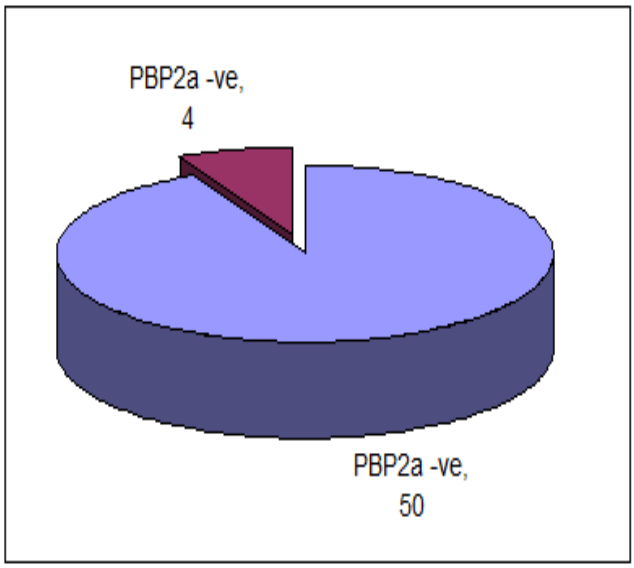
Table 1: Distribution of Methicillin Resistant *Staphylococcus aureus* isolates from various clinical departments (n=50)

Ward	Frequency	%age
Gyane	5	10
Medicine	10	20
Neuro	4	8
Ortho	4	8
Paeds	4	8
Urology	2	4
Surgery	21	42

Table 2: Cefoxitin Inhibition zone and results of *Mec A* gene

mecA results	n	Results of disc diffusion zone(mm)
Positive	54	≤ 21 mm
Negative	76	≥ 22 mm

Fig. 1: Results of Latex Agglutination Test



Out of 54 cefoxitin resistant isolates, 50 were positive as MRSA by LA test while 4 isolates were negative.

Fig. 2: Latex Agglutination Test showing Positive Reaction for PBP2a in MRSA

DISCUSSION

Detection of MRSA with rapid and precise methods must be done as a routine laboratory procedure for therapeutic and epidemiological purposes so that an appropriate antibiotic could be started immediately and spread of these organisms to the community could be avoided. If the detection of MRSA is missed, it may lead to treatment failure and spread of MRSA particularly if suitable infection control measures are not functional. On the other hand, wrong detection of MRSA results in increase health care expenditures following needless isolation and finally it may lead to overuse of glycopeptides such as vancomycin leading to treatment failure[18].

Results of the present study are in accordance with the study done by Oberoi *et al*[18] in India, 2012. Results of their showed that 46 isolates were detected as MRSA by cefoxitin disc diffusion method but 44 strains showed positive reaction for the presence of PBP2a by Latex Agglutination test. i.e., 2 (4.35%) isolates were wrongly identified as MRSA by cefoxitin disc diffusion method. They found that latex agglutination test was rapid and accurate method and could be the best predictor to detect MRSA when the molecular methods are not available.

Several researches conducted by Baddour *et al.,* 2007[19]; Mohanasoundaram and Lalitha, 2008[16]; Dorneanu *et al.,* 2010[9]; Sharma *et al.,* 2011[20] also showed that the conventional methods for detection of methicillin resistance like disc diffusion and MIC are cost-effective but time consuming and are influenced by different environmental conditions. PCR, although it is a good confirmatory test with high sensitivity and specificity but is expensive and is not available in most of the routine laboratories. The PBP2a latex agglutination test is a very rapid and accurate method for detection of *mecA* mediated resistance in *Staphylococcus aureus* and can serve as a good preliminary screen test with high sensitivity and specificity equivalent to PCR. Latex agglutination test can be easily performed in routine Microbiology laboratories. Rahbar *et al,*[21] reported from Iran in 2006 that the latex agglutination kit for detection of PBP2a is an alternative method to PCR for the confirmation of MRSA.

Whereas, Sangeetha *et al.,* in India (2012)[22] reported 48 MRSA isolates detected by cefoxitin disc diffusion test out of 63 isolates of *Staphylococcus aureus*, and same results were obtained by PCR and latex agglutination Kit. They concluded that for small laboratories, performing latex test may not be possible but it can be an alternate option to confirm the doubtful results. Adaleti *et al.,* in Turkey (2007)[12] also showed the similar results.

CONCLUSION

Latex agglutination test was a rapid and accurate method and could be the best predictor to detect MRSA when PCR is not available.

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