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Research Article

SUPPLEMENTATION OF CEFOXITIN DISC DIFFUSION METHOD WITH LATEX AGGLUTINATION TEST FOR THE DETECTION OF MRSA

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ABSTRACT

Background: MRSA is responsible for the largest number of outbreaks in the hospital settings. MRSA causes wide spectrum of infection including sepsis/endocarditis, lower respiratory tract infections etc. Resistance to methicillin is due to the presence of an altered penicillin-binding protein called PBP2a resulting from acquisition of a chromosomal gene called *mec A*. Detection of MRSA is complicated due to its heterogeneous nature. Keeping in mind the increasing rate of infections caused by MRSA there is a need for rapid, accurate and reliable tests for the detection of MRSA so that antibiotic therapy and infection control measures can be initiated. The present study combined the use of cefoxitin disc diffusion as the standard method in supplementation with latex agglutination assay for MRSA detection. **Methods:** cefoxitin disc diffusion method and latex agglutination test. **Results:** By cefoxitin disc diffusion 90 strains were *mecA* positive and were labeled as MRSA and 110 were labeled as MSSA. By latex agglutination test all the 90 MRSA were identified as MRSA whereas of 110 MSSA *S.aureus* strains 109 were detected as MSSA. **Conclusion:** cefoxitin disc diffusion is a good method but it should be supplemented with some other method so that no MRSA is missed. Latex agglutination test on the other hand gave results within 15 min and is able to detect even low levels of PBP2a.

INTRODUCTION

Infections have been one of the major causes of morbidity and mortality worldwide among the human population. Methicillin resistant *Staphylococcus aureus* (MRSA) is responsible for the largest number of outbreaks in the hospital settings. MRSA causes wide spectrum of infection including sepsis/endocarditis, lower respiratory tract infections, skin and soft tissue infections (SSTIs), toxic shock syndrome, bone and joint infections, surgical sites and decubitus ulcers, urinary tract infection, meningitis, epidural abscess, mastitis, neonatal liver abscess, and even chorioamnionitis (Waness, 2010) [1].

During the 1960s, resistance to methicillin emerged. Resistance to methicillin is due to the presence of an altered penicillin-binding protein called PBP2a resulting from acquisition of a chromosomal gene called *mecA*. PBP 2a have low affinity for the β-lactam antibiotics. The *mecA* gene is located within the large mobile staphylococcal chromosomal element called as SCC*mec* (staphylococcal cassette chromosome). *S. aureus* strains that contain SCC*mec* element are termed as MRSA and the strains lacking this element are termed as methicillin-susceptible *S. aureus* (MSSA) (Lowy, 2003) [2].

Detection of MRSA is complicated due to its heterogeneous nature (Chambers, 1998) [3]. Other risk factors for acquisition of MRSA include the administration of multiple antibiotics; modification of nasal bacterial flora when systemic antibiotics are given.

Today a matter of concern for microbiologist is how to overcome the problem of methicillin resistance. Keeping in mind the increasing rate of infections caused by MRSA there is a need for rapid, accurate and reliable tests for the detection of MRSA so that antibiotic therapy and infection control measures can be initiated. There are many molecular and conventional phenotypic methods available in the clinical microbiology laboratories.

The molecular methods are not affordable by every laboratory, so it is essential to evaluate an accurate sensitive method which can provide equivocal results as the molecular methods (Velasco *et al.,* 2005) [4].

The present study combined the use of cefoxitin disc diffusion as the standard method in supplementation with latex agglutination assay for MRSA detection.

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MATERIALS AND METHODS

This prospective study was conducted in the department of Microbiology, Pt.B.D.Sharma PGIMS Rohtak over a period of one year. A total of 200 *S.aureus* strains isolated from various clinical samples (urine, blood, pus, HVS, throat swab and sputum) received from various indoor and outdoor patients were included in the present study.

Antibiotic susceptibility testing using cefoxitin disc 30 µg was performed on Mueller Hinton Agar (MHA) for all the *S. aureus* isolates to detect MRSA and MSSA strain. Afterwards latex agglutination test was applied separately on MRSA and MSSA strains.

Cefoxitin disc diffusion method

By using a sterile forceps 30 µg cefoxitin disc was placed in the center of the MHA plate and incubated at 35°C for 24 h. After incubation the zone of inhibition was measured and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines (2018). For cefoxitin disc, zone diameter ≤21 mm were reported as MRSA and ≥22 mm were reported as MSSA (Datta *et al.,* 2011) [5].

Latex agglutination test

This is a simple and rapid agglutination assay to detect PBP2a from isolates of Staphylococci, as an aid in identifying MRSA. Latex particles were sensitized with a monoclonal antibody of same class IgG subclass but against a human protein showing no reactivity with proteins of *S.aureus*. The *mecA* gene coding for PBP2a causes visible clumps were reported as MRSA (Nakatomi, 1998) [6].

Statistical Analysis

At the end of the study that data was collected, compiled and entered in the MS Excel sheet and further statistical analysis was done using latest version of Statistical Package for Social Sciences and statistical significance.

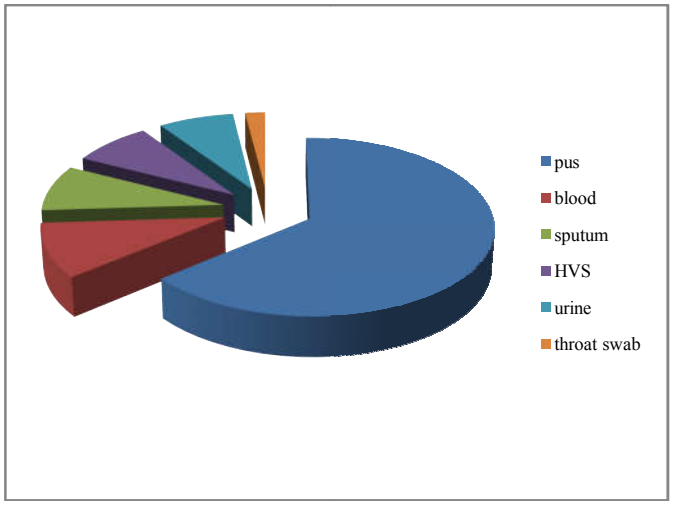
RESULTS

In the present study, 200 isolates of *S.aureus* were recovered from various clinical samples over a period of 1 year. The isolation was maximum from pus (64%) followed by blood (10%) and least from throat swab (2%).

The 200 *S.aureus* isolates were tested for *mecA* gene by cefoxitin disc diffusion in supplementation with latex agglutination assay. Out of the 200 strains, 90 were *mecA* positive and were labeled as MRSA and 110 were labeled as MSSA by cefoxitin disc diffusion test. Thus, the prevalence of MRSA in our institute was 45% (table 1).

Table 1

S.aureus	MRSA (%)	MSSA
200	**90 (45%)**	**110 (55%)**



The sample wise distribution of *S.aureus* is shown in figure 1.

The sample wise analysis of MRSA showed that 70 out of 128 *S.aureus* strains were detected as MRSA from pus, 7 out of 20 from blood, 4 out of 17 from sputum, 5 out of 16 from HVS, 4 out of 15 from urine and 0 out of 4 from throat swab (table 2)

Table 2

Specimen	*S.aureus*	MRSA (%)	MSSA (%)
Pus	128	70 (35)	58 (29)
Blood	20	7 (3.5)	13 (6.5)
Sputum	17	4 (2)	13 (6.5)
HVS	16	5 (2.5)	11 (5.5)
Urine	15	4 (2)	11 (5.5)
Throat swab	4	0 (0)	4 (2)
Total	200	90 (45)	110 (55)

All the 90 MRSA and 110 MSSA *S.aureus* strains were subjected to methicillin sensitivity by latex agglutination test. By latex agglutination test all the 90 MRSA were identified as MRSA whereas of 110 MSSA *S.aureus* strains 109 were detected as MSSA. The sensitivity and specificity of the latex agglutination test were 100% and 99% respectively and the positive predictive value (PPV) and the negative predictive value (NPV) were 98.9% and 100% respectively. The p-value for latex agglutination was 0.919 (non-significant).

DISCUSSION

It is problematic that the present study reports an alarmingly high prevalence (45%) of MRSA infection. Such a high prevalence rate can be attributed to the indiscriminate use of antibiotics, lack of awareness, unethical treatment before coming to the hospital and poor infection control practices. These results were comparable to studies carried out by others (Shilpa *et al*., 2010) [7].

Maximum isolation was from pus samples (64%). Several other studies showed that the highest rate of isolation was from pus samples (Mantri *et al*., 2014) [8]. This can be attributed to contamination of collected specimens with normal flora of skin. The rate of isolation of MRSA was also more from pus samples (35%), which is in concordance with many other studies (Kulshrestha *et al*., 2017) [9]. The high prevalence of MRSA in pus can be attributed to the production of panton valentine leukocidin gene (PVL) by MRSA which is known to be associated with tissue necrosis.

The early and accurate determination of MRSA infection is important for its treatment and prognosis.
In the present study cefoxitin disc diffusion method was taken as the standard method with sensitivity and specificity of

100%. Similar findings have been reported in several other studies (Sheetal *et al*., 2017) [10]. Cefoxitin being an inducer of the *mecA* gene increases the expression of *mecA*-encoded protein PBP2a which is responsible for its higher sensitivity.

The sensitivity of latex agglutination test in the present study was 100% which was similar to other studies (Datta *et al*., 2011) [5]. In the present study latex agglutination test detected all the 90 MRSA isolates and misclassified as MRSA only 1 of 110 MSSA isolates in comparison with cefoxitin disc diffusion test. The false positive result may be attributed to the fact that some *S.aureus* strains which are *mecA* positive produce very small amount of PBP2a which is detected by latex agglutination test. Therefore, MRSA screen test offers a new, valuable tool in the ongoing battle against MRSA.

No statistically significant differences were observed in the two methods. Henceforth, it will be worth mentioning here that both methods have a good sensitivity and specificity.

CONCLUSION

To conclude, cefoxitin disc diffusion is a good method but it should be supplemented with some other method so that no MRSA is missed. Cefoxitin disc diffusion method proved to be a simple, easily available, economical method. Latex agglutination test on the other hand gave results within 15 min and is able to detect even low levels of PBP2a. Therefore, latex agglutination test expensive but independent of environmental variations can be combined with cefoxitin disc diffusion method to detect MRSA in clinical settings.

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