Latex agglutination: A rapid method for MRSA detection

Dr. Parul Parvesh Verma1
Senior Resident, MD Microbiology, Kalpana Chawla Government Medical College, Karnal

Dr. Ashu Gautam2
PG resident, Department of Microbiology, Kalpana Chawla Government Medical College, Karnal

Dr. Ruchika3
Senior Resident, MD Pathology, VMMC Hospital, Delhi

Abstract:
Infection due to *S.aureus* imposes a high burden on health care resources. A growing concern is the emergence of Methicillin Resistant *Staphylococcus aureus* (MRSA) infections. Methicillin resistance is a complex property, and more than one mechanism is involved. The prevalence in India has increased from 12% in 1992 to 80.83% in 1999. The early and accurate determination of Methicillin resistance is of key importance in the treatment of infections caused by Methicillin resistant *S.aureus*. Various methods are available for MRSA detection namely Cefoxitin disc diffusion, Chromogenic agar, Latex agglutination and Oxacillin E-test. Latex agglutination is a rapid agglutination assay detecting PBP2' (also called as PBP2a) in isolates of *Staphylococcus aureus* (MRSA). The purpose of present study is to study latex agglutination assay for the detection of MRSA.

Keywords: MRSA, latex agglutination assay, *S. aureus*

INTRODUCTION:
Infection due to *S.aureus* imposes a high burden on health care resources. *S.aureus* infections are problematic due to frequently occurring antibiotic resistance in *S.aureus* isolates. A growing concern is the emergence of Methicillin Resistant *Staphylococcus aureus* (MRSA) infections in patients with no apparent risk factors. MRSA is now endemic in India. The incidence of MRSA varies from 25% in Western parts of India to 50% in South India. Methicillin resistance is a complex property, and more than one mechanism is involved. Resistance to Methicillin is due to low affinity PBP’s substituting the activities of the normal and essential PBP’s, this low affinity PBP’s are called PBP2a, which is encoded for by the Mec A gene. In addition methicillin resistance is dependent on factors like Methicillin-repressor Mec-1, antirepressor MecR1 and factors essential for expression of methicillin resistance.1,2,3

Community acquired MRSA (CA-MRSA) can spread rapidly among healthy individuals. Outbreaks of CA-MRSA infections have been reported worldwide and are increasingly reported from India. The prevalence in India has increased from 12% in 1992 to 80.83% in 1999.4,5,6 Hospital acquired MRSA (MRSA) is commonly associated with Pneumonia and Bacteremia. MRSA bacteremia is a serious condition which carries the risk of fatality ranging from 23% to 54%. HA-MRSA is commonly found among infants and elderly and presents with the risk factors such as longer hospital stay, urinary catheterization, diabetes, prior antibiotics especially quinolines and acute renal failure.7,8 The early and accurate determination of Methicillin resistance is of key importance in the treatment of infections caused by Methicillin resistant *S.aureus*. Various methods are available for MRSA detection namely Cefoxitin disc diffusion, Chromogenic agar, Latex agglutination and Oxacillin E-test. Latex agglutination is a rapid agglutination assay detecting PBP2’ (also called as PBP2a) in isolates of *Staphylococcus*, as an aid in identifying MRSA.9

The purpose of present study is to study latex agglutination assay for the detection of MRSA.

METHODOLOGY:
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 specimens were included in the present study.
The following samples were included for the present study:

1. Pus
2. Urine
3. Blood
4. High vaginal swabs (HVS)
5. Sputum
6. Throat swab

PROCESSING OF SAMPLES

**Pus samples**

Pus samples or two pus swabs were collected aseptically from each patient. One swab was used for preparing Gram stain and the other was inoculated onto blood agar and MacConkey agar. All plates were then incubated aerobically at 37°C for 18-24 h.

**Urine samples**

About 10-15 ml of urine was collected from patients suspected of urinary tract infection (UTI) with aseptic precautions. The deposit was examined for the presence of leucocytes, red blood cells (RBCs), casts, crystals, and bacteria. The finding of 1 leucocyte per 7 high power fields (HPF) corresponds with 10⁴ leucocytes per ml and the finding of clearly larger numbers than this indicates significant pyuria and culture was put up for those samples. Samples were inoculated onto blood agar and MacConkey agar and incubated at 37°C aerobically for 18-24 h.

**Blood samples**

5-10 ml of blood from patients with signs and symptoms of bacteremia was collected in glucose broth and bile broth bottles. These bottles were then incubated at 37°C for 18-24 h under aerobic conditions. They were sub-cultured after 24 h, 48 h, 72 h, and then on 7th day onto blood agar and MacConkey agar. The inoculated plates were incubated overnight at 37°C aerobically for 18-24 h.

**Throat swabs and sputum**

Two throat swabs and sputum samples were collected from patients suspected of respiratory tract infection. Smears were examined microscopically after Gram staining. Simultaneously, the samples were inoculated onto blood agar and MacConkey agar, and plates were incubated at 37°C for 18-24 h.

**HVS**

Two HVS were collected from the patients having vaginal discharge. From one swab, Gram stained smear was prepared and examined microscopically. Second swab was inoculated onto blood agar and MacConkey agar, and plates were incubated at 37°C for 18-24 h.

**Culture Identification:**

Clinical specimens were inoculated on blood agar and MacConkey agar.

**Blood Agar:**

Colonies of *S. aureus* were round, 1-3 mm in diameter, low convex, smooth, glistening with entire regular margins, densely opaque, of butyrous consistency and easily emulsifiable in normal saline. Colonies of *S. aureus* produced golden yellow pigment and were surrounded by a narrow zone of β-hemolysis.

**MacConkey Agar:**

Colonies of *S. aureus* were smaller as compared to colonies on blood agar and were pink due to lactose fermentation.

**Gram Staining:**

An air dried smear was prepared from each culture plate on a clean, grease free slide. Gram staining was done. Violet colored cocci arranged in clusters were identified as *Staphylococcus* species.

**Biochemical reactions**

**Catalase Test:**

The catalase test was performed with 3% H₂O₂ (hydrogen peroxide) on a clean glass slide. Few colonies of the test organism were taken from the agar plate with a sterile coverslip and touched with H₂O₂. Immediate (within 10 seconds) and vigorous bubbling indicates conversion of the H₂O₂ to water and oxygen gas. Catalase positive isolates were identified as *S.aureus*.

**Coagulase Test:**

This test was done to identify *S.aureus* which produces the enzyme coagulase that causes plasma to clot by converting fibrinogen to fibrin.

**Slide Test Method:**

A drop of normal saline was placed on each end of clean slide. A colony of the test organism was emulsified in each of the drops to make two thick suspensions and then a loopful of plasma was added to one of the suspensions and mixed gently. The other suspension was used as a control. Clumping of organisms within 10 seconds was interpreted as positive slide coagulase test.

**IDENTIFICATION OF MRSA**

All the isolates of *S.aureus* were tested for MRSA by latex agglutination assay.

1) **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*.¹²,¹³
Procedure:

Interpretation: The \textit{mecA} gene coding for PBP2\textsubscript{a} causes visible clumps were reported as MRSA.

PBP2\textsuperscript{a} present in the membranes of MRSA was rapidly extracted by alkaline treatment. A loopful of bacterial cells were suspended in 200 \( \mu \)l of extraction reagent 1 (0.1 mol/L NaOH) and subsequently were be lysed by boiling for about 3 min.

After cooling to room temperature, 50 \( \mu \)l of extraction reagent 2 (0.5 mol/L KH\textsubscript{2}PO\textsubscript{4}) was added to 200 \( \mu \)l of the lysate and samples were mixed well.

After 5 min of centrifugation, 50 \( \mu \)l of the supernatant was used for testing agglutination with latex particles sensitized with monoclonal antibodies and another 50 \( \mu \)l of the supernatant was used for testing with the control latex particles.

The test slides were mixed by rotating them for 3 min, after which agglutination was assessed visually.

Clinical samples were inoculated on Blood agar and MacConkey agar.

From the growth obtained after 24hrs of incubation, gram stain was prepared.

Catalase and slide coagulase test were performed on the basis of gram stain findings.

Strains testing positive for slide coagulase were categorized as \textit{S.aureus}.

**Fig 1:** latex agglutination test for MRSA
Disposal of waste

All the biomedical waste generated during this study in the laboratory will be discarded after proper disinfection or sterilization as per the Biomedical Waste Management and Handling Rules, 2016 and 2018 guidelines.\textsuperscript{14,15}

**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:** Out of the 200 \textit{S.aureus} strains isolated from various samples, 128 (64\%) were from pus, 20 (10\%) from blood, 17 (8.5\%) from sputum, 16 (8\%) from HVS, 15 (7.5\%) from urine and 4 (2\%) from throat swab. The specimen wise distribution is shown in the table no.1:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No. of \textit{S.aureus} isolates</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus</td>
<td>128</td>
<td>64</td>
</tr>
<tr>
<td>Blood</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Sputum</td>
<td>17</td>
<td>8.5</td>
</tr>
<tr>
<td>HVS</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Urine</td>
<td>15</td>
<td>7.5</td>
</tr>
<tr>
<td>Throat swab</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>

From the above observation it was found that \textit{S.aureus} was isolated from all the specimens and more number of \textit{S.aureus} was isolated from the pus sample followed by blood.

![Sample wise distribution of \textit{S.aureus}](image)

Out of the 200 \textit{S.aureus} isolates tested by latex agglutination test 90 (45\%) were MRSA and 110 (55\%) were MSSA as depicted in the table no.2:

<table>
<thead>
<tr>
<th>\textit{S.aureus}</th>
<th>MRSA (%)</th>
<th>MSSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>90 (45%)</td>
<td>110 (55%)</td>
</tr>
</tbody>
</table>

The number of \textit{S.aureus} isolates and the percentage of MRSA and MSSA from different specimens are given in table no. 3:
# Table no.3
Sample wise distribution of MRSA and MSSA

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

From the above table it was inferred that MRSA was isolated from all the specimens except the throat swab and the percentage of MRSA isolated from the pus samples (35%) was highest followed by blood (3.5%), HVS (2.5%), urine (2%) and sputum (2%).

## Discussion:

In the present study, 200 isolates of S.aureus were collected. The site of isolation was maximum from pus (64%) followed by blood (10%) and least from throat swab (2%). Mantri et al (2014) in their study showed that 67.85% S.aureus were isolated in pus samples which is in concordance with the present study. The most frequent isolation of S.aureus from pus can be attributed to contamination of collected specimens with normal flora of skin.

It is a worrisome 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. Comparable results were seen in a study conducted by Shilpa et al (2010), they reported 46% prevalence of MRSA. 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.

Latex agglutination has the advantage of being rapid, giving results in approximately 20 min and is easy to perform and interpret. On the same day after the growth of culture, MRSA can be reported to the physician and empirical treatment can be started accordingly in the benefit of the patient. The only drawback of this assay is the cost.

## Conclusions of this study are described below:

1. The rate of isolation of S.aureus was maximum from pus (64%) followed by blood (10%), sputum (8.5%), HVS (8%), urine (7.5%) and least from throat swab (2%).
2. All the S.aureus isolates were subjected to latex agglutination test and of the 200 S.aureus isolates 90 were MRSA and 110 were MSSA.
3. The prevalence of MRSA was observed in 45% of S.aureus isolates.
4. It was observed that the rate of MRSA isolation was maximum from pus (35%) followed by blood (3.5%), HVS (2.5%), urine and sputum (2%).
5. MRSA infections are difficult to treat, early diagnosis and management is necessary to reduce the morbidity and mortality.

This study focused on finding out a simple, economic and more accessible method with high sensitivity and specificity to identify MRSA. Therefore, latex agglutination test expensive but independent of environmental variations can be used for MRSA detection.

## References


