

Evaluation of Latex Agglutination Test for Rapid Identification of *Staphylococcus aureus* Isolated from Pyogenic Wound Infections at a Tertiary Care Hospital

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ABSTRACT

Background

Staphylococcus aureus infections are increasingly reported worldwide. It is a major clinical problem and imposes significant morbidity and mortality due to widespread emergence of multidrug resistant pathogens like methicillin resistant *Staphylococcus aureus*. Thus, rapid and reliable identification of *Staphylococcus aureus* is essential for timely and effective management of patient.

Objective

The performance of Latex agglutination test (Staphaurex Plus) was compared to conventional method tube coagulase test which is gold standard too for the identification of *Staphylococcus aureus*.

Method

This study was conducted at B.P. Koirala Institute of Health Sciences. Following standard microbiological methods, isolation and identification was done in the Department of Microbiology. MRSA detection was performed following Clinical and Laboratory Standard Institute. All the isolates of *Staphylococci* were subjected for Latex agglutination test and was performed according to manufacturer's instructions using Staphaurex Plus kit.

Result

Out of 377 (methicillin sensitive *Staphylococcus aureus* – 142; methicillin resistant *Staphylococcus aureus* - 233; Coagulase Negative *Staphylococci* –2) isolates of *Staphylococci*, Latex agglutination test was found to be positive in 138 (97.1%) of methicillin sensitive *Staphylococcus aureus* (MSSA) and 220 (94.4%) of methicillin resistant *Staphylococcus aureus* (MRSA). Overall sensitivity, specificity, positive predictive value and negative predictive value of Latex agglutination test was found to be 95.46%, 100%, 100%, 10.52% respectively.

Conclusion

In conclusion, Latex agglutination test is a rapid and reliable test for the identification of *Staphylococcus aureus*.

KEY WORDS

Latex agglutination test, *Staphylococcus aureus*, tube coagulase test

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is associated with wide range of infections and disease syndrome that can vary from superficial to highly invasive or disseminated disease and the most frequently isolated pathogen in clinical specimen worldwide.^{1,2} Among the different methods practiced in clinical laboratories, conventional tube coagulase test for detection of free coagulase is although considered gold standard for identification of *S. aureus* and differentiating it from Coagulase Negative *Staphylococci* (CoNS), is time consuming as it takes 4 to 24 hours to provide results.³

Earlier, several assays based on the detection of specific determinants of *S. aureus*, both clumping factor and protein A were launched.^{4,5} However, false negative results were obtained while testing with methicillin resistant *Staphylococcus aureus* (MRSA) strains.^{6,7} This problem was conquered by development of agglutination assays which incorporated antibodies against capsular polysaccharide 5 and 8 and group specific antigens present on surface of 80% of *S. aureus*.^{6,8,9}

Rapid identification of microbial pathogens improves patient management by providing earlier basis for the choice of optimal antimicrobial agent. Thus, this study was aimed to evaluate the performance of latex agglutination test (LAT) in rapid identification of *S. aureus*.

METHODS

This study was conducted in the department of Microbiology, BPKIHS, Dharan, a tertiary care hospital in eastern Nepal from September 2021 to February 2022. All the specimens (wound swabs and pus aspirates) received in lab during the study period were processed for identification of organisms and were inoculated onto Blood agar and MacConkey agar (HIMEDIA LABORATORIES) and incubated at 35°C for 24 and 48 hours, respectively. No growth on culture media was considered sterile. The bacterial pathogens grown were identified up to species level by standard microbiological techniques like colony morphology, gram stain and several tests like catalase, slide coagulase and tube coagulase.³

Methicillin resistant *Staphylococcus aureus* (MRSA) was determined by Kirby Bauer Disc-Diffusion assay using cefoxitin disk (30 µg) on Muller Hinton Agar (MHA) according to Clinical and Laboratory Standards Institute (CLSI) guidelines.¹⁰ Latex agglutination test (LAT) was performed according to manufacturer's instructions using Remel Staphaurex Plus kit (Lot: 3192621). The Staphaurex plus Test latex consists of yellow latex particles which have been coated with fibrinogen and rabbit immunoglobulin G specific for *S. aureus*. When a drop of reagent is mixed on a card with *S. aureus* organisms, rapid agglutination occurs through the interaction of (i) fibrinogen and clumping factor, (ii) the Fc portion of IgG and protein A or (iii) specific

IgG and cell surface antigens. *Staphylococcus aureus* ATCC 25923 were used as control and tested along with the test strain.

Ethical clearance was obtained from the Institutional Review Committee of BPKIHS (Code No.: IRC/2155/021). Data collected were entered in MS excel 2010 and analysed by using statistical package for social sciences (SPSS) 11.5 version. For descriptive analysis, frequency and percentage was calculated. Chi square test was applied to find out the relationship between tube coagulase and LAT. P-values < 0.05 was considered statistically significant.

RESULTS

Throughout the study period, a total of 375 *Staphylococcus aureus* were isolated. All of the isolates were confirmed by tube coagulase positive. Cefoxitin resistance was found to be in 233 of isolates indicating MRSA (62.1%). Two isolates were identified as Coagulase Negative *Staphylococci* (CoNS). However, these test results were not available while performing the latex agglutination test. Considering tube coagulase test as gold standard, LAT performed for MSSA (142) and MRSA (233) isolates of *S. aureus* and CoNS (2 isolates) is shown in table (1).

Table 1. Frequency of Tube Coagulase test and LAT (n=377)

	Tube Coagulase test, n (%)		LAT positive, n (%)	
	Positive	Negative	Positive	Negative
MSSA (n=142)	142 (100.0)	0	138 (97.1)	4
MRSA (n=233)	233 (100.0)	0	220 (94.4)	13
CONS	0	2	0	2
Total	375	2	358	19

Association of LAT for identification of *S. aureus* with tube coagulase test was significant (p value < 0.001) which showed overall sensitivity of 95.4%, and specificity of 100% with Positive predictive value (PPV) 100% and Negative predictive value (NPV) 10.5% (table 2).

Table 2. Tube Coagulase test and LAT (n=377) for identification of *Staphylococci*

Test	Test status	Tube coagulase test		P value	Remarks
		Positive	Negative		
LAT	Positive	358	0	< 0.001	Significant
	Negative	17	2		
Total	375	2			

The sensitivity of latex agglutination test for detection of MSSA was 97.1% and PPV was 100%. Similarly, Sensitivity and PPV of latex agglutination test for detection of MRSA were 97.1% and 94.4% respectively.

DISCUSSION

Staphylococcus aureus is a significant pathogen commonly isolated in clinical specimen. The incidence has increased in the past decade, and this increase has paralleled the rising rates of emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) infections globally, which has presented additional challenges for the management of infection. This surge in antimicrobial resistance further delays wound healing and the infection becomes more worst which increases hospital stay, prolongs trauma care, and high medical costs. It is important to have a clear understanding of the most likely etiologic agent to decide on appropriate antibiotic therapy. Treatment of these infections has been more difficult despite the advancements in diagnostic and treatment techniques.

There are different conventional methods for detection of *Staphylococcus aureus* like slide and tube coagulase test, urease and mannitol test. Identification of this major pathogen is confirmed by one of the traditional gold standard test, tube coagulase test which is time consuming.^{2,3} This often forces clinical microbiology laboratories to use more rapid alternatives. Thus, latex agglutination test can also be done according to availability of the test kits for rapid identification. Several rapid tests are used commonly to make a presumptive identification of *S. aureus* including Staphaurex Plus kit. Many studies have evaluated the accuracy of latex agglutination test kit as rapid, reliable and easy detectors of *S. aureus* by comparing among commercially available test kits.¹¹⁻¹³ On literature search, similar data has not been found from our country, Nepal.

In our study, the tests were done on pure fresh culture isolates obtained routinely from culture of specimens received in diagnostic microbiology laboratory. All the isolates tested positive for tube coagulase, were confirmed as *S. aureus*. However, these test results were not available while performing the LAT. Latex agglutination test was found to be positive in 358 (95.46%) of isolates. Moreover, two isolates of Coagulase Negative *Staphylococci* tested negative by both tube coagulase and latex agglutination test. Therefore, considering tube coagulase test as gold standard, latex agglutination test performed to detect *S. aureus* was significant. Thus, the overall sensitivity and specificity of latex agglutination test for identification of *S. aureus* were 95.4% and 100% while positive predictive value and negative predictive value were 100%, 10.5% respectively. Sensitivity of latex agglutination test for MSSA strains was 97.1%, and for MRSA strain was 94.4% respectively. Negative predictive value could not be predicted well as we had only two isolates of Coagulase Negative *Staphylococci*.

Several studies in the past described the varying range of sensitivity and specificity by comparing latex agglutination

test kit from different manufacturers like the Dry Spot Staphytest Plus test (Oxoid), the Pastorex Staph Plus test (Bio-Rad), the Slidex Staph-Kit and Slidex Staph Plus test (bioMérieux), the Staphaurex Plus test (Remel) and the Staphylase Test (Oxoid). Our finding is lower than the report of Griethuysen et al. showing sensitivity of 96.7% to 100% and specificity of 95.1% to 100% for overall *S. aureus* identification. However, it is comparable to the study done by Weist et al. reporting sensitivity and specificity of 88.9% to 100% and 91.3% to 99.1%, Gupta et al. reporting sensitivity and specificity of 95.4% to 100% and 88.2% to 99.3% and Andriessse et al. reporting sensitivity and specificity of 95.2% to 99.2% and 98.8% to 100% respectively.¹²⁻¹⁵ Moreover, positive predictive value and negative predictive value determined were 97.8% to 99.8% and 66.9% to 100% from Germany, 99.4% to 100% and 91.3% to 98.4% from Netherlands.^{13,15} Similarly, study from Massachusetts done in 1982 documented positive predictive value of 100% and negative predictive value of 91% while, study from India in 2018 revealed positive predictive value and negative predictive value of 96.9% and 100% by testing with only one test kit.^{16,17} Likewise sensitivity and positive predictive value of latex agglutination test for methicillin sensitive *Staphylococcus aureus*, documented earlier were 96.1% to 100% and 98.8% to 100%.¹²⁻¹⁶ While MRSA considered alone as a parameter subjected for latex agglutination test, is variably sensitive (91.6% to 98.8%).^{12,15} Moreover, our finding is lower than the sensitivity of latex agglutination test as reported by previous study from Korea (97.9%) done in 1999 but comparable with another study done in 2010 reported sensitivity of 94.1%.^{18,19} Overall latex agglutination test was highly sensitive for the reason that in addition to clumping factor and protein A these assays also detect group-specific antigens and capsular polysaccharides.¹⁷⁻²⁰

The result of test done by Staphaurex Plus kit could not be compared with other similar test kit from other manufacturers.

CONCLUSION

Overall this study reveals that the latex agglutination test seems to be useful for identification of *Staphylococcus aureus*. Moreover, Latex agglutination test appears to be accurate, reliable, rapid and easy to perform test where *Staphylococcal* infection is very common.

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