Evaluation of different tests for detection of *Staphylococcus aureus* using coagulase (*coa*) gene PCR as the gold standard

HK Tiwari,[1] D Sapkota[1] and MR Sen[2]

Department of Microbiology, [1]Universal College of Medical Sciences, Bhairahawa, Nepal, [2]Institute of Medical Sciences, Banaras Hindu University, Varanasi, UP- 221005, India.

Corresponding author: Dr. Hare Krishna Tiwari, Associate Professor, Department of Microbiology, Universal College of Medical Sciences, Bhairahawa, Nepal, e-mail: hktiwari_2005@rediffmail.com

ABSTRACT

A total of 288 staphylococcal specimens isolated from different clinical specimens were selected for the evaluation of tests used to detect *Staphylococcus aureus*. The coagulase (*coa*) gene PCR was preformed, which confirmed 288 specimens as S. *aureus* and 51 specimens as coagulase negative staphylococci (CoNS). All the specimens were subjected to slide coagulase test, Slidex Staph plus test and tube coagulase test. Sensitivity, specificity, positive predictive value and negative predictive values of were calculated using *coa* gene PCR as gold standard for the detection of *S. aureus.* The tube coagulase test showed very good sensitivity (98.7%), specificity (98.1%), PPV (99.5%) and NPV (94.4%) than other methods. Slidex Staph plus test showed fairly good sensitivity and specificity. Slide coagulase test has good specificity but poor sensitivity. Therefore we recommend that tube coagulase test be done routinely for the detection of *S. aureus* in microbiology laboratory.

Keywords: *S. aureus*, MRSA, tube coagulase test, *coa* gene PCR.

INTRODUCTION

Staphylococcus aureus is a common aetiological agent in nosocomial and community infections, therefore exact identification of *S. aureus* isolates is essential for microbiology laboratories.[1] During recent years the proportion of infections due to *S. aureus* isolates resistant to methicillin (MRSA) has soared worldwide.[2] In comparison to methicillin sensitive *S. aureus* (MSSA), MRSA strains are highly pathogenic and cause high degree of morbidity and mortality in the affected patients.[3] Unlike coagulase negative staphylococci (CoNS), *S. aureus* secretes free plasma coagulase which is not only a virulence factor but also an important criterion for distinguishing it from CoNS. There are several standard methods like mannitol fermentation test, coagulase tests, agglutination test for discrimination of *S. aureus* from other staphylococci.[4] Other commercially available agglutination based tests are available which can promptly detect *S. aureus*.[5] However, these tests are not cost effective for clinical laboratory of developing countries. In countries like ours *S. aureus* is differentiated from CoNS mostly by slide coagulase test. Therefpre, tube coagulase test still remain a test of choice for *S. aureus* identification because of its high sensitivity and specificity.[6,7]

The present work evaluates tube coagulase test, slide coagulase test, and Slidex Staph plus test for *S. aureus* detection considering coagulase gene PCR as the reference method.

MATERIALS AND METHODS

Bacterial strains and its identification: This study was conducted at the Department of Microbiology and S.S. Hospital of the Institute of Medical Sciences, BHU during 2002 and 2005. A total of 288 staphylococcal strains isolated from pus, urine, blood, sputum, respiratory secretions, endotracheal tubes, catheter tips, and drain tubes of different outpatients and inpatients were included in the study. The specimens were inoculated onto Blood Agar, MacConkey agar, and CLED agar (for urine only) and incubated at 37^0C overnight. Staphylococci were identified by observing colony characteristics, cell morphology and arrangement, O/F test, and catalase test.[7] Mannitol fermentation test was done to further confirm *S. aureus.* Using growth on Blood Agar, all the strains were subjected to the following tests.

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Table-1: Performance of tube coagulase, slide coagulase, and Slidex Staph plus tests in discriminating 273 *S. aureus* strains and 51 coagulase negative staphylococcal strains

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42	+	+	+	-
15	+	+	-	-
5	+	+	-	+
2	+	-	+	-
1	+	-	-	+
3	-	-	+	+
1	-	+	-	+
47	-	-	-	-

A Biometra DNA thermocycler was programmed for the initial denaturation, 4 min at 94°C; 35 cycles with a 1 min denaturation step at 95°C, a 1 min annealing step at 54°C and a 1 min extension step at 72°C and 2 min extension step at 72 °C and a holding step at 4°C until the sample was analyzed. The PCR products were electrophoresed, stained with 10 ìM ethidium bromide and visualized by using UV transillumination.

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Quality Control: *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 14990 were used as positive and negative control respectively.

RESUTLS

Of the 288 staphylococcal strains, 237 were *coa* gene PCR positive with the PCR products of 1456, 1150 and 710 bp size (Fig. 1). Rest 51 strains were *coa* gene negative. The results obtained by subjecting 237 *S. aureus* and 51 CoNS strains to tube coagulase, slide coagulase, and Slidex Staph plus tests are depicted in Table-1.

Performance of different testing methods for detection of *S. aureus* were analyzed for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) considering *coa* PCR as gold standard. Tube coagulase test was found to be very good test to detect *S. aureus* with 98.7% sensitivity, 98.1% specificity, 99.5% PPV and 94.4% NPV followed by Slidex Staph plus and slide coagulase test (Table-2). Slide coagulase test has shown a good specificity but a very low sensitivity.

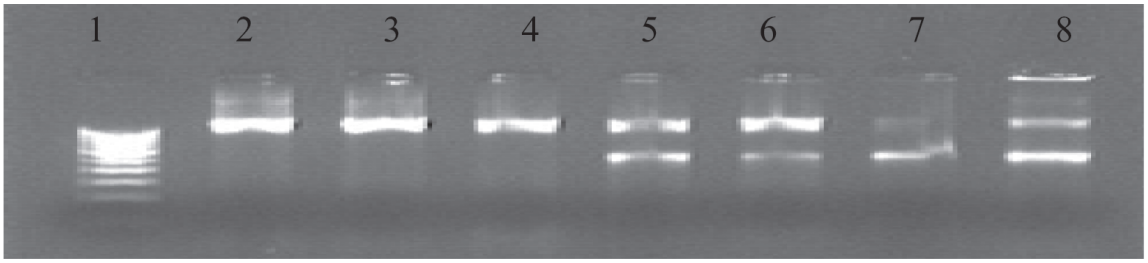
Table-2: Efficacy of tube coagulase, slide coagulase, and Slidex Staph plus tests to detect *S. aureus*

Tests	Sensitivity	Specificity	PPV	NPV
Tube Coagulase	98.73	98.04	99.57	94.44
Slide coagulase	75.10	92.16	97.80	44.33
Slidex Staph Plus	91.14	94.12	98.63	69.56

DISCUSSION

In current study evaluation of slide coagulase test, tube coagulase test and Slidex Staph Plus test was done considering the coagulase (*coa*) gene PCR as gold standard for the identification of *S. aureus*. Slide coagulase test showed low sensitivity by failing to detect 59 *S. aureus* strains. Slidex Staph Plus showed relatively good sensitivity and specificity; however, the test failed to detect 12 MRSA and 7 MSSA and gave 3 false positive results. Griethuysen *et al*[9] have reported similar findings with 98.2% sensitivity and 98.9% specificity of Slidex Staph Plus test. Tube coagulase has demonstrated the highest sensitivity (98.7%) and specificity (98.1%); it failed to identify only 3 *S. aureus* strains and reported only one CoNS as coagulase positive. Luijendijk *et al*[10] have evaluated free-coagulase test (Bacto coagulase plasma; Difco Laboratories, Detroit, Mich.), bound-coagulase test, and the Pastorex Staph plus (Sanofi Diagnostics Pasteur, SA, Marnes-La-Coquette, France) for the detection of *S. aureus*. They found 98.0% sensitivity with free-coagulase test and 99.0% with bound coagulase test and 100.0% with Pastorex Staph plus. Since geographical differences can correlate with antigenic variation of capsular polysaccharides and surface glycopolysaccharides of *S. aureus* and can therefore affect the outcome of an evaluation of an identification test for *S. aureus*, a study has been carried out in three different centers in three European countries.[11]

Current study therefore suggests that



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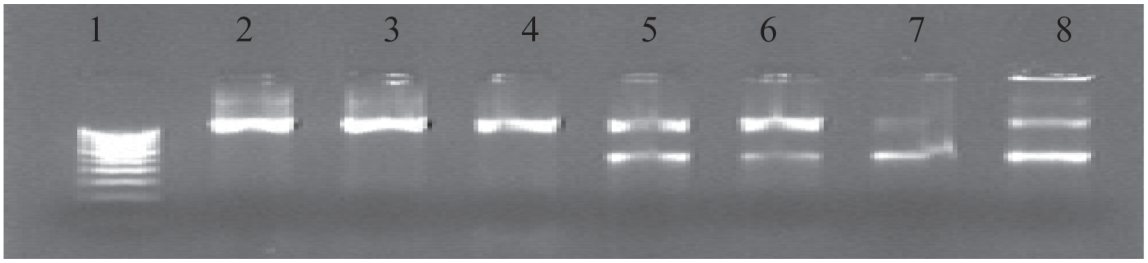

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Slide coagulase test: A test colony were mixed with a drop of saline and plasma and clumping of plasma were observed immediately.[4]

Latex agglutination test (Slidex Staph Plus): The test was performed according to the manual supplied by the manufacturer (Biomerurix India Ltd).

Quality Control: *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 14990 were used as positive and negative control respectively.

RESUTLS

Of the 288 staphylococcal strains, 237 were *coa* gene PCR positive with the PCR products of 1456, 1150 and 710 bp size (Fig. 1). Rest 51 strains were *coa* gene negative. The results obtained by subjecting 237 *S. aureus* and 51 CoNS strains to tube coagulase, slide coagulase, and Slidex Staph plus tests are depicted in Table-1.

Performance of different testing methods for detection of *S. aureus* were analyzed for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) considering *coa* PCR as gold standard. Tube coagulase test was found to be very good test to detect *S. aureus* with 98.7% sensitivity, 98.1% specificity, 99.5% PPV and 94.4% NPV followed by Slidex Staph plus and slide coagulase test (Table-2). Slide coagulase test has shown a good specificity but a very low sensitivity.

Table-2: Efficacy of tube coagulase, slide coagulase, and Slidex Staph plus tests to detect *S. aureus*

Tests	Sensitivity	Specificity	PPV	NPV
Tube Coagulase	98.73	98.04	99.57	94.44
Slide coagulase	75.10	92.16	97.80	44.33
Slidex Staph Plus	91.14	94.12	98.63	69.56

DISCUSSION

In current study evaluation of slide coagulase test, tube coagulase test and Slidex Staph Plus test was done considering the coagulase (*coa*) gene PCR as gold standard for the identification of *S. aureus*. Slide coagulase test showed low sensitivity by failing to detect 59 *S. aureus* strains. Slidex Staph Plus showed relatively good sensitivity and specificity; however, the test failed to detect 12 MRSA and 7 MSSA and gave 3 false positive results. Griethuysen *et al*[9] have reported similar findings with 98.2% sensitivity and 98.9% specificity of Slidex Staph Plus test. Tube coagulase has demonstrated the highest sensitivity (98.7%) and specificity (98.1%); it failed to identify only 3 *S. aureus* strains and reported only one CoNS as coagulase positive. Luijendijk *et al*[10] have evaluated free-coagulase test (Bacto coagulase plasma; Difco Laboratories, Detroit, Mich.), bound-coagulase test, and the Pastorex Staph plus (Sanofi Diagnostics Pasteur, SA, Marnes-La-Coquette, France) for the detection of *S. aureus*. They found 98.0% sensitivity with free-coagulase test and 99.0% with bound coagulase test and 100.0% with Pastorex Staph plus. Since geographical differences can correlate with antigenic variation of capsular polysaccharides and surface glycopolysaccharides of *S. aureus* and can therefore affect the outcome of an evaluation of an identification test for *S. aureus*, a study has been carried out in three different centers in three European countries.[11]

Current study therefore suggests that

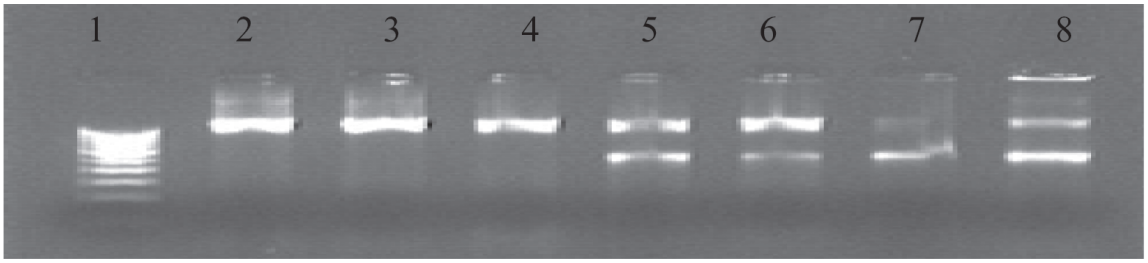

Fig. 1. Detection of the coa gene by PCR. Lane 3-8 different test strains showing coa positive PCR bands. Lane 2 coa PCR positive control. Lane 1 has 1000 kbp DNA ladder)

tube coagulase test is superior to not only slide coagulase test but also Slidex Staph plus test. Although tube coagulase test provides results only after 4-24 hr and is little cumbersome while Slidex Staph plus test is rapid and easy to perform, this disadvantage of tube coagulase is certainly outstripped by its better efficacy. As for slide coagulase, it should always be complimented by tube coagulase test. We therefore recommend that tube coagulase test be performed on regular basis in routine clinical microbiology laboratory so that we can correctly differentiate *S. aureus* from CoNS.

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