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Evaluation of Pastorex Staph Plus Rapid Agglutination Test to Confirm *Staphylococcus aureus* Isolated from Clinical Specimens in a Tertiary Care Teaching Hospital

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Authors' contributions

This work was carried out in collaboration between all authors. Author DD designed the study, wrote the protocol and wrote the first draft of the manuscript. Author PG managed data analyses of the study author AC managed the literature searches and wrote the final draft of the manuscript. Author KKH has guided during the whole study. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Staphylococcus aureus* is one of the most common pathogen isolated. Accurate and rapid identification of *S. aureus* and MRSA is of great importance for proper care of the patient. Keeping this in mind, we had used Pastorex Staph plus rapid agglutination test to confirm *S. aureus*.
Aim: To evaluate Pastorex Staph plus rapid agglutination test for confirming *Staphylococcus aureus* isolated from clinical specimens.
Study Design: Cross-sectional type.
Methodology: This study was conducted in a tertiary care hospital in Eastern India. It included all clinical samples, where *Staphylococcus* spp. was isolated. The phenotypic confirmation of the

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isolate was done by slide coagulase test, tube coagulase test, mannitol fermentation test and Pastorex Staph plus rapid agglutination test. Methicillin Resistant *Staphylococcus aureus* (MRSA) strains were identified as per CLSI 2017 guideline. Statistical analyses of the results were done to evaluate the validity, diagnostic accuracy & reliability of the tests. Tube coagulase test was taken as the gold standard test.

Results: Among the total 83 *Staphylococcus* spp. isolates, 31 isolates (37%) were *Staphylococcus aureus*. Among these *S. aureus* isolates, only 1(3.2%) isolate was MRSA. Statistical analysis showed that, in identification of *Staphylococcus aureus*, Pastorex Staph plus test had highest sensitivity (100%), specificity (98%) and diagnostic accuracy (98.4%) with Cohen's Kappa coefficient as 0.97.

Conclusion: Considering the sensitivity, specificity, accuracy and Cohen's kappa coefficient, it was concluded that Pastorex Staph plus rapid agglutination test can be used as rapid and effective method to diagnose *S. aureus*.

Keywords: Pastorex Staph plus; S. aureus; Coagulase Negative Staphylococcus; tube coagulase; mannitol fermentation.

1. INTRODUCTION

Staphylococcus aureus is one of the most common pathogenic bacteria isolated from clinical specimens. It has long been recognized as an important pathogen in human disease [1]. Moreover, Methicillin resistant *Staphylococcus aureus* (MRSA) is endemic in India and is a dangerous pathogen for hospital acquired infections [2].

The identification and detection of *S. aureus* or MRSA strains in laboratory, by either conventional or molecular methods, are quite troublesome because both methods have their own advantages and disadvantages depending on the equipment and expertise available in the laboratory setting. [3] usually, misidentification of *Staphylococcus aureus* as coagulase-negative *Staphylococcus* (CoNS) can result in a costly search for other pathogens or unwarranted broad-spectrum empiric antimicrobial coverage [4].

In developing countries, confirmation of *Staphylococcus aureus* strains is done routinely by slide coagulase test, tube coagulase test and mannitol fermentation test. It was reported that 10% *Staphylococcus* strains were misidentified by conventional method [5]. Unfortunately, in our country, many laboratories identify *Staphylococcus aureus* only by slide coagulase test and the results of which are frequently erroneous thereby resulting in misidentification of other *Staphylococcus* spp. as *Staphylococcus aureus*. Other laboratories rely routinely on the tube coagulase test because it is considered a reference method and is highly sensitive [6]. However, its effectiveness is influenced by several factors, like source and lot-to-lot variation of plasma, incubation time, degree of clotting, reversal of positive results to negative, length of time to perform the test (4-24 hours), etc. These problems, together with the relative delay (24 hours) in the availability of definitive results, had led to the requirement of rapid reliable methods. Several commercial kits are being introduced for the rapid (within 1 minute) identification of *S. aureus* in primary culture. These kits usually are based on the coating of latex particles or sheep erythrocytes with fibrinogen to detect clumping factor alone, the use of immunoglobulin G (IgG) to detect protein, a combination of both methods, or a combination of the two together with use of monoclonal anti-bodies to detect surface immunogens or capsular polysaccharide [7,8,9]. Bio-Rad Pastorex ™ Staph Plus (code 56356 from Bio Rad) test is one of such rapid latex agglutination tests. The complete kit includes control, disposable cards and sticks [10].

Keeping all these points in mind, the present study was conducted to evaluate Pastorex Staph plus rapid agglutination test for confirming *Staphylococcus aureus* isolated from clinical specimens. The main objectives of our study were: (1) To evaluate the validity of Pastorex Staph plus rapid agglutination test, slide coagulase test and mannitol fermentation test taking tube coagulase test as the gold standard test; (2) To compare the diagnostic accuracy of Pastorex Staph plus rapid agglutination test with the conventional identification techniques like slide coagulase and mannitol fermentation tests to identify *Staphylococcus aureus;* (3) To know whether Pastorex Staph plus rapid agglutination test can be used as a rapid reliable diagnostic test for identification of *Staphylococcus aureus*.

2. MATERIALS AND METHODS

2.1 Study Design

This cross-sectional type of study was carried out in the Microbiology Laboratory of a tertiary care teaching hospital in Eastern India in the year 2017. All the Gram positive, catalase positive cocci occurring in pairs, short chains or clusters isolated from routine clinical specimens were subjected to slide coagulase test, tube coagulase test , mannitol fermentation test and Pastorex Staph plus rapid agglutination test to differentiate *Staphylococcus aureus* from other *Staphylococcus* spp.

2.2 Procedure of the Tests

The colonies were processed for slide coagulase test, tube coagulase test and mannitol fermentation test as per conventional microbiological techniques [11]. Tube coagulase test was considered confirmatory test for *S. aureus* in our study. Hence, isolates showing clot formation at 1 hour, 2 hour, 4hour or 24 hour were considered as *S. aureus* [11]. *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228 were used as controls.

All the *Staphylococcus* isolates were screened for methicillin resistance using Cefoxitin disc (30 µg) in Mueller Hinton Agar as per standard Kirby Bauer disc diffusion method and the reading was taken after 18-24 hours of aerobic incubation at 35ºC. *S. aureus* isolates showing zone of inhibition ≤ 21 mm and CoNS isolates showing zone of inhibition ≤ 24 mm, were interpreted as MRSA and MRCoNS strains respectively. This method was according to CLSI 2017 guideline. [12] *S. aureus* ATCC 25923 and *S. aureus* ATCC 43300 were used as mecA negative and mecA positive controls respectively.

2.2.1 Pastorex staph plus rapid agglutination test [10]

The Pastorex Staph-plus (Bio-Rad, France) was used. The procedure was done as per the instructions given in the kit insert. A few colonies of the isolate were placed into a marked black circle on the Pastorex Staph-plus reaction card. A drop of the latex reagent was added inside circle of the card and colonies inside were thoroughly mixed with a wooden applicator stick. The card was rotated and examined for 20 seconds. A positive reaction was defined as clumping of the latex particles with substantial clearing of the milky background. A negative reaction was defined as no clumping of latex particles that occurred without substantial clearing of the milky background.

2.3 Statistical Analysis

Collected data were compiled and recorded on Microsoft excel sheet (Microsoft, Redwoods, WA, USA). Validity of the tests like slide coagulase test, mannitol fermentation test and Pastorex Staph plus agglutination test were expressed by sensitivity and specificity by taking tube coagulase test as gold standard [5]. Kappa co-efficient was also computed, using online GraphPad software, to see up to what extent the reading of two different methods agreed beyond which we would expect by chance alone [13,14].

3. RESULTS

In our study, a total of 83 *Staphylococcus* spp. were isolated from 71 patients, as few patients had several samples growing *S.aureus*. 63% of our patients were male while 37% were females (Fig. 1). In our study most common age group was 40 - 49 years followed by 30-39 years (Table 1). Pus was the most common sample from where *Staphylococcus* species were isolated (55%) (Fig. 2) Distribution of specimens is depicted in Fig. 2. Among 83 isolates of *Staphylococcus* spp., 31 isolates (37%) were *Staphylococcus aureus* while 52 isolates (63%) were Coagulase Negative *Staphylococcus* (CoNS) (Fig. 3). A comparative analysis for identification of *Staphylococcus* spp. by slide coagulase test, Pastorex Staph plus agglutination test and mannitol fermentation test are shown in Table 2 & Table 3. Slide coagulase, Pastorex Staph plus and Mannitol fermentation tests showed comparable sensitivity but differed in specificity. Among these three tests, Pastorex Staph plus showed highest sensitivity (100%) and specificity (98%). Sensitivity of slide coagulase test and mannitol fermentation test was same (96.8%). However Specificity of mannitol fermentation test was better than slide coagulase test. All the three tests showed good agreement and Pastorex Staph plus rapid agglutination test showed almost perfect agreement. Moreover diagnostic accuracy of Pastorex Staph plus rapid agglutination was found to be very good (98.4%) (Table 3).

Among the *S. aureus* isolates, only 1(3.2%) isolate was found to be MRSA. In contrast to this, most of the Coagulase Negative *Staphylococcus* isolates (61.5%) were methicillin resistant (Table 4).

Table 1. Age wise distribution of patients

Age (Years)	No. of patients	Percentage (%)
<10	2	2.8
10-19	4	5.6
20-29	6	8.5
30-39	20	28.2
40-49	23	32.4
50-59	10	14
>59	6	8.5
Total	71	100

Table 2. Finding wise distribution of the tests

Test	Positive	Negative
Slide coagulase	40	43
Pastorex staph plus	32	51
Mannitol fermentation	35	48

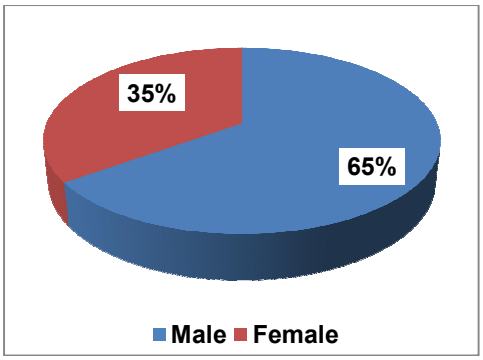

Fig. 1. Gender wise distribution of patients

4. DISCUSSION

S. aureus is responsible for a variety of clinical conditions including superficial skin and soft tissue infection, osteomyelitis, arthritis, central lines related bacteremia and infective endocarditis, etc. with high morbidity and mortality [15]. They occur frequently in hospitalized patients and have severe consequences, despite giving antibiotic therapy. Due to an increasing number of infections caused by the methicillin-resistant *S. aureus* strains, which are now most often multi-drug-resistant, the therapy has become problematic [1]. In spite of so much importance of *S. aureus*, especially the methicillin resistant *S. aureus* strains, as a significant cause of nosocomial infections, enough attention is not paid to its diagnosis. The identification of *S. aureus* in most routine laboratories is, at best, treated casually, without due consideration [5]. In a study by David P K et al., they stated that the ideal identification of *Staphylococcus aureus* from clinical isolates requires a battery of tests and this is costly in resource limited settings [16]. In many developing countries, the tube coagulase test is usually considered confirmatory for *S. aureus* and is routinely done using either human or sheep plasma. But, single phenotypic test cannot be relied upon solely to identify *S. aureus*, rather a combination of phenotypic tests are needed for accurate identification of *S. aureus* [17]. Moreover, tube coagulase is a time consuming test and may take 4 to 24 hours, thereby increasing the duration of turn-around time. Hence, it won't be wrong to state that rapid and accurate identification of *S. aureus* isolates is of utmost importance for timely management of various infections. Unfortunately, in developing country like India rapid latex agglutination tests are not routinely used in laboratory for rapid identification of *S. aureus* isolates.

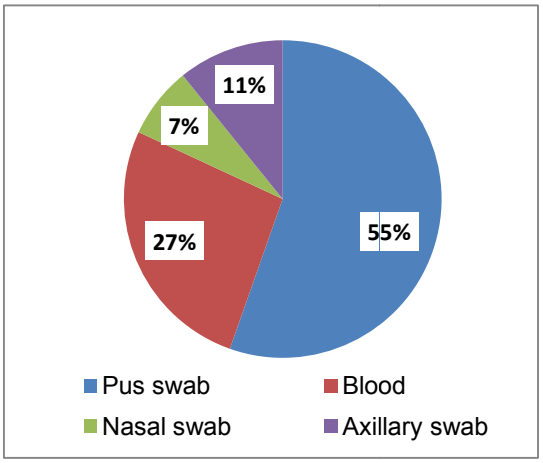

Fig. 2. Distribution of specimens

Table 3. Comparison of three tests for identification of *S. aureus*

Test	Sensitivity (%)	Specificity (%)	PPV# (%)	NPV## (%)	Accuracy	Cohen's kappa coefficient
Slide coagulase	96.8	80.8	75	97.7	86.8	0.73
Pastorex Staph plus	100	98	96.9	100	98.4	0.97
Mannitol fermentation Test	96.8	90.3	85.7	97.9	92.8	0.85

 # Positive predictive value; ## Negative predictive value

Table 4. Methicillin resistant and sensitive profile of the isolates

Isolate	Methicillin resistant	Methicillin sensitive
S. aureus	1(3.2%)	30(96.8%)
CoNS	32(61.5%)	25(38.5%)

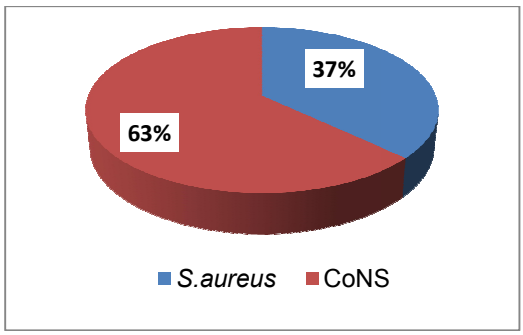

Fig. 3. Species wise distribution of *Staphylococcus*

Taking into consideration all these aspects, in the present study, we had used Pastorex Staph plus, a rapid latex agglutination test, for identification of *Staphylococcus aureus*. This kit was designed to allow simultaneous detection of three components, namely, the fibrinogen affinity factor (also referred to as bound coagulase or "clumping factor"), protein A (which possesses an affinity for the crystallisable fragment (Fc) of IgG immunoglobulins) and capsular polysaccharides of *Staphylococcus aureus*. The reagent in this kit is made of latex particles sensitised by fibrinogen and IgG as well as specific monoclonal antibodies raised against capsular polysaccharides of *Staphylococcus aureus*. The combination of fibrinogen, IgG and anti-capsular monoclonal antibodies in the same reagent allows the recognition of highly encapsulated strains of Staphylococcus aureus as well as poorly encapsulated strains. For highly encapsulated strains, anti-capsular polysaccharide antibodies agglutinate the bacteria. For strains that have lost their polysaccharide capsule, the bacteria are agglutinated by fibrinogen and IgG [10]. However, geographical differences can correlate with antigenic variation of capsular polysaccharides and surface glyco-polysaccharides of *S. aureus* and can therefore affect the outcome of an evaluation of and identification test for *S. aureus* [18].

Similar to a study done by Bello C.S.S et al. [5], we had also considered tube coagulase test as gold standard and evaluated the role of Pastorex Staph plus agglutination test for confirmation of *Staphylococcus aureus* as in India rapid agglutination tests are not routinely used for confirmation of *Staphylococcus aureus*. In the present study, 83 *Staphylococcus* isolates were subjected to slide coagulase test, tube coagulase test, mannitol fermentation test and Pastorex Staph plus agglutination test. In our study, isolation of *Staphylococcus* spp. was most common in age group of 40 - 49 years followed by 30-39 years. Unlike our study, Bhatt et al. showed higher isolation of *Staphylococcus aureus* in paediatric patients of 0-10 year age group (24%) [19].

The slide coagulase test is based on the characteristic presence of bound coagulase i.e. clumping factor in *S. aureus*. However, a high rate of false negatives (about 10-15%) has been reported by this test [20,21]. In contrast to this, in our study we observed a high rate of false positives (12%). Ten Coagulase Negative *Staphylococcus* isolates were wrongly identified as *S. aureus* by slide coagulase test. In our study, we observed good sensitivity (96.8%) for slide coagulase test but specificity was low (80.8%). In contrast to our study, in a study in Nepal by Tiwari H K et al., specificity (92.1%) observed was better than sensitivity (75%) for slide coagulase test. They also recommended that tube coagulase test should be performed on regular basis in routine clinical microbiology laboratory so that we can correctly differentiate *S. aureus* from CoNS [22]. Both sensitivity and specificity of slide coagulase test were lower than that of Pastorex Staph plus agglutination test, thereby making the latter a more reliable test.

In the present study, Pastorex Staph plus rapid agglutination test showed very good sensitivity (100%) and specificity (98%). Similar to our study, few other studies had shown that Pastorex Staph plus agglutination yields very good results. As per a study in Berlin, by Weist K et al., on evaluation of six agglutination tests for identification of *Staphylococcus aureus*, it showed that Pastorex Staph plus agglutination test had very good sensitivity (98.7%) and specificity 98.0%) [23]. Luijendijk et al. had evaluated free-coagulase test (Bacto coagulase plasma; Difco Laboratories, Detroit, Mich.), bound coagulase test and the Pastorex Staph plus (Sanofi, 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 [24]. In

the study done by Bello C.S.S et al. they also found that Pastorex Staph plus agglutination test had good sensitivity (96%) and specificity (98%) in identification of *Staphylococcus aureus* [5]. Another study demonstrated very good sensitivity (98.2%) and specificity (98.8%) for Pastorex Staph plus agglutination test while evaluating role of a fourth generation latex agglutination kit for the identification of *Staphylococcus aureus* [25].

As far as kappa agreement is concerned, Landis and Koch suggested that, kappa agreement greater than 0.75 represents excellent agreement; a kappa below 0.40 represents poor agreement and a kappa of 0.40 to 0.75 represent intermediate to good agreement [13]. In our study, kappa of slide coagulase test was 0.73, thereby representing intermediate to good agreement, while kappa of Pastorex Staph plus (0.97) and Mannitol Fermentation test (0.85) represented excellent agreement.

Subramanian A et al. reported that the sensitivity and specificity for Mannitol Fermentation test, in identification of *S. aureus*, was 97.86% and 100% respectively [26]. In contrast to their finding, our study showed that Mannitol Fermentation test has lower specificity (90.3%) than sensitivity (96.8%). Even though, mannitol fermentation test had an excellent kappa agreement and showed somewhat similar sensitivity and specificity with Pastorex Staph plus agglutination test, it must be kept in mind that its turn-around time is much higher; thereby making the latter a more rapid test.

In our study, we observed that prevalence of MRSA was low (3.2%) compared to prevalence of Methicillin resistant Coagulase negative *Staphylococcus* (61.5%). Unlike to our study, Bhatt et al. reported 19% cases of Methicillin resistant Staphylococcus aureus (MRSA) [19]. Correct identification of MRSA is essential for appropriate treatment strategies and sufficient infection control measures for prevention of nosocomial infection. Therefore it is very important to identify *Staphylococcus* spp. as wrong identification of CoNS as *S. aureus* may lead to unnecessary use of antibiotics.

Thus, we find that slide coagulase test does not give confident results and hence should be used supplemented with other tests like tube coagulase test, mannitol fermentation test and rapid agglutination test for proper identification of *S. aureus*. However, the turn-around time for tube coagulase test and mannitol fermentation test is much higher than rapid agglutination test (like Pastorex Staph plus agglutination test). Hence, in the field of medical science where every single moment is crucial for treating a patient, rapid and accurate agglutination test like Pastorex Staph plus agglutination test can be adopted for identification of *S. aureus.*

5. LIMITATIONS

We had not done genotypic methods to confirm identification of *S. aureus* isolates and MRSA strains due to cost constrains.

6. CONCLUSION

Pastorex Staph plus rapid agglutination test showed highest sensitivity (100%) and specificity (98%) when compared with slide coagulase test & mannitol fermentation test, taking tube coagulase test as the gold standard. Slide coagulase test should not be used as sole criterion for identification of *S. aureus* as it has high false positive rate. As far as diagnostic accuracy is concerned, Pastorex Staph plus rapid agglutination had highest diagnostic accuracy of 98.4%. Practice of doing only slide coagulase test for identification of *S. aureus* should be abolished in microbiology laboratories in our country and it should always be supplemented with tube coagulase test and mannitol fermentation test. Tube coagulase test is still the gold standard test for confirming *S. aureus* in developing country like India due to low cost of the test but it takes 4 hours to 24 hours for confirmation. Therefore, taking into account the sensitivity, specificity, accuracy, Cohen's kappa coefficient and turn-around time, Pastorex Staph plus rapid agglutination test can be used as rapid and effective method to diagnose *S. aureus* avoiding the problems of false positive result of CoNS and to reduce the turn-around time to initiate therapy.

CONSENT

As per international standard or university standard written patient consent has been collected and preserved by the authors.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the institutional ethics committee and have therefore been performed in accordance with the ethical

standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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