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SENSITIVTY OF DIFFERENT PHENOTYPIC TESTS USED FOR DETECTION OF STAPHYLOCOCCUS AUREUS IN COAGULASE TEST

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ABSTRACT

Objective: To evaluate performance (sensitivity & specificity) of a range of phenotypic tests which are currently used in tertiary care hospitals for the indentification of S aureus and to identify an optimal phenotypic test that is reliagle and cost effective and can be used with confidence for confirmation of S. aureus.

Material and Methods: The present study was conducted at clinical microbiology laboratory department of Pathology of Northwest General Hospital and Research Centre, Peshawar from January 2012 to September 2013. The study group consisted of 300 samples were collected from different clinical sources i.e. patient's blood, body fluids, pus swabs, wound swabs, urine and sputum. During sampling safety methods were adopted and data were collect regarding age, sex, types of specimens and present health condition were also recorded.

Results: All clinical samples evaluated with slide coagulase (SCT), mannitol salt fermented test (MSA) and DNase test the result were 95%, 87% and 86%, respectively. Combination of these tests with different sera used in SCT their sensitivity results increased considerably. The human plasma when used in SCT test the positive results were 95%, but when used in combination with MSA test and DNase test then sensitivity increased to 98% and 96%, respectively. Similarly, while using horse plasma, 90% were slide coagulase positive and combination of SCT with mannitol test and DNase test depicted 94% and 91% positive results respectively. In similar manner, when cow plasma was used, slide Coagulase test showed 85% sensitivity and in combination with mannitol fermentation test and DNase test depicted 87% and 86% sensitivity, respectively.

Conclusion: It is concluded that there is no single phenotypic test that can give 100% reliable identification of S. aureus and we have to use combination of tests for maximum reliable results. The combination of slide coagulase test with mannitol fermentation test provide the maximum reliable results while using human plasma followed by using horse and cow plasma.

Key Words: Staphylococcus aureus, Phenotypic test, Human Plasma, Horse and Cow Plasma.

INTRODUCTION

Staphylococcus aureus (S. aureus) is a common bacterial pathogen responsible for causing nosocomial and community acquired infections. Identification of S. aureus is done by performing different biochemical tests i.e. catalase test, coagulase test, mannitol fermentation test and DNase test. None of these have confirmatory status and have different sensitivity level. Staphylococcus aureus (S. aureus) is a Gram-positive coccus shaped facultative anaerobe and a member of the Staphylococcaceae family. In Greek, Staphylococcus means "cluster of grape". S. aureus is the most important species of Staphylococci[1]. S. aureus is not always pathogenic; however, some serotypes are responsible for causing severe human diseases i.e. bacteremia, pneumonia, cerebrospinal meningitis, osteomyelitis endocarditis empyema toxic shock syndrome, urinary tract infections and nosocomial infections[2]. Pathogenic strains of S. aureus produce toxins and cell surface proteins that suppress immune system. S. aureus is an important community Gram-positive pathogenic bacterium, which is increasingly resistant to most commonly used antibiotics. Due to prophylactic long courses, high cost and inappropriate uses of antibiotics may lead to increased resistance to antibiotics and incidence of drug reaction[3].

Coagulase test is used routinely to identify and differentiate S. aureus from other Staphylococci. S. aureus produce coagulase enzyme which can be detected using slide coagulase test (SCT) and tube Coagulase test (TCT). Tube coagulase is a time consuming and may take 4 to 24 hours. A coagulase test (clot fibrin) determines pathogenic from non-pathogenic Staph-

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ylococci. S. aureus produce two forms of coagulase (i.e., bound coagulase and free coagulase) (Bello and Qahtani, 2006). With the exception of public and private sector clinical laboratories in Pakistan, majority of the laboratory rely on slide coagulase test for differentiation of S. aureus from coagulase negative Staphylococci. Although coagulase test is invaluable for identification of S. aureus but a 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.

S. aureus produce an enzyme called deoxyribonuclease enzyme which can use DNA as a source of carbon and energy for growth. A medium containing DNA and indicator is used. The DNase test was found least sensitive for identification of S. aureus as compared to coagulase and MSA tests (Lagacé-Wiens et al., 2007). Several commercially available kits were used for the rapid identification of S. aureus. About 10 to 15% of S. aureus may yield a negative result with the slide coagulase test and 2 to 5% yield a negative with the tube coagulase test[4,5].

True S. aureus identification is a routinely diagnostic problem in over clinical care centre. Molecular biological diagnostic methods such as nucleic acid detection or protein detection may solve most of the problems related to microbiological procedure and give quick and rapid identification of bacteria[6]. Most of the pathogenic microorganisms are rapidly identified through DNA identification methods that provide definitive identification of pathogenic microorganisms. Polymerase Chain Reaction (PCR) is a molecular biology analytical technique, which helps to identify Staphylococci at the species level, using different target sites of the DNA[7,8]. Due to high cost of molecular diagnosis in Pakistan, it is preferred to use optimal phenotypic test. Nucleic acid detection or protein detection is 10-20 times more expensive than optimal phenotypic test. The latter is cost effective and easily available in diagnostic clinical care centres.

This complex situation clearly demands for in depth knowledge of phenotypic tests routinely used for detection of S. aureus. In same connection the present study was designed to check the overall performance of phenotypic tests compare with molecular test used for true identification of S. aureus.

MATERIAL AND METHODS

The study was conducted at clinical microbiology Department of Pathology of Northwest General Hospital & Research Centre, Peshawar, KPK, Pakistan from January 2012 to September 2013. Samples were collected from different clinical sources i.e. patient's blood, body fluids, pus swabs, wound swabs, urine and sputum. Three hundred samples collected from tertiary care hospitals of KPK i.e. North West General Hospital & Research Centre (NWGH), Rehman Medical Institute (RMI), Khyber Teaching Hospital (KTH), Lady Reading Hospital (LRH), Hayatabad Medical Complex (HMC) and City Medical Centre, Peshawar. Data regarding age, sex, types of specimens and present health condition were also recorded.

After collections, blood culture isolates were processed in Bactec B12 media and within five (5) days, positive blood culture was subculture on Blood Agar (CM55 and SR50-OXOID) and Chocolate Agar (CM55 and SR50-OXOID) and then incubated at 37°C for 24 hours. Other clinical isolates (pus, wound swab, sputum, urine) were cultured on Blood Agar base (CM55 and SR50-OXOID), and Chocolate Agar Base (CM55 and SR50-OXOID), and then incubated at 37°C for 24 hours in order to get sufficient growth on plates and then these colonies were sub cultured to get purity plates to identify Staphylococci species in the clinical samples.

Ten suspected colonies were collected, each from blood agar and chocolate agar and then purified, stored and subsequence analysis for further identification. All the clinical samples were first processed to get pure culture of S. aureus, using selective media such as MSA agar, DNase agar and blood agar. All isolates were identified on the basis of morphological (Gram Staining), biochemical (catalase test, coagulase test, DNase test and mannitol fermentation test).

RESULTS

This study evaluated the performance of commonly used phenotypic tests to identify Staphylococcus aureus. These tests included slide coagulase test (SCT), deoxyribonuclease (DNase) test and mannitol salt agar (MSA) tests. A total of 300 previously characterized clinical isolates of S. aureus were studied.

Coagulase test is commonly used in clinical laboratories to differentiate Staphylococci into coagulase

Table 1: Samples taken from different Tertiary Care Centre

Care Centre	Pus	W. Swab	Blood	Fluid	Sputum	Urine	Total samples
Northwest Hospital	92	45	10	04	07	03	161
Rehman Medical Institute	07	03	04	05	01	01	21
Hayatabad Medical Complex	13	09	02	05	00	01	30
Khyber Teaching Hospital	11	04	02	02	01	00	20
Leady Reading Hospital	09	06	03	04	02	00	24
City Medical Centre	20	16	03	04	00	01	44
Total samples	164	95	27	24	15	07	300

Table 2: Comparison of human, horse and cow plasma (in percentage); used in Coagulase phenotypic tests for identification of S. aureus

S. No.	Plasma with positive (%)
1	Human (95%)
2	Horse (90%)
3	Cow (85%)

Table.3: Identification of S. aureus isolates using human plasma by various phenotypic markers and their positivity rate

S. No	Test	Total samples	Positive (%)
1	SCT*	300	285 (95%)
2	MSA†	285***	210 (70%)
3	DNase‡	285***	180 (60%)

*** Only slide coagulase positive samples were evaluated for MSA fermentation &DNase production

Key: SCT* = Slide coagulase test

MSA† = Mannitol salt agar test

DNase‡ = Deoxyribonuclease test

Table. 4: Detail of clinical samples with positive ratio (in percentage) for Identification of S. aureus using Horse plasma

S. No.	Test	Total samples	Positive (%)
1	SCT*	300	270 (90%)
2	MSA†	270***	189 (70%)
3	DNase‡	270***	162 (60%)

*** Only slide coagulase positive samples were subjected for MSA &DNase

Key: SCT* = Slide coagulase test

MSA† = Mannitol salt agar test

DNase‡ = Deoxyribonuclease test

positive and coagulase negative Staphylococci. In the present study, the 300 clinically reported S. aureus isolates were investigated with plasma obtained from human, horse and cow to compare the sensitivity of plasma from different sources and determine its usefulness in clinical laboratories. The details are given in Table 2, 3 and 4 respectively.

In slide coagulase test, 285 (95%) isolates were positive by the slide coagulase test. Amongst these, 288 (96%) isolates were tube coagulase positive. Twelve (04%) isolates were tube coagulase negative after incubation at 4 and 24 hours as shown in Table 3 and 4 respectively.

Similarly these 300 isolates were evaluated with cow plasma. Amongst these, 255 isolates (85%) were slide coagulase positive. The remaining 45 isolates (15%) produced a negative slide coagulase reaction. Amongst 300 clinically S. aureus isolates; human plasma gave 95%, horse plasma showed 90% and cow plasma gave 85% sensitivity.

Coagulase positive isolates with human plasma were sub-cultured on to Mannitol Salt agar plates. Out of 285 isolates, only 210 (70%) were able to ferment mannitol. Using horse plasma, out of the 270 isolates, 189 (70%) was mannitolfermentor. Mannitol salt agar using cow plasma, identified 178 (70%) positive clinical isolates out of 255 isolates (Table 4.2, 4. and 4.4 respectively). Similarly by using human plasma; out of 285 coagulase positive isolates, only 180 (60%) samples show DNase activity. Out of the 270 isolates, 162 (60%) samples were DNase test positive with horse plasma. DNase test using cow plasma identified 153 (60%) positive clinical isolates out of 255 isolates.

Identification of S. aureus was improved by combination of various phenotypic tests. The sensitivity for the identification increased when coagulase was combined with DNase and/or MSA tests. Combination of MSA and coagulase test (human plasma) shows 98% sensitivity, and combination of DNase and coagulase test (human plasma) showed 96% sensitivity. The sensitivity of the combination of MSA and coagulase test using horse plasma was 94%. The sensitivity of DNase and coagulase test using horse plasma was 91%. The sensitivity, in case of combinations of MSA with coagulase test using horse and cow plasma, DNase with coagulase test using horse and cow plasma was 87% and 86% respectively. The best combination for the identification of S. aureus was MSA test with DNase test and coagulase test using human plasma, which gave optimum results.

DISCUSSION

Staphylococcus aureus (S. aureus) is amongst the transient flora of human skin and anterior nares[9]. The organism is responsible for a variety of clinical conditions including superficial skin and soft tissue infection, osteomylitis, arthritis, central lines related bacteraemia and infective endocarditis with high morbidity and mortality[10]. Hence, rapid and accurate identification of S. aureus is of paramount importance for timely and effective management of such infections.

Due to a high risk of blood borne viral infection; human plasma is not routinely recommended for coagulase test[11,12]. The results of the present study show that; coagulase test using human plasma gave excellent results as compared to the literature reported. So it is recommend human plasma to be used in coagulase tests; however safety laboratory measures should be taken as human plasma may contain blood borne viruses. McDonald and Chapin, observed that human plasma gave good result in tube coagulase test[13] Konemann, reported that human plasma didn't give good results always, because human plasma contains coagulase reacting factor and anti-Staphylococcal antibodies[14]. Lagace Wiens et al reported variation in results; using plasma from different sources. Efficiency of plasma varies with setting and performance of plasma can

influence the efficiency of slide coagulase tests and tube coagulase test[15]. Marcos JY et al also investigated that citrate or EDTA anticoagulant plasma affects the performance of tests[16]. Citrated plasma only affects the performance of coagulase reaction of Enterococci but does not affect the performance of coagulase producing organisms such as S. aureus. This study obtained best results for human plasma using citrate as anticoagulant.

The plasma of different species i.e. rabbit, bovine, pig, human etc were used in coagulase reaction for the identification of S. aureus. It was noted that the mixture of pig and rabbit plasma improve the reliability as compared than either plasma[17]. Human plasma shows more sensitive than sheep plasma for the tube coagulase test (91% and 81%, respectively) but both had low specificity (11% and 7%, respectively). The specificity and sensitivity of tube coagulase test (human plasma) was improved when DNase and mannitol salt agar test were introduced as a tri combination test for identification of S. aureus (75% specificity and 100% sensitivity), while introducing the sheep plasma in the tri combination test, the sensitivity and specificity was 67% and 100%, respectively. Human plasma was more sensitive as compared to horse and cow plasma (sensitivity of 95%, 90%, and 85%, respectively). Thus in this setting, it is unlikely that horse plasma and cow plasma will replace human plasma for routinely used coagulase tests, because human plasma provides better sensitivity as compared to horse and cow plasma. Furthermore; the overall sensitivity of human plasma is in agreement with on this previously published report[18].

For identification of S. aureus, single phenotypic test sensitivity was checked in the current study. In agreement with the previous studies; it was observed that tube coagulase test was more sensitive phenotypic test for S. aureus identification than slide coagulase test (SCT). Further it was found that coagulase tests (both slide and tube) have better results in term of sensitivity than MSA and DNase tests described the same results that coagulase tests are better than MSA and DNase tests[19]. In current study 70% samples were positive on MSA test. Its sensitivity was higher than DNase tests, but lower than coagulase tests Piper J, Sperber WH found 76.5% and 71% positive results for S. aureus through MSA test, respectively. MSA test alone is not a conclusive test but with the conjunction of other tests, its sensitivity can be improved[20,21].

In current study; DNase test provided positive test in 60% of samples. The DNase test was found least sensitive for identification of S. aureus as compared to coagulase and MSA tests. Taponen S et al reported that DNase test show 75% sensitivity. Their results are higher than our study; but they are agreeing with the fact that DNase tests are inferior test than coagulase and MSA tests[22].

It has been observed that no single phenotypic test is providing 100% correct results. Their correctness can be increased if these phenotypic tests are used in combination. It was observed that combination of SCT with MSA test and SCT with DNase test improved the positive results of the clinical samples[23]. Furthermore; this improvement was best seen in combination of MSA test and SCT with human plasma and least seen in combination of SCT and DNase test with cow plasma. Similarly Wood CA et al described that coagulase test is invaluable phenotypic test for identification of S. aureus and can be used in combination with other phenotypic tests[24]. The findings of this suggest that at least two phenotypic tests preferably SCT+MSA, used in clinical laboratory to accurately identify S. aureus.

CONCLUSION

The best phenotypic test for identification of Staphylococcus aureus is coagulase test if used alone, while in combination it provides improved results with MSA test. Combination of different phenotypic tests for identification of S. aureus increases the percentage of positive tests. The combination of slide coagulase test and MSA test provides the best results as compared to other combinations such as slide coagulase and DNase tests.

RECOMMENDATIONS

It is recommended that human plasma may be used in routine coagulase tests for the identification of S. aureus in diverse clinical specimens.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Khattak MS: Idea.

Bilal M: Overall supervision.

Rizwan M: Manuscript writting

Ahmad S: Bibliography.

Meer A: Statistics.

Ullah I: Follow up.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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