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Microbiology Section

Evaluation of Hiaureus™ Coagulase Confirmation Kit in Identification of *Staphylococcus aureus*

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ABSTRACT

Introduction: *Staphylococcus aureus* is a facultative anaerobic Gram positive coccal bacterium whose incidence ranges to different infections. It is a cause of various uncomplicated skin infections, abscesses, septicaemia/bacteraemia, gastroenteritis, endocarditis, toxic shock syndrome and food intoxications. Various methods with varied time, sensitivities, specificities and costs are available, but may not be used as a reliable test for the identification and differentiation of *S. aureus*. Therefore, there is a need to evaluate newer tests.

Aim: To compare the conventional tests with a commercial available kit for reliable, cost effective identification and confirmation of *S. aureus.*

Materials and Methods: The current prospective study was conducted in the Department of Clinical Pathology, Haffkine Institute for a period of six months. A total of 341 clinical isolates of staphylococci isolated from pus, urine, blood culture and sterile body fluids were subjected to conventional tests like Tube Coagulase Test (TCT) using Rabbit Plasma (RP) and Human Plasma (HP), culture media such as Mannitol Salt Agar (MSA) and Deoxyribonuclease (DNase) media in parallel to Hiaureus™ Coagulase Confirmation Kit (HACCK), a commercially available kit for identification of *S. aureus*. Amplification of the *fem*A gene was used as a comparative reference point test to calculate the sensitivity, specificity and concordance values of the conventional tests.

Results: Amongst the coagulase based tests, HACCK was 100% sensitive and specific. The TCT using RP was 98.58% sensitive while TCT using HP was less sensitive (95.37%). A total of 100% specificity was observed for TCT using RP while TCT using HP was 96.68% specific. The MSA and DNase media were 97.86% vs 96.44% and 96.67% vs 91.67% sensitive and specific respectively. The combination tests had varying sensitivity and specificity ranges. The HACCK demonstrated 100% concordance with *fem*A amplification and was labelled as an ideal perfect test (κ=1) with MSA as an alternative test for *S. aureus* identification.

Conclusion: The HACCK can be used as an exclusive, reliable and cost effective test for identification of *S. aureus*. Alternatively, in view of the cost factor MSA either as a single test or in combination with TCT using HP could be used as screening tests and confirm discordant results with HACCK.

Keywords: Deoxyribonuclease, Mannitol, Plasma, Polymerase chain reaction, Rabbit

INTRODUCTION

Staphylococcus aureus, a pathogenic Gram positive bacterium is a ubiquitous commensal bacterium on human skin and mucous membranes [1]. Being present in up to 80% of healthy individuals, it is responsible for various infections in hospital and community settings. It is the most common cause of Skin and Soft Tissue Infections (SSTIs), necrotizing pneumonia, septic arthritis, endocarditis and osteomyelitis [2].

S. aureus is generally considered a virulent and pathogenic strain amongst staphylococci. The infections caused by *S. aureus* is related to a consortium of virulence factors that allow it to adhere to the surface, invade or avoid the immune system and cause harmful effects to the host [3-5]. One of the earliest described virulence factors is the coagulase enzyme, which forms the basis of coagulase test, is widely used to distinguish *S. aureus* from other staphylococci i.e., Coagulase Negative Staphylococci (CoNS). There are two forms of coagulase enzyme viz., bound and free coagulase. The bound coagulase clump the bacteria together thereby shield themselves from the immune system and together produce an elevated quantity of toxins. The secreted free coagulase aids in the formation of a fibrin clot which protects the bacterium from phagocytosis and facilitates a protective environment effectively from the immune system [6]. Studies have reported the incidence of infections caused by CoNS which are commensals of skin and mucous membranes and interfere in diagnosis. Thus, it is imperative to distinguish between *S. aureus* and CoNS, considering the augmented incidence of infections caused by *S. aureus* and CoNS worldwide [7,8].

The other markers that differentiate *S. aureus* from CoNS is the ability to ferment mannitol sugar and breakdown of DNA which is achieved by use of MSA and DNase agar respectively. MSA is a specialized media that selects the growth of staphylococci bacteria due to its high salt concentration in addition to mannitol fermentation which differentiates between *S. aureus* and CoNS [9,10]. *S. aureus* are known to produce DNase enzyme that breaks down DNA into smaller fragments. Moreover, the DNase activity of *S. aureus* have been shown to have a strong correlation with coagulase activity [11]. Therefore, the DNase activity of *S. aureus* is used as a tool for identification of *S. aureus*. The other methods include specialized media such as Baird Parker Agar, Vogel Johnson Agar and various chromogenic media, latex agglutination tests based on detection of protein A and/or clumping factor and automated systems [12,13]. In such settings, coagulase test that is considered as a gold standard amongst the conventional tests remains the test of choice [14]. However, these tests vary in their sensitivities, specificities, time of identification and costs. Also, it has been documented time and again that none of the existing tests can be used as a single test for the identification and differentiation of *S. aureus*, thereby, providing an impetus to evaluate newer tests. HACCK (HiMedia Laboratories, Mumbai, India) is a commercial kit which can be used for identification of *S. aureus* based on coagulase test principle. Consequently, the study was designed to compare the coagulase tests by RP and HP against the commercial kit with other conventional methods in identification of *S. aureus* and differentiation from CoNS.

MATERIALS AND METHODS

Bacterial Isolates and Controls

This prospective study was carried out for a period of six months from February to July 2015 in Department of Clinical Pathology, Haffkine Institute. During this study period, a total of 341 clinical isolates of genera *Staphylococcus* was collected from Sir Jamshedjee Jeejeebhoy (JJ) Hospital, a tertiary care hospital. The inclusion criteria for collection of the isolates were based on Gram staining results i.e., Gram positive cocci in clusters. All other isolates i.e. Gram negative bacteria and Gram positive cocci in clusters with mixed flora were excluded from the study. The sources of these isolates included pus, urine, blood culture and sterile body fluids. The isolates were identified as *Staphylococcus* genera by Furazolidone (100 mcg) antibiotic disc wherein, the isolates exhibited zone diameter greater than 15 mm [15]. *S. aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228 were used as positive and negative control respectively.

Identification of *S. aureus* by Conventional Methods

All the clinical isolates were tested by TCT using RP and HP, MSA and DNase agar media for identification and differentiation of *S. aureus* from CoNS. The HACCK was also used as a phenotypic test.

TCT

The TCT was performed using commercially available RP (HiMedia Laboratories, Mumbai, India) and 1:10 diluted HP (National Plasma Fractionation Centre, Mumbai, India). Pure colonies from an 18-hour-old culture were inoculated into 0.5 ml of respective plasmas, incubated at 37°C and observed for clot formation for four hours. In case of no clot observed at four hours, the test was continued with an overnight incubation at room temperature. A final observation at 24 hours was noted [16]. Prior to coagulase testing, the plasma was checked for its coagulase activity using ATCC cultures which include *S. aureus* ATCC 25923, *S. aureus* ATCC 29213 and *S. aureus* ATCC 43300 that were coagulase positive and S. *epidermidis* ATCC 12228, *Staphylococcus saprophyticus* ATCC 15305 and *Staphylococcus haemolyticus* ATCC 29970 that were coagulase negative. The plasma was kept in 1.5 ml sterile cryovials and stored at -80°C till further use.

HACCK

HACCK is a coagulase based test for rapid identification of *S. aureus* and differentiation from CoNS. The kit contents included tubes with dehydrated medium, suspension fluid and a dropper. The test was carried out using manufacturer's instructions. Briefly, the content of the tube was rehydrated with the sterile suspension fluid. The culture was inoculated in the tube and incubated at 37°C. The tubes were observed for clot formation at regular intervals up to four hours. A positive result was indicated by clot formation. The tube was incubated overnight at room temperature in case of no clot formation until four hours and interpreted as either positive or negative based on clot formation [16,17].

Growth on MSA and DNase Agar Media

The isolates were streaked on MSA media. Growth of yellow colonies on MSA after 24 hours of incubation at 37°C was an indicative of positive result [9]. The DNase test was performed by spotting and incubating the isolates for 24 hours at 37°C on DNase agar with Toluidine blue. Appearance of the pink zone of clearance around the growth of bacteria was an indicative of a positive result [16].

Identification of *S. aureus* by PCR

The detection of *S. aureus* by PCR amplification was used as a reference point test. A monoplex PCR targeting the *fem*A gene was carried out for identification of *S. aureus* using the primers *fem*A-F 5'-CGATCCATATTTACCATATCA-3' and *fem*A-R 5'- ATCACGCTCTTCGTTTAGTT-3' [18]. An aliquot of 2 µl of the extracted DNA was used as a template in a 25 µl of PCR mixture containing 1X PCR buffer, 2.5 mM $MgCl_2$ (HiMedia Laboratories, Mumbai, India), 0.2 mM dNTPs mix (HiMedia Laboratories, Mumbai, India), 1U of Taq DNA polymerase enzyme (HiMedia Laboratories, Mumbai, India) with 0.2 µM of each primer. The amplification was performed with the following thermal cycling profile; 1 cycle of initial denaturation at 94°C for 3 min, followed by 30 cycles of amplification (denaturation at 94°C for 30 s, annealing at 56°C for 45 s and extension at 72°C for 30 s) with a final extension at 72°C for 5 min on Prima 96™ PCR machine (HiMedia Laboratories, Mumbai, India). The PCR products were subjected to Agarose Gel Electrophoresis (1.5% Agarose, 1X Tris-Acetate-EDTA) and were resolved along with a 100-bp molecular size ladder. The amplicons were visualized and photographed using a Gel Doc system (Bio-Rad Laboratories, California, USA) [18].

STATISTICAL ANALYSIS

The PCR results were compared to the conventional methods for identification of *S. aureus.* The data were analysed by a 2×2 contingency table. The sensitivity and specificity were calculated using the diagnostic test evaluation calculator available from MedCalc website [19]. For categorical assessment of the agreement of the conventional tests and coagulase confirmation kit with the PCR assay, Cohen's kappa coefficient (κ) was calculated using a free tool available from Graph Pad software website [20,21].

RESULTS

Identification of *S. aureus* using PCR

The identification of *S. aureus* by targeting the *fem*A gene was used as a reference point test for comparison. Among the 341 staphylococci isolates, 82.40% (n=281) were positive for *fem*A gene while 17.60% (n=60) strains were negative.

Identification of *S. aureus* by conventional methods

TCT and HACCK

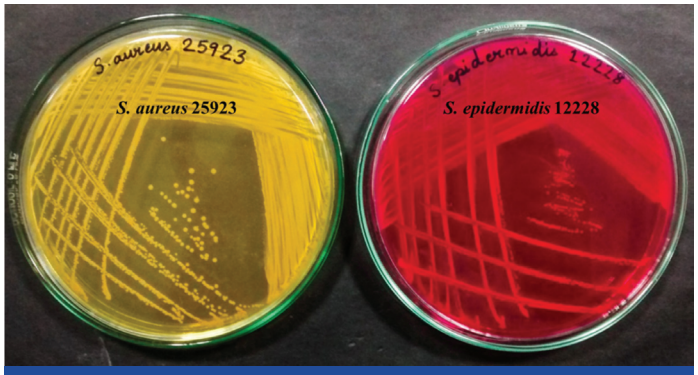
Of the 281 *fem*A positive isolates, 98.58% (n=277) and 95.37% (n=268) exhibited clot formation in the TCT using RP and HP. A 100% (n=281) positivity was observed among *fem*A positive isolates by HACCK. Likewise, the 60 *fem*A negative isolates were negative by the TCT using RP and the HACCK. However, 96.67 (n=58) isolates were negative in TCT using HP as two *fem*A negative isolates showed clot formation.

Growth on MSA and DNase agar media

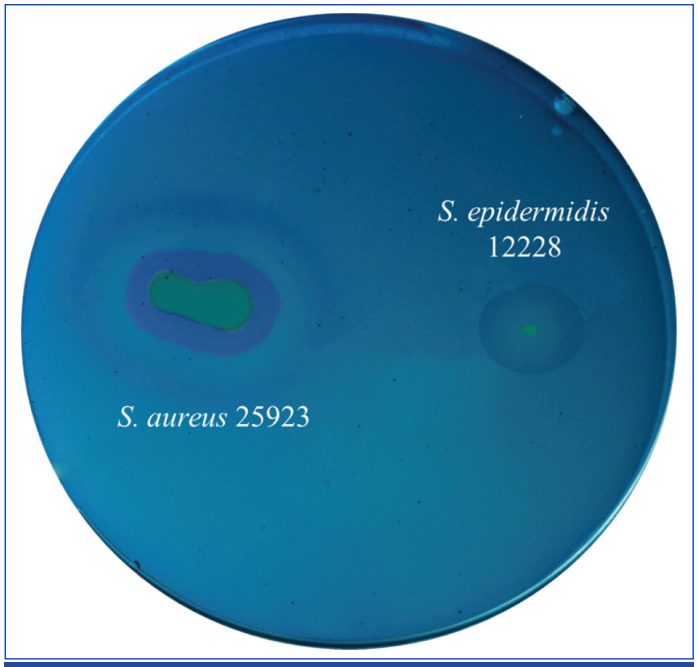
In the mannitol fermentation test using MSA media, 97.86% (n=275) *fem*A positive isolates were positive, while the remaining 2.14% (n=6) *fem*A positive isolates were negative. Of the *fem*A negative isolates, 100% (n=60) of isolates produced pink coloured colonies on MSA and were therefore, marked as CoNS [Table/Fig-1]. In the DNase test, DNase activity was exhibited among 96.44% (n=271) *fem*A positive isolates while 3.56% (n=10) of isolates were negative in this test. Besides, 8.33% (n=5) of *fem*A negative isolates produced pink zones of clearance and the remaining 91.67% (n=55) of *fem*A negative isolates were negative in this test [Table/Fig-2].

Efficiency of Combination of Tests

Among the total 341 isolates, a complete concordance of 90.91% (n=310) was observed amongst the conventional tests and HACCK in comparison to *fem*A PCR assay of which 75.95% (n=259) isolates were identified as *S. aureus* and 15.54% (n=53) were marked as CoNS by all the tests. Discordant results were observed in 8.54% (n=29) isolates using the tests. Therefore, the combination of tests was evaluated.



[Table/Fig-1]: Representation of Mannitol Salt Agar (MSA).
Yellow colour colonies – mannitol fermentation positive
Pink colour colonies – mannitol non-fermentation negative



[Table/Fig-2]: Representation of Deoxyribonuclease (DNase) agar.
Appearance of pink zone of clearance around the growth of bacteria – DNase activity positive
Appearance of no pink zone of clearance around the growth of bacteria – DNase activity negative

RP with MSA and/or DNase media

About 96.80% (n=272), 95.73% (n=269) and 94.66% (n=266) of *fem*A positive isolates were identified as *S. aureus* by combination tests of RP+MSA, RP+DNase and RP+MSA+DNase combination respectively in comparison to PCR [Table/Fig-3]. Likewise, 100% (n=60), 91.67% (n=55) and 91.67% (n=55) tested negative for *S. aureus* by either tests of RP+MSA, RP+DNase and RP+MSA+DNase combination respectively in comparison to PCR [Table/Fig-3].

HP with MSA and/or DNase media

In conflict to RP, 93.95% (n=264), 93.95% (n=264) and 92.53% (n=260) of isolates were labelled as *S. aureus* by HP+MSA, HP+DNase and HP+MSA+DNase combination respectively in comparison to PCR [Table/Fig-3]. In the same way, 96.67% (n=58), 88.33% (n=53) and 88.33% (n=53) were identified as CoNS by either tests of HP+MSA, HP+DNase and HP+MSA+DNase combination respectively in comparison to PCR [Table/Fig-3].

Hiaureus™ Coagulase Confirmation Kit with MSA and/or DNase media

Of the total 281 *fem*A positive isolates, 97.86% (n=275), 96.44%, (n=271) and 95.02% (n=267) of the isolates were identified as *S. aureus* using the combination of HACCK+MSA, HACCK+DNase and HACCK+MSA+DNase respectively [Table/Fig-3]. On the other hand, 100% (n=60), 91.67% (n=55) and 91.67% (n=55) of the isolates did not test positive for *S. aureus* using either tests of HACCK+MSA, HACCK+DNase and HACCK+MSA+DNase combination respectively in comparison to PCR [Table/Fig-3].

Phenotypic methods	*fem*A positive isolates (n=281)		*fem*A negative isolates (n=60)	
	% TP	% FN	% FP	% TN
RP	98.58	1.42	0.00	100.00
HP	95.37	4.63	3.33	96.67
HACCK	100.00	0.00	0.00	100.00
MSA	97.86	2.14	0.00	100.00
DNase	96.44	3.56	8.33	91.67
RP+MSA	96.80	3.20	0.00	100.00
RP+DNase	95.73	4.27	8.33	91.67
RP+MSA+DNase	94.66	5.34	8.33	91.67
HP+MSA	93.95	6.05	3.33	96.67
HP+DNase	93.95	6.05	11.67	88.33
HP+MSA+DNase	92.53	7.47	11.67	88.33
HACCK+MSA	97.86	2.14	0.00	100.00
HACCK+DNase	96.44	3.56	8.33	91.67
HACCK+MSA+DNase	95.02	4.98	8.33	91.67
MSA+DNase	95.02	4.98	8.33	91.67

[Table/Fig-3]: Identification of *S. aureus* by conventional methods in comparison to *fem*A PCR as reference point test (n=341).
RP – Rabbit Plasma, HP – Human Plasma, HACCK - Hiaureus™ Coagulase Confirmation Kit, MSA – Mannitol Salt Agar, DNase – Deoxyribonuclease media, TP – True Positive, FN – False Negative, FP – False Positive, TN – True Negative

MSA and/or DNase Media

The combination of two medias identified 95.02% (n=275) of isolates as *S. aureus*. Correspondingly, 91.67% (n=55) of isolates were misidentified as *S. aureus* by MSA+DNase test in comparison to PCR assay [Table/Fig-3].

Assessment of Sensitivity and Specificity

The results were statistically analysed using a 2×2 contingency table to calculate the specificity and sensitivity for evaluation of individual tests and combination of tests for identification of *S. aureus* in comparison with PCR amplification of *fem*A gene [Table/Fig-4]. Amongst the coagulase based tests, HACCK was 100% sensitive and specific in the detection and differentiation of *S. aureus* from CoNS. The TCT using RP was 98.58% sensitive while TCT using HP was comparatively less sensitive i.e., 95.37%. Conversely, 100% specificity was observed for TCT using RP while TCT using HP had a specificity rates of 96.67%. The MSA and DNase media had a sensitivity rate of 97.86% and 96.44% and specificity rate of 100% and 91.67% respectively. Among the combination tests, HACCK+MSA showed better sensitivity (97.86%) as compared to RP+MSA (96.80%). The other combination tests had a good sensitivity rate, but low specificity rate with an exception of HP+MSA with low sensitivity and moderate specificity rate (93.95% vs 96.67%).

Calculation of Cohen's kappa coefficient (κ) and concordance rates

The Cohen's kappa (κ) value and concordance rates were calculated for every test in comparison to *fem*A PCR. Among the tests, HACCK showed 100% concordance with the PCR assay (κ=1) and labelled as a perfect test. Four different tests i.e., RP, MSA, RP+MSA and HACCK+MSA were labelled almost perfect test to have concordance rate nearing 100% with varying κ values. The remaining tests were labelled either strong or moderate tests [Table/Fig-5].

Methods for *S. aureus* detection	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RP	98.58	100.00	100.00	93.75
HP	95.37	96.67	99.26	81.69
HACCK	100.00	100.00	100.00	100.00
MSA	97.86	100.00	100.00	90.91
DNase	96.44	91.67	98.19	84.62
RP+MSA	96.80	100.00	100.00	86.96
RP+DNase	95.73	91.67	98.18	82.09
RP+MSA+DNase	94.66	91.67	98.15	78.57
HP+MSA	93.95	96.67	99.25	77.33
HP+DNase	93.95	88.33	97.42	75.71
HP+MSA+DNase	92.53	88.33	97.38	71.62
HACCK+MSA	97.86	100.00	100.00	90.91
HACCK+DNase	96.44	91.67	98.19	84.62
HACCK+MSA+DNase	95.02	91.67	98.16	79.71
MSA+DNase	95.02	91.67	98.16	79.71

[Table/Fig-4]: Calculation of Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of conventional tests in comparison to *fem*A PCR.
RP – Rabbit Plasma, HP – Human Plasma, HACCK - Hiaureus™ Coagulase Confirmation Kit, MSA – Mannitol Salt Agar, DNase – Deoxyribonuclease Media, TP – True Positive, FN – False Negative, FP – False Positive, TN – True Negative
Sensitivity = TP/ (TP+FN)*100, Specificity = TN/ (FP+TN)*100, PPV = TP/ (TP+FP)*100, NPV = TN/ (TN+FN)*100

Methods for *S. aureus* detection	κ at 95% CI	Concordance with PCR (%)	Strength of agreement
HACCK	1	100	Perfect
RP	0.961	98.83	Almost perfect
MSA	0.942	98.24	Almost perfect
RP+MSA	0.914	97.36	Almost perfect
HACCK+MSA	0.942	98.24	Almost perfect
HP	0.859	95.6	Strong
DNase	0.853	95.6	Strong
RP+DNase	0.836	95.01	Strong
HP+MSA	0.825	94.43	Strong
HACCK+DNase	0.853	95.6	Strong
MSA+DNase	0.819	94.43	Strong
RP+MSA+DNase	0.81	94.13	Strong
HACCK+MSA+DNase	0.819	94.43	Strong
HP+DNase	0.63	87.81	Moderate
HP+MSA+DNase	0.741	91.79	Moderate

[Table/Fig-5]: Kappa coefficient test for agreement between two tests.
RP – Rabbit Plasma, HP – Human Plasma, MSA – Mannitol Salt Agar, DNase – Deoxyribonuclease Media
κ at 95% CI (None: 0.0 – 0.2, Minimal: 0.21 – 0.39, Weak: 0.40 – 0.59, Moderate: 0.60 – 0.79, Strong: 0.80 – 0.89, Almost Perfect: 0.90 – 0.99 and Perfect: 1)

DISCUSSION

Accurate and rapid detection play a vital role in the proper management of infections caused by *S. aureus*. In the present study, we attempted to evaluate the conventional methods for detecting *S. aureus* and differentiating from other staphylococci in parallel to a commercially available kit. Of the total 341 isolates, 82.40% of the isolates were identified as *S. aureus* based on the presence of *fem*A gene, a chromosomally encoded factor, occurring naturally in *S. aureus* [22]. This gene has been implicated in cell wall metabolism and is present in large amounts in actively growing cultures [18,23].

Coagulase test, the most common conventional test, is either performed as a slide coagulase test or by TCT. 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 [12,24]. This may be due to masking of the clumping factor by the capsular polysaccharides or due to misidentification of certain clumping factor producing CoNS; thereby, reducing its efficiency, reliability and sensitivity [12,25,26].

The plasma has a component called the Coagulase Reacting Factor (CRF) which binds to the Staphylococcal Coagulase (SC) and forms SC-CRF complex resulting in the conversion of fibrinogen to fibrin clot which forms the basis of coagulase test. RP, pig plasma and HP have been deemed to be the most suitable plasmas for coagulase testing with optimal concentration of CRF, high sensitivity, specificity and low reaction time [27-29]. However, pig plasmas are not widely available and therefore, RP and HP are ideal candidates for testing purposes [30].

In the present study, TCT was performed using RP and HP. The TCT using RP had a sensitivity and specificity rate of 98.58% and 100% respectively which were in agreement with other studies [31,32]. High sensitivity, less reaction time, easy acquisition of rabbits, non-laborious breeding and bleeding makes them as an appealing choice. Nevertheless, the locally available RP may be of poor quality while the commercially available ones are expensive, making it unaffordable [29]. Consequently, HP is used as an alternative in TCT. The HP was 95.37% sensitive and with similar rates from 91% to 95% in comparison to *fem*A PCR [12,29]. However, the use of HP is not perfectly efficacious despite of an overall high sensitivity. HP may contain anti-Staphylococcal agents which may interfere in the identification process and result in false negative outcomes [29]. Also, the lab personnel are at risk of contracting undetected HIV/AIDS, Hepatitis B and C [17,29,33]. Therefore, there is a need for an efficient test that may be helpful to overcome the drawbacks of TCT using RP or HP. This is the first reported study of HACCK to be used for identification of *S. aureus*. The HACCK exhibited 100% sensitivity, 100% specificity and 100% concordance with *fem*A PCR.

In the present study, 81.23% isolates were mannitol positive while 18.77% were mannitol negative. Theoretically, coagulase positive strains of *S. aureus* are usually mannitol fermenters. However, 2.14% (n=6) *fem*A positive isolates were mannitol negative. Kateete et al., have reported 6% *S. aureus* as MSA negative [29]. Shittu et al., have also reported MSA negative Methicillin Resistant *S. aureus* (MRSA). These false negative results may be due to genetic variation and/or lack of capability of *S. aureus* to ferment mannitol sugar [34]. Although, false positive results were not observed for MSA test in the present study, there are reports of some CoNS being mannitol fermenters [29,34]. This may be explained by the fact that these isolates can hydrolyse mannitol sugar in the media which forms the basis of identification of *S. aureus* isolates [35,36]. Therefore, the false negative or positive results can lead to wrong diagnosis or a delay in isolation and subsequent identification of *S. aureus* [29,34]. Another frequently used test for the detection of *S. aureus* is the DNase test. In the present study, 10 *fem*A positive isolates were negative for DNase activity while few *fem*A negative isolates were positive for the same. Similar findings have been reported earlier too with no explanation for these findings and thus, requires confirmation using an additional test [29,37-39]. In addition, there may be technical difficulties with regards to interpretation in case of weak positive reactions. Thus, DNase cannot be used as a single test for diagnosis of *S. aureus* which was evident with low concordance rate with PCR among other individual tests. The evaluation of combination tests revealed that HP with MSA and/or DNase had a low sensitivity similarly reported by Kateete et al., previously [29]. The combination of RP with MSA and/or DNase has not been reported elsewhere. A comparatively higher sensitivity was noted in the present study. Similarly, assessment of the HACCK with MSA and/or DNase is first of its kind and showed highest sensitivity percentage in comparison to RP and HP.

The HACCK test correctly identified all *fem*A positives and *fem*A negatives as *S. aureus* and CoNS respectively. In view of Cohen Kappa (κ) value, the HACCK was considered as a perfect test for identification of *S. aureus*. In addition to 100% specificity, sensitivity and concordance rate further advantages were observed. A greater percentage of isolates showed clot formation by the HACCK in less than three hours as against TCT using RP and HP (data not shown). The kit can be stored at room temperature with an extended shelf life, ready to use with minimal preparation time and reduced contamination sources. The number of tubes was reconstituted specifically for the number of samples to be tested which reduced the overall consumption of the kit. The TCT using RP or HP required a time consuming preparation time which included reconstitution of RP, thawing of HP from the frozen state to room temperature and dilution of the same. Also, RP once re-constituted can be stored for a maximum of 14 days at 2-4°C. The calculated cost of HACCK and TCT using RP for a single test was Rs.130-140 and Rs.150-160 respectively. The TCT using HP was cheaper at Rs.60-70 of a single test. The MSA, which had an almost perfect concordance rate of 98.24% with PCR may be used as an alternative test at a cost of 20-30 Rs. for a single test. Thus, MSA could be considered as a second line test for diagnosis of *S. aureus* and differentiation from CoNS. Also, keeping in mind the cost factor, MSA in combination with TCT using HP could be used as screening tests for diagnosis and confirm discordant results with the highly sensitive HACCK.

LIMITATION

The observation of clots by coagulase test was not checked between 4-24 hours. It has been reported that, *S. aureus* strains produce an enzyme called fibrinolysin along with coagulase, which can dissolve the clot formed from 4-24 hours and produce a false negative test when observed after 24 hours.

CONCLUSION

In view of the sensitivity, specificity and Cohen's kappa value, the HACCK can be used as an effective tool with high level of confidence to diagnose *S. aureus* infections, overcome the problems of false positive result of CoNS species and reduce turnaround time for drug susceptibility testing and initiating therapy for the patient.

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