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ORIGINAL ARTICLE

Sa*select*: a Well-Performing Chromogenic Medium for Primary Isolation & Identification of *S. aureus* & CoNS Directly from Clinical Samples

[1]Marwa K. Sallam*,[2] Mervat M. Khalaf, [3]Rana A. Essawy [3]

[1]Medical Microbiology & Immunology Department, Faculty of Medicine, Cairo University
[2]Intensive Care Medicine Department, Faculty of Medicine, Cairo University
[3]General Histology Department, Faculty of Dentistry - MSA University

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Corresponding Author:
Marwa K. Sallam
Medical Microbiology & Immunology Department, Faculty of Medicine, Cairo University
Tel.: 01145700335
drmicro91@yahoo.com

ABSTRACT

Background*: Since the importance of Staphylococcus aureus and Coagulase negative staphylococci (CoNS) as the most common pathogens sought in clinical microbiology laboratories, the need for highly sensitive and highly specific culture based methods has been aroused. To improve the recovery and identification of S.aureus and CoNS, several chromogenic mixtures have been developed which allow identification of the bacteria within 24 hours of incubation on the basis of colonial morphology and distinctive color patterns.* ***Methodology****: In the present study we used SaSelect, DNase test and coagulase test for direct detection of staphylococci from clinical specimens.* ***Objectives****: To asses the performance of the chromogenic medium (SaSelect) for identification & isolation of S. aureus & CoNS directly from clinical samples and to compare the performance of (SaSelect) to the conventional methods for identification & isolation of S. aureus & CoNS directly from clinical samples.* ***Results****: SaSelect was found to be a well-performing chromogenic medium that significantly improved the detection of staphylococci, especially S. aureus, compared to conventional culture (P <0.001).* ***Conclusion:*** *SaSelect is a well performing culture medium for the primary isolation of various staphylococci in comparison to conventional methods. It was highly sensitive and specific especially for isolation of S. aureus.*

INTRODUCTION

Staphylococci are a diverse group of bacteria that resemble part of the normal human flora. However, they can also cause diseases, ranging from minor skin infections to life threatening bacteremia. They are classified into coagulase – positive staphylococci (*S. aureus*) and CoNS according to their ability to produce coagulase[1].Within the genus *Staphylococcus*, *S. aureus* is the most important human pathogen, while the CoNS play roles mainly in opportunistic infections[2,3]. CoNS, particularly *S. epidermidis*, are among the most frequently isolated-bacteria in the clinical microbiology laboratory. They have emerged as important nosocomial pathogens during the last few decades[4]. They are considered as a major cause of infections in ICUs patients[5]. The incidence of staphylococcal nosocomial infections in ICUs is 4-5 times greater than in general wards[6].

Culture-based detection methods are cost-effective and useful, especially when various microbe species are examined. Disadvantages of conventional culture, however, are the need for additional tests for accurate species identification and the difficulty in differentiating various microbes if different species produce similar colonies, as may be the case with staphylococci. Furthermore, swarming or rapidly growing bacteria, such as Gram-negative bacilli, may cover or overgrow all other species present in the specimen unless selective supplements are used [7].

Isolation of *S. aureus* is usually accomplished with the use of conventional media such as blood agar. The disadvantage of such media is the need of confirmatory tests to differentiate *S. aureus* from colonies with identical colony appearance or when swarming colonies of Proteus cover those of *S. aureus* on such ordinary media [8]. Moreover, performing identification tests, such as biochemical tests, coagulase and DNase tests, on all colonies resembling staphylococci can be time-consuming and labor intensive [9]. In addition to the need for a more skilled expertise to confirm the diagnosis[7], it has been reported that recovery of *S. aureus* using culture methods, requires 1 to 4 or more days for accurate detection and identification of *S. aureus* [10]. All these factors cause delay of reaching the accurate diagnosis by the clinician to start the appropriate treatment for the case [11].

To improve the recovery and identification of various microbes, several chromogenic mixtures have been developed[9]. These media allow presumptive identification on the basis of colonial morphology and distinctive color patterns[10].

The use of chromogenic media can potentially reduce the number of confirmatory tests that are necessary for the detection of *S. aureus*[12]. The ideal characteristics of any candidate chromogenic medium are the detection of *S. aureus* with high sensitivity and specificity at least comparable to conventional media after 18 to 24 h of incubation [9].

Although rapid detection of *S. aureus* in clinical specimens is essential for appropriate patient care, the recovery and identification of other staphylococci is also important, especially from catheters, other foreign body samples and blood cultures [13].

This study aimed to assess the performance of the chromogenic medium **(Sa*Select*)** for identification and isolation of *S. aureus* & CoNS directly from clinical samples. Also to compare the performance of the chromogenic medium (Sa*Select*) to the conventional methods for identification and isolation of *S. aureus* & CoNS directly from clinical samples.

METHODOLOGY

The current study was conducted on 200 patients admitted to Critical Care Department (3rd unit) of Kasr El-Ainy hospitals, Cairo University during the period from February 2016 through August 2016. The study population comprised 108 male and 92 female with an age range between 19 to 84 years.

Sample collection:

Different clinical samples were collected under complete aseptic conditions using sterile containers, swabs, suction catheters, syringes. The isolates were obtained by cultivation of different clinical specimens including: wound swabs, urine samples, respiratory tract secretions and pus samples. All swabs were transported in charcoal transport medium. Samples of urine were collected in sterile dry, wide-necked, leak-proof containers. The sample port was cleaned with a swab saturated with 70% isopropyl alcohol and allowed to dry. A sterile lock syringe was inserted into the port at 90° angle and turned half a turn clockwise, and then a urine sample was slowly drawn [14].

All specimens were labeled with the date, patient's name, patient's number, time of collection and specimen type, and then transported immediately to the laboratory of Medical Microbiology and Immunology Department, Faculty of Medicine, Cairo University.

Cultivation of samples:

Direct plating on Sa*Select* medium (BioRad, USA) and MSA (Oxoid, United Kingdom) was done to test for direct isolation of *S. aureus* and CoNS directly from the clinical samples. Primary plating on blood agar (Oxoid, United Kingdom) was added to our routine testing regimen so as not to miss any *Staphylococcus* in the specimen; being a non-selective enriched medium.

All plates were incubated aerobically at 37°C aerobically and examined after 24h of incubation. Incubation was occasionally prolonged to 48h for some plates only, in order to enhance differences between colony morpho-types.

Identification of isolates as belonging to *Staphylococcus* genus:

Presumptive identification of the genus Staphylococcus:

a. On blood agar: Any yellow round concave colonies surrounded by clear beta hemolysis zone or non-hemolytic white round concave colonies after 24h to 48h of incubation were considered positive growth [15].
b. On Sa*Select* media: The criteria for presumptive identification of different staphylococci growing on Sa*Select* were defined as follows; [7]:
   - *S. aureus*: pink to orange colonies.
   - *S. epidermidis*: small white/ faint pink colonies.
   - *S. intermedius*: bulky purple-gray colonies.
   - *S. saprophyticus*: turquoise colonies.
   - *S. simulans*, *S. cohnii*, or *S. xylosus*: light-blue colonies .
   - *S. lugdunensis* or *S. sciuri*: yellow colonies.
c. On Mannitol Salt Agar (MSA):
   - All yellow colonies were considered *S.aureus*, while pink colonies were considered CoNS [16].
   - Negative growth was incubated for total 48h for further confirmation.

Confirmatory identification of the genus Staphylococcus:

Suspected staphylococci colonies gown on the three media were subjected to the following confirmatory tests.

a. Gram stain: Staphylococci appear as Gram positive cocci arranged in grape like clusters, occurring characteristically in groups but also singly and in pairs [17].
b. Catalase test: It was done according to Bailey and Scott[18]:
c. Oxidation/Fermentation (O/F) test: To differentiate staphylococci from other catalase positive and Gram positive cocci, O/F test (Merk Millipore Company, USA) was done. Saccarolytic colonies were considered staphylococci for further identification[18].

Differentiation of *S. aureus* and CoNS:

All suspected colonies grown on the three media were subjected to the following tests,

Slide coagulase test:

- A coarse clumping of bacteria, visible to the naked eye within 10 seconds (agglutination), was considered positive result indicating *S.aureus*.

– Absence of clumping or any reaction taking more than 10 seconds to develop was considered negative result and was confirmed by Tube coagulase test.

Tube – coagulase test :

– In positive cases: The plasma will coagulate, resulting in a clot and this indicatesS.aureus isolate.
– In negative cases: The plasma remains liquid all through the 18 hours and this indicates CoNS isolate.

DNase test:

Colonies from primary recovery plates presumptively identified as staphylococci were inoculated onto DNase plates were supplied as dehydrated medium (Oxoid, United Kingdom),

– A zone of clearing around the streak was considered positive DNase activity, indicative of S. aureus.
– Absence of zone of clearance around the streak was considered negative DNase activity, indicative of CoNS.

S.aureus identification was confirmed by fulfilling the following Criteria[19]**:**

– Gram stain and morphological picture of staphylococci.
– Catalase positive.
– Facultative anaerobe and glucose fermentative.
– Positive slide and/or tube coagulase test
– Positive DNase test.

While it was considered CoNS if it had the following Criteria[19]:

– Gram stain and morphological picture of staphylococci.
– Catalase positive.
– Facultative anaerobe and glucose fermentative.
– Negative slide and tube Coagulase test.
– Negative DNase test.

Statistical analysis methods:

Data were statistically described in terms of frequencies (number of cases) and percentages. Comparison between the study modalities was done using McNemar test while agreement was tested using kappa statistic. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows.

RESULTS

Out of 200 samples, 142 staphylococcal isolates were isolated from 138 (69%) different clinical samples. Sixty two samples (31 %) were negative for any growth or had growth of organisms other than staphylococci.

The number of staphylococci isolated directly from clinical specimens differed on the three media as follows; 142 staphylococcal isolates recovered on blood agar, 141 isolates were recovered on Sa*Select* medium while only 110 isolates were recovered on MSA.

Accordingly, the sensitivity of both media for identifying and isolating staphylococci was evaluated as shown in table (1).

Table 1: Sensitivity of Sa*Select* and MSA for isolation and identification of staphylococci species:

Total number of staphylococcal isolates	Media	Number of true-positive isolates for staphylococci species	Number of false-negative isolates for staphylococci species	Sensitivity* (%)
142	Sa*Select*	141	1	99.3 %
	MSA	110	32	77.4%

*P value = < 0.001

The 142 staphylococcal isolates were further subjected to confirmatory tests (slide/tube coagulase test and DNase test) to differentiate *S.aureus* from CoNS, as shown in table (2).

Table 2: Number of *S. aureus* and CoNS isolates grown on blood agar and confirmed by slide/tube coagulase and DNase tests:

Clinical isolates	Number	%
Positive *S.aureus*	75	52.8%
Positive CoNS	67	47.2%
Total number of isolates	142	100%

Table 3: Sensitivity and specificity of SaSelect and MSA for detection of *S. aureus*

	***S. aureus* isolates**				**Medium**	
	Positive 75		**Negative 67**		**Specificity**	**Sensitivity**
	True +ve	**False -ve**	**True -ve**	**False +ve**		
Sa*Select* 75 orange colonies	**75**	0	67	**0**	100%	100%
MSA 56 yellow colonies	55	20	66	1	98.5 %	73.3%
P value					> 0.999	< 0.001

As shown in table (3), Sa*Select* revealed 75*S.aureus* isolates as orange/pink colonies out of the 75 *S.aureus* isolates recovered on blood agar and confirmed by slide/tube coagulase tests and DNase test. Sa*Select* did not miss any positive isolate for *S.aureus;* moreover all the orange/pink colonies grown on Sa*Select* were proved to be *S. aureus* by slide/tube coagulase tests and DNase test; making both the sensitivity and specificity for isolating and identifying *S.aureus* using this medium after a total of 48h of incubation to be 100%.

MSA revealed 56 yellow isolates, 55 isolates of them were confirmed to be *S. aureus*by slide/tube coagulase tests and DNase test, while one isolate turned to be CoNS making the specificity of this media for isolation and identification of *S.aureus* after a total of 48h of incubation to be 98.5%. Out of the 75 positive isolates of *S. aureus* recovered on blood agar and confirmed by slide/tube coagulase tests and DNase test, MSA missed 20positive *S.aureus* isolates making the sensitivity for isolating and identifying *S. aureus* using this medium after a total of 48h of incubation to be 73.3%.

Therefore, there is a significant difference in sensitivity of detection of *S.aureus* between the two media (P. value: <0.001) while the difference in specificity was not statistically significant (P value: >0.999).

Table 4: Sensitivity and specificity of SaSelect and MSA for detection of CoNS

	CoNS isolates				**Medium**	
	Positive 67		**Negative 75**		**Specificity**	**Sensitivity**
	True +ve	**False -ve**	**True -ve**	**False +ve**		
Sa*Select* 66 Different colors colonies	**66**	1	75	**0**	100%	98.5%
MSA 54 pink colonies	53	14	74	1	98.2%	79.1%
P value					> 0.999	< 0.001

As shown in table (4), out of the 67 isolates of CoNS were recovered from blood agar plates and confirmed to be slide/tube coagulase negative and DNase negative, 66 isolates were recovered from Sa*Select* with different colors of colonies and all of them were confirmed to be slide/tube coagulase negative and DNase negative but only one sample yielded no growth. The specificity and sensitivity for isolating and identifying CoNS using Sa select medium were 100% and 98.50% respectively after 48 hours of incubation.

MSA revealed 54 pink isolates, 53 isolates of them were confirmed to be CoNS by slide/tube coagulase tests and DNase test while one isolate turned out to be *S. aureus* making the specificity of this media to be 98.2%. Out of 67 isolates of CoNS were recovered from blood agar and confirmed by slide/tube coagulase tests and DNase test, MSA missed 14 positive isolates making the sensitivity for isolating and identifying CoNS using this media after 48 hours of incubation to be 79.1%.

Therefore, there is a significant difference in sensitivity of detection of CoNS between the two media (P. value: <0.001) while the difference in specificity was not statistically significant (P value: > 0.999).

DISCUSSION

Our results are in consistence with the results of a study conducted in Microbiology &Immunology Department, Assiut University, Egypt by Hassan *et al*., [20], in which staphylococci were recovered in 77 (58.3%)

specimens out of 132 specimens collected from different ICU patients. Also, similar results were reported by a study done in Khartoum State, Sudan by Osman *et al.* [21], in which out of the 135 bacteria isolated from clinical specimens, 79 (58.5%) were identified to be *staphylococcus* species.

In the present study, differentiation between *S. aureus* and CoNS was done using slide coagulase test, tube coagulase test and DNase test. Out of the 138 staphylococci positive samples, *S.aureus* isolates were detected in 71 (51.4%) samples, while CoNS were detected in 63 (45.6%) samples with four samples (2.8%) turned up to have colonies from both types, *S. aureus* and CoNS at the same time. This finding made the number of identified staphylococci isolates to be 142.

In the current study, Sa*Select* medium revealed 141(99.3%) staphylococcal isolates out of the 142 identified staphylococci after 24h. It worth mentioning that; no further growth was detected after extending the incubation period to 48h. Therefore, Sa*Select* medium showed high sensitivity of 99.3%in detecting staphylococci directly from clinical samples after 24h incubation, compared to the gold standard conventional medium; blood agar.

Similar results were reported by Hirvonen *et al.*, [7] in which Sa*Select* showed high sensitivity (99.2%) and high specificity (99.9%) in detecting staphylococci directly from clinical samples. In Roberts &Scopes [22] Sa*Select* showed a sensitivity of 83.2% in detecting and identifying staphylococci directly from clinical samples.

In the present study, MSA detected 110 staphylococci isolates out of the 142, with no further growth after extending the incubation time to 48h.This made the sensitivity for isolating staphylococci using this media after 48h of incubation to be 77.4% compared to blood agar. There is a significant difference in sensitivity for detection of staphylococci between MSA and Sa*Select* medium (P. value: 0.000).

In the present study, Sa*Select* detected 75 *S. aureus* isolates as orange/pink colonies out of the 75 *S. aureus* isolates. Sa*Select* did not miss any positive isolate for *S. aureus;* moreover all the orange/pink colonies grown on Sa*Select* were proved to be *S. aureus* by slide/tube coagulase tests and DNase test; making both the sensitivity and specificity for isolating and identifying *S. aureus* using this medium after a total of 48h of incubation to be 100%.

Similar results were reported by Hirvonen*et al.*[7], in which the performance of Sa*Select* medium was compared to another two chromogenic media in growing and identifying *S. aureus*. Sa*Select* showed the highest sensitivity (100%) and the highest specificity (100%) among them all with no false positive results.

In the present study, MSA specificity for isolation and identification of *S. aureus* was 98.5% and the sensitivity was 73.3%. There is a significant difference in sensitivity of detection of *S. aureus* between the two media (P. value: < 0.001).

Similar result was reported by Bakr & Selim, [23] where MSA showed a low sensitivity (73.49%) and specificity of 95.45% for isolation and presumptive identification of *S. aureus* after 48h. The authors referred this low sensitivity to the high salt component of MSA that might have inhibited some staphylococci strains. Kateete*et al.*,[19] reported the sensitivity and specificity of Mannitol salt agar/DNase/tube coagulase combination to be 67% and 100% respectively after 48h of incubation.

Our results are comparable to other studies**,** who reported a low sensitivity of MSA, 71% [24] and 76.5%[25], after 48h of incubation in comparison to chromogenic media.

On the other hand, a study was conducted in Germany using well-defined strain collection of *S. aureus* isolates, Kipp *et al*.,[16] reported low sensitivity of MSA (66%) after 24h, slightly higher sensitivity (89%) of MSA for isolation of *S. aureus* after 48h but remarkably higher sensitivity (94%) only after extension of time of incubation.

In the current study, Sa*Select* detected 66 isolates of CoNS with different colors of colonies out of the 67 isolates of CoNS and only one sample yielded no growth, making the sensitivity for isolating and identifying CoNS using this media after a total of 48h of incubation to be 98.50%.

Our results are comparable to Hirvonen *et al.*[7] where Sa*Select* showed a high sensitivity of 96.6% for isolation of CoNS and reported a high specificity (99.9%) after 24h of incubation.

Also, Sa*Select* provided excellent assistance in the case of mixed growth of different staphylococci. Four specimens showed abundant growth of *S.aureus* and *S.epidermidis*, which could be distinguished only on Sa*Select* but not on conventional media after 24h of incubation. In these cases, *S. aureus* isolates did not produce the characteristic yellow pigment on MSA, however, distinctive orange/pink colonies on Sa*Select*. The colonies of *S. epidermidis* were white to pale pink. Thus, Sa*Select* provide great assistance with the differentiation of staphylococci in polymicrobial specimens.

Hirvonen*et al.*[7] showed that among the polymicrobial wound specimens containing mixed growth of *S. aureus* and *Proteus* spp., or *P. aeruginosa*, *S.aureus* was recovered and identified more quickly with Sa*Select* (after 24h of incubation) than using conventional culture (after 48 to 72h of incubation).On conventional media, rapidly growing Gram-negative bacilli covered the colonies of *S. aureus*, which could not be isolated unless sub-cultured into additional plates. It was explained that the specific chromogenic substrates in Sa*Select* allowed rapid and reliable

identification of *S. aureus*, decreasing the need for further testing.

The conventional methods are considered too slow for use in the routine clinical microbiology laboratory and because *S. epidermidis* is the predominant pathogen among CoNS, many clinical laboratories routinely report all CoNS as *S. epidermidis* without performing any biochemical testing other than a coagulase test. Clearly, this is not strictly correct and, given the emergence of CoNS as major nosocomial pathogens, may result in confusion and misinterpretation of culture results [27]. As the CoNS isolates produced different shades of pink, blue, purple, yellow, or white colonies on Sa*Select* medium, variation in species detection was observed.

In our study, other CoNS, i.e., yellow colonies for *S. lugdunensis* and *S. sciuri*, were also well differentiated from *S. aureus* by Sa*Select*. This can be useful for preliminary identification of the two species. In a global endocarditis study, *S. lugdunensis* was reported as the second most common CoNS pathogen [26].

S. saprophyticus shows characteristic bulky turquoise colonies on Sa*Select* which are easily recognizable after 24h of incubation. So, Sa*Select* can be helpful for the preliminary screening of *S. saprophyticus* in urine specimens, in case of frequent urinary tract infections.

The cost of Sa*Select* is higher than that of nonselective conventional media. However, there is some advantage gained with the use of this medium. The more rapid visualization of the specific pigmentation of *S. aureus and* CoNS allows working through cultures more quickly; therefore, slide/tube coagulase tests can be substantially reduced or eliminated[28]. In our study, the total time taken for identification of staphylococci, either *S. aureus* or CoNS, did not exceed 24 hours, while the needed time for identification of staphylococci using the conventional method could exceed 48 hours up to 72 hours in some clinical samples. MSA alone cannot be used for the identification of *S. aureus*[29]. There is no single phenotypic test (including the TCT) that can provide reliable results in the identification of *S. aureus*, and a combination of tests should be used for the correct identification of isolates.

CONCLUSION

Sa*Select* is a well performing culture medium for the primary isolation of various staphylococci showing high sensitivity & specificity, in comparison to conventional methods. Although chromogenic media may be more expensive than conventional media, their use in primary plating of specimens may have a great advantages, since the need for identification tests for various isolates decreases and presumptive results are obtained sooner. Sa*Select* was highly sensitive and specific especially for isolation of *S. aureus.*

Although the sensitivity and specificity of Sa*Select* for detection of, e.g. *S. epidermidis*, *S. intermedius*, *S. saprophyticus* proved to be high, the requirement for additional identification tests could not be excluded.

Recommendations:

- Larger number of specimens as well as larger number of isolates should be used to confirm the use of Sa*Select* in the following settings:
- Isolation & identification of staphylococci directly from clinical samples instead of conventional methods.
- Isolation & identification of staphylococci in case of polymicrobial specimens.
- Screening for staphylococci in case of urinary tract infections.
- Screening for staphylococci in case of endocarditis.
- In case of CoNS, further evaluation of their isolation & identification is highly recommended through using more strains of CoNS and combining that with applying further tests on a reasonable number of CoNS strains to confirm their species.

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