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Original Article

Evaluation of CHROMagar and Pastorex Test in Identification of *Staphylococcus aureus*

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ABSTRACT

Background: Diagnosis of *Staphylococcus aureus* (*S. aureus*) is very important to help in treatment of different clinical conditions. Rapid detection tests are the aim of many microbiological laboratories.
Aim of Work: This study aimed to compare the efficacy of CHROMagar staph aureus media (CSAM) to that of conventional media in the detection and identification of *S. aureus*.
Patients and Methods: This study was carried out on 50 individuals attending surgical and internal medicine departments, Ain Shams University Hospitals, in the period from October 2007 to December 2008. They were suffering from different pyogenic infections. All samples were cultured on conventional media (Columbia blood agar and chocolate blood agar) and on CSAM. Isolated colonies were identified by catalase, coagulase (tube and slide) and Pastorex Staph plus agglutination test. Antibiotic sensitivity test was performed for all *S. aureus* isolates using the disc diffusion method.
Results: CSAM revealed better detection of *S. aureus* (90%) than the conventional culture media (80%). Pastorex Staph plus agglutination test had higher specificity and sensitivity than coagulase test. In addition, *S. aureus* isolates were able to resist many antibiotics.
Conclusion: CSAM is recommended for detecting *S. aureus* especially in cases of mixed infections. A higher sensitivity obtained when CSAM is followed by Pastorex Staph plus

Keywords: CSAM, pastorex staph plus, conventional media, *s. aureus*, identification, antibiogram

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INTRODUCTION

S. aureus is a Gram positive pathogen that causes severe suppurative infection, therefore its isolation from infectious lesions is necessary but it may be missed when clinical sample is mixed with flora as well when the colonies masked by swarming *Proteus* or *Pseudomonas* colonies *(Carricajo, et al. 2001)*. CHROMagar *Staph. aureus* media is a chromogenic media incorporating chromogenic enzymatic substrates and a variety of antimicrobial agents, have become available for detection of *S. aureus*, including methicillin-resistant strains *(Louie, et al. 2006)*. *Staphylococcus aureus* can grow on CSAM (Chrom agar microbiology, Pari, France). Unlike colonies of other *staphylococcus* species, the *S. aureus* colonies are pink, which yields higher detection rate with better sensitivity than conventional media *(Gaillol, et al. 2000)*. The Pastorex Staph-Plus latex agglutination test (Bio-Rad, Marnes-la-Coquette, France) is a rapid latex agglutination test, based on the detection of clumping factor, Staphylococcal protein A and capsular polysaccharides. The latex agglutination reagent was mixed with either colonies taken from blood agar subcultures or pink colonies grow on CSAM *(Personne, et al. 1997; Compernolle, et al. 2007)*.

Antimicrobial susceptibility testing can be performed by disc diffusion method on colonies directly isolated from CSAM *(Carricajo, et al. 2001)*.

AIM OF THE WORK

This study was conducted to compare between efficacy of CSAM and conventional media in detection and identification of *S. aureus*.

SUBJECTS AND METHODS

A total of 50 individuals, (26 males and 24 females) attending the surgical and internal medicine departments, Ain Shams University Hospitals in the period from October 2007 to December 2008, were enrolled in this work. They were suffering from different infectious lesions as surgical wound infection (21), skin lesions (10), burn (6), sore throat (5), lower respiratory tract infection (4) and urinary tract infection (4).

Every patient was subjected to full history taking besides, thorough clinical examination and laboratory investigations e.g. hemoglobin assay and white blood cell count.

Clinical specimens were collected according to site of lesions e.g. swabs (from skin lesions, burn, sore throat and surgical site infections), urine, sputum, bronchoalveolar lavage, tracheal or gastric aspirates from pneumonic patients. All samples were examined microbiologically by Gram's stain to detect morphology of staphylococci. Thereafter, all samples were cultured aerobically on Colombia blood agar, Chocolate agar and CSAM.

CSAM is a selective and differential medium designed for the detection and identification of *S. aureus* without the need of further testing.

Composition (g/L): Agar 15, peptones and salts 75 and special chromogenic mix 2.5.

The formulation includes selected peptones; as nutrients and mixture of chromogenic substrates that release an insoluble colored compound when hydrolyzed by specific enzymes released from *S. aureus* leading to growth of the microorganism in pink colonies. The addition of selective agents to the medium inhibits growth of *S. epidermidis*, gram negative bacteria and yeasts.

Samples were cultured on plates and incubated aerobically at 37°C for 24-48 hrs.

S. aureus suspected colonies on (chocolate agar and blood agar) and pink colonies (on CSAM), were identified by Gram's stain, catalase test, sugar fermentation tests, coagulase tests (slide and tube coagulase tests) and Pastorex Staph plus latex agglutination test.

Pastorex Staph plus latex agglutination test (code 56356) composed of: Fifty test kit , one dropper bottle of 1ml of red latex sensitized by fibrinogen, IgG monoclonal antibodies directed against capsular polysaccharides of *S. aureus*,0.02% sodium merthiolate and less than 0.1% sodium azide.

Negative control composed of: One dropper bottle of 1ml of negative control reagent of red latex sensitized by bovine albumin solution contains 0.02% sodium merthiolate and less than 0.1% sodium azide.

Sixteen disposable agglutination cards and 150 rods were also supplied.

Procedure: Latex reagents were homogenized by shaking. A drop of latex test reagent was deposited into one of the circles of the agglutination card and a drop of negative control latex reagent was dropped in another circle. From the Gram positive, catalase positive colonies 1-3 colonies taken by loop or plastic stir rod and emulsified in the drop of latex agent for 10 seconds. The same was repeated for the negative control latex. Homogenization was done by gentle rotation of the card. Results were recorded within 30 seconds of beginning the card rotation.

Positive reaction detected by formation of aggregates with the reagent test only, visible to the naked eye under normal lighting within 30 seconds of beginning the card rotation. While negative reaction detected as the suspension did not produce any aggregates and retains its milky appearance.

Antimicrobial Susceptibility Test: Different commercially prepared discs (6mm in diameter) with different antibiotic contents (Oxoid-England) were used; Ampicillin (10µg), penicillin G (10 IU), Amoxicillin/clvaulonic 2:1 (30µg), cephradine (30µg), fusidic acid (10µg), Methicillin (5µg), Erythromycin (15µg) and vancomycin (5µg).

Colonies of the isolated strains of *S. aureus* were picked up by the loop and then suspended in saline to make suspension equivalent in density to opacity standard (McFarland standard 0.5) (1 x 10^8 CFUmL). Sterile swab was inoculated into the suspension and squeezed from excess fluid against the side of the tube and then rubbed over plate of Muller Hinton agar. After period of diffusion of two hours at 4°C, the agar plates were incubated overnight at 37°C. Antimicrobial effect was assessed by measuring the diameter of the growth inhibition zone. Results were interpreted according to clinical laboratory standard institute *(Cheesbrough, 2004)*.

Statistical Analysis:

Data were analyzed using SPSS 10 for Windows (Statistical Package for the Social Sciences).

1. Descriptive statistics: Mean standard deviation, minimum, maximum and range of numerical data and the frequency and percentage of non-numerical data.
2. Chi square test to compare between two groups regarding non numerical variables.
3. Wilcoxon signed rank test (Z) was done for non parametric comparison between two non numerical variables.

RESULTS

Fifty specimens were collected from 50 patients were suffering from different infectious diseases (26 males and 24 females).

S. aureus were isolated from 45 of the 50 (90%) clinical specimen using CSAM with non-statistically significant difference between different clinical samples using Chi square test (Table 1). Forty (40) out of the 45 CSAM isolates were detected after incubation for only 24 hours, while the other 5 isolates were detected after 48 hours. On the other hand, *S. aureus* detected in 40 out of 50 specimens (80%) cultured on conventional media (Columbia blood agar and chocolate agar). There was no statistically significant difference in sensitivity between CSAM and conventional media using Wilcoxon signed rank test (Table 2).

Fourty four isolates were positive for Pastorex Staph. chrom coagulase test, while 43 isolates of them were positive by slide and tube coagulase test. The Pastorex Staph chrom coagulase test revealed 100% sensitivity, specificity of 85.71%, positive predictive value (PPV) of 97.73% and negative predictive value (NPV) was 100%, as shown in Table(3).

S. aureus isolates were highly sensitive to cephradine, fusidic acid and vancomycin with sensitivity percentages of 95%, 92.5% and 87.5%, respectively. The sensitivity to Amoxicillin + clavulonic acid and Erythromycin was equivocal (50%). On the other hand, penicillin and Ampicillins showed the lowest sensitivities (10% and 15%, respectively).

Table 1: Percentage of *S. aureus* from clinical specimens on CSAM.

Clinic al specimens	Positive results Chrom agar *Staph. aureus* medium	Negative results Chrom agar *Staph. aureus* medium	Total	χ^2
Surgical wound infections	20	1	**21**	
Skin infections (boils)	10	—	**10**	
Burn infections	5	1	**6**	4.603
Urinary tract infections	3	1	**4**	P: 0.466
Respiratory tract infections	3	1	**4**	Non significant
Sore throat	4	1	**5**	
Total	**45 (90%)**	**5 (10%)**	**50**	

Table 2: Sensitivity of conventional media Vs CSAM.

Clinical specimens	Conventional media	Chrom agar *Staph.aureus* media	Z
Surgical wound infections	18	20	
Skin infections (boils)	9	10	
Burn infections	5	5	
Urinary tract infections	2	3	
Respiratory tract infections	3	3	
Sore throat	3	4	
+ve culture	40 (80%)	45 (90%)	1.604
-ve culture	10(20%)	5(10%)	P=0.109
Total culture	**50 (100%)**	**50 (100%)**	NS

Table 3: Coagulase (slide and tube) Vs Pastorex Staph plus tests.

	Slide and tube coagulase test (+ve) (n=43)	Slide and tube coagulase test (-ve) (n=7)
+ve Pastorex coagulase test	43 (100%)	1 (14.29%)
…….	……	……
-ve Pastorex coagulase test	0 (0%)	6 (85.71%)
P	<0.001	
Significance	HS*	
Sensitivity	100%	
Specificity	85.71%	
PPV	97.73%	
NPV	100%	

Table 4: Antibiotic sensitivity pattern of *Staphylococcus aureus* positive specimens.

Antibiotic	*Staph. aureus* strains			Total
	Sensitive	Intermediate	Resistant	
Ampicillin	6 (15%)	2 (5%)	32 (80%)	**40**
Penicillin	4 (10%)	1 (2.5%)	35 (87.5%)	**40**
Amoxicillin + clavulonic acid	20 (50%)	1 (2.5%)	19 (47.5%)	**40**
Cephradine	38 (95%)	1 (2.5%)	1 (2.5%)	**40**
Fusidic acid	37 (92.5%)	1 (2.5%)	2 (5%)	**40**
Methicllin	12 (30%)	4 (10%)	24 (60%)	**40**
Erythromycin	20 (50%)	8 (20%)	12 (30%)	**40**
Vancomycin	35 (87.5%)	1 (2.5%)	4 (10%)	**40**

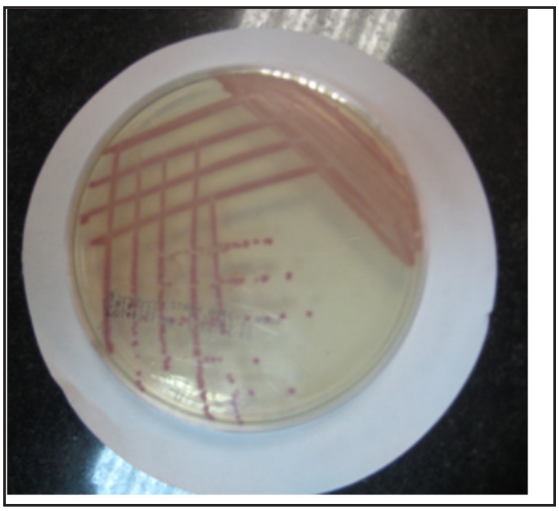

Figure 1: *S. aureus* on CSAM appear as pink colonies.

DISCUSSION

S. aureus causes severe suppurative infections associated with high morbidity and mortality. CSAM enables easier detection of *S. aureus* by their pink color *(Carricajo, et al. 2001)*. Pink colonies grown on Chrom agar Staph aureus media can be rapidly confirmed to be *S. aureus* by using agglutination kits, such as Pastorex Staph plus which simultaneously detects the clumping factor, protein A and capsular antigens of *S. aureus (Carricajo, et al. 2001)*.

This study was carried out on 50 patients (26 males and 24 females) suffering from different infectious diseases e.g. surgical wound infection (21), skin lesions (10), burn (6), sore throat (5), respiratory tract infection (4) and urinary tract infection (4). *S. aureus* isolates were detected in 40 out of 50 specimens (80%) cultured on conventional media (Columbia blood agar and chocolate agar). While, it was detected in 45 specimens (90%) cultured on CSAM with a non-statistically significant difference in sensitivity between them. Forty out of the 45 *S. aureus* isolates grown on CSAM were detected after incubation for 24 hours, while the other 5 isolates were detected after 48 hours. Thus it could be suggested that longer incubation of CSAM plates may reveal more sensitive results. On comparing the results obtained from culture on conventional media (80%) versus (90%) on CSAM, it is clear that sensitivity of CSAM is higher but the small sample size made the difference non-statistically significant. These results were in accordance with the recorded by *Carricajo et al. (2001)*, who reported that the sensitivity of CSAM and conventional media were 98.5% and 91.8%, respectively. Besides, CSAM improved the ability to detect *S. aureus* by recovering 12 isolates missed by conventional media as recorded in the study of *Flayhart et al. (2004)*. Overall, the sensitivity and specificity of CSAM in their study were 99.5 and 98%, respectively.

In addition, *Perry et al. (2003)* compared the *S. aureus* ID (a chromogenic agar medium for detection of *S. aureus*) to CSAM and conventional media in detection of *S. aureus*. After 18-20 hours of incubation, 96.8% of strains formed green colonies on *S. aureus* ID compared with 91.1% of strains forming pink colonies on CSAM. A total of 94.3% of strains were recovered within 18-20 hours with conventional media. The sensitivity was increased after 48 hours of incubation to be 98.7, 96.2 and 95.6% for *S. aureus* ID, CSAM and conventional media, respectively. As well, *Denis et al. (2003)* evaluated the performance of CSAM for isolation of methicillin resistant *S. aureus* (MRSA) from surveillance swabs in 860 patients. MRSA strains were isolated from CSAM (n = 60) and blood agar (n=59), respectively. The sensitivities of the different agars were 72% for CSAM and 46% for blood agar, with a non-statistically significant difference. The median times to MRSA identification were two days for blood agar (range 2-4 days) and three days for CSAM (range 2 to 4 days). They concluded that CSAM is convenient for MRSA detection from surveillance culture, but its performance seems similar to conventional Columbia blood agar medium.

The current study showed that (44) specimens were positive for Pastorex Staph. chrom coagulase test, while (43) specimens of them were positive by slide and tube coagulase test. The sensitivity of Pastorex Staph chrom coagulase test was 100%, specificity was 85.71%, positive predictive value (PPV) was 97.73% and negative predictive value (NPV) was 100%. In this regards, *Compernolle et al. (2007)* found that the Pastorex chrom agglutination test was faster and more specific than other two chromogenic tests in the detection of MRSA. In addition, *Fonsale et al. (2004)* reported 98.1% sensitivity and 100% specificity in identification of *S. aureus*

by Staph Chrom tests in comparison to other tests. In this work, the antibiotic sensitivity pattern of *S. aureus* strains isolated from patients was performed using disc diffusion method. *Staph. aureus* showed 95% sensitivity towards cephadrine and 92.5% to fusidic acid, while showed 60% resistance against methicillin. *S. aureus* isolates resisted penicillin in a percentage of 87.5% and 80% agaist Ampicillin. Besides, the MRSA were 60% which is more than the observed (40%) by *El-Gendi et al. (2004)*, which means that MRSA strain is increasing in our community. In other researches, *Compernolle et al. (2007)* found MRSA in 72% and *Van Griethuysen et al. (2001)* reported MRSA as one third of *S. aureus* isolated in their study. This difference in results is due to difference in communities as well points to the antibiotics-misuse in the Egyptian community.

CONCLUSION

CSAM is excellent in detecting *Staph. aureus* especially in cases of mixed infections. Despite being costy, CSAM is still precious in emergency infection for rapid and easy diagnosis especially in MRSA, oxicillin resistant *S. aureus* and vancomycin resistant *S. aureus* strains (VRSA). CSAM plates may give more sensitive results if incubated for 48 hours.

Pastorex Staph plus is a rapid test, reliable and easy to use. Its perfect sensitivity makes it suitable as an accurate test for *S. aureus* identification in the clinical laboratory than conventional (slide and tube) coagulase test. When CSAM is followed by Stpah Chrom coagulase testing (Pastorex Staph plus), it yields better results.

RECOMMENDATION

So we recommend the use of CSAM and Pastorex Staph plus agglutination test for rapid and easy diagnosis of *S. aureus* infections in critical cases especially in mixed and hospital acquired infections.

In addition, further researches to assess CSAM and Pastorex Staph plus agglutination test for MRSA and VRSA are required.

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ملخص البحث

تقييم الوسط اللونى واختبار باستروكس فى تشخيص البكتريا العنقودية الذهبية

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تسبب البكتريا العنقودية الذهبية العديد من الأمراض الصديدية مثل التهاب الحلق و الألتهاب الرئوى و عدوى الجروح والتهاب المسالك البوليه وعدوى المستشفيات وغيرها من العدوى التى قد تصل إلى حد الوفاة. تشخيص البكتريا العنقودية الذهبية شديد الأهمية حيث يترتب عليه تحديد المضاد الحيوى المناسب. وقد تعددت الدراسات الخاصة بتشخيصها ومن أحدثها استخدام الوسط اللونى (CSAM) الذى يعطى اللون الوردى (pink) للبكتريا العنقودية الذهبية وكذلك اختبار التلزن اللونى (Pastorex Staph plus).

الهدف من الدراسه: أجريت هذه الدراسة لمقارنة قدرة وحساسية الوسط اللونى بالمزارع العادية لعزل والتعرف علي البكتريا العنقودية الذهبية.

الأشخاص و طر ق البحث: أجريت هذه الدراسة من أكتوبر ٢٠٠٧ إلى ديسمبر ٢٠٠٨ على خمسين شخصاً تضمنت ٢٦ رجلا و٢٤ سيدة ترددوا على أقسام الأمراض الباطنيه و الجراحه و كانوا يعانون من أمراض صديدية مختلفه. أخذت عينات من صديد جروح هؤلاء المرضى أو مسحات من الحلق أو من البول أو من البصاق. زرعت كل عينة على الآجار الدموى الكولومبى (Columbia blood agar) والآجار الشيكولاتى (Choclate blood agar) وآجار الوسط اللونى(CSAM).

تم تحديد البكتريا العنقودية باستخدام: صبغة الجرام، اختبار التخمر السكرى، اختبار التلزن اللونى (Pastorex Staph plus tests) ، الاختبار التجميعى (Coagulase) و اختبار حساسية للمضادات الحيوية المختلفة.

النتائج: قد أسفرت الدراسة عن أن مزارع الوسط اللونى (CSAM) أكثر حساسية من المزارع العادية، حيث أن البكتريا العنقودية الذهبية تظهر بلون وردى واضح بين مستعمرات المزارع الأخرى. حيث تم عزل ٤٥ حاله (٩٠٪) على مزارع الوسط اللونى(CSAM) فى مقابل ٤٠ حاله (٨٠٪) على المزارع العادية. كما أسفرت الدراسة عن ارتفاع معدل حساسية التلزن اللونى (Pastorex Staph plus tests) عن الاختبار التجميعى العادى الروتينى. أسفرت المزرعه لأختبار حساسية المضادات الحيوية أن البكتريا العنقودية الذهبية لها مقاومة لمعظم المضادات الحيوية.

الخلاصه: يوصي بأستخدام مزارع الوسط اللونى(CSAM) متبوعاً باختبار التلزن اللونى (Pastorex Staph plus tests) لتشخيص البكتريا العنقودية الذهبية خاصة فى حالات العدوى بأكثر من ميكروب و فى حالات عدوى المستشفيات.