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Optimization the Best Methods of *Staphylococcus Aureus* Identification in Pregnant Women

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Abstract

Many bio-chemical and molecular assays should be used to obtain the right identification of *Staphylococcus aureus*. Molecular assay is the best method for identification. The aim of this study was to optimize the best methods of Staphylococcus *Aureus* identification in pregnant women. This study has been done by collection of 100 samples from pregnant women (aged from 20- 30 years) were collected from Al-Karama Teaching Hospital and Al- Zahra Teaching Hospital. Only 50 (0.5 %) clinical samples of positive culture bacteria for *Staphylococcus aureus* by using different biochemical and molecular tests. PCR assay was the best method for identification. Usually, many assays should be done to ensure the right identification and exclude the false positive results.

Keywords: Staphylococcus aureus, Pregnant Women, UTI

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تحديد أفضل طرق التعرف على المكورات العنقودية الذهبية لدى النساء الحوامل المصابات بالتهاب المجاري البولية

أيات علي محمود [1]

المستخلص

تشير التجربة الحالية الى انه يجب استخدام العديد من الاختبارات البيوكيميائية والجزيئية للحصول على التشخيص الصحيح للمكورات العنقودية الذهبية. الفحص الجزيئي هو أفضل طريقة للتشخيص. تهدف الدراسة الحالية الى عزل وتشخيص بكتريا المكورات العنقودية من ادرار النساء الحوامل المصابات بالتهابات الجهاز البولي. كما تهدف هذه الدراسة ايضا الى توضيح اهم وادق طريقة للتشخيص والتي تكون نسبة التشخيص الخاطئ فيها قليلة اذا ما قورنت بالطرق الاخرى. تمت هذه الدراسة من خلال جمع 100 عينة من النساء الحوامل (من سن 20- 40 سنة) تم جمعهم من مستشفى الكرامة التعليمي ومستشفى الزهراء التعليمي. استخدمت طرق بيوكيميائية وجزيئية لغرض تحديد وتشخيص المكورات العنقودية. اوجدت النتائج ان 50 (0.5%) عينة سريرية فقط من البكتيريا إيجابية الزرع للمكورات العنقودية الذهبية باستخدام الاختبارات البيوكيميائية والجزيئية المختلفة. كما كان فحص PCR أفضل الطرق لتحديد بكتريا المكورات العنقودية. عادة، ينبغي إجراء العديد من الاختبارات لضمان التحديد الصحيح واستبعاد النتائج الإيجابية الخاطئة

الكلمات المفتاحية: بكتريا المكورات العنقودية، التهاب المجاري البولية، النساء الحوامل

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معلومات البحث
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1. Introduction

Staphylococcus aureus is a gram-positive, cocci grape-shaped, naturally occurring bacterium that can cause infections. It has different virulence factors that related to its pathogenesis [1]. These virulence factors include antigens (microcapsule), enzymes (Coagulase, Lipase Catalase, DNase, Hemolysins, Leukocidins, Proteases) and toxins like: enterotoxins, exfoliative toxins, and which are increasingly seen among pregnant ladies [2,3]. Adverse effects of UTI treatment in both pregnant mother and the embryo represent the biggest challenge. Pregnancy-related urinary tract infections (UTIs) are prevalent in pregnant women, and these infections vary from lower to upper UTIs [4]. It usually lives on the skin and the upper respiratory

tract [5]. S .aureus is a dangerous pathogen, which causes many clinical manifestations. It is common in hospital and community and its treatment is so difficult because it has the ability for multi-drug resistant such as Methicillin-resistant Staphylococcus aureus MRSA [6]. However, S. aureus has many types of virulence factors that include the cytolysis toxins, enzymes and enterotoxins [7], Cytotoxic enterotoxins could kill cells by changing the apical membrane permeability of the mucosal (epithelial) cells of the intestinal wall. They are mostly pore-forming toxins (mostly chloride pores), secreted by bacteria, that assemble to form pores in cell membranes. This causes the cells to die [8,9,10]. This study aimed to optimize the best methods of *Staphylococcus Aureus* identification in pregnant women infected with UTI.

2. Materials and Methods

2-1. Samples collection: - 100 urine samples collected from pregnant women who had UTIs were analyzed. These samples were used to isolate and identify *S. aureus*. Many different biochemical and molecular tests were used to optimize the best methods for *S. aureus* identification. These methods include Manitol salt agar (MSA), The tube coagulase test, CHROMagar™ Staph aureus, Dnase test, In addition, molecular diagnoses methods, including conventional PCR, were also used.

2-2. Bacterial DNA extraction: Bacterial DNA was extracted by Presto ™ Mini gDNA bacterial kit. Bacterial colonies were firstly cultured and activated on blood agar and then they cultured on Manitol salt agar for isolation. Loop-full was taken and suspended in heart brain infusion agar (5ml in tube) and incubated for 24h in 37C. Then Presto ™ Mini gDNA bacterial kit, Geneaid was used for DNA extraction. The solutions must be prepared before starting DNA extraction, these solutions were prepared according to Presto ™ Mini gDNA bacterial kit.

2-3. Primer of Human *Staphylococcus aureus* Enterotoxin Genes: - Primer of human *Staphylococcus aureus* enterotoxin genes (Bioneer, Korea) that utilized in this study [11].

3. Results and Discussion

In this presented study, 100 samples from pregnant women (aged from 20-40 years) were collected. These samples were isolated from pregnant women patients with UTI and identified by different biochemical tests. These results are shown in Table (1).

Table (1):- Biochemical and molecular tests of Staphylococcus aureus

	Bio-molecular tests	Positive results	
1	Manitol salt agar (MSA)	50+	
3	Tube coagulase test	50+	
4	Chromogenic agar	50	
5	DNase agar	50	
8	PCR	50	

(+):- More than 50 clinical samples give positive result in this test

All the 50 clinical samples of positive results of *S. aureus* in this presented study were considered as a *S. aureus* after give a positive result for all tests that were utilized in this study. Therefore, all these samples (50) that give one or two negative results for any test were excluded in this study.

Despite MSA was considered as a selective media for *Staphylococcus aureus* identification, but MSA fermentation usually must be followed by other identification methods of *Staphylococcus aureus*. Therefore, other biochemical identification methods must be used because this media might give a false positive result [12]. MSA may give a high false positive for identification *S. aureus* due to the misuse of various classes of antibiotics in emergency cases and thereby contribute to increased resistance[13,14]. The tube coagulase test (TCT) result is also +50 samples and giving variable results.

S aureus and *S intermedius* are coagulase positive. Other studying was showed that TCT may give false positive result, so this test should be done in duplicate to be ensured for the right result [15]. TCT was depended on many parameters such as age, gender, clinical information, hospitalization departments [16]. On other hand, another study also found that coagulase test will never make a diagnostic error [17]. On the other hand, the coagulase test is not 100% sensitive and the negative predictive value is lowered. While on the other hand, some researches were showed that TCT result (99.2%) with 100% specificity [18,19]. Dnase test also was considered one of biochemical test that was used in this presented study. This test gave 50 samples as showed in Figure (1). *S aureus* was identified by their ability to produce deoxyribonuclease enzyme leading to making halo zone around the growth culturing. In another study, three types of biochemical test (coagulase, deoxyribonuclease and DNase) were used for identification of *Staphylococcus aureus* which demonstrated that (1%) coagulase-positive isolates were DNase-negative and (18 %) coagulase negative isolates were DNase-positive [20]. Also some research concluded that the best test for phenotypic 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 [21]. 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. In other study, some strains of *staphylococci* could produce positive deoxyribonuclease- such as *Staphylococcus epidermidis* strains [22,23]. As referred above, other commercial tests included MAS, TCT and DNase tests may give a false positive result, so many other methods should be used for right identifications [24].

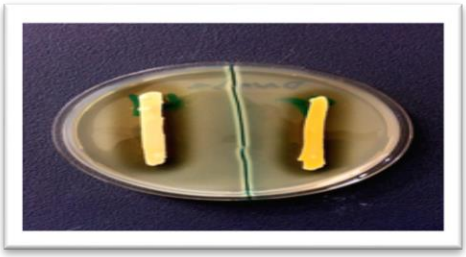

Figure (1): shown the clear zone around the colonies due to the ability of *S. aureus* to produced Dnase enzyme

The final biochemical test that had been used in this study was chromogenic agar. Generally, this new selective media was developed to facilitated identification of many types of bacteria. Chromogenic detection medium firstly developed in 1079 for *E. coli* and later Rambach™ Agar for *Salmonella* detection. Each type of bacteria has a specialized type of chromogenic media and give a different color of colonies such as *Escherichia coli* (Green-blue colonies), *Salmonella* (red colonies) while *staphylococcus aureus* was given rose to mauve colonies on CHROMagar™ Staph aureus and blue to colorless for other types [25]. This recent study was trying to use many biochemical methods for reaching to the right identification and avoiding misidentification. For this reason, CHROMagar™ *Staph aureus* as was showed in Figure (2) which were giving only 50 samples without giving false positive results (only 50 samples, not variable results).

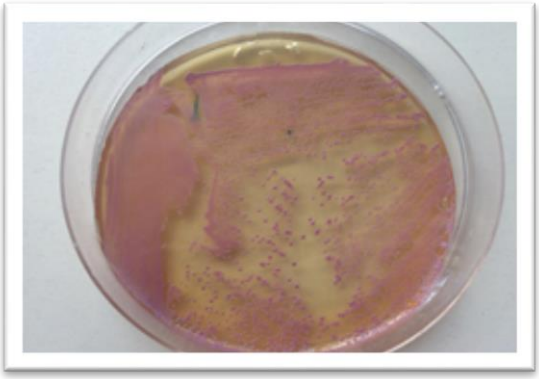

Figure (2): shown the mauve colonies of *S. aureus* on CHROMagar Staph

Molecular diagnoses methods: - Genomic DNA extraction form bacterial cells using Gram-Positive Bacteria protocol by genomic DNA purification kit (Bioneer/ Korea). Amplification of essential gene regions by **Conventional PCR for detection of enterotoxin.** The positive culture of *S. aureus* are 50 samples out of 100, these results were depended on PCR result for its specificity and accuracy. As showed in Figure (3), gel electrophoresis PCR of *Staphylococcal* enterotoxins for one strain. In this figure, one strain of *S. aureus* could produce more than one enterotoxin**.** The gel electrophoresis results showed: - sea (127pb), sec (271bp), sed (319bp) and tsst (455bp) [26,27,28].

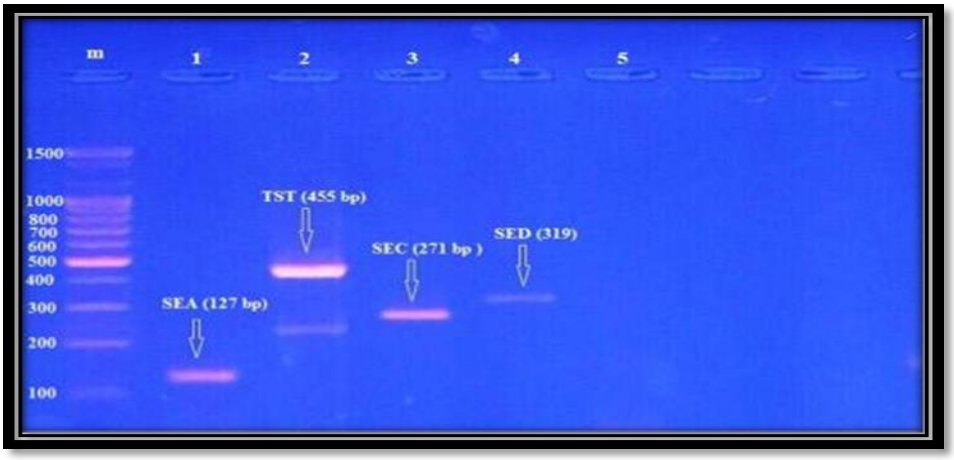

Figure (3): The gel electrophoresis PCR of staphylococcal enterotoxins for one strain

Conclusions

In this study, conclusion could be listed as following points: -

- Identification of *staphylococcus aureus* must be done by both biochemical, molecular (PCR) and by immunological tests such as ELISA assay.
- MSA may give false positive result, so this test should be followed by other biochemical and molecular tests.
- TCT, chromogenic test gives the right identification.
- TCT should be done in duplicate manner for ensuring.

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