Comparison between Cefoxitin disk diffusion, Crome agar and EPI-M Screening Kit for Detection of Methicillin- Resistant *Staphylococcus aureus*

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Abstract

Specimens have been collected from one hundred and seventeen patients residing in local hospitals, 33 with burns and 84 wound injuries,. Three different methods ,Cefoxitin disk diffusion, EPI-M Screening Kit and Crome agar (MeReSa agar)with selective supplement were used to detect methicillin-resistant *Staphylococcus aureus* (MRSA) . A comparison was made between these 3 methods according to the results. It was found that the results of the Cefoxitin disk diffusion test were compatible with the results of culturing on Crome agar, while those obtained from the EPI-M Screening kit were not accurate and some of them gave false negative results.

Key words: MRSA, Burns, wounds, EPI-M, Cefoxitin, MeReSa agar

المقارنة بين ***Cefoxitin disk diffusion*** ,***Cromeagar*** و ***EPI-M Screening kit*** لاجل التحري عن بكتريا المكورات العنقودية المقاومة للمثسلين

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الخلاصة:

تم جمع العينات من 117 شخص راقدين في المستشفيات المحلية، 33 مصاب بحروق و 84 شخص مصاب بجروح. استخدمت ثلاثة طرق*Cefoxitin disk diffusion*،*EPI-M Screening Kit* و *Crome agar (MeReSa agar)* مع الملحق الخاص بالوسط، للتحري عن بكتريا المكورات العنقودية المقاومة للمثسلين ***(MRSA)*** وقورنت هذه الطرق بالاعتماد على نتائج استخدام كل طريقة. لقد وجد ان نتائج طريقة *Cefoxitin disk diffusion* و الزرع على وسط *Crome agar* كانتا متطابقتين، بينما النتائج الحاصلة من استخدام *EPI-M Screening kit* لم تكن دقيقة و اعطت لقسم منها نتائج سلبية خاطئة.

1. Introduction

The genetic plasticity of *S. aureus* has resulted in the emergence of varying degrees of antibiotic resistance and virulence patterns [1].In the 1950s many strains of *S. aureus* produced penicillinases to overcome penicillin. Methicillin, a ß-lactam drug, was introduced thereafter and became available in the late 1950s as a drug of choice to treat penicillin-resistant Staphylococcal infections. One year after the launch of methicillin Methicillin

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resistant *S.aureus* (MRSA) has emerged as nosocomial pathogens in the early 1960s [2]. Resistance to most ß-lactams antibiotics in staphylococci is mediated by an altered penicillin-binding protein, PBP, which is encoded by *mec* genes [3,4]. The ß-lactams antibiotics including penicillin, methicillin, and even cephalosporins are ineffective if the bacterium possesses *mecA* gene.Many risk factors were associated with acquiring MRSA infection such as prolonged hospitalization and antimicrobial therapy, nasal colonization has also been identified as a risk factor for infection and carriage of MRSA in various healthcare settings [5] .Infections caused by methicillin- or oxacillin- resistant *Staphylococcus aureus* (MRSA) are widespread in most countries[6,7] because hospital and clinic environments are fertile grounds for microorganism proliferation, often breeding bacterial species highly resistant to mainstream antibiotics. Nosocomial infections contribute to unacceptable morbidity and mortality in health care settings [8].Immunocompromised patients are much more susceptible to invasive form of infection[9]it has become a major public health issue, with concern expressed by patients and members of public about the clinical implications [10].Hospital employees' hands are an important route of transmission of MRSA [7,11].Studies have shown that some MRSA isolates can indeed survive in dust or on synthetics for more than 5 weeks [12,13].

2. Material and Method

Specimens collection

Clinical specimens were collected from 117 burn and wound injuries patients hospitalized in Al-Kindy teaching hospital and Medical city complex-burn specialized hospital by using sterile cotton swabs with Amies transport medium. Amies transport medium was used to preserve the viability of microorganisms in the specimen without allowing multiplication [14].

Isolation and Identification

All samples were inoculated on the blood agar(LAB/U.K) , incubated at 37ºC for 24 hours, the isolates that suspected to be *Staphylococci* were transferred to mannitol salt agar(HiMedia/India) ,which is considered as selective and differential media, it was used for purification and identification of *Staphylococcus aureus* by their ability to ferment mannitol to form acidic products that lower the pH of the medium, when this occurs, the phenol red pH indicator in the media turns from red to yellow [15]. Gram stain, Catalase and DNase tests were performed , the isolates which gave positive results in all these tests were subjected to Coagulase tube test for identification of *S. aureus* before starting with the three methods; Cefoxitin disk diffusion (Bioanalyse/Turkey), EPI-M Screening Kit (HiMedia/India) and Crome agar (HiMedia/India) for MRSA identification. 27 isolates which were gram positive ,cluster shape microscopically , gave positive results to catalase and DNase tests. Twenty two were coagulase positive and the other five were coagulase negative. 19 isolates (17 are coagulase positive and 2 coagulase negative) were then randomly chosen to test the accuracy of the three test used for the identification of MRSA.

3. Results and Discussion

11isolates out of 33 burn cases and 16 isolates out of 84 wound injuries cases were *S. aureus* according to morphology and biochemical tests, five *S. aureus* strains found in wounds gave negative results with coagulase tube test, they may be atypical strains with absence of coagulase enzyme [16,17] The percentage of *S. aureus* in burns specimens 33.3%($11/33 \times 100$)was greater than that in wounds specimens 19% ($16/84 \times 100$). Burns units in hospitals have become major reservoir for *S. aureus* that have the special characteristics for spreading quickly in hospital environment, that's why percentage of *S. aureus* in burn injuries is greater than in wound injuries[18].

19 out of 27 *S. aureus* isolates were detected and confirmed to be MRSA by using three different methods (table 1).Ten isolates of coagulse positive *S. aureus*(numbered 1-10) were isolated from burn injuries , while seven coagulase positive *S. aureus* (numbered 11-17)were isolated from wounds and number 18 and 19 were coagulase negative found in wound injuries. The isolates were activated by the enrichment medium Brain heart infusion broth (BHIB) (HiMedia/ India) before performing the three tests and it is found that this has an effect on the results of Crome agar through giving green colour instead of bluish-green (Figure 2 A), and give false negative results with EPI-M Screening Kit (Figure 1 A). Therefore, the enrichment medium was replaced by Tryptone Soya broth (HiMedia/ India) because the trypton soya broth is more luxurious enrichment medium than the brain heart infusion broth

according to the information provided by HiMedia Laboratories .This change of the enrichment media, gave the corresponding positive results shown in figure 2B (bluish-green colour) and figure 1B

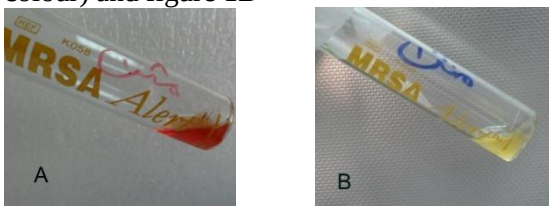

Figure 1- EPI-M Kit (A) Negative result (B) Positive result

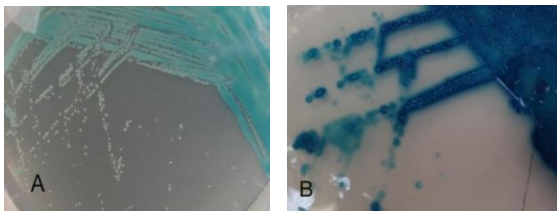

Figure 2- MRSA on HiCromeMeReSa (A) Isolates activated by BHIB, (B) Isolates activated by trypton soya broth

In the Cefoxitin disk diffusion test the diameters of inhibition zones for 19 *S. aureus* isolates were less or equal to 21mm and they were considered as oxacillin-resistant isolates according to CLSI,M100-S17, 2007 [19].

EPI-M Screening Kit gave positive results and false negative results when BHIB was used. When the activation was replaced by trypton Soya broth, some of the false negative results have changed to positive results (Figure 1 B), this gave an indication that the EPI-M Kit is not accurate.

Table 1- Three tests for MRSA identification

Isolates	Cefoxitin disk diffusion (Oxacillinresistant)	EPI-M ScreeningKit	Crome agar
***S.aureus*1**	+	+	+
***S.aureus* 2**	+	+	+
***S.aureus*3**	+	+	+
***S.aureus*4**	+	+	+
***S.aureus*5**	+	+	+
***S.aureus*6**	+	+	+
***S.aureus*7**	+	+	+
***S.aureus*8**	+	+	+
***S.aureus*9**	+	-	+
***S.aureus*10**	+	-	+
***S.aureus*11**	+	+	+
***S.aureus*12**	+	+	+
***S.aureus*13**	+	+	+
***S.aureus*14**	+	+	+
***S.aureus*15**	+	+	+
***S.aureus*16**	+	-	+
***S.aureus*17**	+	-	+
***S.aureus*18**	+	+	+
***S.aureus*19**	+	+	+

By using Crome agar (HICromeMeReSa agar base with MeReSa selective supplement FD229) all the isolates gave positive results for being MRSA (Figure 2 A), because only the *S. aureus* has the ability to cleave the chromogenic mixture in the medium giving the bluish-green coloured colonies [20] and only the methicillin-resistant isolates can grow with the presence of methicillin in the medium.

As a conclusion from the experiment of the present study and for the first time according to our knowledge, Crome agar and Cefoxitin disk diffusion which indicate oxacillin resistant were identical and gave same results, while EPI-M Kit gave inaccurate results.

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