A comparison study for biofilm formation ability in Nosocomial MRSA using two detection methods

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Abstract

Nosocomial infections with MRSA (Multidrug resistance *Staphylococcus aureus*) is a significant problem during medical care for hospitalized patients. Biofilm formation made this problem worse due to increase time for cure and costs for treatment. This study focused light on MRSA isolates and aimed to determine the best method used for biofilm formation detection methods. In this study clinical isolates included MRSA isolated from Post-operation infections (hernia and caesarean section), Skin trauma infected wounds, Burns infections and Urinary tract infections (UTI). Results showed that the isolates obtained from UTI and Burns infections were more aggressive in that most of them were able to form biofilm. Also, using the two methods for biofilm formation detection; visible evaluation and spectrophotometer optical density estimation, is the best way for obtaining an accurate results to be depended in MRSA biofilm formation ability.

Keywords: *Nosocomial infections, Staphylococcus aureus, Antibiotics Resistance, MRSA, Biofilm Formation, biofilm detection.*

دراسة لمقارنة قابلية تكوين الغشاء الحيوي في المكورات الذهبية المستشفوية والمقاومة للمضادات الحيوية بأسخدام طريقتين للتحري

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الخلاصة

تعد الاصابات المستشفوية ببكتريا المكورات العنقودية الذهبية متعددة المقاومة للمضادات الحيوية Multidrug resistance *Staphylococcus aureus* مشكلة مهمة خلال العلاج الطبي للمرضى الراقدين للعلاج في المستشفيات. وأن قابلية تكوين الغشاء الحيوي biofilm formation جعلت من هذه المشكلة أسوأ وذلك لزيادة تكلفة العلاج والوقت اللازم له. لقد سلطت هذه الدراسة الضوء على هذه العزلات وهدفت الى تحديد أفضل طريقة للتحري عن قابلية تكوين الغشاء الحيوي بين طريقتين تم أختبارهما أثناء البحث.وتضمنت العزلات السريرية عزلات MRSA المعزولة من مرضى الاصابات مابعد العمليات (عمليات الفتق والولادات القيصرية) والجروح والاصابات الجلدية والحروق والتهابات المجاري البولية UTI. أظهرت النتائج بأن العزلات المأخوذة من التهابات الحروق والمجاري البولية كانت شرسة وعدوانية وكان لمعظمها القابلية على تكوين الغشاء الحيوي. كذلك فان أستخدام الطريقتين, التقييم البصري وأستخدام المطياف الضوئي في قياس الكثافة الضوئية معا تعد أفضل طريقة للحصول على نتائج دقيقة ليتم اعتمادها لتأكيد قابلية بكتريا المكورات العنقودية الذهبية المقاومة للمضادات الحيوية MRSA لتكوين الغشاء الحيوي للحصول على أفضل نتائج من ناحية الحساسية والنوعية.

الكلمات المفتاحية: *الاصابات المستشفوية, المكورات العنقودية الذهبية, مقاومة المضادات الحيوية, تكوين الغشاء الحيوي, التحري عن الغشاء الحيوي.*

Introduction

Staphylococcus aureus considered a very important nosocomial bacteria because of the wide range of virulence factors produced by these pathogens. Moreover, these bacteria are able to develop antibiotic resistance and become Multidrug resistance *Staphylococcus aureus* (MRSA), due to genetic information exchange using plasmids that encode for different antibiotics resistance. Recently the most important virulence factor reported in Nosocomial MRSA is biofilm formation [1].

Biofilm is the slim growing of *Staphylococcus aureus* as group of bacterial cells forming a layer upon other layer of bacterial growth, during the course of colonization process of the invasive infection. The biofilm extracellular matrix components contains polysaccharides polymer, protein (teichoic acid), and DNA, these factors play a major role in pathogenicity of bacteria because biofilm help to share nutrients, shield from the immune system of hosts by escape phagocytosis process and avoid antibiotics effect, hence infections with these MRSA isolates make treatment very difficult and in most cases patients need surgical interference [2 ، 3].

The ability to form biofilms help MRSA to adhere tightly to infection surfaces, catheter devices, medical tools and other surfaces in hospitals making these bacteria a significant medical care problem [1]. This research focused on the nosocomial MRSA isolates that are able to produce biofilm and caused infection in patients post hospitalization, and aimed to determine the best method used for biofilm formation determination between two methods involved in the study.

Methods and Materials

Subjects: Patients involved in this study included the cases of infected burns area, post operation infected wounds, UTI and post trauma skin infections. No antibiotics was taken as treatment by patients before sampling. Patients were examined by specialized physicians in Al-Hussein Hospital/ Al-Muthanna Governorate.

Samples Collection: The clinical samples were collected from infected areas of skin and post-operation wounds infections using disposable swabs provided by Dragon company/China after sterilization of the surrounding area for swabbing process using the ordinary disinfectants to get rid of normal microbiota [4]. Midstream urine samples were collected using disposable containers provided by

Dragon Company /China, as recommended by [1]. While samples from burns were collected by the physicians because of the critical situation of cases.

MRSA Isolates: A total of 39 isolates were identified from the collected samples of the subjects involved in this study, (table 1). Isolates were confirmed as *Staphylococcus aureus* MRSA using standard bacteriological procedures and culturing on Hi-Crome MRSA Agar Base Medium (Hi-Media company/India). This medium was supported with Cefoxitin and MeReSa to be a selective medium for MRSA isolation process. Isolation and confirmation process was accomplished according to [5] and the cases were confirmed to be infected with MRSA regardless of being pure or mixed infections with another pathogen.

Table (1): Distribution of MRSA isolates on clinical cases

Medical case	No. of MRSA Isolates Collected
Post-operation infections (hernia and caesarean section)	11
Skin trauma infected wounds	6
Burns infections	12
Urinary tract infections UTI	10
Total cases No.	39*

*: The MRSA cases included pure and mixed infections with another pathogen.

Biofilm formation ability detection

Two methods for detection were utilized in the present study, in both methods; all MRSA isolates were cultured in the disposable microtiter plates provided by (Dragon company/China). This assay was according to the protocol in [2, 6 and 7]. Brain Heart Infusion BHI medium was used to culture all isolates for overnight with glucose 1% supplementation. After growth, confirmed by turbidity, cultured

media were diluted with new fresh media that distributed in 96 well microtiter plate in a percentage 1:100. Three wells for each isolate were inoculated (triplet wells). After incubation overnight, cultured media in wells were discarded and staining was done using (0.1%) crystal violet provided by (Sigma Company/ USA). Fixation is the next step by Sodium acetate (0.2%) provided by B.D.H company/UK. The control wells were applied in each strip, filled with media only without inoculation [6].

- **First method for Visible Biofilm Detection:**

After drying, microtiter plates were examined visibly, if there was a visible line in the wall and bottom of the well; the isolate was considered with high ability, if the purple colour was marked only as small dot and light line in the wall, the isolate was considered as moderate ability. Clear wells or similar to controls were considered as unable or with no biofilm formation ability. This method was accomplished according to [1].

- **Second method for Optical Density Biofilm Detection:**

After drying, ELISA reader was used for optical density estimation (OD) at 570 nm. The device was provided by Bio-TeK Company/ U.S.A. and recently calibrated. Detection procedure was performed in triplicate (three runs), then the obtained data results were averaged and classified according to the bacterial adherence range provided by [6 and 7] as (no, moderate, high) when OD value were (<0.120, 0.120-0.240, >0.240) respectively.

Statistical Analysis

Percentages were calculated and performed using Microsoft excel 2010.

Ethical approval

All approvals were obtained before starting work in the Al-Hussein Teaching Hospital in Al-Muthanna Governorate, all patients' ethical consents for samples collection were respected.

Results

Table (2) shows the results obtained in this study, whereas, the outcomes of both methods used for biofilm formation detection were presented as numbers of isolates with High, Moderate and not able to form biofilm. Total number and percentages were calculated for each type of isolate obtained for both methods' results. Figure (1).

Table (2): Biofilm Formation abilities of the two methods distributed on the Medical cases

Medical case	Total No. of MRSA Isolates	First Method No. (%)			Second Method No. (%)		
		High	Moderate	Not able	High	Moderate	Not able
Post-operation infections (hernia and caesarean section)	11	4 (36.4%)	5 (45.4%)	2 (18.2%)	5 (45.4%)	6 (54.5%)	0
Skin trauma infected wounds	6	1 (16.6%)	5 (83.3%)	0	3 (50%)	2 (33.3%)	1 (16.6%)
Burns infections	12	9 (75%)	2 (16.6%)	1 (8.3%)	8 (66.6%)	2 (16.6%)	2 (16.6%)

Urinary tract infections UTI	10	8 (80%)	2 (20%)	0	7 (70%)	2 (20%)	1 (10%)
Total	39	22	14	3	23	12	4
Percentage from total No. (39 Isolates)	-------	56.4%	35.8%	7.7%	58.9%	30.7%	10.3%

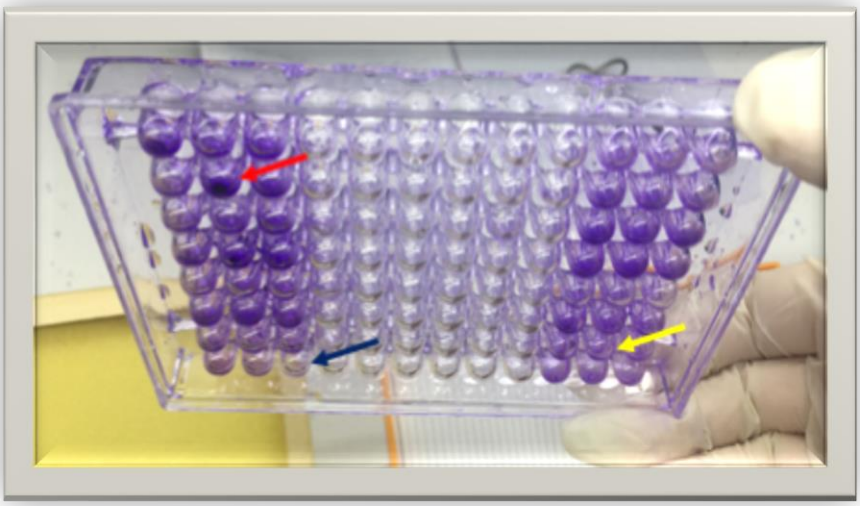

Figure (1): Microtiter plate for Biofilm formation detection. Red arrow shows High ability, Yellow arrow shows moderate ability and Blue arrow shows no ability to form biofilm by the isolate.

Discussion

Staphylococcus aureus was and still stand as a problem in medical care for hospitalized patients regardless the cause of their need to stay in hospital, MRSA, the most aggressive type of *Staphylococcus aureus,* have developed a dangerous style of pathogenicity that enable them to survive in the infected area, and resist both antibiotics treatment and the immune system defences. Multi-antibiotics resistance and biofilm formation are very important virulence factors of colonization and adherence [6].

Manian, *et al.* 2003 [8] stated that recently the nosocomial Methicillin-Resistant *Staphylococcus aureus* represents a challenge for hospitals and caused an increase in the time required for cure and medical care costs.

This study results showed that most of the isolates obtained from UTI and Burns infections recorded the highest percentages of positive biofilm formation, since the obtained percentages were 75% and 80% respectively as shown in Table (2). That can explain the deterioration of these cases and their need for recurrent medical interference and treatment, due to the aggressive MRSA isolates involved in these infections.

These findings were in agreement with, Mahmoudi, *et al. (*2019), who found that more than (94%) of burns isolates were capable of producing biofilm [9]. Also in the study of Al-Hamadany, (2019), biofilm forming isolates were more than 60% of UTI isolates as stated by the author [2].

Giulieri, (2020) and others suggested that nosocomial isolates must be examined on the genetic level since they were more pathogenic comparing with other isolates and explained that patients infected with these isolates are more susceptible to get bacteraemia and endocarditis, hence, the need to understand the

molecular mechanism to control the transmission at the hospital and in the community [10].

Concerning this study aim, to investigate which is the best method to be used for detection of biofilm formation ability, the method that depended on the OD reads showed good sensitivity in measuring, as the results were more accurate in determining the moderate type of isolates from the High ability isolate, despite of that they were all positive and able to form biofilm. These findings are in consent with those of Halim, 2018 and his colleagues [11]. This similar study recommended the method of microtiter plate culturing and staining then reading the OD with spectrophotometer as a best method to get more accurate results of biofilm formation detection for MRSA isolates.

From another point view, the method of detection that used the visual determination of biofilm formation showed a good specificity due to avoiding reads error that can occur due to devices wrong calibration and help in finding contamination if happened since the position of the deposited biofilm after fixation will be changed obviously. These finding are supported by the results of [2], since the method that was used in the detection was the same of the present study first method.

For the present study results, using both methods in detection of biofilm formation can be the best way for obtaining an accurate results to be depended in MRSA biofilm formation ability confirmation. That opinion is also recommended by the authors Achek, *et.al* (2020), who used both visible and OD reads in determination of positive isolates for biofilm formation during their research [12].

Conclusions

The present study proved that MRSA isolates that isolated from patients with Burns infections and UTI were more aggressive than other pathogenic isolates, in addition using both methods for biofilm formation detection is the best way for obtaining an accurate results to be depended in MRSA biofilm formation ability confirmation, for best sensitivity and specificity.

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