

Biofilm formation by Methicillin resistant *Staphylococcus aureus* and its relation to antibiotic resistance in Thi-qar province/Iraq

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Abstract

Methicillin resistant *Staphylococcus aureus* is one of the most dangerous pathogens in the community and the hospital environment for its high resistance to antibiotics and the production of a number of virulence agents such as toxins and biofilm. The study aims to determine the ability of local isolates to produce the biological membrane and its relationship to resistance to antibiotics. A cross sectional study include (37) isolates of methicillin-resistant *S. aureus* from the burn department at Al-Hussein Teaching Hospital in Thi Qar province/Iraq for the period April-October 2015, The capacity of bacteria to produced biofilm was done by micro plate technique and the antibiotic susceptibility test for vancomycin, amikacin, ciprofloxacin, norfloxacin, cefotaxime and amoxicillin-Claviolate using the diffusion technique of antibiotic disks. The study showed that 64.9% of the MRSA isolates were able to form biofilm, while the isolates were fully resistant to the used beta-lactam antibiotics. But, the 94.6% of the isolates were sensitive to the vancomycin. There was no significant statistical relationship between the antibiotic resistance and the ability of bacteria to produce the biofilm except for the ciprofloxacin. The study showed that the local MRSA isolates have a high ability to produce the biological membrane and antibiotic resistance with the exception of the vancomycin with a relationship between the resistance to ciprofloxacin and the production of the biological membrane by bacteria. Therefore, the study recommends the use of vancomycin in medical sites to treat the infections caused by MRSA to prevent the spread and development of these resistant strains.

Keywords: Biofilm, Staphylococci, antibiotic resistance

Introduction

Staphylococcus aureus is a gram positive cocci may present as normal flora in skin and mucus membrane especially in the nose but it considered as an important medical pathogen

associated with the different types of community and hospital acquired infections range from mild skin infections to severe infections such as toxic shock syndrome and pneumonia,

this bacterium able to produce several types of toxins and virulence factors that help in initiation and development of infections e.g. hemolysin and coagulase. In addition to this. *S. aureus* developed resistance rapidly to the different classes of antibiotics like β -lactams and aminoglycosides.^(1,2,3)

Methicillin resistant *Staphylococcus aureus* (MRSA) was firstly reported in Europe in 1960s,^(4,5,6). Its became a significant pathogens causing a variety of nosocomial life-threatening infections such as ventilator-associated pneumonia, catheter related infections, sepsis, endocarditis and soft tissue infections that lead to increases in mortality and morbidity among hospitalized patients and increase the cost of treatment, the asymptotically colonized healthcare workers are the major sources of MRSA in the hospital environment. There have been an increasing number of outbreaks of MRSA infections in hospitals reported from many countries. Today, the infections by MRSA isolates are reported in most communities not only in hospitals with high resistance to many antibiotics classes^(1,5,6,7).

The most important problem in treatment of MRSA is the development of multi-drug resistant (MDR) isolates in hospital and community acquired infections specially to many commonly used antibiotics and the ability of MRSA to develops resistance to vancomycin which is used to management of MRSA infections for decades are reported in many countries. So, the MDR isolates

appearing lead increase difficulty in treatment, morbidity, mortality and increase requirement to screening the anti-biogram of MRSA in different medical settings and communities. Clinical infections are most common in patients in hospital intensive care units, nursing homes, and other chronic care facilities; however, MRSA are emerging as an important community acquired pathogen as well. Although there are some reports on the prevalence of vancomycin resistant *S. aureus* (VRSA) and vancomycin intermediate *S. aureus* (VISA), most MRSA isolates are susceptible to vancomycin and teicoplanin; therefore resistance increase to these antibiotics results in the limitation of treatment options and also the requirement of a new class of antibiotics^(7,8).

The biofilm is complex sessile cells communities embedded in the polymers from exopolysaccharides matrix produced by themselves, it help bacteria to adhesion on environmental surfaces and host tissue to initiates infections. The adhesion of biofilm and its development provides an ideal environment for germs and overlap with each other to help in the exchange of genetic material and metabolites between them, Also, biofilm appear to be represents an important microbial survival mechanism used by bacteria to resist of the non-desired conditions like desiccation, antimicrobial agents etc⁽⁹⁾.

The researchers was recorded many MRSA are a biofilm formation bacteria in different medical settings which is increase the persistence of bacteria and

their ability to resistance of antibiotics and the action of immune system e.g. phagocytosis, some host factors aid in biofilm formation such as adhesive factors and capsules⁽¹⁰⁾.

The study aimed to determine the ability of local isolates of MRSA to form biofilm and its relation to antibiotics resistance.

Methodology

Bacterial isolates

A cross sectional study including (37) isolates of Methicillin resistant *S. aureus* were obtained from the patients with burn infections in Al-Hussein teaching hospital Thi qar province/Iraq in the period from April to October / 2015. the diagnosis of bacteria were done by using standard biochemical techniques.

Antibiotic susceptibility test

The test was achieved by disk diffusion technique with the following antibiotics vancomycin (VAN), amikacin (AK), ciprofloxacin (CIP), norfloxacin (NOR), cefotaxime (CTX), Co-amoxyclav (AMC) and methicillin (MET) discs provided by (Bioanalyze-Turkey)⁽¹¹⁾.

Biofilm formation

S. aureus isolates were inoculated in test tubes each of which containing (3)

ml of trypticase soya broth (TSB) and incubated at a temperature of 25°C for 24 hours.

B .bacterial culture washed by TSB diluted 1: 3 three times using the centrifuge (3000 rpm).

C.The number of cells in each tube calculated by microscopic examination using Petroff-Hausser chamber for the concentration ranges between (5×10^5 - 1×10^6) CFU / ml.

D. A volume of (200 μ l) of culture from each isolate tube was add to rounded bottom microtiter plate then incubated at 25 °C for 24 hours, wells with TSB only was used as control.

E. The culture was removed from wells and washed three times by normal saline. the plates incubated at 60 °C for one hour, then the wells was stained by crystal violet for 5 minutes, the plate washed with running water to get rid the remnants of pigment, plates were incubated at a temperature 37 ° C for 30 minutes to dry.

F. The absorbency of formed biofilm was read by Microplate reader (Biotech-USA) at wavelength of 492 nm⁽¹²⁾.

Statistics

The statistical analysis was performed by using SPSS program (version 19).

Results

Table (1) shown the isolates ability to form biofilm as the 24 isolates represents (64.9%) have had the ability to produce the biofilm on plastic surfaces of micro titer plate.

Table (1): Biofilm formation by MRSA isolates.

Biofilm formation	Frequency	Percent (%)
Positive	24	64.9
Negative	13	35.1
Total	37	100

The MRSA isolates were completely resistant (100%) to β -lactam drugs in this study including cefotaxime and Co-amoxyclav, highly resistance was shown to ciprofloxacin and norfloxacin in percent (67.6% , 73%), respectively. Amikacin showed moderate activity against MRSA were (59.5%) of isolates expressed resistance. While, vancomycin was the most active antibiotic used, 2 isolates represents (5.4 %) were resistant to VAN with high significant differences under ($P < 0.001$) in compared with other antibiotics as shown in table (2).

Table (2): Antibiotics Susceptibility pattern for MRSA isolates from burn infections.

Antibiotics	Susceptible		Resistant	
	No.	%	No.	%
Vancomycin	35	94.6	2	5.4
Amikacin	15	40.5	22	59.5
Ciprofloxacin	12	32.4	25	67.6
Norfloxacin	10	27	27	73
Cefotaxime	0.0	0.0	37	100
Co-amoxyclav	0.0	0.0	37	100

The results showed no significant correlation between biofilm production and resistance of bacteria to antibiotics (VAN, AK, NOR, CTX and AMC) under the (P<0.05). While, there were significant differences between sensitivity pattern of CIP and the capacity of MRSA to produced biofilm under the probability of less than 0.05, as shown in Table (3).

Table (3): Relationship between antibiotic susceptibility and biofilm formation

Biofilm antibiotic		Positive	Negative	Total
VAN	S*	22	13	24
	R**	2	0.0	13
AK	S	9	6	15
	R	15	7	22
CIP	S	5	7	12
	R	19	6	25
NOR	S	5	5	10
	R	19	8	27
CTX	S	0.0	0.0	0.0
	R	24	13	37
AMC	S	0.0	0.0	0.0
	R	24	13	37

*susceptible ** resist

Discussion

MRSA now represents an important health problem worldwide with increase incidence of these bacteria in different types of infections dramatically including community and hospital acquired MRSA infections which ranging from skin and soft tissue infections to serious sepsis and pneumonia^(13,14).

MRSA isolates showed high resistance to antibiotics under study, especially beta-lactam drugs. This may be due to the increased transmission of resistance genes between these bacteria and other species found in the hospital environment. The increased use of

antibiotics in the community and the hospital made these bacteria in great contact with antibiotics, While, vancomycin were the most effective antimicrobial agents against MRSA isolates which may be due to low rate of using of this antibiotic in the community or in medical sites. This study is consistent with a number of other studies that have shown that MRSA isolates are highly resistant to many antibiotics and highly sensitive to vancomycin. (7,15)

In this study, MRSA showed increase ability to biofilm production, and this is evidenced by a number of

studies as observed high capacity of these bacteria to produce biofilm in different environments, especially in the hospital, and this may be due to the ability of the bacteria to produce a number of adhesion factors which helps in the initiation of first step biofilm formation and the isolates of bacteria began to show high capacity to produce extra-cellular polysaccharides which are the main components of the biological membrane^(16,17). Ciprofloxacin is widely used in eradication of biofilm of many bacteria^(18,19,20), this resistance antibiotic showed significant correlation with biofilm formation may because the relation between genes that responsible for resistance and those that help in production of adhesive factors or polysaccharide production as where these genes may be located under the effects of the same regulatory gene.

The increase development of antibiotic resistance with increased ability to biofilm production, as demonstrated by the study, is a major problem especially in the hospital environment. Attached bacteria (sessile cells) to the membrane are inactive which increase resistance. The low metabolism of sessile cells in biofilm reduces the deadly effect of antibiotics. Many of these antibiotics are highly effective against high-metabolizing cells, and since free cells in this study have shown high resistance to most of the widely used antibiotics in community. Also, The biofilm acts as a diffusion barrier that prevents the antibiotic from reaching to embedded cells or reducing the concentration of the antibiotics in biofilm^(9,21,22). This has compounded problem by the development of chronic infections and

increased mortality and morbidity rates. As it reduced the options for the use of antibiotics. So, the patients needs a high concentration of active anti-MRSA antibiotics to eliminate the bacteria in the membrane.

Conclusion

The study showed that the local isolates of MRSA have a high ability to produce the biological membrane and antibiotic resistance with the exception of the vancomycin with a relationship between the resistance to ciprofloxacin and the production of the biological membrane by bacteria.

Recommendation

the study recommends the use of vancomycin in medical sites to treat the infections caused by MRSA to prevent the spread and development of these resistant strains

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إنتاج الغشاء الحيوي بواسطة المكورات العنقودية الذهبية المقاومة للمضاد مثسيلين و علاقته بالمقاومة للمضادات الحياتية في محافظة ذي قار/ العراق

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الخلاصة

تعد المكورات العنقودية الذهبية المقاومة للمضاد مثسيلين من أكثر الممرضات خطورة في المجتمع و بيئة المستشفى وذلك لقدرتها العالية على مقاومة المضادات الحياتية و إنتاج عدد من عوامل الضراوة مثل السموم و الغشاء الحيوي، تهدف الدراسة إلى تحديد قدرة عزلات المكورات العنقودية الذهبية المقاومة للمضاد مثسيلين المحلية على إنتاج الغشاء الحيوي و علاقته بالمقاومة للمضادات الحياتية، تم جمع (٣٧) عزلة من المكورات العنقودية الذهبية المقاومة للمثسيلين من قسم الحروق في مستشفى الحسين (ع) التعليمي في محافظة ذي قار/العراق للفترة من ابريل إلى أكتوبر /٢٠١٥. إذ تم قياس قدرة البكتريا على إنتاج الغشاء الحيوي باستخدام اختبار الصفائح الدقيقة، و اجري اختبار الحساسية للمضادات الحياتية فانكوميسين، اميكاسين، سايبورفلوكساسين، نورفلوكساسين، سيفوتاكسيم و اموكسيسيلين-كلافولينات باستخدام تقنية الانتشار لأقراص المضادات. أظهرت الدراسة إن (٦٤,٩%) من العزلات كانت منتجة للغشاء الحيوي، فيما كانت العزلات مقاومة بصورة كاملة لمضادات البيبتالكتام المستخدمة بينما كانت (٩٤,٦%) من العزلات حساسة للمضاد فانكوميسين وقد لوحظ عدم وجود علاقة بين المقاومة للمضادات الحياتية المستخدمة و القدرة على إنتاج الغشاء الحيوي باستثناء المضاد سايبورفلوكساسين. أثبتت الدراسة أن عزلات المكورات العنقودية الذهبية المقاومة للمضاد مثسيلين المحلية لها قدرة عالية على إنتاج الغشاء الحيوي ومقاومة المضادات الحياتية باستثناء المضاد فانكوميسين مع وجود علاقة بين المقاومة للمضاد سايبورفلوكساسين و إنتاج الغشاء الحيوي بواسطة البكتريا. و بذلك يوصي بضرورة استخدام المضاد فانكوميسين في المواقع الطبية لمعالجة الإصابات الناتجة عن المكورات العنقودية الذهبية المضادة للمثسيلين لمنع انتشار و تطور هذه السلالات المقاومة