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Detection of mec A gene in *Staphylococcus aureus* isolated from wounds infections

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Abstract

This study aimed to detect the occurrence of *mecA* gene in *S.aureus* isolates by PCR technique. The study included a total of 25 *S. aureus* was collected from patients with a wound infection. Identification of *S. aureus* was based on growth on manitol salt agar, catalase test, DNase test and coagulase test. Antibiotic Susceptibility test by disc diffusion method was done for all *S. aureus* isolates against four β- lactam antibiotics and the resistance percentage was as the following: 92% for methicillin, 32% for oxacillin, 60% for amoxicillin – clavulanic acid and 24% for ampicillin – sulbactam. PCR technique was used for the detection of *mecA* gene in *S. aureus* isolates and results showed that 24% of isolates were *mecA* positive, while 76% of the isolates were *mecA* negative.

كشف عن جين (mec A) في المكورات العنقودية الذهبية المعزولة من أخماج الجروح

سروى عزيز خالد نهاد عبد الحسين جعفر

الخلاصة

هدفت الدراسة الى كشف عن جين mecA في عزلات المكورات العنقودية الذهبية باستخدام تقنية (PCR). تضمنت الدراسة جمع (25) عزلة من بكتريا المكورات العنقودية الذهبية من المرضى الذين يعانون من أخماج الجروح. اعتمد في تشخيص المكورات العنقودية الذهبية على نموها في وسط مانتول الملحي ،اختبار أنزيم الكتاليز ،الاختبار محلل الدنا واختبار أنزيم الخثرة. تم اجراء اختبار حساسية العزلات البكتيرية للمضادات الحيوية بطريقة الأقراص حيث استخدم أربع مضادات نوع بيتالاكتام في هذا الاختبار وكانت النسب المئوية للمقاومة كما يلي: 92% للمثيسيلين، 32% للاوكساسيلين ، 60 % للأموكسيلين – حامض الكلافيولانيك و 24% لأمبسيلين –سلباكتام. استخدمت تقنية تفاعل البلمرة المتسلسل (PCR) للكشف عن وجود جين mecA في عزلات المكورات العنقودية الذهبية وقد أظهرت النتائج أن24 % من العزلات كانت تمتلك هذا الجين، في حين ان 76 % كانت غير حاوية على هذا الجين.

الكلمات المفتاحية : العنقوديات المقاومة للمثيسيلين، أخماج الجروح ، جينmec A.

Introduction

Wounds infections is result from dynamic interaction between the host and the pathogens .All wound are contaminated with a variety of microorganisms, the most commonly pathogens associated with wound infections are *S.aureus, Pseudomonas aeruginosa ,Streptococcus species* and anaerobes [(1)]. Some bacteria have pyrogenic exotoxin (exfoliative toxin , panton valentine leukocidin and toxic shock syndrome toxin) that cause wound infection such as *S.aureus* [(2)]. *S.aureus* is a widespread pathogens that associated with broad spectrum of disease including wound infection, endocarditis, pneumonia, urinary tract infection and food poisoning [(3)]. Drug resistance increasing among *S.aureus* strains and Methicillin resistant *S.aureus* (MRSA) spread is a global threat [(4)]. Resistance of staphylococci to methicillin and all β-lactam antibiotics is associated with the low affinity of a penicillin-binding protein, PBP2a, which is not present in susceptible staphylococci, PBP2a are under chromosome control, this protein is encoded by the *mecA* gene [(5)]. MRSA strains have a higher ability to cause epidemic and outbreak infections than methicillin –sensitive *S.aureus* (MSSA) [(6)]. For a long time MRSA infections were limited to hospitalized patients, during the 1990s reports of community – associated MRSA among healthy individual [(7)]. MRSA cause community and hospital infections, Community – acquired –MRSA (CA-MRSA) is most commonly cause skin infections and is distinguish from Hospital- Aquired-MRSA (HA-MRSA) in clinical, epidermiological and microbiological feature [(8)]. The aim of this study is the detection of *mec A* gene by using polymerase chain reaction (PCR) in *S.aureus* isolated from wound infection.

Materials and Mehtod

Samples and Identification of *S.aureus* strains: A total of 25 isolates collected by sterile swab from patients with a wound infection from 1-86 years of age in Tikrit hospital. The swabs were cultured on blood agar and then incubated for 24 h aerobically at 37□C. The isolates of *S.aureus* were identified by grow in manitol salt agar, catalase test, DNase test, tube and slide coagulase test [(9)].

Antibiotic susceptibility test

The sensitivity of *S.aureus* to methicillin and other β-lactam group of antibiotic was tested by the disk diffusion method (CLSI) [10]. Muller –Hinton agar was inoculated with suspension of 24-hour culture of *S.aureus*, density of o.5 MacFaland. After 15 min antibiotic disks were placed: methicillin (10µg / disc), oxacillin (5µg/ disc), Amoxicillin – clavulanic (20/10µg/disc), ampicillin – sulbactum (10/10µg / disc) and incubated for 18-24 hour at 37 □C.

Detection of *mecA* genes by Polymerase Chain Reaction (PCR)

DNA was extracted from *S.aureus* isolates as described by Onasanya et al [11]. The primers used for detection of *mec A* gene were Mec A 1(5'-AAA ATC GAT GGT AAA GGT TGG C-3') and Mec A 2 (5'-AGT TCT GCA GTA CCG GAT TTG C -3') which amplify a (533) base pair(bp) fragment specific for *mec A* genes as described by Naji [12]. Primers were synthesized by Solarbio company, China., are dissolved with sterile deionized water to give final concentration of each primer in 100 picomoles / µl, the amount of water added to each primer based on the data sheet which is sent from the provider. For preparation 10 picomoles work solution primer, 10µl of stock solution (100 picomoles) was added to 90µl sterile deionized water, and then it was used depended on the procedure of gene amplification. PCR reaction were performed in (20µl), the PCR reactions components are shown in according to the recommendation of Clinical Laboratory Standard Institute

Table (1):- The PCR conditions program as shown in Table (2).

Component	Volume (µl)
Go TaqGreen Master Mix	10
Nuclease Free Water	3
DNA Template	5
Forward Primer (10 picomoles)	1
Reverse Primer(10 picomoles)	1
Total volume	20

Table (2):- Program conditions for amplification of *mec A* genes.

Stage	Temperature C	Time	Cycle number
Initial denaturation	95	5 min	1
Denaturation	95	1min	30
Annealing	58	1min	
Extension	72	1min	
Final extension	72	5 min	1

After the reaction was complet, (10µl) of PCR products was loaded in (1.5%) agarose gel containing red safe followed by electrophoresis and visualized by using UV transilliuminator, then photographed by using digital camera**.**

Results and discussion

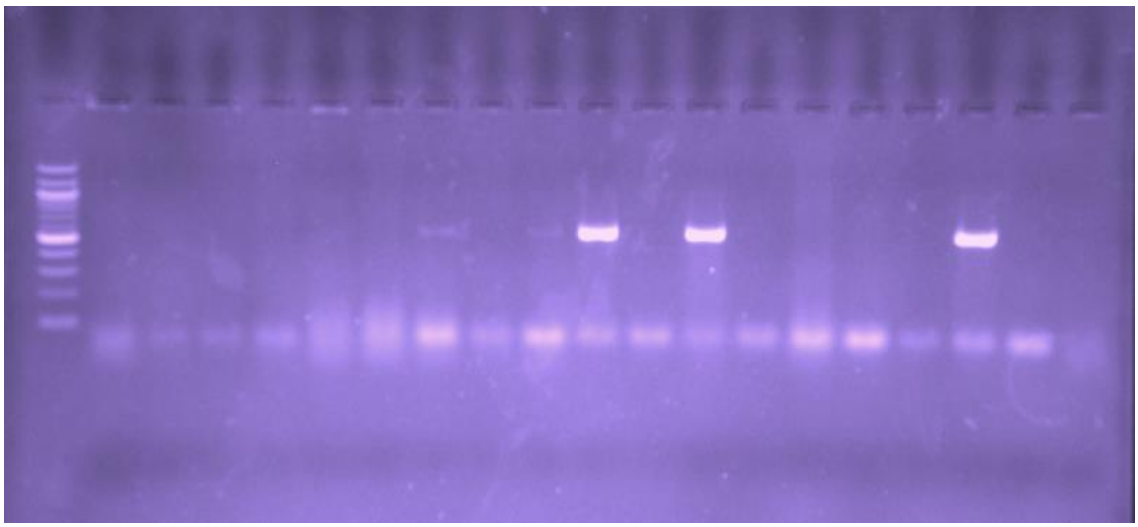
Antibiotic susceptibility test by disk diffusion method for 25 isolates of *S.aureus* was done against four β-Lactam antibiotics [10]. The resistance percentage of *S.aureus* isolates varied for β- lactam antibiotics used in this study as shown in table 3.

The results revealed that resistance was 92 % for methicillin, 32 % for oxicillin, 60% for amoxicillin – clavulanic acid and 24 % for ampicillin - sulbactam. This study showed that the higher resistance by the isolates of *S.aureus* is to methicillin.

Table (3):- Resistance percentage of *S.aureus* to β-Lactam antibiotics .

Antibiotic	No. of resistant isolates	% of resistance
Methicillin	23	92
Oxacillin	8	32
Amoxicillin – clavulanic acid	15	60
Ampicillin – sulbactam	6	24

In this study all isolates 25 of *S.aureus* were analyzed by PCR to detect *Mec A* gene. The results showed that (6) isolates (%24) were have the *mec A* gene, while (19) isolates (%76) were lacking the *mec A* gene. Figure (1) and (2) shows gel electrophoresis of PCR amplification products of *mec A* gene.



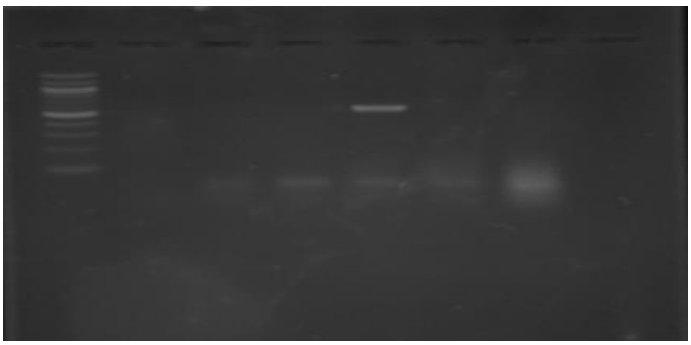


Figure (1) and (2): Gel electrophoresis of PCR amplification for *mecA* gene in 25 isolates of *S.aureus* ; Ladder : The DNA molecular weight marker (100 bp ladder); 7, 9, 10, 12, 17 and 23 are *mecA* positive isolates. The methicillin resistance mechanism in *mecA*-positive isolates was due to the production of PBP2a by *mecA* gene. Methicillin resistance is associated with a large genetic element known as Staphylococcal cassette chromosome *mec* (SCC *mec*).The SCC *mec* contain two components:1- The mec gene complex which consists of *mec A* that responsible for resistance to methicillin and other β-lactam antibiotic, 2- The ccr gene complex that contain of cassette chromosome recombinase (ccr) genes which encoding recombinases mediating integration and excision of SCC mec into and from the chromosome and surrounding genes [(7)]. Mahmood found that only 24 (%57.14) from total 42 clinical *S.aureus* isolates showed *mec A* gene positive in Erbil, Iraq[(13)]. Naji Reported that 31 (62%) from a total 50 clinical *S.aureus* isolates were *mec A* positive. The most commonly known carrier of the *mecA* gene is the bacterium known MRSA [(12)].Other study found that *S. aureus* is responsible for the 81% of infections and 61% of these were methicillin resistant, the Central Public Health Laboratory, UK found that 61% of nosocomial *S.aureus* infections in the 96 hospitals studies were methicillin resistant [(14)]. MRSA is a major problem in many contries in world such as United States, because the pathogens may develop resistance to many antibiotic that is important to treat their infections [(15)]. Hu et al and Bhatta et al, in their study reported MRSA isolates in pus and wound infection by detection of *mecA* gene using PCR technique [(16, 4)]

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