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Molecular characterization of *mec*A gene in Methicillin-Resistant *Staphylococcus aureus*

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Abstract:

Background & objectives: It is clear now that methicillin-resistant *Staphylococcus aureus* (MRSA) strains considered as one of the most bacteria responsible for different diseases among humans and animals. The present study aimed to detect the molecular profile of methicillin-resistant *S. aureus* isolated from skin abscess patients in Nassyriah City, Southern Iraq.
Methods: During the period of from June 2014 to February 2015, 120 *S. aureus* were isolated from abscess patient in two governmental hospitals, and subjected to conventional Polymerase Chain Reaction which used for the amplification of 310 bp *mec*A gene. Three PCR products of *mec*A were named primarily (THQR1, THQR2, and THQR3) were selected and subjected to partial DNA sequencing for the *mec*A gene to follow up their possible relationship between these local isolates and what recorded globally in Genbank. **Results**: Only 64 *S. aureus* isolates were diagnosed phenotypically as MRSA (53.33%), and 88/120 (73.33%) of *S. aureus* were positive for the targeted gene. The Three PCR products of *mec*A were registered in Genbank under the official accession numbers of (KY468502, KY468503 and KY468504, respectively). The constructed phylogenetic tree showed that *S. aureus* KY468502 and KY468504 were highly relative to each other in comparison with *S. aureus* KY468503 that revealed a close relatedness to *S. aureus* TN/CN/1/12, *mec*A gene beta-lactam, and *mec*A gene isolated in USA.
Interpretation & conclusions: The present study results confirmed the importance of *mec*A gene in MRSA detection and highlighted the increasing manner of its prevalence in Iraq, furthermore, the importance of molecular techniques as an epidemiological tool.
Key words: Gene sequencing; Methicillin-resistant; *Staphylococcus aureus*; Phylogenetic tree; *mec*A gene of *S. aureus*

الخصائص الجزيئية لجين *mec*A في بكتريا المكورات العنقودية الذهبية المقاومة للمشيلين

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الخلاصة:

الخلفية والأهداف: أصبح واضحا الآن أن المكورات العنقودية المقاومة للمشيلين تعتبر من أكثر أنواع البكتريا المسئولة عن مختلف الأمراض للإنسان والحيوانات. هدفت الدراسة الحالية للكشف عن الخصائص الجزيئية لبكتريا المكورات العنقودية المقاومة للمشيلين المعزولة من مرضى الخراج الجلدي في مدينة الناصرية , جنوب العراق.

طرق العمل: خلال الفترة من شهر حزيران 2014 إلي شباط 2015 تم عزل 120 عزلة من بكتريا المكورات العنقودية من مرضي الخراج في اثنين من المستشفيات الحكومية وقد تم إخضاعها لتقنية تفاعل السلسلة المتبلمرة التقليدي والذي استخدم لتضخيم الجين *mec*A وبطول 310 زوج قاعدي. تمت

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التسمية الأولية لثلاثة من نواتج التضخيم للجين بـ THQR1 و THQR2 و THQR3 وتم استخدامها لحساب التسلسل الجيني الجزئي للجين لأجل متابعة علاقاتها المحتملة مع ما مسجل عالميا في بنك الجينات.

النتائج: تم تشخيص 64 عزلة من بكتريا المكورات العنقودية الذهبية فقط مظهريا على أنها مقاومة للمثيسيلين (53,33%) وكانت 88 /120 قد أظهرت كشفا موجبا للجين *mec*A (73,33 %). تم تسجيل نواتج الترحيل الثلاث للجين في بنك الجينات تحت أرقام الانضمام الرسمية KY468502 و KY468503 و KY468504 على التوالي. أظهرت الشجرة التطورية التي تم بناؤها أن بكتريا *S. aureus* للسلالتين KY468502 و KY468504 كانتا ذات علاقة تطورية متقاربة لبعضهما مقارنة مع السلالة KY468503 التي أظهرت تقاربا مع بكتريا *S. aureus* لسلالات TN/CN/1/12 و *mec*A gene beta–lactam و *mec*A gene المعزولة في الولايات المتحدة الأمريكية.

التفسيرات والاستنتاجات: أكدت نتائج الدراسة الحالية على أهمية استخدام الجين *mec*A في الكشف عن المكورات العنقودية الذهبية المقامة للمثيلين وتم تسليط الضوء على الزيادة المطردة لانتشارها في العراق , بالإضافة إلى التأكيد على أهمية التقنيات الجزيئية كأدوات للدراسات الوبائية.

Introduction:

Staphylococcus aureus is usually a member of normal skin flora and the nasal cavity, almost can be responsible for different superficial and deep skin infections; bones; heart; and lung infections (Schito, 2006). In the early of 1960_s; semi-synthetic β-lactam resistant pencillins, such as mecithillin and oxacilllin were introduced, and declining of multi-drug resistance *S. aureus* was noticed clearly (Shanson, 1981). Unfortunately, after about one decade later, strains resistant to these pencillins, especially, methicillin-resistance of *S. aureus* (MRSA) emerged with increased manner (Jensen and Lyon, 2009). Nowadays, MRSA became as one of the most potential pathogen worldwide with the emergence of community acquired (CA-MRSA) and hospital acquired (HA-MRSA) strains. As a fact accompli, it is difficult to differentiate between these two MRSA types, since CA-MRSA could spread into hospitals (Jarvis *et al*., 2007 ; Wannet *et al*., 2003). The molecular origin of methicillin resistant is due to the presence of *mec*A gene, which is a part of Staphylococcal cassette chromosome *mec* (SCC_{mec}). About eleven different SCC_{mec} types are reported, and continue to be used in the classification of MRSA strains (Rahimi *et al*., 2014 ; Rahimi and Karimi, 2015). The current study designed to determine the molecular profile of *mec*A gene among MRSA isolated from skin abscess patients in Nassyriah city, Iraq.

Materials and Methods:

Samples collection: A total of 120 *S. aureus* isolates were used in the present study which randomly collected from outpatients with skin abscesses in two governmental hospitals during the period from June 2014 to February 2015, in Nassyriah city, Iraq.

Laboratory methods: All *S. aureus* isolates used in this study were taken from pus swabs and diagnosed depending on Gram's stain; cultural characteristic on Blood agar (BA) base and Manitol salt agar (MSA), followed by conventional biochemical tests (Harley and Prescott, 2002). The diagnosis was confirmed by API system (BioMerieux/France).

Antibiotic susceptibility test for MRSA isolates: All MRSA isolates were subjected to antibiotic susceptibility by using disc diffusion method as mentioned by(Bauer *et al*., 1966), with 5µg of methicillin and 10 µg oxacillin (Bioanalyse, Turkey). The inhibition zone diameters were measured and interpreted according to (CLSI, 2009).

Detection of *mec*A gene by Polymerase Chain Reaction: *S. aureus* previously extracted DNA was used for the amplification of *mec*A gene. A volume of 20 µl PCR reaction mixture consisting of 10 µl master mix, 1.25 µl of each forward and reverse primers specific for the target gene, 5 µl of DNA template, and the volume was completed by adding nuclease free water. A 310-bp fragment of the *mec*A was amplified primers; *mec*A- F: 5′ GTA GAA ATG ACT GAA CGT CCG ATA A -3′ and *mec*A- R: 5′ CCA ATT CCA CAT TGT TTC GGT CTA A -3′. The mixture was briefly centrifuged and the tubes was transferred into PCR apparatus (ESCO, India) which has been programmed with the following conditions: an initial denaturation step for 4 minutes at 94°C with one cycle, 30 cycles of amplification were performed as follows: denaturation

at 94°C for 45 seconds, annealing at 50°C for 45 seconds and extension at 72°C for 1 minute, followed by a final extension step at 72°C for 2 minutes (Jonas *et al*., 2002).

DNA sequencing: Three PCR products of *mec*A genes that represent methicillin-resistant *S. aureus* strains, were selected for sequencing, and forward and reverse primers for the target gene were sent outside Iraq to be sequenced (Macrogen, Korea). Basic Local Alignment Search Tool analysis (BLAST) was lead to blast algorithm. The samples sequences which designated as (THQR1, THQR2, and THQR3) were edited, aligned, and compared with the reference sequences using BioEdit sequence Alignment Editor Software Version 7.1 (DNASTAR, USA) (Hall, 1999). A phylogenetic tree for each gene sequence was constructed by using MEGA7 software (Kumar *et al*., 2016).

Results:

Phenotypic characterization of methicillin-resistant *Staphylococcus aureus* (MRSA): Out of 120 MSA positive *S. aureus* isolated from skin abscess patients, 64 isolates showed resistant to methicillin and 120 to oxacillin discs which represent a resistant rate of 53.33% and 100%, respectively.

Detection of *mec*A gene: All *S. aureus* isolates were subjected to molecular detection of *mec*A gene. Among the assayed *S. aureus*, 88/120 amplified the targeted gene, which represent a percentage of 73.33%, with a molecular weight of approximately 310 bp (Figure 1).

DNA sequencing: The three selected phenotypic and molecular MRSA isolates were subjected to partial DNA sequencing for *mec*A gene. A FASTA format files containing the local strains sequences were used to assess a molecular relationship between Nassyriah city, Southern Iraq, isolates and other global sequences to find out the possible closely related strains. *S. aureus* THQR1, THQR2, and THQR3 isolates (with a query lengths of 253, 320, and 277 nucleotide, respectively), showed a phylogeny percentage of 99% when compared by BLAST algorithm. The Three PCR products of *mec*A *S. aureus* isolates were granted Genbank accession numbers of (KY468502, KY468503 and KY468504, respectively). A phylogenetic tree with all sequences were constructed. Figure 2, illustrates the maximum likelihood tree for the locally three *S. aureus* MRSA isolates when aligned with similar sequences around the world. The analysis involved four nucleotide sequences of *S. aureus mec*A genes which were under the following accession numbers: TN/CN/1/12, *mec*A gene beta-lactam, *mec*A gene, and NCTC8325, in which all of them were isolated in USA. A different homology and similarities were noticed between the local MRSA isolates and the American ones.

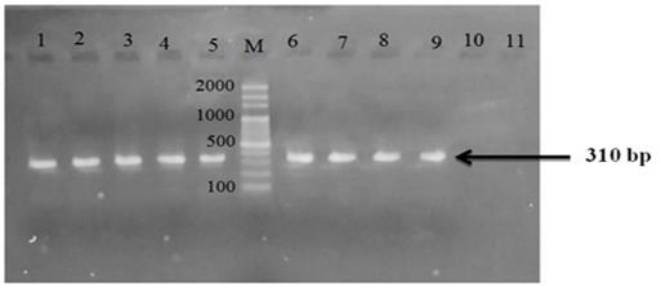

Fig. 1: PCR amplification of 310 bp *mec*A gene by 1.4% agarose gel electrophoresis, where M: ladder, lane(1-9): positive results. Lane (10 and 11): negative.

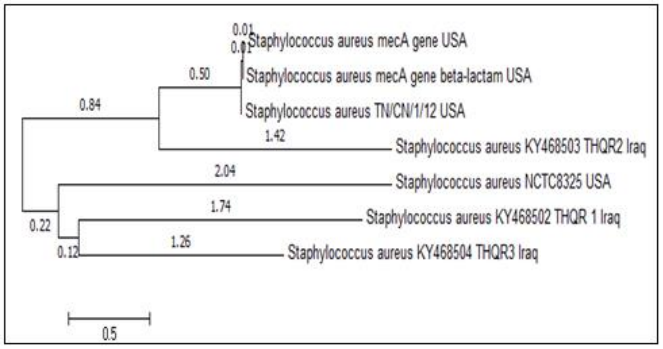

Fig. 2: A dendrogram showing the neighbor joining phylogenetic tree of Thi-Qar *mec*A isolates and the related strains from Genbank.

Discussion

According to the growing evidences which considered methicillin-resistant *Staphylococcus aureus* (MRSA), as one of the most significant pathogen that has emerged in the last four decades, a global consensus was assessed about the medical importance of MRSA detection for both patients care and a proper use of infection control resources (AL-Ruaily and Khalil, 2011). As a whole comparison between phenotypic and PCR-based methods for detection of MRSA, and as shown in the present study results, it is still leaning towards the sensitivity of molecular techniques.

However, an important opinion indicates that methicillin-resistant can be due to not only to the presence of the *mec*A gene alone; but by a cluster of this gene and *ica* gene (Memmi *et al*., 2008). A lot of local similar studied targeted *mec*A gene, as a molecular indicator of MRSA in different parts of Iraq, with variable *S. aureus* samples. A study in Kurdstan region, Northern Iraq, recorded a MRSA prevalence of 51.7% (Hussain, 2016). Other study in Iraq, revealed a slightly, high MRSA rates with 93.4% and 73.2% for Baghdad and Wasit Provinces, respectively (Al-Dahbi and Al-Mathkhury, 2013 ; Al-Mayahie *et al*., 2015). According to the phenotypic resistant, a study in Thi-Qar Province, revealed a MRSA occurrence of 67.46% (Degaim *et al*., 2015); which seems to be higher than the results obtained by the present study. Methicillin resistant rates continue to be confusing when highlighted the similar studies conducting in neighboring countries, such as what recorded in Saudi Arabia and Iran with a MRSA rates of 86.7% and 56.5%, respectively (AL-Ruaily and Khalil, 2011 ; Ghasemiam and Mirzaee, 2016) . The differences of MRSA prevalence among the regions in Iraq, or even worldwide, may be explained by the variation of geographical distribution, *S. aureus* sample sources, and types and accuracy of techniques used. The sequence types of the locally targeted *S. aureus* KY468502 and KY468504 isolates seems to be more relative, since they originate from a single ancestral root and branched as a monophyletic sister cladding form. However, these two isolates were relatively, far from *S. aureus* KY468503, which was closely related to *S. aureus* TN/CN/1/12, *mec*A gene beta-lactam, and *mec*A gene isolates, followed by *S. aureus* NCTC8325. The genetic diversity appears to be important in determining evolutionary relationships, following the epidemiology of specific bacterial isolates. Furthermore, an overview about invasiveness and virulence of some isolates especially in hospitals can be assessed (Onasanya *et al*., 2003). Little studies were estimated the *S. aureus* resistant to methicillin according to published researches in Iraq. A variable genetic relatedness of some clinically isolated *S. aureus* isolates was documented (Othman *et al*., 2014).

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