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Amplification and sequencing of *hla* and *sea* genes in *Staphylococcus aureus* isolated from outpatients in Nassyriah City

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Abstract:

An increasing concern about the role of *Staphylococcus aureus* as an opportunistic pathogen that can affect different parts of human body. The pathogenic role of this bacterium is associated with its ability to secrete a highly effective virulence factors. Among these, the production of hemolysin a (hla) and enterotoxin A (sea). The aim of the current study was to investigate the occurrence and molecular profile of *hla* and *sea* genes in *S. aureus* isolated from Otitis media outpatients in Nassyriah city, Iraq during the period from August 2014 to January 2015. A total of 100 *S. aureus* isolate were assayed for the presence of *hla* and *sea* genes by Polymerase chain reaction technique. The study results showed that 68/100 and 52/100 isolate were positive for the two targeted genes, respectively ($P \leq 0.05$). five of *S. aureus* isolates (two for *hla* assigned IQSH1 and IQSH2 in which they granted the official Genbank accession numbers of KY468500 and KY468501, and three for *sea* assigned ZWS1, ZWS2 and ZWS3) were subjected to partial DNA sequencing to reveal their relative to similar isolates in Genbank. The phylogenetic tree that was created in MEGA7 software showed that there were different molecular relationships among the local *S. aureus* isolates with similar ones around the world. These findings may be lead to better understanding about the importance of *S. aureus* epidemiology in Iraq which began to increase steadily.

Keywords: Gene sequencing, *Staphylococcus aureus*, Virulence factors.

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التضخيم وحساب التسلسل لجيني *hla* و *sea* في المكورات العنقودية الذهبية المعزولة من المرضى الخارجيين في مدينة الناصرية

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الخلاصة:

ما زال القلق يتزايد حول دور المكورات العنقودية الذهبية كممرضات انتهازية تستطيع أن تصيب أجزاء مختلفة من جسم الإنسان. الدور المرضى لهذه البكتريا متعلق بقابليتها على إفراز عوامل الضراوة عالية التأثير. منها محلل الدم نوع a و السموم المعوية نوع a. هدفت الدراسة الحالية للكشف عن ال تواجد والطور الجزيئي لجيني *hla* و *sea* في بكتريا *Staphylococcus aureus* المعزولة من مرضى التهاب الأذن الوسطى في مدينة الناصرية , جنوب العراق خلال الفترة من شهر آب 2014 إلى كانون الثاني 2015 .

تم الكشف عن وجود جيني *hla* و *sea* في 100 عزلة تعود لـ *S. aureus* باستخدام تقنية تفاعل السلسلة المتبلمرة. أظهرت نتائج الدراسة أن 68 / 100 و 52 / 100 عزلة كانت موجبة لكلا الجينين على التوالي ($P\leq0.05$). تم إخضاع خمسة عزلات *S. aureus* (اثنان لجين *hla* تم تسميتهما IQSH1 و IQSH2 واللتان حصلتا على رقمي الانضمام الرسمي لبنك الجينات KY468500 و KY468501 ، وثلاث عزلات لجين *sea* تمت تسميتها ZWS1 و ZWS2 و ZWS3) لحساب تسلسل DNA الجزئي للكشف عن علاقتها مع العزلات المشابهة في بنك الجينات.

أظهرت الشجرة التطورية التي تم بنائها باستخدام برنامج MEGA7 وجود علاقات جزيئية مختلفة لعزلات *S. aureus* المحلية مع نظيراتها حول العالم. هذه النتائج قد تؤدى إلى فهم أفضل عن أهمية وبائية بكتريا *S. aureus* في العراق والتي بدأت في الزيادة باطراد.

1. Introduction:

As a clinical fact, *Staphylococcus aureus* can be considered as one of the major pathogen in both human and animal medicine [1]. It is clearly now that morbidity and mortality rates in hospitals is largely due to Staphylococci bacteremia [2]. When *S. aureus* gains its initial foothold into the host, it can cause a wide range of infections ranging from mild skin and soft tissue infections to life-threatening ones such as endocarditis and brain abscesses [3,4,5]. The main pathogenicity of *S. aureus* depends on a number of virulence factors, especially surface and cellular proteins, proteases and toxins [6]. Molecular typing of pathogenic bacteria including, *S. aureus* can be useful for supporting infection control measures, investigating suspected outbreaks, and preventing nosocomial transmission [7]. The present study aimed to investigate the molecular characterization of *hla*, and *sea* genes in some *S. aureus* isolates in Nassyriah city, Southern Iraq.

2. Materials and Methods:

Ethical approval: This research was approved by the Science College Ethics Committee, Thi-Qar University, Thi-Qar Province, Iraq.

Samples collection, Isolation and identification: The present study was carried out on 100 isolated *Stapylococcus aureus* which was collected from patients with Otitis media whom consulted the ENT unit in Al-Habobi Teaching Hospital in Nassyriah city, Iraq during the period from August 2014 to January 2015. Identification of all *S. aureus* isolates was done depending on Mannitol salt agar (MSA) (LAB, UK) cultural characteristic, microscopic, and biochemical tests [8]. Bacterial diagnosis was confirmed by API system (BioMerieux, France).

Detection of *hla*, and *sea* genes by Polymerase Chain Reaction: *Staphylococcus aureus* isolates were subjected to the detection of alpha-hemolysin (*hla*), and

enterotoxin A (*sea*) genes by conventional PCR technique using specific primer pairs (Table 1). The amplification was conducted in a thermal cycler (ESCO, India) with the programs described by Safaei *et al*. [11], and Alfatemi *et al*. [12] for the selected genes, respectively. **DNA sequencing:** Five PCR products of *S. aureus*, distributed to two *hla* and three *sea* genes, were selected for sequencing and forward and reverse primers for each gene were sent outside Iraq to be sequenced (Macrogen, Korea). Basic Local Alignment Search Tool analysis (BLAST) was lead to blast algorithm (www.ncbi.nlm.nih.gov/BLAST). The sample sequences designated as (IQSH1, IQSH2, ZWS1, ZWS2, and ZWS3) were edited, aligned, and compared with the reference sequences using BioEdit sequence Alignment Editor Software Version 7.1 (DNASTAR, USA) [13]. A phylogenetic tree for each gene sequences was constructed by using MEGA7 software [14]. **Statistical Analysis:** The results of the present study were statistically analyzed by using SPSS program Version 16. *P* values below or equal to 0.05 was considered significant.

Table 1: Primer sequences of *hla*, and *sea* genes of *Staphylococcus aureus*.

Gene	Primer sequence (5′-3′)	Product size(bp)	Reference
hla	F: CTG ATT ACT ATC CAA GAA ATT CGA TTG R: CTT TCC AGC CTA CTT TTT TAT CAG A	209	[9]
sea	F: TTGGAAACGGTTAAAACGAA R: GAACCTTCCCATCAAAAACA	120	[10]

3. Results and Discussion:

Phenotypic characterization of *Staphylococcus aureus* isolates: Three hundred and fourty ear swabs were brought to the laboratory for diagnosis, 100/340 specimens showed a positive culture as *S. aureus* on MSA which recorded a total percentage of *S. aureus* infection with 29.41%.

Detection of *hla* and *sea* genes: Each of the 100 of *S. aureus* isolated from Otitis media patients, that were accurately identified according to the previous methods were used for DNA extraction, the results were detected by agarose gel

electrophoresis. Molecular detection of the two selected genes among all *S. aureus* isolates showed that 68/100 amplified *hla* gene, with a molecular weight of approximately 209 bp (Figure 1 A)**.** The amplification of *sea* gene showed that 52/100 *S. aureus* isolates gave a positive results for the targeted gene with a molecular weight of approximately 120 bp (Figure 1 B)**.** The two genes distribution was statistically significant ($p \leq 0.05$). The present study attempted to shed light on the prevalence of two of *S. aureus* virulence factors by molecular detection of alpha-hemlysin (*hla*), and enterotoxin A (*sea*) genes isolated from Otitis media outpatients in Nassyriah City, Southern Iraq. Despite the ongoing debate about the role of different *S. aureus* virulence factors, a lot of reports tends to believe that alpha-hemolysin, which is a pore toxin encoded by the *hla* gene, continuo to play a major role in *S. aureus* pathogenesis [15,16]. Locally, only a few *hla* prevalence in *S. aureus* investigations are available, such as the finding of Hassuny [17] in Hilla city (Central Iraq), and Shallal [18] in Basrah city (Southern Iraq), who recorded a variable percentages of 22.22% and 9.09%, respectively. These finding seems to be lower than the results of the present study. However, the variability of this gene distribution among *S. aureus*, globally, continue to be dissimilar and this is obviously, reflected by different reports around the world; such as what were reported in Indonesia, Nigeria, and France [19,20,21]. These differences may be due to many factors; such as the sources and number of the clinical samples used, geographical distribution, and the sensitivity of different techniques used. In the second bank of research, the present study targeted the distribution of *sea* gene in which, approximately, half of *S. aureus* isolates amplified this gene. In Basrah city, Jassim *et al*. [22], recorded a low *sea* prevalence with 9.09%. On the other hand, high rates of this gene was recorded in Hilla city with a percentage of 87.5% [23]. The two previous local reports seems to be not compatible with the present study results. These differences may be due to basically to the differences in size and source of *S. aureus* samples since this bacteria can be isolated from almost all parts the body. **Gene sequencing:** The two selected phenotypic and molecular alpha hemolysin isolates were subjected to partial DNA sequencing for *hla* 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 universal sequences submitted to Genbank to find out the possible genotypic differences. *S. aureus*

IQSH1and IQSH2 (the official Genbank accession numbers of KY468500 and KY468501), showed a phylogeny percentages of 97% and 98% when compared by BLAST algorithm. As illustrated in Figure 2, the evolutionary analysis with six *S. aureus hla* gene isolates, Both of the local *S. aureus* isolates showed that they were closely relative. On the other hand, the present study investigate the phylogeny analysis of other three *S. aureus* entertoxin, *sea* sequences. These sequences showed BLAST identity of (100%), so the Genbank submission seems to be not necessary. Unlike the distribution of the previous *hla* isolates, The results showed a variable relation between *S. aureus* ZWS1, ZWS2, and other similar sequences (Figure 3). The molecular phylogenetic analysis for *hla* gene sequences reveals a closely relation between IQSH1 and IQSH2 isolates. The only strain relative to the study targeted sequences was *S. aureus* AP017320.1 isolated in Russia. However, the rest of the similar strains, in which isolated in Japan, South korea, and Australia seems to relatively close to the local isolates. high prevalence of *hla* gene recorded in the present study may be supported by the opinion that propose that about 95% of *S. aureus* isolates could harbored *hla* and/or *hlb* genes [24]. The *sea* gene isolate ZWS2 appears to closely related to the two strains isolated in India. Nevertheless, ZWS3 was not far away from them as the case of ZWS1. Loudly speaking, almost consensus that genotying of *S. aureus*, especially the expression of their toxins, might be useful in assessing epidemiological strains [25].

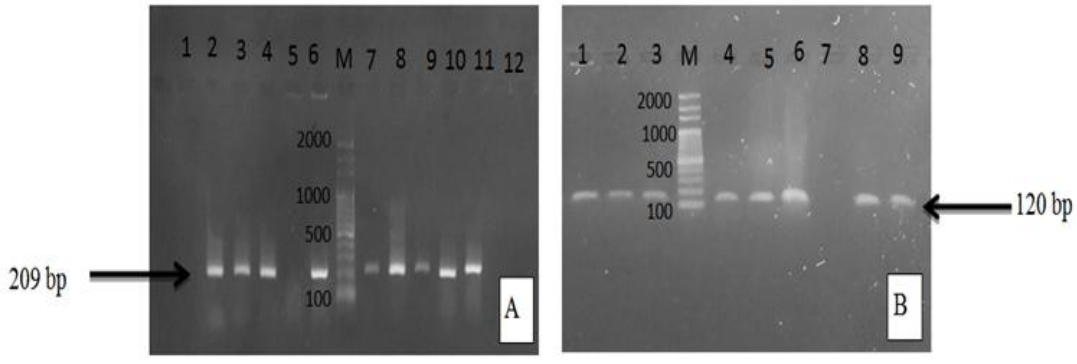

Figure 1: (A) Agarose gel electrophoresis of 209 bp *hla* gene PCR product including (Lane 2,3,4,6,7,8,9,10,11) positive, (Lane 1, 5 and 12) negative. **(B)** Agarose gel electrophoresis of 120 bp *sea* gene PCR product including (Lane 1,2,3,4,5,6,8,9) positive, (Lane 7) negative. M Ladder.

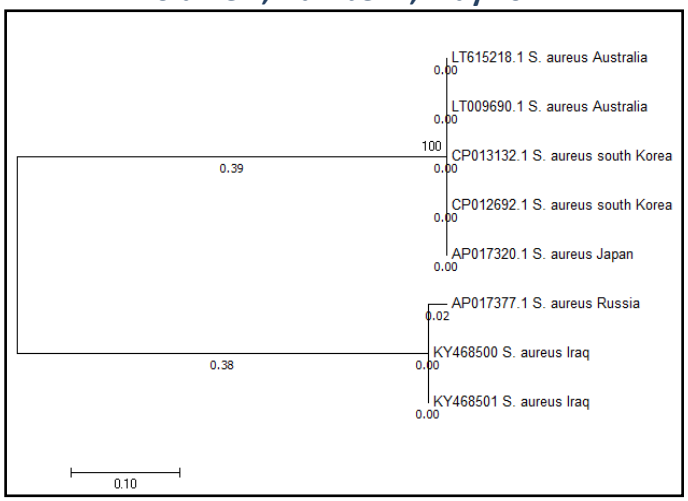

Figure 2: Phylogenitic diversity of the locally *S. aureus* sequences isolates (IQSH1 and IQSH2) by Maximum Composite Likelihood approach.

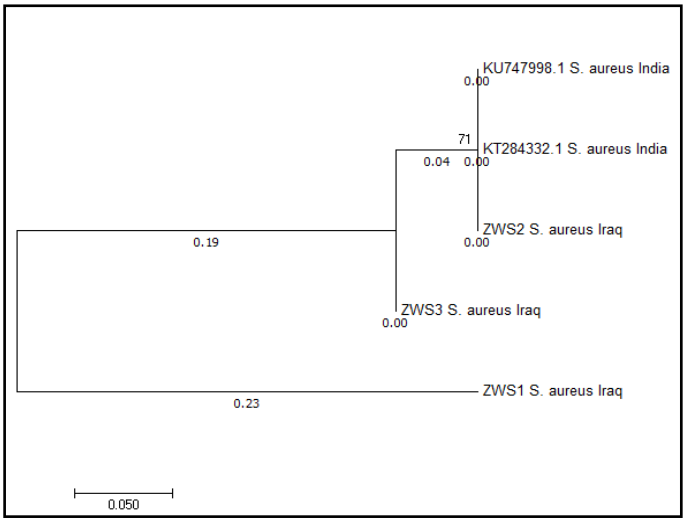

Figure 3: Phylogenitic diversity of the locally *S. aureus* sequences isolates (ZWS1, ZWS2 and ZWS3) by Maximum Composite Likelihood approach.

Conclusions: The present study highlighted the role of *Staphylococcus aureus* virulence factors on its prevalence. Furthermore, the importance of the molecular techniques in epidemiology study researches.

4. References:

[1] Pantosti A. Methicillin-resistant *Staphylococcus aureus* associated with animals and its relevance to human. Microbiol, 2012; 3: 137.

[2] Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, *et al*. Invasive Methicillin-Resistant *Staphylococcus aureus* infection in the United States. J Am Med Assoc, 2007; 298(15): 1763-71.

[3] Al-Talib H, Hassan H, Yean CY, Al-Ashawal SM, Ravichandran M. Fatal necrotizing pneumonia caused by Panton-Valentine leukocidin-producing hospital acquired *Staphylococcus aureus*: a case report. JPN J Infect Dis, 2011; 64: 58-60.
[4] Dryden MS. Skin and soft tissue infection: microbiology and epidemiology. Int J Microbial Agents, 2009; 34(S1): S2-S7.
[5] Yamada K, Yanagihara K, Hara Y, Araki Y, Morinaga Y, Matsuda Y, *et al.* Clinical features of bacteremia caused by methicillin-resistant *Staphylococcus aureus* in a tertiary hospital. Tohoku Exp Med, 2011; 224: 61-7.
[6] Denges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. Clin Microbial, 2000, 13(1): 16-34.
[7] Shittu A, Oyedara O, Abegunrin F, Okon K, Raji A. Characterization of methicillin-susceptible and resistant Staphylococci in the clinical setting: a multicentre study in Nigeria. BMC Infect. Dis, 2012; 12: 286.
[8] Harley JP, Prescott LM. Laboratory Exercises in Microbiology. 5th ed. New York. McGraw-Hill: 2002. Pp.466
[9] Mehrotra M, Wang G, Johnson WM. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxin, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbial, 2000; 38(3): 1023-35.
[10] Betley M, Mekalanos JJ. Nucleotide sequence of the type A Staphylococcal enterotoxin gene. J Bacteriol 1988; 170: 34-41.
[11] Safaei HR, Pirasteh H, , Pournasiri Z, Dormanesh B. Study the enterotoxigenixity of *Staphylococcus aureus* isolated from the urine samples of pediatric with UTIs. Biomed Pharmacol J, 2015; 8: 111-18.
[12] Al-Fatimy SMH, Mohammed MM, Hadi N, saraie HSE. Analysis of virulence genes among methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Jund J Microbiol, 2012; 7(6): 1-7.
[13] Hall TA. Bioedit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98nt. Nucl Acids Symb ser, 1999; 41: 95-8.
[14] Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol and Evol, 2016; 33:1870-74.
[15] Hildebrandt J. Pore-forming virulence factors of *Staphylococcus aureus* destabilize epithelial barriers-effects of alpha-toxin in the early phases of airway infection. AIMS Micobiol, 2015; 1(1): 11-36.

[16] Taveres A, Nielsen JB, Boye K, Rohde S, Paulo AC, *et al*. Insights into Alpha-Hemolysin (Hla) Evolution and Expression among *Staphylococcus aureus* Clones with Hospital and Community Origin. Plos one, 2014; 9(7): e98364.
[17] Hassuny AA. Detection of Virulence Factors hla and hlb of *Staphylococcus aureus* using PCR technique. Med J Babyl, 2014; 11(4): 1000-6.
[18] Shallal SH. Occurance of *Enterobacter sakazakii* and *Staphylococcus aureus* in infant milk formula and other sources with molecular study of their virulence genes. PhD thesis. Basrah University. Science College. Biology Department. Pp. 52-68.
[19] Ariyanti D, Salasia SO, Tato S. Characterization of Haemolysin of *Staphylococcus aureus* Isolated from Food of Animal Origin. Indones J Bio, 2011; 16(1): 32-7.
[20] Kolawole DO, Adeyanju A, Schaumburg F, Akinoyoola AL, Lawal OO, *et al*. Characterization of Colonizing *Staphylococcus aureus* Isolated from Surgical Wards' Patients in a Nigerian University Hospital. PLOS one, 2013; 8(7): e68271.
[21] Ferry T, Thomas D, Genestier A, Bes M, Lina G, *et al*. Comparative Prevalence of Superantigen Genes in *Staphylococcus aureus* Isolates Causing Sepsis With and Without Septic Shock. Clin Infect Dis, 2005; 41: 771-7.
[22] Jassim HA, Bakir SS, Alhamdi KI, Albadran AE. Polymerase chain reaction (PCR) for detection superantigenicity of *Staphylococcus aureus* isolated from psoriatic patients. Int J Microbiol Res Rev, 2013; 1(1): 22-7.
[23] Ewadh RM, Aldraghi WA, and Abis AG. Genetic Study of The Etiology of Some Bacterial Pathogens in People with Inflammation of The Eye and to Investigate The Prevalence of The SEA gene. Iraq J Bio, 2014; 13(2): 23-34.
[24] Grumann D, Nubel U and Broker BM. *Staphylococcus aureus* toxins - Their functions and genetics. Infect Gen Evol, 2014; 21: 583-92.
[25] Nishi J, Yoshinaga M, Miyanohara H, Kawahara M, Kawabata M, *et al*. An epidemiological survey of methicillin-resistant *Staphylococcus aureus* by combined use of *mec*-HVR genotyping and toxin genotyping in a universal hospital in Japan. Infect Control Hosp. epidemiol, 2002; 23: 506-10.