VIRULENCE POTENTIAL OF *S. aureus* ISOLATED FROM IMPORTED AND LOCAL CHICKEN DEPENDING ON THE PHENOL SOLUBLE MODULINS (*PSMMEC*) IN DUHOK PROVINCE, KURDISTAN REGION OF IRAQ

Nacheervan Majeed Ghaffar

College of veterinary medicine, Duhok research center, University of Duhok

(Received 4 July 2020 ,Accepted 14 July 2020)

Key word: (SCC), *PSM*, *mecA*

Corresponding Author: Nacheervan.ghaffar@uod.ac

ABSTRACT

Staphylococcus aureus is considered as one of the major foodborne pathogens in human and animals which can lead to a wide range of diseases, including food poisoning. The toxins of *S. aureus* play an important role in disease pathogenesis, contributing to both injury of the host tissues and the immune response. One of these toxins is phenol-soluble modulin (*PSM*) peptides which has the ability of immune invasion and considered as a cytolytic toxin. Commonly, mobile genetic elements (MGE) of *S. aureus* that carrying antibiotic resistance gene do not carry the virulence genes, however, *PSMmec* has been identified within the methicillin resistance staphylococcus-encoding MGE SCCmec. This study was conducted for six months, over-all 200 whole chicken carcasses were collected including (100) local chicken and (100) imported one from supermarkets in Duhok province. The samples for *S. aureus* were cultured on mannitol salt agar and then were confirmed using colony morphology, biochemical test like, catalase test and coagulase test, in addition to the species specific primer (*nuc* gene) for PCR. The PCR positive samples were selected and used in this study. The aim of this study was to evaluate the virulence potential of *S. aureus* isolated from imported and local chickens depending on *PSMmec*-complex PCR (spanning *PSM*, *xylR* and *mecR* genes) and *mecA*. The results of this study shows that 46 isolates out of 57 from imported chickens were carried *mecA* (methicillin resistant isolates) from

these 28 isolates harbored *PSMmec* gene. Regarding to the local chicken, only 2 isolates out of 18 carries together both *PSMmec* and *mecA*. According to the *PSM*-complex tested in this study, *S. aureus* isolates from imported chickens have *SCCmec* elements (SCCmec, II (2A) and IID, while the local chicken isolates have just SCCmec IID. Isolates resistance to methicillin with *PSM* may contribute to staphylococcal virulence. The outcomes of present study suggest that isolates from imported chicken were more virulent comparing with local isolates. This study needs further confirmation by amplification of SCC elements and sequencing them to determine the proper genetic structures of these regions.

INTRODUCTION

Staphylococcus aureus is the most common foodborne pathogen distributed all over the world. These microorganism carriers a virulence gene called phenol-soluble modulins (*PSMs*), comparing with other virulence factors, this gene is α-helical peptides and has the ability to play a role as pro-inflammatory cytolytic toxins (1). Antibiotic resistance genes are normally located on mobile genetic elements (*MGEs*) like plasmids, genomic islands and, transposons (2). The methicillin resistance gene (*mecA*) is specifically situated in the staphylococcal cassette chromosome-mec (SCCmec). At least four main and many sub-types of SCCmec elements are introduced, their sizes ranging from 21 to 67 kb, these elements are characterized by two important gene complexes (*mec* and *ccr*) and transposons as an accessory gene loci (2).

PSM gene has been described by (3), In methicillin-resistant *S. aureus* (MRSA). Kaito et al., 2008, found that the *PSM-mec*, partly overlaps the open reading frame of a putative virulence-modulating, "*fudoh*" (4). It was determined that *PSM-mec* is found within SCC*mec* elements genes which include the genes responsible for *mecA*, recombinase genes, regulatory elements and inconsistently other resistance genes (5–7). In these elements, the merely known related virulence factor *is PSM-mec*. This gene has been identified in *S. aureus* SCC*mec* types II, III and VIII, SCC*mec* types II and III in *S. epidermidis* as well as found in *S. cohnii, S. saprophyticus*, *S. sciurii* and *S. vitulinus* (3). The virulent of *S. aureus* infection is mostly determined by the ability of *S. aureus* toxins to make an infection. For example, *S. aureus* may produce phenol-soluble modulins (*PSMs*), toxic shock syndrome toxin-1, enterotoxins, leukocidins, a-toxin and other toxins (8–10). Many of them have the ability to destroy immune

cells, thus contributing significantly to the immune evasion capacity of *S. aureus*. Many *S. aureus* toxins are encoded on the bacterial core genome such as a-toxin and *PSMs* (1,11).

This study was aimed to screen *S. aureus* isolated from imported and local chicken for the presence of *PSM-mec* complex associated with *mecA* in order to obtain more data on its distribution and to determine the virulence potential of these isolates as this gene is considered as a virulence determinant that connects the transcriptional regulation, virulence in staphylococci.

MATERIAL AND METHODS

This study was conducted for six months from March, 2019 to September 2019.

Sample collection

A total of 200 whole chickens were scanned for the detection of *S. aureus* (100 of local chickens and 100 of imported chickens). Local fresh chicken were collected from the Duhok chicken abattoir in Summel, Duhok province. While, the whole imported chicken carcasses were collected from different supermarkets in Duhok city (Turkish origin). These carcasses were collected and transferred immediately in a cold box to the Microbiology Laboratory at College of Veterinary Medicine for microbiological analysis.

Isolation and identification of *S. aureus*

The whole chicken was transferred to a sterile plastic bag then 400 ml of buffer peptone water (BPW) was added to the bag. The carcass was rinsed and washed thoroughly for about 2 minutes. For isolation, 10 ml of chicken rinse were collected aseptically and mixed with 90 ml of BPW. The broth was then incubated for 24 h at 37°C. A loop of the broth culture was streaked onto the mannitol salt agar (MSA) and then incubated aerobically for 18-24 h at 37°C. *S. aureus* colonies were firstly examined based on colonial morphology. The suspected *S. aureus* colonies were then selected and streaked onto MSA to get pure colonies of *S. aureus*. Gram-staining and catalase test were then applied for colonies that have a typical morphological feature. Gram and catalase positive isolates were further confirmed for *S. aureus* biochemically using coagulase test and also using species- specific primer for PCR.

Molecular methods

Ttotal DNA extraction

DNA was extracted from *S. aureus* isolates according to (12). Shortly, DNA was prepared by resuspending 2-3 colonies in 100 µl of deionized autoclaved water and mixed very well. Bacterial suspensions were boiled in a heat block for 20 min.The suspension was then centrifuged at 10,000 xg for 1 min. The supernatant was collected to new eppendorf tubes and then used as the DNA template for subsequent tests. DNA quality and concentrations were assessed using NanoDrop 2000C spectrophotometer (Theremo Scientific). The ratio 260/280 nm was in the range of 1.55 to 2.3 for all DNA samples used. The DNA samples were stored at -20°C for further analysis.

PCR technique

Details of the nucleotide sequences of the primes used for PCR amplification are provided in Table (1). Approximate gene and primer localizations, based on positions within staphylococcus genomes can be seen in (13).

Table 1: Nucleotide sequences of primers used in this study

Primer name	Sequence (5'—3′)	Annealing temperature	references
***nuc* gene**	F:AGCGATTGATGGTGATACGG R:ATACGCTAAGCCACGTCCAT	55°C	**14**
PSMmec	F:CGAAAGCCTGAATGCAAGTCT R:GGATTTCACTGGTGTTATTACAAGC	70°C	**13**
XylR(R) to ***PSMmec(F)***	F:CGAAAGCCTGAATGCAAGTCT R:AAGCGTCATCTTCTCATTTAGTTGA	55°C	**13**
PSMmec(R)* to *mecR(F)	F: CCAGAAAGTAAACAACGATATTCACC R:GGATTTCACTGGTGTTATTACAAGC	55°C	**13**
mecA	F: GTAGAAATGACTGAA CGTCCGATAA R: CCAATTCCACATTGTTTCGGTCTAA	55°C	**15**

Current study all PCR reactions were achieved in (0.2 ml) PCR tubes, crystal hot start master mixes 2x was used for this PCR (Jena Bioscience, Germany).

A total of 20 µl reaction volumes were prepared according to the manufacturer's instructions. Shortly, each reaction involved the following components: 10 µl of master mix (2X), 1.5 µl of each forward and reverse primer (10pmol/µl), and template of DNA (~ 2 µl) according to the concentration (50ng/µl) and dH2O was added to be completed to 20 µl. PCR amplification was carried out in a PCR System 9700 Thermocycler (Applied Biosystems).All the isolates used in this study were further been confirmed using thermo-stable nuclease gene (*nuc*) in detection of *S. aureus* (14).

PCR primers used in this study shows in (Table1). First PCR Reaction conditions of *PSMmec* primer included an initial denaturation (2 min at 96°C) followed by 35 cycles (20 sec at 96°C, 20 sec at 70°C and 20 sec at 72°C). A second PCR covered the region from *xylR* to *PSMmec* (*PSMmec* and *xylR*), while the third one spanned the region from (*PSMmec* to *mecR*). Both second, third and *MecA* PCR reactions included denaturation for (2 min at 96°C) followed by 35 cycles (20 sec at 96°C, 20 sec at 55°C and 70 sec at 72°C), the details can be seen in Table 2 (16).

Table2: The PCR primer condition of all the genes used in this study

<table>
<tr><th rowspan="2">Primer Name</th><th colspan="5">PCR conditions</th></tr>
<tr><th>Initial Denaturation</th><th>Denaturation</th><th>Annealing Temperature</th><th>Extension</th><th>Final Extension</th></tr>
<tr><td>nuc gene</td><td>2 min at 94°C</td><td>30 sec at 96°C</td><td>55°C</td><td>2min at 72°C</td><td>72 °C 10 min</td></tr>
<tr><td>PSMmec</td><td rowspan="3">2 min at 96°C</td><td rowspan="3">20 sec at 96°C</td><td>70°C</td><td rowspan="3">20 sec at 72°C</td><td rowspan="3">72 °C 10 min</td></tr>
<tr><td>XylR(R) to PSMmec(F)</td><td rowspan="2">55°C</td></tr>
<tr><td>PSMmec(R) to mecR(F)</td></tr>
<tr><td>mecA</td><td>10 min at 94°C</td><td>45 s at 94°C</td><td>55°C</td><td>72°C for 75 s</td><td>72 °C 10 min</td></tr>
<tr><td colspan="2"></td><td colspan="3">Repeated for 35 cycles</td><td></td></tr>
</table>

Gel electrophoresis technique:

PCR products were established by electrophoresis in a 1.5% (w/v) agarose gel using 1X TAE buffer with nucleic acid dye Syber® Safe (Invitrogen). Seven microliters of each PCR product were loaded into each well. The size of the PCR products was showed by loading 7 µl of 100bp DNA ladder (Jena Bioscience, Germany). The fragment sizes were then visualised and photographed under UV light figure (1).

RESULTS AND DISCUSSION

From 200 (100 local and 100 imported chicken) whole chicken samples were examined for the present of *S. aureus*, 108 isolates were found to be positive for staphylococcus by culture method (80 from imported frozen chicken and 28 from local chicken), however, only 68 from imported and 22 from local chicken were noticed to be *S. aureus* species depending on the coagulate test. Moreover, these samples were further been confirmed using *S. aureus* specific

gene (nuc gene) by conventional PCR, from these only 57 and 18 were indicated as *S. aureus* in both imported and local chicken, respectively, these results have been published in a separate study (14), the same isolates have been used to implement this study.Identification of the Staphylococcus cassette chromosome mec (SCCmec) of this study isolates will mainly depend on the previous studies outcomes (3,13,16,17), inorder to recognise the possible classification of MGEs of *S. aureus* isolated in this study. In present study, a PCR reaction that covering the area from *xylR* to *PSMmec* indicate that, out of 57 imported isolates only 31 isolates were found to be positive for this region, and 2 out of 18 isolates from the local chickens were give positive results for this locus.In 32 isolates *PSMmec*, were found negative (20 from imported and 12 from local) isolates, from these *xylR* was also absent. Results of the third PCR *mecR/PSMmec* were only found in 15 imported isolates, in addition to the present of *mecA*, *PSMmec/xylR* and *PSMmec* (Table 3), while none of the local isolates were give positive results for the third PCR (*mecR/PSMmec*), (Table 3). The PCR results are shown in Figure (1)

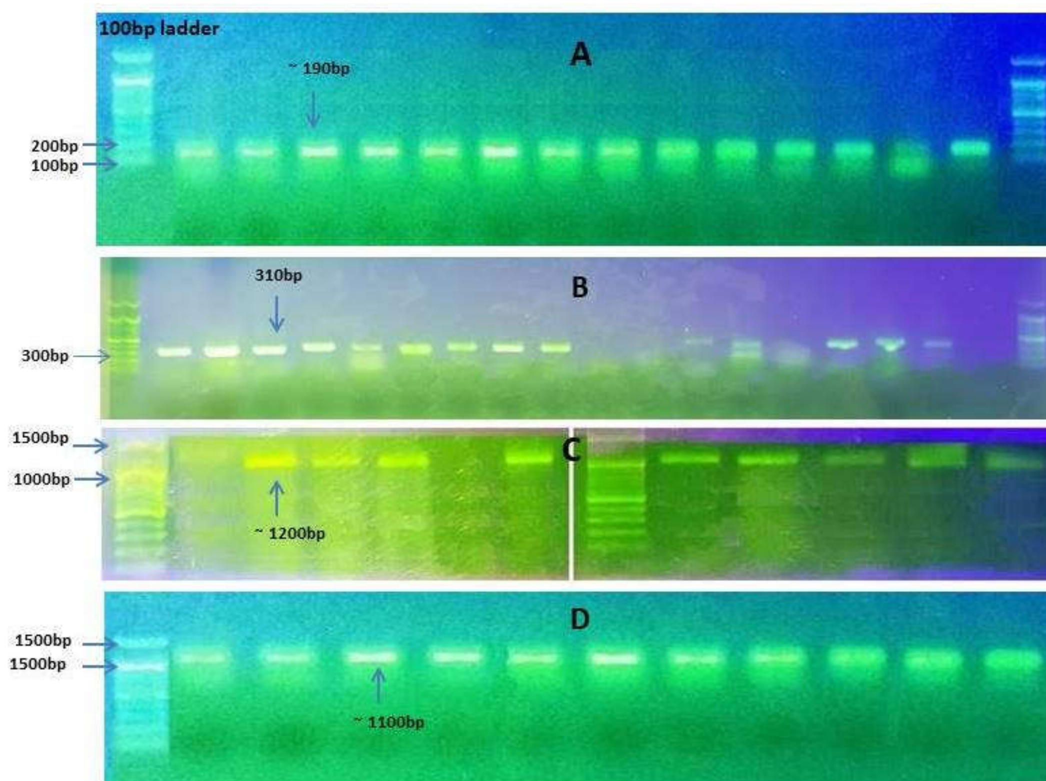

Figure 1: PCR assay for detection of *PSMmec* gene (A), methicillin resistance gene (*mecA)* (B), *PSMmec/xylR* region (C) and *mecR/PSMmec* region (D) in *S. aureus* isolates from imported and local chicken. Lane (1) 100bp DNA ladder (Jenabiosciences, Germany).

Table3: Description of PCR results of PSM complex

Species of bacteria	Host and origin	No. of isolates tested	*PSMmec*	*PSMmec /xylR*	*mecR / PSMmec*	*mecA*	Expected SCC*
S. aureus	Imported chicken	20	-	-	-	+(13/20)	
S. aureus	Imported chicken	19	+(17/19)	+(15/19)	+(15/19)	+(16/19)	SCCmec I, II(2A)
S. aureus	Imported chicken	18	+(17/18)	+(16/18)	-	+(17/18)	SCCmec (I, IID)
S. aureus	Local chicken	12	-	-	-	+(6/12)	
S. aureus	Local chicken	6	+(2/6)	+(2/6)	-	+(2/6)	SCCmec(IID)

*According to the genes tested the *S. aureus* isolated for imported chickens have SCC elements SCCMECI, II (2A) and IID, while the local chickens have just SCCmec IID. This classification is done depending on the study results of (3,6,7) , however, this must be confirmed by sequencing of SCCs in the future study.

On the basis of available MRSA genome sequences, the P*SMmec* gene is found mostly within SCCmec determinants of types II and III (18). It has been noticed that CC12-MRSA, WA-MRSA-59 harbored SCCmec elements containing *mecR* but missing *xylR*. However, this study revealed that some of the imported chickens isolates may carries SCCmec type (IIA) and IID elements having *xylR* but lacking *mecR* this classification depends on the outcome of (3,13,16).

It was found that the *PSMmec* is associated with *mecI* and/or *xylR*, in another word it can be found in the isolates harboring Classification of Staphylococcal Cassette Chromosome mec (SCC*mec)* types II, II-A, II-B, II-D, III, and VIII or types II-A/B and III as irregular elements related to SCC*mec*. However, *PSMmec* was not limited just in *S. aureus*. The other staphylococcus species such as *S. pseudintermedius*, *S. epidermidis*, *S. hominis S. fleuretti, S. saprophyticus, S. vitulinus* and *S. simulansmay* may also carry *PSMmec.* Present of this gene is not only restricted in one host species, it was noticed in different isolates from different host like in cattle, turkeys, pigs, goats, sheep, cattle, cats and human (5,7,13).

The *PSMmec* was not found in SCCmec types I, IIC, IIE, IV, V or XI and in SCC elements missing the *mec* complex (13,16). Accordingly, this study also revealed that 13 isolates of *S. aureus* from imported chicken were just carries *mecA*, this is suggests that these isolates may carry either SCCmec type I or II. Some *S. aureus* isolates of the current study were missing *PSMmec*, this is an indication of either absent of SCC*mec* elements, or carrying SCC*mec* types I, IIC, IIE, IV and V as stated by (13,16). Virulence factors association with mobile genetic elements (MGEs) deliberating drug resistance detected in *E. coli* (2,19) and enterococci (20), however, the connection of *PSMmec* to SCCmec in staphylococci, is rather unique (21). The α-type PSM peptide has a cytolytic and proinflammatory action and plays an important role in MRSA infection. In a study conducted by (22), found that 7.6% of all MRSA strains carried the psm gene from the isolates collected from blood, sputum, wound, and trachea. Joo and coworkers (2011) (23) state that *PSMs* have cytolytic activities on the neutrophils, red blood cells, and white blood cells and impact on pathogenesis of bacteremia, skin and soft tissue infections. According to their surfactant like properties, *PSMs* have also been suggested to influence biofilm maturation and detachment(21–24).

The presence of *PSM*mec in MRSA strains can be a key factor for the pandemic spread and abundance success of SCC*mec* elements. Therefore, this study suggest that the *S. aureus* isolated from imported chicken may be more virulent than the isolates from local chicken due the present of *PSMmec* complex which were from types I, II(2A) and IID as listed in Table (3).

Isolates resistance to methicillin with *PSM* contribute to staphylococcal virulence. Accordingly, this study shows that this pattern is more obvious in imported chicken isolates rather than local chickens. The role of methicillin resistance groups in the pathogenicity of staphylococcal has also studied by (3), they identified that the majority of serious antibiotics and virulence resistance elements may be associated in staphylococcal MGEs. Our study shows that in similarity to the previous belief, *S. aureus* can handle both resistance and virulence factors on MGEs, consequently, this combination will help to transfer of two important factors for causing human disease in one genetic episode.

الضراوة المحتملة لبكتريا المكورات العنقودية الذهبية *S. aureus* المعزولة من الدجاج المحلي والمستورد بالاعتماد على Phenol Soluble Modulin (*PSMmec*) في محاظة دهوك، أقليم كوردستان العراق

نيجرفان مجيد غفار[١]

[١]كلية الطب البيطري، مركزدهوك للابحاث، جامعة دهوك،أقليم كوردستان العراق

الخلاصة

تعد المكورات العنقودية الذهبية *Staphylococcus aureus* أحد أهم البكتريا المسببة للتسمم الغذائي لدى الانسان والحيوان. الذيفانات التي تنتجها *S. aureus* تلعب دورا مهما في الامراضية، والتي تتضمن تلف انسجة المضيف والتأثيرعلى الاستجابة المناعية. أحد هذه الذيفانات هو ذيفان بروتيني يدعى Phenol Soluble Modulin (PSM)، والذي له القدرة على اختراق الجهاز المناعي ويعتبر أيضا من الذيفانات المحللة للخلايا. غالبا العوامل الجينية المتنقلة Mobile genetic elements للمكورات العنقودية الذهبية والتي تحتوي على مورثات مقاومة لمضادات الحيوية لاتحتوي على مورثات الفوعة ماعدا (PSM) والتي تم تشخيصه في المكورات العنقودية الذهبية المقاومة للميثيسلين والتي تشّفر للعامل الجيني المتنقل Staphylococcal Cassette Chromosome (SCC). تم فحص ٢٠٠ عينة لحوم دواجن كاملة بواقع ١٠٠ عينة من الدجاج المحلي و ١٠٠ عينة من الدجاج المستوردة. تم زراعة العينات على أوساط المانيتول الملحي ليتم تأكيد شكل المستعمرات وأجراء فحص صبغة غرام ، فحص الكتاليز وباستخدام تقنية PCR،حيث العزلات الموجبة لمورث (*nuc*) تم الاعتماد عليها لتكملة هذه الدراسة .هذه الدراسة تهدف إلى تقييم الضراوة المحتملة للمكورات العنقودية الذهبية المعزولة من الدجاج المحلي والمستورد إعتمادا على تفاعل البلمرة المتسلسل ل *PSMmec*-complex والتي تغطي الجينات (*mecR*, *xylR* and *psm*)بالاضافة إلى المورث *mecA* . أظهرت هذه الدراسة بأنه ٤٦ عزلة من أصل ٥٧ عزلة من المكورات العنقودية الذهبية في الدجاج المستورد تحمل المورث *mecA* ، وأن ٢٨ من هذه العزلات تحمل المورث*PSMmec*. اما بالنسبة للمكورات العنقودية الذهبية المعزولة من الدجاج المحلي، أظهرت هذه الدراسة أنه فقط عزلتين من ضمن 18 عزلة تحمل المورثات *mecA* و *PSMmec* معا. تبعا لاختبار PSM-complex الذي إجراءه على عزلات *S. aureus* المعزولة من الدجاج المستورد، تبين ان هذه العزلات قد تحمل عوامل SCC من نوع SCCmec I, II (2A) وIID. بينماعزلات الدجاج المحلي كانت تحمل فقط النوع SCCmecIID. العزلات المقاومه للميثيسلين والتي تحمل المورث PSM من الممكن أن تلعب دورا مهما في ضراوة المكورات العنقودية. النتائج المتحصلة في هذه الدراسة تقترح أنه العزلات في الدجاج المستورد أكثرضراوة من الدجاج المحلي. هذه النتائج قد تكون اكثر تأكيدا إذا ما استخدم تضخيم عوامل SCC وتقنية سلسلة االدنا DNA sequencing لتحديد الخارطة الجينية لهذه المناطق.

REFERENCES

1. **Wang R, Braughton KR, Kretschmer D, Bach THL, Queck SY, Li M, et al**.(2007). Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat Med. 13(12):1510–4.
2. **Ito T, Okuma K, Ma XX, Yuzawa H and Hiramatsu K**(2003). Insights on antibiotic resistance of Staphylococcus aureus from its whole genome: Genomic island SCC. Drug Resist Updat. 6(1):41–52.
3. **Queck SY, Khan BA, Wang R, Bach THL, Kretschmer D, Chen L, et al**(2009). Mobile genetic element-encoded cytolysin connects virulence to methicillin resistance in MRSA. PLoS Pathog. 5(7):1–12.
4. **Kaito C, Omae Y, Matsumoto Y, Nagata M, Yamaguchi H, Aoto T, et al**.(2008). A novel gene, fudoh, in the SCCmec region suppresses the colony spreading ability and virulence of Staphylococcus aureus. PLoS One. 3(12).
5. **Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, et al**.(2001). Structural Comparison of Three Types of Staphylococcal Cassette Chromosome. Society. 45(5):1323–36.
6. **Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, et al**.(2011). Detection of staphylococcal cassette chromosome mec type XI carrying highly divergent mecA, mecI, mecR1, blaZ, and ccr genes in human clinical isolates of clonal complex 130 methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 55(8):3765–73.
7. **Ito T, Hiramatsu K, Oliveira DC, De Lencastre H, Zhang K, Westh H, et al**. (2009). Classification of staphylococcal cassette chromosome mec (SCCmec): Guidelines for reporting novel SCCmec elements. Antimicrob Agents Chemother. 53(12):4961–7.
8. **Dinges MM, Orwin PM and Schlievert PM** (2000). Exotoxins of Staphylococcus aureus. Clin Microbiol Rev. 13(1):16-34.
9. **Foster TJ**(2005). Immune evasion by staphylococci. Nat Rev Microbiol. 3(12):948–58.
10. **Otto** M (2014). Laporan Praktikum Isolasi Dna Dan Teknik Pcr. Nama Juwita Rika Nailuvar Sinaga. 32–7.

11. **Gray GS and Kehoe M** (1984). Primary sequence of the α-toxin gene from Staphylococcus aureus Wood 46. Infect Immun. 46(2):615–8.

12. **Aranda KRS, Fagundes-Neto U and Scaletsky ICA**(2004). Evaluation of multiplex PCRs for diagnosis of infection with diarrheagenic Escherichia coli and Shigella spp. J Clin Microbiol. 42(12):5849–53.

13. **Monecke S, Engelmann I, Archambault M, Coleman DC, Coombs GW, Cortez de Jäckel S, et al**.(2012). Distribution of SCCmec-associated phenol-soluble modulin in staphylococci. Mol Cell Probes. 26(2):99–103.

14. **Abdulrahman RF**(2020). Detection of Staphylococcus aureus from Local and Imported chicken in Duhok Province/ Kurdistan Region of Iraq Using Conventional and Molecular Methods. BasJVetRes. 2(19):accepted.

15. **McClure JA, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W, et al**. (2006). Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. J Clin Microbiol. 44(3):1141–4.

16. **Monecke S, Skakni L, Hasan R, Ruppelt A, Ghazal SS, Hakawi A, et al**.(2012). Characterisation of MRSA strains isolated from patients in a hospital in Riyadh, Kingdom of Saudi Arabia. BMC Microbiol. 12(1).

17. **Novick RP, Schlievert Pand Ruzin A**.(2001). Pathogenicity and resistance islands of staphylococci. Microbes Infect. 3(7):585–94.

18. **Berube BJ, Sampedro GR, Otto M and Wardenburg JB**.(2014). The psmα locus regulates production of Staphylococcus aureus alpha-toxin during infection. Infect Immun. 82(8):3350–8.

19. **Raad I, Alrahwan A and Rolston K**.(1998). Staphylococcus epidermidis : Emerging Resistance and Need for Alternative Agents . Clin Infect Dis. 26(5):1182–7.

20. **Mehlin C, Headley CM and Klebanoff SJ**.(1999). An inflammatory polypeptide complex from Staphylococcus epidermidis: Isolation and characterization. J Exp Med. 189(6):907–17.

21. **Lowy F.** (1998). The chromosome, as well as the extrachromosomal el- ements. 6 These genes are transferred between staphy- lococcal strains, species, or other gram-positive bacte- rial species through the extrachromosomal elements. 7. N Engl J Med. 339:520–

32.

22. **Eshaghi M, Bibalan MH, Pournajaf A, Gholami M and Talebi M (**2017). Detection of New Virulence Genes in mecA-positive Staphylococcus aureus Isolated from Clinical Samples: The First Report from Iran. Infect Dis Clin Pract. 25(6):310–3.
23. **Joo HS, Cheung GYC and Otto M.** (2011). Antimicrobial activity of community-associated methicillin-resistant Staphylococcus aureus is caused by phenol-soluble modulin derivatives. J Biol Chem. 286(11):8933–40.
24. **Klingenberg C, Rønnestad A, Anderson AS, Abrahamsen TG, Zorman J, Villaruz A, et al**.(2007). Persistent strains of coagulase-negative staphylococci in a neonatal intensive care unit: Virulence factors and invasiveness. Clin Microbiol Infect. 13(11):1100–11.