Tikrit Journal of Pure Science 21 (3) 2016 *ISSN: 1813 – 1662 (Print)*
E-ISSN: 2415 – 1726 (On Line)

Detection of Some Staphylococcal Enterotoxin Genes in MRSA Strains Using PCR Techniques

Suha M. Abed[1] , Waad M. Raoof[2] , Akeel H. A. Assie[1] , Zeina *S.* M. Al-Hadeithi[3] , Farooq Ibrahim[4]
[1] *Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq*
[2] *College of Pharmacy, University of Tikrit, Tikrit, Iraq*
[3] *College of Pharmacy, University of Alnnahrin, Baghdad, Iraq*
[4] *Research Center and bio technologies, University of Alnnahrin, Baghdad, Iraq*

Abstract
In view of the increasing interest in the Methicillin-resistant *Staphylococcus aureus* (MRSA). The extracted DNA yield was observed using the phenol-chloroform method, it ranged from (1.6-1.8) and concentration ranged from 100 to 800 ng/µl. Five classical enterotoxin genes were investigated in 20 isolates using multiplex PCR method after it had been molecularly identified into methicillin resistant using *mec A* (which is the key genetic component of methicillin resistance) and *fem A* genes in a duplex PCR technique. A multiplex PCR test based on the simultaneous amplification of the five genes genes; *sea* 102bp, *seb* 164bp, *sec* 451bp, *sed* 278 bp and *see* 209bp was conducted to directly detect the toxin gene content. Our results had showed that most of MRSA samples harbored at least one enterotoxin gene. Multiple toxin gene combinations were also observed. Using this PCR assay we found that among the MRSA strains obtained (n=20). The most commonly found gene was the enterotoxin A *sea* (n: 18, 90%), which was found alone and together with other toxin genes.

Keywords: *Staphylococcus aureus*, PCR, MRSA, Enterotoxin toxin, Multiplex PCR.

Introduction
The staphylococcal enterotoxins are recognized agents of intoxication staphylococcal food poisoning syndrome and may be involved in other types of infections with sequelae of shock in humans and animals. Serologically, five toxin groups have been recognized and designated staphylococcal enterotoxin A (SEA), SEB, SEC, SED, and SEE. Minor epitope differences in the SEC group have resulted in a further subdivision into SEC1, SEC2, and SEC3. SEA, SED, and SEE share immunological determinants, as do SEB and SEC1 and streptococcal pyrogenic exotoxin A (SPEA) ([1], [2], [3], [4], [5]). These toxins are for the most part produced by *Staphylococcus aureus* although other species have also been shown to be enterotoxigenic. ([6])

Staphylococcal enterotoxins (SEs) are exoproteins which, when ingested, induce gastro-enteric syndrome in humans and can cause toxic shock. Staphylococcal food poisoning outbreaks are characterized by vomiting and diarrhea and occur quite frequently worldwide; hence, the detection of enterotoxins is epidemiologically essential ([4]). The SEs are emetic toxins and are classified as members of the pyrogenic toxin superantigen family because of their biological activities and structural relatedness ([7]). These proteins (MW: 35 kDa) are not inactivated by heating to 100 ℃ for 15–30 minutes ([8]). Five classical enterotoxin types, i.e., SEA through SEE and many new types of SEs or superantigens (SAgs), i.e., SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER, and SEU have been reported ([9], [10],[11]). Apart from having pathogenic versatility, S. aureus can adapt rapidly to the selective pressure of antibiotics, with the emergence and spread of methicillin-resistant S. aureus (MRSA) isolates being a relevant example. MRSA was first described in 1961, the year in which methicillin was marketed ([12]).

The main goal of this study was to investigate the presence of some genes encoding staphylococcal enterotoxins (SEs) by polymerase chain reaction (PCR) in MRSA isolates.

Material and Methods
Bacterial Strains
The study included 20 local isolates of methicillin resistant *Staph. aureus* collected from human skin cases provided from Baghdad University, college of science, Biotechnology department. Those isolates were identified at the species level using morphological characteristics and biochemical tests and were detected as methicillin resistant using molecular ways.

Specimen processing and DNA extraction
Genomic DNA was extracted from staphylococcal cultures manually using the the phenol-chloroform method that included organic solvent; CTAB, phenol/ chloroform/ isoamyl alchohol. This default extraction protocol is presented by reference ([13]) with some modification.

Duplex PCR
Fem A gene was used for molecular confirmation of *Staph. aureus* and *mec* A was used to detect the methicillin resistant strains directly without the need to proceed the step of antibiotic disc diffusion methods as referred by ([14]) following the procedure published by ([15]). The PCR cycling conditions were as follows: PCR reaction was conducted with (BiONEER. KOREA) containing 1U Taq DNA polymerase; 250 µM deoxynucleoside triphosphates; (10 mM Tris-HCl [pH 9], 30 mM KCl); 1.5 mM MgCl2 in a 25µl reaction mixture containing of template DNA and 10 pmol of each primer (forward and reverse). Amplification program was 1 cycle at 94°C for 1 min; 35 cycles of 94°C for 1min, 63°C for 1min, 72°C for 1min; 72°C for 10min. The reactions were performed on an Applied Biosystems. The

amplified product was subjected to gel electrophoresis that were to: Five-microliter aliquots were loaded onto agarose gel electrophoresis gels (2% agarose, 1x TBE; 50 V for 2 hours) and stained with 0.5 mg/ml of ethidium bromide for 20-30 minutes after electrophoresis.

Multiplex PCR Conditions

The nucleotide sequences of all primers used in this study for the multiplex PCR and their respective amplified products are listed in the table below:

Table (1) Nucleotide sequences, gene locations, and anticipated sizes of PCR products for the MRSA gene-specific oligonucleotide primers as described by reference ([16]).

Gene	Primer	Oligonucleotide sequence	Size of amplified product (bp)	Multiplex PCR set
sea	GSEAR-1 GSEAR-2	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102	A
seb	GSEBR-1 GSEBR-2	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164	A
sec	GSECR-1 GSECR-2	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	451	A
sed	GSEDR-1 GSEDR-2	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTCGTTC	278	A
see	GSEER-1 GSEER-2	AGGTTTTTTCACAGGTCATCC CTTTTTTTTCTTCGGTCAATC	209	A

A multiplex PCR amplification of enterotoxin genes in MRSA isolates was employed using five pairs of SE primers according to the instruction of AcuuPower PCR PreMix from (BiONEER, Korea), with slight modifications to the procedure written by ([17]).

Multiplex Primer Reaction

contained 250 µM deoxynucleoside triphosphates; 2.5U of *Taq* DNA polymerase (10 mM Tris-HCl [pH 9], 30 mM KCl); 1.5 mM MgCl2; 10 pmol (each) of *sea*, *seb*, *sec*, *see* and *sed* primers and 10 to 50 ng of template DNA. The volume of this mix was adjusted to 50 µl with sterile water.

Table (2) Details of PCR Reactions Including Concentration and Volume

Material	Final concentration	Volume of 1 tube
PCR premix	1X	10µl
Forward primer	10 pmol/µl	2 µl (0.4 µl ×5)
Reverse primer	10 pmol/µl	2 µl (0.4 µl ×5)
Template DNA	50 ng	4 µl
Deuinezed D.W	-	32µl

Multiplex PCR Program

DNA amplification was carried out in a Labnet thermocycler with the following thermal cycling profile: an initial denaturation at 94°C for 5 min was followed by 35 cycles of amplification (denaturation at 94°C for 2 min, annealing at 57°C for 2 min, and extension at 72°C for 1 min), ending with a final extension at 72°C for 7 min ([17], [18]). PCR products (10 µl) were resolved in a 2% agarose gel in 1x TBE buffer 80 V for 2 hours or approximately 200 min to separate the different amplification products efficiently. The gel was stained with ethidium bromide and photographed using gel documentation system ([19], [20]). The molecular weights of bands were estimated by using standard molecular weight marker purchased from BIONEER Co.

Results and Discussion

The specimens used in this study were obtained from different clinical cases of localized skin infections. The results of DNA extraction had revealed relative purity of (1.6-1.8) and concentration ranged from 100 to 800 ng/ml. Results of samples analyzed by the Nano-Drop method revealed an absorption ratio of 260/280 nm, which represented the desired purity of extracted DNA. Our results for the manual extraction applied method are consistent with([21]) who declared that the phenol-chloroform extraction method was so cost effective and reliable with relatively high DNA concentrations.

Phenotypic methods take several days for identification and antimicrobial susceptibility testing of staphylococcal isolates therefore we conducted duplex PCR to directly distinguish the MRSA strains from the Staphylococcal isolates agreed with ([22]) had who published that (PCR) for the amplification of the *mec-A* is presently considered the gold standard for the detecting methicillin resistance in *S. aureus* comparing with the performance of phenotypic methods in the detection of methicillin resistance that is not consistent, time consuming and also encounter difficulties in detecting all the resistant isolates. This study approaches to investigate toxin gene content by performing multiplex PCR technique (irrespective of whether the strain produces the toxin or not). A single PCR reaction (multiplex PCR amplification) was employed using five pairs of SEs (classical enterotoxins) following the general principles described by ([15]). Amplicon sizes ranged from 102 to 451 bp, differing by at least 60 bp to facilitate electrophoretic separation. It is worthy to mention that isolate number 20 was not listed in the figure bellow however it is included in the table results.

ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

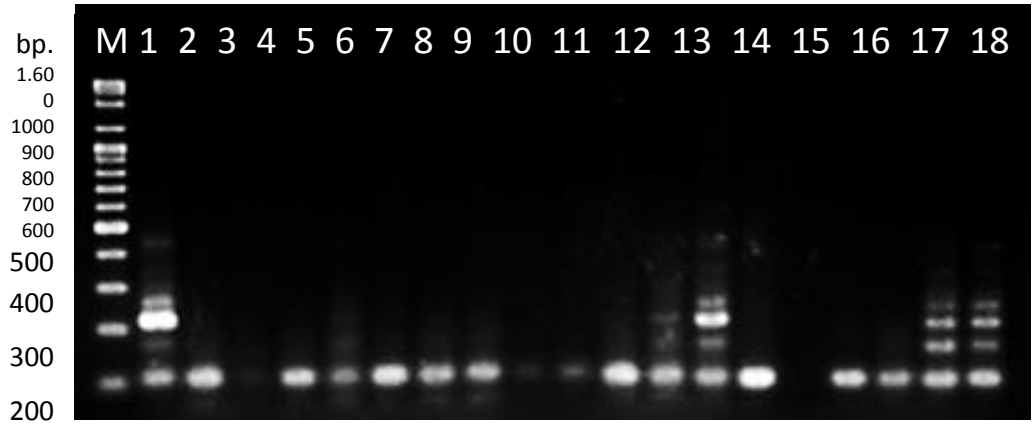

Fig. (4-24). 2%Agarose gel electrophoresis of the Set A multiplex PCR amplification products obtained from analysis of 19 tested strains. MW = molecular weight marker 100 bp ladder (BIONEER).

Table (3) Distribution of toxin genes in the selected MRSA isolates (set A)

Isolate number	Gene combination				
	sea	***seb***	***sec***	***sed***	***see***
MRSA1	+	+	+	-	+
MRSA2	+	-	-	-	-
MRSA3	-	-	-	-	-
MRSA4	+	-	-	-	-
MRSA5	+	-	-	-	-
MRSA6	+	-	-	-	-
MRSA7	+	-	-	-	-
MRSA8	+	-	-	-	-
MRSA9	+	-	-	-	-
MRSA10	+	-	-	-	-
MRSA11	+	-	-	-	-
MRSA12	+	+	-	+	-
MRSA13	+	+	-	+	+
MRSA14	+	-	-	-	-
MRSA15	-	-	-	-	-
MRSA16	+	-	-	-	-
MRSA17	+	-	-	-	-
MRSA18	+	+	+	+	+
MRSA19	+	+	+	+	+
MRSA20	+	-	-	-	-

Using the multiplex PCR assay for the enterotoxin genes, we found that among the MRSA strains obtained (n=20). The most commonly found gene was the enterotoxin A *sea* (n: 18, 90%), which was found alone and together with other toxin genes. Agreed with ([23]) who demonstrated in their research that the *sea* gene was the predominant enterotoxin gene In the Jordanian clinical *Staph. aureus* isolates. Our results showed that most of MRSA samples harbored at least one virulence gene. Multiple toxin gene combinations were also observed. Our findings of the toxin gene content of MRSA isolates agreed with ([24]) who reported that *Staphylococcus aureus* strains often carry in their genomes virulence genes that are not found in all strains and that may be carried on discrete genetic elements.

Most, if not all, staphylococcal superantigens are encoded by accessory genetic elements that are either mobile or appear to have been mobile at one time. These identified elements include plasmids, transposons, prophages, and the pathogenicity islands and that explains our results ([25]). Another study had stated that Staphylococcal enterotoxins (SEs) may play an important role in staphylococcal disease states in addition to their ability to cause food poisoning. The genetics of *Staph. aureus* enterotoxin production have been well studied, and genes coding for these toxins have been localized on the chromosome for SEB and SEC (pathogenicity island SaPI 3 and 4, on bacteriophage vectors for SEA (on a bacteriophage having at least two integration sites within the chromosome), and on plasmids for SED (on a large penicillinase plasmid designated pIB485) ([26]). Although SEE and SEA are the most closely related enterotoxins, SEE genes have not been shown to be phage associated, and hybridization data suggest that genes for SEE may be linked to a defective converting phage carrying SEA-like coding sequences ([27], [1], [28]) and this might explain our results, since some isolates showed couple of bands while others had only showed one or two. The SEs could be able to indicate the origin of the *Staph. aureus* strains because it was observed that a higher ratio of isolates from bovine produced SEC and those from human produced mainly SEA ([29]).

Some strains of *Staph. aureus* producing one or both of two immunologically distinct exfoliative toxins, exfoliative toxin A (ETA) or ETB (have been associated with a series of impetiginous staphylococcal diseases referred to as staphylococcal scalded skin syndrome (characterized by the splitting of the epidermis and exfoliation). Although ETA and ETB have identical biological activity and a degree of genetic similarity, the gene coding for ETA is chromosomal whereas the gene coding for ETB is plasmid linked ([30], [29], [1]). Another study by ([31]) stated that the eta gene encoding ETA is located on a prophage that is integrated into the *Staph. aureus* chromosome which might explain our PCR results for ETa since some of the isolates showed band while others did not. This research focuses on multiplex systems in which each primer pair targets a single locus. From these results, and from our own

experience with multiplex PCR, it has become evident that Single template PCR Reaction is ideal for conserving costly polymerase and templates in short supply and can be applied in Pathogen Identification. Multiplex PCR is a variant of PCR in which two or more loci are simultaneously amplified in the same reaction ([32]). It was first developed in 1988 by Chamberlain *et al.*, ([33]). A number of review and research articles have provided detailed descriptions of the key parameters that may influence the performance of standard (uniplex) PCR however, fewer publications discuss multiplex PCR. In diagnostic laboratories the use of PCR is limited by cost and sometimes the availability of adequate test sample volume. To overcome these shortcomings and also to increase the diagnostic capacity of PCR, a variant termed multiplex PCR has been described. In multiplex PCR more than one target sequence can be amplified by including more than one pair of primers in the reaction. Multiplex PCR has the potential to produce considerable savings of time and effort within the laboratory without compromising test utility. Since its introduction, multiplex PCR has been successfully applied in many areas of diagnostics nucleic acid but it is a multifaceted procedure that has to be planned and optimised thoroughly to achieve robust and meaningful results ([34], [35], [36]). From these results, and from our own experience with multiplex PCR, it has become evident that Single template PCR Reaction is ideal for conserving costly polymerase and templates in short supply and can be applied in Pathogen Identification.

References

1. Ataee R. A., Ataee M. H., Alishiri Gh. H., Esmaeili D., Staphylococcal Enterotoxin C in Synovial Fluid of Patients With Rheumatoid Arthritis, *J. of Iran Red Crescent Med.* 2014,16 (10): 1-6.

2. Duquenne M., Fleurot I., Aigle M., Darrigo C., Boreze´e-Durant E., Derzelle S., Bouix M., Deperrois-Lafarge V., and Delacroix-Buchet A., Tool for Quantification of Staphylococcal Enterotoxin Gene Expression in Cheese, *J. of Appl. Environ. Microbiol.*, 2010, 76 (5): 1367-1374.

3. Becker K., Roth R. and Peters G., Rapid and Specific Detection of Toxigenic *Staphylococcus aureus*: Use of Two Multiplex PCR Enzyme Immunoassays for Amplification and Hybridization of Staphylococcal Enterotoxin Genes, Exfoliative Toxin Genes, and Toxic Shock Syndrome Toxin 1 Gene, *J. of Clin. Microbiol.* 1998, 36 (9): 2548-2553**.**

4. Sharma N. K., Rees C. E. D. and Dodd Ch. E. R., Development of a Single-Reaction Multiplex PCR Toxin Typing Assay for *Staphylococcus aureus* Strains, *J. of Appl. Environ. Microbiol*. 2000, 66 (4): 1347-1353.

5. Naffa R. G., Bdour S. M., Migdadi H. M. and Shehabi A. A., Enterotoxicity and genetic variation among clinical *Staphylococcus aureus* isolates in Jordan, *J. of Med. Microbiol.* 2006, 55:183-187.

6. Pinchuk I. V., Beswick E. J. and Reyes V. E., Staphylococcal Enterotoxins, *J. of Toxins* 2010, 2, 2177-2197.

7. Dinges M. M., Orwin P. M. and Schlievert P. M., Exotoxins of *Staphylococcus aureus, J. of Clinic. Microbiol. Rev*. 2000, 13 (1): 16-34.

8. Kayser F. H., Bienz K. A., Eckert J., Zinkernagel R. M., Medical Microbiology, Thieme Stuttgart, New York, 2005, p229.

9. Blaiotta G., Ercolini D., Pennacchia C., Fusco V., Casaburi A., Pepe O. and Villani F., PCR detection of staphylococcal enterotoxin genes in Staphylococcus spp. strains isolated from meat and dairy products. Evidence for new variants of seG and seI in *S. aureus* AB-8802, *J. of Appl. Microbiol.*, 2004, 97: 719-730.

10. Chiang Y-Ch., Liao W-W., Fan Ch-M., Pai W-Y, Chiou Ch-Sh. and Tsen H-Y., PCR detection of Staphylococcal enterotoxins (SEs) N, O, P, Q, R, U, and survey of SE types in Staphylococcus aureus isolates from food-poisoning cases in Taiwan, *Int.J. of Food Microbiol.*, 2008, 121: 66-73.

11. Morandi S., Brasca M., Andrighetto C., Lombardi A. and Lodi R., Phenotypic and Genotypic Characterization of Staphylococcus aureus Strains fromItalian Dairy Products, *J. of Int. Microbiol.*, 2009, 10: 7.

12. Ortega E., Abriouel H., Lucas R. and Gálvez A., Multiple Roles of Staphylococcus aureus Enterotoxins: Pathogenicity, Superantigenic Activity, and Correlation to Antibiotic Resistance, *J. of Toxins* 2010, 2, 2117-2131.

13. Onasanya A., Mignouna H.D.and Thottappilly G., Genetic fingerprinting and phylogenetic diversity of *Staphylococcus aureus* isolates from Nigeria, *African J. of Biotechnol* 2003, 2 (8): 246-250.

14. Tang Y-W. And Stratton C. W., Advanced Techniques in Diagnostic Microbiology, Springer, New York, USA, 2006.p 426,429.

15. Kareem N.H., Sabbah M. A., Ibraheem A. N., Said A.M., Development of SYBR Green Real time PCR for Identification Methicillin-Resistance *Staphylococcus aureus* (MRSA), *J. of Biotech. Research Center*, 2013, 7 (1): 67-71.

16. Mehrotra M., Wang G. and Johnson W. M., Multiplex PCR for Detection of Genes for *Staphylococcus aureus* Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance, *J. of Clinic. Microbiol.* 2000, 38 (3):1032.

17. Demír C., Aslantas O., Duran N., Ocak S., Ozer B., Investigation of toxin genes in *Staphylococcus aureus* strainsisolated in Mustafa Kemal University Hospital, *J. of Turk. Med Sci.* 2011, 41 (2): 343-352.

18. Strommenger B., Kettlitz C., Werner G., and Witte W., Multiplex PCR Assay for Simultaneous Detection of Nine Clinically Relevant Antibiotic Resistance Genes in *Staphylococcus aureus, J. of Clinic. Microbiol*. 2003, 41 (9): 4089-4094.

19. Oliveira D. C. And H. de Lencastre, Multiplex PCR Strategy for Rapid Identification of Structural Types and Variants of the *mec* Element in Methicillin-Resistant *Staphylococcus aureus*, *J. of Antimicrob. Agents and Chemotherap*. 2002, 46 (7): 2155-2161.

20. Hameed N. M., In vitro Evaluation of the Activity of *Aspergillus niger* Extract on the Viability of *Staphylococcus aureus and* Protoscolices of Hydatid cysts, Master thsesis, College of Medicine, University of Tikrit, 1993.

21. Bhutia K. O., Singh T. S., Biswas S., Adhikari L., Evaluation of phenotypic with genotypic methods for species identification and detection of methicillin resistant in *Staphylococcus aureus*, *International J. of Appl. and Basic Med. Research*, 2012, 2 (2): 84-91.

22. Anand T., Kalaiselvan A., Gokulakrishnan K., Chandramohan S., Elambarathi P., Analysis of Genetic Variation by Methyl Orange in Rhizobium Using RAPD–PCR, *Int. J. Pharm. Tech. Res*. 2012, 4 (3): 1101-1109**.**

23. Tan S. Ch. and Yiap B. Ch., DNA, RNA, and Protein Extraction: The Past and the Present, *J. of Biomed. And Biotechnol*. 2009: 1-10.

24. Moore P. C. L. and Lindsay J. A, Genetic Variation among Hospital Isolates of Methicillin-Sensitive *Staphylococcus aureus*: Evidence for Horizontal Transfer of Virulence Genes, *J. of Clinic Microbiol. 2001*, 39 (8): 2760-2767.

25. Yarwood J. M., McCormick J. K., Paustian M. L., Orwin P. M., Kapur V. and Schlievert P. M., Characterization and Expression Analysis of *Staphylococcus aureus* Pathogenicity Island 3, Implications for The Evolution of Sphylococcal Pathogenicity Islands, *J. of Biol. Chemist*., 2002, 277 (15):13138-13147.

26. Karahan M., AcÇık M. N. and Çetinkaya B., Investigation of Toxin Genes by Polymerase Chain Reaction in Staphylococcus aureus Strains Isolated from Bovine Mastitis in Turkey, *J. of Foodborne Path. And Dis*. 2009, 6 (8): 1029-1035.

27. Jacobsson G., Invasive *Staphylococcus aureus* infections, Sahlgrenska Academy University of Gothenburg, Sweden, 2009.

28. Bayles K. W. and Iandolo J. J., Genetic and Molecular Analyses of the Gene Encoding Staphylococcal Enterotoxin D, *J. of Bacteriol*., 1989, 171 (9):4799-4806.

29. Khudor M. H., Abbas B. A. AND Idbeis H. I., Detection of Enterotoin Genes of *Staphylococcus aureus* Isolates From Raw Milk, *Basrah J. Vet. Res.* 2012, 11 (1): 245-264.

30. Oliveira L., Rodrigues A. C., Hulland C., Ruegg P. L., Enterotoxin production, enterotoxin gene distribution, and genetic diversity of *Staphylococcus aureus* recovered from milk of cows with subclinical mastitis, *Am. J. of Vet. Res.* 2011, 72 (10): 1361-1368.

31. Sareyyüpoğlu B., Müştak H. K., Cantekin Z. and Dıker K. S., Molecular detection of exfoliative toxin in *Staphylococcus intermedius* isolates from dogs with pyoderma, *J. of Ankara Üniv. Vet. Fak. Derg*, 2013, 60: 15-19.

32. Holochová P., Růžičková V , Dostálová L, Pantůček R, Petráš P and Doškař J, Rapid detection and differentiation of the exfoliative toxin A-producing Staphylococcus aureus strains based on ϕ ETA prophage polymorphisms, *J. Diag. Micro. Bio.* 2009, 10 (8): 248-252.

33. Henegariu O., Heerema N.A., Dlouhy S.R., Vance G.H. And Vogt P.H., Multiplex PCR: Critical Parameters and Step-by-Step Protocol, *Bio Techniques*, 1997, 23 (3): 504-511.

34. Elnifro E. M., Ashshi A. M., Cooper R. J., AND Klapper P. E., Multiplex PCR: Optimization and Application in Diagnostic Virology, *J. of Clinic. Microbiol. Rev.*, 2000, 13 (4): 559-570.

35. Sasaki T., Tsubakishita S., Tanaka Y., Sakusabe A., Ohtsuka M., Hirotaki Sh., Kawakami T., Fukata T., and Hiramatsu K., Multiplex-PCR Method for Species Identification of Coagulase-Positive Staphylococci, *J. of Clinic. Microbiol. JCM.* 2010, 48 (3): 765-769.

36. Sint D.., Raso L. and Traugott M., Advances in multiplex PCR: balancing primer efficiencies and improving detection success, *J. of Methods in Ecol. and Evolu*., 2012, 3: 898-905.

Tikrit Journal of Pure Science 21 (3) 2016 *ISSN: 1813 – 1662 (Print)*
E-ISSN: 2415 – 1726 (On Line)

تحديد بعض جينات التسمم المعوي في بعض سلالات بكتيريا المكورات العنقودية الهبية المقاومة للمثيسيلين بإستخدام تقنية تفاعل البلمره المتسلسل

سهى ماهر عبد [1] ، وعد محمود رؤوف [2] ، عقيل حسين العاصي [1] ، زينه سيف الدين محمد[3] ، فاروق إبراهيم محمد [4]

[1] قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

[2] كلية الصيدلة ، جامعة تكريت ، تكريت ، العراق

[3] كلية الصيدلة ، جامعة النهرين ، بغداد ، العراق

[4] مركز البحوث والتقانات الاحيائية ، جامعة النهرين ، بغداد ، العراق

الملخص

نظراً لتزايد الاهتمام حول سلالات بكتريا المكورات العنقودية الذهبية المقاومة للمثيسيلين, تم استخلاص المادة الوراثية باستخدام الطريقة الاعتيادية (طريقة الفينول–كلوروفوم) حيث تراوحت تراكيز المستخلص ونقاوته بين (1.8–1.6 800–100 ng/µl) تم التحري عن الجينات التي تشفر لأنواع الذوفانات المعوية الخمسة الرئيسة لعشرين عزله بعد ان تم تشخيصها على المستوى الجزيئي الى سلالات مكورات عنقوديه ذهبيه مقاومه للمثيسيلين باستخدام البادئات *fem A* و *mec A* (إذ يعّد الأخير مفتاح المكون الجيني الرئيس لمقاومة المثيسيلين) في تقنية تفاعل انزيم البلمره المزدوج وذلك بأستخدام طريقة تفاعل البلمره المتسلسل حيث تعتمد تقنيه تفاعل البلمره المتعدد على تضاعف الخمس جينات سويةً : *sea* 102bp, *seb* 164bp, *sec* 451bp, *sed* 278 bp and *see* 209bp. أظهرت نتائج دراستنا بأن اغلب السلالات قد احتوت على الأقل على واحد من هذه الجينات. لوحظ أيضاً وجود مزيج من الجينات. كان جين الذوفان المعوي نوع *sea* هو الأكثر تواجد ضمن عيناتنا (من أصل 20 عزله أعطى نسبة 90%).