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Molecular Detection of Enterotoxin Genes sea, seb, sec, sed and see in Staphylococcus aureus Isolated from Different Clinical Sources in Erbil City

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ABSTRACT

Staphylococcus aureus is important pathogens that can produce wide range of toxins.Enterotoxins are among those staphylococcal virulence factors that can cause infection and diseases to human. *S. aureus* strains show variability in possessing enterotoxin *SEs* genes, most strains possess one or more of the *SEs* genes, while some strains possess none of them. The current study aimed to use PCR technique for detection the presence and prevalence of *SEs* genes (*sea, seb, sec, sed and see*) of S. aureus, also the distribution of those genes among different strains. In this study a total of thirty positive isolates collected from patients who were admitted to Rizgary Teaching Hospital during the period from January 2020 to March 2020. Samples collected from different clinical sources: from burns, surgical wounds blood, sputum, and urine samples. Only those isolate included which identified as positive using cultural, microscopical, biochemical tests, and confirmed by VITEK2 compact system. PCR technique was used for the detection of enterotoxin gene *sea to see* in *S. aureus* isolates and the results showed that 13 (43.33%), 3 (10%), 16 (53.33%), 20 (66.67%) and 0 (0%) of the isolates were positive for *sea, seb, sec, sed* and *see* genes respectively, also results showed variability among strains in having *SEs* genes.

Keywords: *Staphylococcus aureus,* enterotoxin genes *SEs,* Molecular detection (PCR).

1. INTRODUCTION

S. aureus is a gram-positive, coccal bacterium, causes many healthy consequences when infect human, ranging from moderate to severe infections depending on the site of infection. Toxic shock syndrome, pneumonia, bacteremia, osteomyelitis, meningitis, osteomyelitis, acute endocarditis, myocarditis and pericarditis are examples of severe infections. While, impetigo, furuncles, cellulitis and postoperative wound infections, are examples of some diseases that *S. aureus* implicated in (Schmidt, 2018, G. Abril et al., 2020).

A series of virulence factors produced by *S. aureus,* among them: surface associated adhesions, enzymes, exotoxins, enterotoxins, toxic shock syndrome toxin-1 (TSST-1), hemolysins (Hlα and Hlβ), Panton-Valentine leucocidin (PVL), and fibronectin-binding proteins A and B (Oliveira et al., 2018, Li et al., 2019). Enterotoxins (SEs) are small peptides, approximately 26 to 29 kDa in size, characterized by heat-stability and considered as superantigens (Sags). When SEs enter human body through ingestion, induce gastroenteric syndrome and toxic shock syndrome (Benkerroum, 2018, Krakauer, 2019).

Generally, SEs can be classified into two main groups, classical and new SEs. Classical groups are widely recognized groups of enterotoxin SEs which include SEA to SEE. Beside those 5 widely recognized groups there are new types of SEs like: SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER, and SEU that have been reported (Argudín et al., 2010, G. Abril et al., 2020).

Currently, different of methods has been employed to characterize *S. aureus* isolates. Polymerase chain reaction (PCR) is among the techniques used to identify toxin genotypes, gene amplification, by, is widely used currently as a very successful and reliable tool to detect *SEs*, such as toxin genes *sea–see*. PCR is used for detecting *SEs* genes in staphylococcal strains because of several advantage like being a simple, rapid, and accurate technique (G. Abril et al., 2020). The present study aimed to determine the frequency of *SEs* genes (*sea, seb, sec, sed,* and *see*) isolated from different clinical sources, and the distribution of these genes among different strains of *S. aureus.*

2. MATERIALS AND METHODS

2.1. Samples collection

In this study a total of thirty isolates of *S. aureus* were obtained in different clinical sources from patients who admitted to Rizgary Teaching Hospital-Erbil during period from January 2020 to March 2020. Samples were taken from different sites: burns, surgical wounds, blood, sputum, and urine.

2.2. Identification of the isolates

Samples were tested using microscopical, morphological, biochemical tests and VITEK2 compact system, only positive *S. aureus* samples are included in the present study (MAHROUS* et al., 2020).

2.3. Genomic DNA extraction

Genomic DNA was extracted from pure cultures *via* the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan) according to the manufacturer's instructions; extract was eluted with 100 µl elution buffer. Extracts were stored at – 20 °C before PCR run. DNA's concentration measurements, and purity assessment was performed using NanoDrop 2000c spectrophotometer (ThermoFisher Scientific, USA) at 620 nm.

2.4. Polymerase chain reaction (PCR) for amplification of *sea to see* genes

2.4.1. Primer design:

The extracted genomics DNA from all isolates was tested to detect the presence of *sea to see* gene. Six sets of specific primers were used based on previous researches and checked in NCBI blast software program (Valizadeh and Amini, 2016, Leke et al., 2017). The first primer *16s rRNA* used to detect the presence of *S. aureus* genome, while the other five primers used to detect the *SEs* genes (*sea* to *see*) sequences of the primers and their amplified size are shown in table (1).

Table (1): Primers and their sequences used for detection of *16s rRNA* and five *SEs* genes and their base pairs.

Enterotoxin genes	Primer sequences (5'-3')		Size of amplified product (bp)	Reference
16s rRNA	*Forward*	GTGCCAGCAGCCGCGGTAA	876	(Salisbury et al., 1997)
	Reverse	AGACCCGGGAACGTATTCAC		
sea	*Forward*	GGTTATCAATGTGCGGGTGG	102	(Mehrotra et al., 2000, Valizadeh and Amini, 2016)
	Reverse	CGGCACTTTTTTCTCTTCGG		
seb	*Forward*	GTATGGTGGTGTAACTGAGC	264	(Mehrotra et al., 2000, Valizadeh and Amini, 2016)
	Reverse	CCAAATAGTGACGAGTTAGG		
sec	*Forward*	GCCTAGCATCTGAAGCCACAA	660	(Leke et al., 2017)
	Reverse	ACACGACTGTGCCGTATTATCAGA		
sed	*Forward*	CTAGTTTGGTAATATCTCCT	317	(Leke et al., 2017)
	Reverse	TAATGCTATATCTTATAGGG		
see	*Forward*	TAGATAAAGTTAAAACAAGC	170	(Leke et al., 2017)
	Reverse	TAACTTACCGTGGACCCTTC		

2.4.2. PCR protocol and condition

The amplification of these genes was done using specific primers for each gene as shown in table (1). PCR amplification for detection of *SEs* genes was conducted using 1 µl of elute in a 25 µl as a final volume. Following the addition of 12.5 µl of 2× HotStart Taq Master Mix (RED AMPLICON, Germany), then 1 µl of each primer added to the tube, the volume completed with free nuclease water to 25 µl. Thermal cycler (Gradient thermal cycler Alpha Cycler PCRmax series) used for amplification. Most conditions for all six primers were the same; the only differences were in their annealing temperature, and number of *16srRNA* cycles. Steps, temperature, and number of cycles are listed in table (2).

Table (2): shows PCR protocol including: steps, temperature, time and number of cycles used for each gene.

Enterotoxin genes	Initial denaturation		Denaturation		Annealing		Elongation		Final extension		No. of cycles
	Temp	Time	Temp.	Time	Temp	Time	Temp	Time	Temp	Time	
16srRNA	95°C	5min.	94°C	30 sec.	55°C	30 sec.	72°C	30 sec.	72°C	2min	25
sea	94°C	2min.	94°C	30 sec.	57°C	30 sec.	72°C	1min.	72°C	3min	30
seb	94°C	2min.	94°C	30 sec.	54°C	30 sec.	72°C	1min.	72°C	3min	30
sec	94°C	2min.	94°C	30 sec.	61°C	30 sec.	72°C	1min.	72°C	3min	30
sed	94°C	2min.	94°C	30 sec.	48°C	30 sec.	72°C	1min.	72°C	3min	30
see	94°C	2min.	94°C	30 sec.	50°C	30 sec.	72°C	1min.	72°C	3min	30

2.4.3. Detection of PCR products

After amplification, 5µl of PCR product was analyzed using electrophoresis (BioTech-USA) in 1.2% at 100 V for 45 minutes. Safe stain (EvaGreen Fluorescent Gel Stain-Jena Bioscience) used to visualize the band, 100 bp. DNA marker used as ruler and visualized under UV trans-illuminator (Benchtop UV-Transilluminator-BioTech-USA). Results documented using 16 megapixel camera for photography (Valizadeh and Amini, 2016).

2.5. Statistical analysis

One Way ANOVA (Tukey's multiple comparisions) were performed for variables and $P<0.05$ values were regarded as significant differences.

3. RESULTS:

3.1. Isolation and Identification of *S. aureus*

All thirty isolates that included in the present study were those samples that were able to grow on mannitol salt agar (selective media for Staphylococcus). Circular, smooth, yellow to golden colonies rose on blood agar with various degrees of hemolysis (mostly beta hemolysis). Under light microscope, smears of S. aureus isolates appeared as purple single, diplo, and gram positive cocci. All isolates were positive for catalase, coagulase, and DNase (Morello et al., 2010). All isolates were identified as *S. aureus* by VITEK2 compact system with over 99% probability percentage.

3.2. Molecular detection of *SEs* genes (*sea* to *see*)

Results of the present study among thirty positive samples of *S. aureus* using PCR for detecting the *SEs* genes showed that 13 (43.33%), 3 (10%), 16 (53.33%), 20 (66.67%) and 0 (0%) of the isolates were positive for *sea, seb, sec, sed* and *see* genes respectively, as shown in figures (1) to (5).

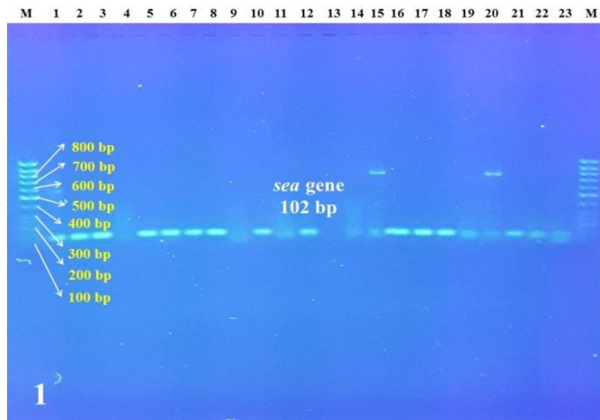

Figure (1): PCR amplification of *sea* gene in *S. aureus*. Isolates number 1-3,5-8,10,12, 16-18,and 21 were positive while isolate number 4,9,11, 13-15,19,20,22, and 23 were *sea* negative. M: ladder.

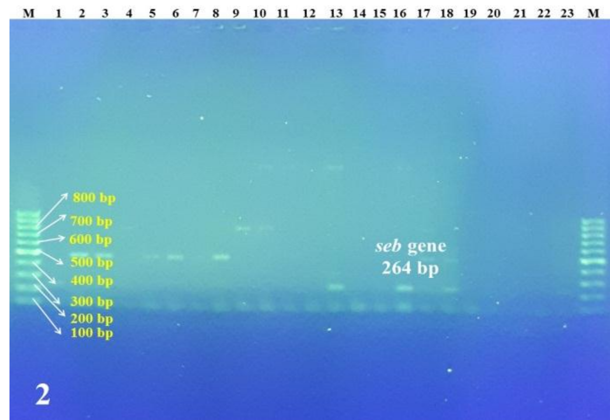

Figure (2): PCR amplification of *seb* gene in *S. aureus*. Isolates number 13, 16, and 18 were positive while other isolates were negative for this gene. M: ladder.

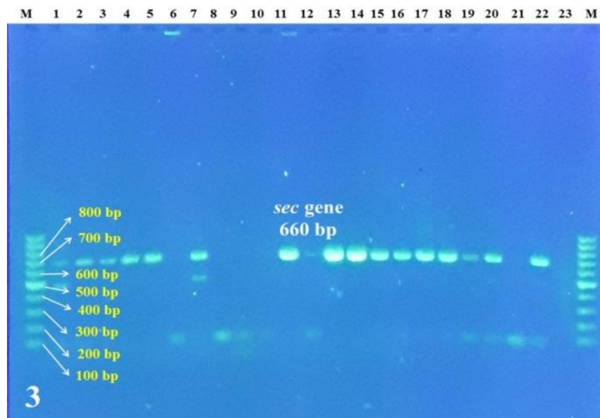

Figure (3): PCR amplification of *sec* gene in *S. aureus*. Isolates number 1-5, 7, 11, 13-20, and 22 were positive while isolate number 5, 7-10, 21and 23 were *sec* negative. M: ladder.

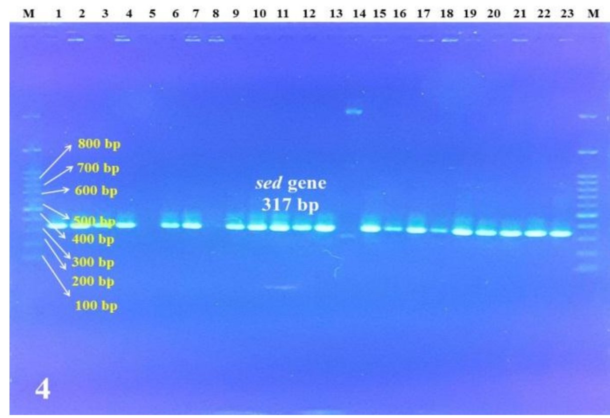

Figure (4): PCR amplification of *sed* gene in *S. aureus*. Isolates number 1-4, 6, 7, 8-12, 14-23, were *sed* positive while isolate number 5, 8, and 13 were *sed* negative. M: ladder.

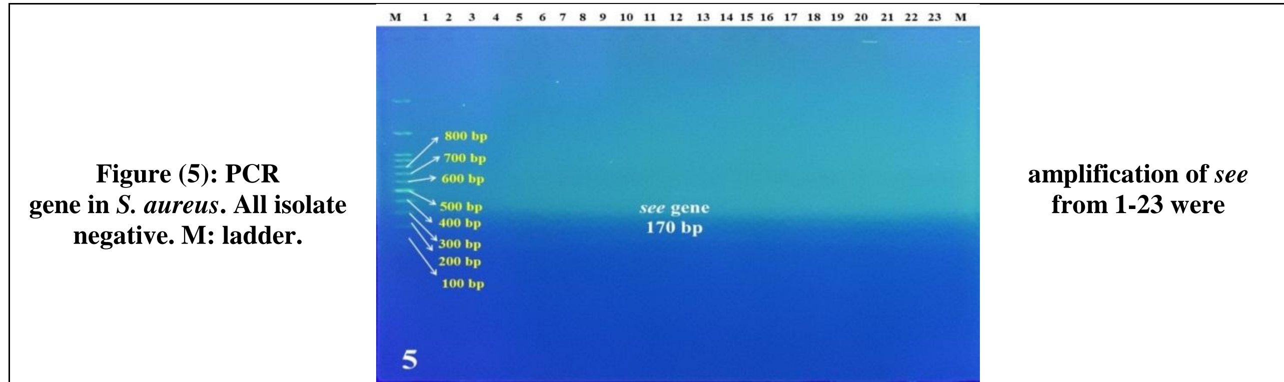

Figure (5): PCR amplification of *see* gene in *S. aureus*. All isolate from 1-23 were negative. M: ladder.

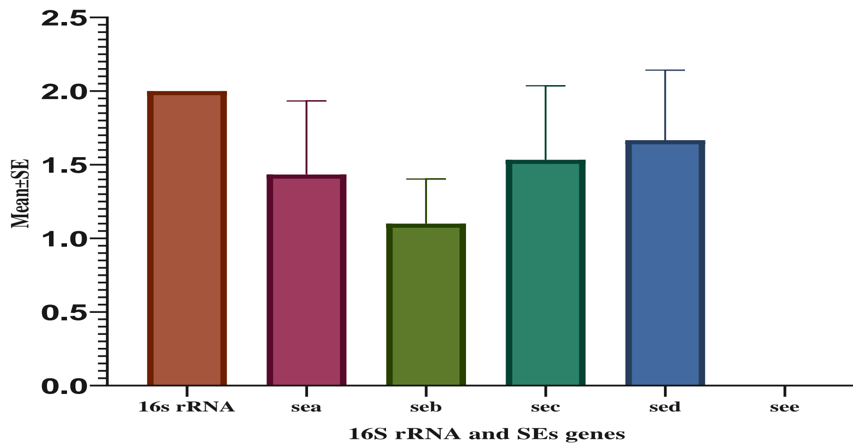

Figure (6): A chart shows results and frequency of 16srRNA and *SEs* genes (*sea* to *see*) according to the present study, P Value <0.0001.

As shown in figure (6), SEs genes detected in different frequencies, according to the statistical analysis, there are highly significant differences between 16srRNA and SEs genes with (p<0.0001). *sed* the most prevalence, followed by *sec, sea, and seb*, while see not detected at all.

Regarding the number of *SEs* genes in different *S. aureus* strains, 10 strains (33.3%) possessed one SEs gene, 12 strains possessed two *SEs* genes, and 6 strains (20%) possessed three SEs genes, while none of the strains possessed four or five *SEs* genes together as shown in table (3).

Table (3): Strains of *S. aureus* with number of *SEs* genes.

No. of *SEs* genes in *S. aureus* strains	One *SEs* gene	Two *SEs* genes	Three *SEs* genes	Four *SEs* genes	Five *SEs* genes
No. of Isolates	10 (33.3%)	12 (40%)	6 (20%)	0 (0%)	0 (0%)

4. DICUSSIONS:

Regarding the statistical analysis, results showed a highly significant difference between *16srRNA*, *SEs* genes and their distribution among different strains of *S. aureus* (p<0.0001), as shown in figure (6).

The most prevalence enterotoxin genes was *sed and sec* with frequencies of (66.67%) and (53.33%) respectively, Detecting *sed* gene in present study with such high frequency is approved by one of a most recent study carried out

in 2020 (Macori et al., 2020). High frequency of *sed* gene in clinical samples shows the importance and critical role of this gene in producing broad spectrum of diseases.

In an approach for detecting SEs genes in Iraq using PCR technique, Al-Khafaji and his colleague did research in 2019, their results showed that (48%, 28%, 40%, 36% and 12%) of isolates have *sea* gene, *seb* gene, *sec* gene, *sed* gene and *see* gene respectively (Al-Khafaji et al., 2019). Despite some differences in the frequencies of SEs genes that may be resulted from source of sampling as they took samples from high vaginal swap from women with vaginosis, their results somehow approve the current research. Also in 2019, Sultan and Ltef detected *sea* to *see* genes with following frequencies respectively: (48.31%), (44.94%), (6.74%), (3.37%) and (16.85%) (Sultan and Latef, 2019). Differences in frequencies with present research may be resulted from including food samples in their research beside the geographical distribution as they carried their research in Anbar governorate. The present study didn't detect any *see* gene, such a result is reported in other studies, Xie and his colleagues in their research revealed that among 108 isolates, the *see* gene was not detected (Xie et al., 2011). Regard to absences of *see* gene in all isolates that may be carried on the plasmids which are not present in our isolates.

Results of the present study showed variability in *S. aureus* for possessing *SEs* genes as shown in table (3), statistical analysis showed a high significant differences among the strains ($p<0.0001$). Not all strains have all *SEs* genes, such results approved by previous researches which declared that each bacterial strain may or may not carry one or more genes encoding these toxins (Vasconcelos et al., 2011, Bokaeian et al., 2016, Macori et al., 2020). The variations in the prevalence between our results and other studies, that have been carried-out in Iraq and other countries, can be attributed to several factors, including the geographical distribution of the strains, the size of the samples tested, the sampling mode (human clinical samples, food or milk samples), the seasons in which the sampling was done, the methods of isolation used, and the antibiotics used by the patients (Teramoto et al., 2016, Bokaeian et al., 2016, Moura et al., 2019). Avila-Novoa and his colleagues in their research in 2018, declared that differences in the presence of classical S. aureus enterotoxin types in the studies indicates a geographic variation in the distribution of enterotoxigenic *S. aureus* strains. This variation may reflect the distinct ecological reservoirs present in different countries or may simply result from differences in the sensitivity, and specificity of the techniques employed to detect the enterotoxins (Avila-Novoa et al., 2018).

5. CONCLUSIONS:

Based on the results of the present study, it can be concluded that not all strains of *S. aureus* have all *SEs* genes. The frequency of *SEs* genes within same strain of *S. aureus* is different, *sed* and *sec* genes are most common in *S. aureus* strains in Erbil city, while *see* is rare. Absences of *see* gene in all isolates that may be carry on the plasmids which are not present in our isolates.
PCR detection for encoded toxic genes in *S. aureus* has many advantages, besides being somehow simple, rapid, and it is very specific in detecting *SEs* genes and can detect several genes at the same time. More researches including bigger sample size are recommended.

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Author's Contributions

Alia T. Abdulrahman and Nigar S. Abdulla performed sample collection and practical works in the hospital.
Dr. Zirak F.A. Abdulrahman supervised the whole research and statistical analysis.
Rastee H. Saeed performed the part of molecular test PCR in the molecular lab.
Ahmed N. Hassan participated in molecular test PCR and writing the research.

6REFRENCES:

1. AL-KHAFAJI, N., KADHUM, S. & AL-DAHMOSHI, D. H. 2019. PCR-based investigation of enterotoxin profile among Staphylococcus Aureus isolated from women with vaginosis.
2. ARGUDÍN, M. Á., MENDOZA, M. C. & RODICIO, M. R. 2010. Food poisoning and Staphylococcus aureus enterotoxins. *Toxins,* 2**,** 1751-1773.

3. AVILA-NOVOA, M., IÑIGUEZ-MORENO, M., GONZÁLEZ-GÓMEZ, J. P., ZACARÍAS-CASTILLO, E., GUERRERO, P., PADILLA-FRAUSTO, J., NAVARRO VILLARRUEL, C. & GUTIÉRREZ-LOMELÍ, M. 2018. DETECTION OF ENTEROTOXIN GENES OF Staphylococcus aureus ISOLATES FROM FOOD CONTACT SURFACES IN THE DAIRY INDUSTRY OF JALISCO, MEXICO. *Biotecnia,* 20**,** 72-78.
4. BENKERROUM, N. 2018. Staphylococcal enterotoxins and enterotoxin-like toxins with special reference to dairy products: An overview. *Critical Reviews in Food Science and Nutrition,* 58**,** 1943-1970.
5. BOKAEIAN, M., SAEIDI, S. & HASSANSHAHIAN, M. 2016. Molecular Detection of Staphylococcus aureus Enterotoxin A and B Genes in Clinical Samples from Patients Referred to Health Centers in Zahedan City. *Research in Molecular Medicine,* 4**,** 44-46.
6. G. ABRIL, A., G. VILLA, T., BARROS-VELÁZQUEZ, J., CAÑAS, B., SÁNCHEZ-PÉREZ, A., CALO-MATA, P. & CARRERA, M. 2020. Staphylococcus aureus Exotoxins and Their Detection in the Dairy Industry and Mastitis. *Toxins,* 12**,** 537.
7. KRAKAUER, T. 2019. Staphylococcal Superantigens: Pyrogenic Toxins Induce Toxic Shock. *Toxins,* 11**,** 178.
8. LEKE, A., GOUDJIL, S., MULLIE, C., GROGNET, S. & BIENDO, M. 2017. PCR detection of staphylococcal enterotoxin genes and exfoliative toxin genes in methicillin-resistant and methicillin-susceptible Staphylococcus aureus strains from raw human breast milk. *Clinical Nutrition Experimental,* 14**,** 26-35.
9. LI, H., ANDERSEN, P. S., STEGGER, M., SIEBER, R. N., INGMER, H., STAUBRAND, N., DALSGAARD, A. & LEISNER ,J. J. 2019. Antimicrobial Resistance and Virulence Gene Profiles of Methicillin-Resistant and -Susceptible Staphylococcus aureus From Food Products in Denmark. *Frontiers in Microbiology,* 10.
10. MACORI, G., BELLIO, A., BIANCHI, D. M., CHIESA, F., GALLINA, S ,.ROMANO, A., ZUCCON, F., CABRERA-RUBIO, R., CAUQUIL, A., MERDA, D., AUVRAY, F. & DECASTELLI, L. 2020. Genome-Wide Profiling of Enterotoxigenic Staphylococcus aureus Strains Used for the Production of Naturally Contaminated Cheeses. *Genes,* 11**,** 33.
11. MAHROUS ,*H., MOHAMED, K. F., ORABI, A. & MOHAMED, S. R. 2020. MOLECULAR INVESTIGATION OF ANTIMICROBIAL RESISTANCE PROFILE AND VIRULENCE OF S. AUREUS ISOLATED FROM MASTITIC MILK IN EGYPTIAN DAIRY FARMS AND ITS PUBLIC HEALTH IMPLICATION. *Journal of Critical Reviews,* 7**,** 2655-2662.
12. MEHROTRA, M., WANG, G. & JOHNSON, W. M. 2000. Multiplex PCR for Detection of Genes forStaphylococcus aureus Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance. *Journal of Clinical Microbiology,* 38**,** 1032-1035.
13. MORELLO, J., GRANATO, P. & MIZER, H. 2010. *Lab Manual and Workbook in Microbiology: Applications to Patient Care*, McGraw-Hill Education.
14. MOURA, E. O., RANGEL, A. H. N., MACÊDO, C. S., URBANO, S. A., NOVAES, L. P. & LIMA JÚNIOR, D. M. 2 .019Enterotoxin-encoding genes in Staphylococcus aureus from buffalo milk. *Pesquisa Veterinária Brasileira,* 39**,** 587-591.
15. OLIVEIRA, D., BORGES, A. & SIMÕES, M. 2018. Staphylococcus aureus Toxins and Their Molecular Activity in Infectious Diseases. *Toxins*.252 ,10 ,
16. SALISBURY, S. M., SABATINI, L. M. & SPIEGEL, C. A. 1997. Identification of Methicillin-Resistant Staphylococci by Multiplex Polymerase Chain Reaction Assay. *American Journal of Clinical Pathology,* 107**,** 368-373.
17. SCHMIDT, T. 2018. Antimicrobial Resistance in Staphylococci at the Human-animal Interface.
18. SULTAN, F. & LATEF, S. 2019. Prevalence of Staphylococcus aureus toxins genes in clinical and food isolates in Iraq.
19. TERAMOTO, H., SALAHEEN, S. & BISWAS, D. 2016. Contamination of post-harvest poultry products with multidrug resistant Staphylococcus aureus in Maryland-Washington DC metro area. *Food Control,* 65**,** 132-135.
20. VALIZADEH, E. & AMINI, K. 2016. Identification of Staphylococcus Aureus Enterotoxin Genes Using Multiplex PCR. *Babol-Jbums,* 18**,** 26.32-
21. VASCONCELOS, N. G., PEREIRA, V. C., ARAÚJO JÚNIOR, J. P. & DA CUNHA, M. D. L. R. S. 2011. Molecular detection of enterotoxins E, G, H and I in Staphylococcus aureus and coagulase-negative staphylococci isolated from clinical samples of newborns in Brazil. *Journal of Applied Microbiology,* 111**,** 749-762.

22. XIE, Y., HE, Y., GEHRING, A., HU, Y., LI, Q., TU, S.-I. & SHI, X. 2011. Genotypes and toxin gene profiles of Staphylococcus aureus clinical isolates from China. *PloS one,* 6**,** e28276-e28276.