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Prevalence of *Staphylococcus aureus* toxins genes in clinical and food isolates in Iraq

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Abstract:

Objectives: This study aims to determination of the prevalence and presence of genes encoding exfoliative toxins (*eta and etb*), toxic shock syndrome toxin-1 (*tst*), enterotoxins (*sea, seb,sec, sed* and *see*) and methicillin resistance (*mecA*) in *S. aureus* isolates that isolated from clinical samples and food samples in Iraq using multiplex PCR technique, and to determine the relationship between toxins genes distribution with antimicrobial resistance.

Methods: From July 2017 to December 2017, 113 specimens were collected from different sources of clinical and food specimens. Conventional and molecular methods were accomplished for identify of *S. aureus*, sequencing technique was used to confirm the diagnosis of *S. aureus* enterotoxins genes and statistical analysis was done by Chi square test for determination the relationship between toxins genes distribution with antimicrobial resistance.

Results: Results of routine methods were demonstrated discrepancy with results of molecular method which showed all 89 (100%) isolates were harboring *femA and mecA* gene (MRSA), while toxins genes were distributed on *S. aureus* isolates as following: *tst* (86.51), *eta* (5.61), *etb* (2.24), *sea* (48.31), *seb* (44.94), *sec* (6.74), *sed* (3.37) and *see* (16.85). Out of 89 *S. aurues* isolates, 86 (96.62%) isolates were toxigenic and 66 (76.74%) isolates were harbored more than one gene of superantigenic toxins genes. Statistically, toxins genes showed significantly associated with MRSA isolates, but there is no association with VISA and VRSA isolates.

Conclusion: High prevalence of toxins genes between MRSA isolates, that making local *S. aureus* isolates high virulent and difficult in therapy. Results showed that conventional bacteriological methods are nonspecific for identification and epidemiological study of *S. aureus* strains because of their phenotypic and genotypic variations.

Keywords: Multiplex PCR, *Staphylococcus aureus*, Toxins.

INTRODUCTION

Staphylococcus aureus is a common pathogen related with serious community and hospital acquired diseases and has for a long time been considered as a major problem of Public Health. Moreover, *S. aureus* is often present in foods and it is among the major causes of foodborne bacterial intoxications worldwide (1). The pathogenicity of *Staphylococcus aureus* is due to the toxins, invasiveness and antibiotic resistance. It can cause a wide range of disease severity, with clinical presentation varying from minor skin infections to the seriously life-threatening(2). One of the significant characteristics of *S. aureus*, its ability to secrete toxins which involving in severity diseases that including: exfoliative toxins, toxic shock syndrome toxin-1 and staphylococcal enterotoxins which implicated in staphylococcal scalded skin syndrome, toxic shock syndrome and staphylococcal food poisoning (SFP), respectively (3). Each of these toxins is known to have potent effects on cells of the immune system, and encoded by *eta*,*etb* genes (exfoliative toxins A and B), *tst* gene (toxic shock syndrome toxin-1) and *sea, seb, sec, sed, see* genes (staphylococcal enterotoxins) (4).

S. aureus infections has become more difficult in treatment because of the emergence of multidrug-resistant strains and that emerged as a serious problem for health care professionals worldwide, methicillin resistant *S. aureus* (MRSA) shows resistance against almost all β-lactam antibiotics (5)that enhance their virulence or enable them to cause particular clinical syndromes (6).

The introduction of PCR method will help provide the information required for appropriate therapy and infection control during outbreaks of *S. aureus* because that technique only will identify *S. aureus* strains harboring the resistant and toxins genes(7).The aim of the present study was to determination of the prevalence and presence of genes encoding exfoliative toxins, toxic shock syndrome toxin-1 and enterotoxins in *S. aureus* strains that isolated from different clinical infections and food specimens.

MATERIALS AND METHODS

Collection of samples

From July 2017 to December 2017, 113 specimens were collected. Seventy clinical specimens were obtained from patients of three hospitals in Baghdad, clinical samples included: wound swab (5), blood (14), endocarditis (6), bone marrow (4), urine (14), abscesses (6), ear swab (8), throat swab (3), sputum (2), environmental sample(1), nasal swab (6) and skin swab (1). Forty three food specimens were collected from popular restaurants and Baghdad markets, food samples included: beef burger (2), cheese (8), chips (1), falafel (5), ice cream (1), kubba (3), meatball (1), minced meat (9), raw milk (3), salad (3), spice (1) and uncooked kebab (6).

Culture media and biochemical tests

Clinical specimens were cultured on 5% blood agar and mannitol salt agar then incubated in aerobic conditions at 37°C for 24 hours. Coagulase positive *staphylococci* were isolated from food as follows: 25 g of each sample were diluted with 225 ml of pepton water and mixing gently, 0.1 ml from this solution was cultured on Baird parker agar then incubated in aerobic conditions at 35°C for 48 hour (8). Both clinical and food isolates were examined for gram stain, oxidase, catalase, coagulase tube and API Staph system. Finally, all 89 isolates were cultured on CHROMagar *S. aureus* (isolation and identification of *S. aureus* in one single step with high specificity and sensitivity after 24 hours incubation).

Antibiotics susceptibility test

The pattern of antibiotic susceptibility of clinical and food isolates were determined by Kirby-Bauer method on Muller-Hinton agar (Biomark) (9). The plates were incubated for 18-24 hours at 37° C. Following the incubation, the diameters of all zones of inhibition were measured by millimeter and compared these data with a standard zone of growth inhibition according to Clinical and Laboratory Standards Institute (10).

Primers design

They were synthesized by (Eurofins Genomics company/Germany). The primers were designed to target the coding regions of the genes, measures were taken to avoid areas of homology within the enterotoxins structural genes. The primer sequences used in the multiplex and uniplex PCRs were published previously (7) and are described in table (1).

Genomic DNA extraction

From an overnight blood agar culture, pure colony was sub-cultured in 10 ml of brain heart infusion broth overnight, in aerobic conditions, at 37°C before extraction of total DNA (11), genomic DNA from the growth cultures was extracted and purified by using the manufacturer protocol (Geneaid "Presto", Korea). The integrity of genomic DNA were confirmed by agarose gel electrophoresis with 1 % concentration for one hour at 70 volt.

Estimation of DNA concentration and purity

The nanodrop spectrophotometer device (NAS-99) was used to measure the DNA concentration and purity (ratio of absorbance at 260nm / absorption at 280nm).

Detection of selected *Staphylococcus aureus* genes by using Multiplex PCR technique

The two sets of genes were amplified in one multiplex PCR run. For each sample, all forward and reverse primers (0.5 µL of forward primer (10 Pm/µL), 0.5 µL of reverse primer (10 Pm/µL)) of set A of genes that including: *sea, seb,sec, sed* and *see* genes were added in to one tube "Gold multiplex PCR Premix" that contains 5 µL of lypholized mixture (Bioneer, Korea),other contents were added which are 1µL DNA template (50-500 ng) and 14 µL DNase-RNAse free deionized distilled water, in the same way and contents all forward and reverse primers of set B of genes that including: *femA*, *mecA*, *eta*, *etb* and *tst* gene (7) were added in to other tube of "Gold multiplex PCR Premix". Primers and amplification conditions of multiplex PCR were described in Table (1) and (2), respectively. PCR was based on *femA* gene as an internal positive control. After finished of PCR amplification, the end products were electrophoresed with 2 % concentration of agarose gel for 2 hours at 70 volt. Identification of *S. aureus* enterotoxins genes were confirmed by sequencing technique.

Statistical analysis

Statistical analysis was performed using Chi square test for determination the relationship between toxins genes distribution with antimicrobial resistance.

Table (1): Oligonucleotides primer used in this study

Gene	Primers' Sequences (5'→3')	Tm (°C)	Product size (bp)	Location Withingene.	Accession no.
sea	F:GGTTATCAATGTGCGGGTGG	59.4	**102**	349–368	M18970
	R:CGGCACTTTTTTCTCTTCGG	57.3		431–450	
seb	F:GTATGGTGGTGTAACTGAGC	57.3	**164**	666–685	M11118
	R:CCAAATAGTGACGAGTTAGG	55.3		810–829	
sec	F:AGATGAAGTAGTTGATGTGTATGG	57.6	**451**	432–455	X05815
	R:CACACTTTTAGAATCAACCG	53.2		863–882	
sed	F:CCAATAATAGGAGAAAATAAAAG	51.7	**278**	492–514	M28521
	R:ATTGGTATTTTTTTTCGTTC	47.0		750–769	
see	F:AGGTTTTTTCACAGGTCATCC	55.9	**209**	237–257	M21319
	R:CTTTTTTTTCTTCGGTCAATC	52.0		425–445	
femA	F:AAAAAAGCACATAACAAGCG	51.1	**132**	1444–1463	NC00779
	R:GATAAAGAAGAAACCAGCAG	53.2		1556–1575	
mecA	F:ACTGCTATCCACCCTCAAAC	57.3	**163**	1182–1201	X52593
	R:CTGGTGAAGTTGTAATCTGG	55.3		1325–1344	
eta	F:GCAGGTGTTGATTTAGCATT	53.2	**93**	775–794	PGWZ0100752
	R:AGATGTCCCTATTTTTGCTG	53.2		848–867	
etb	F:ACAAGCAAAAGAATACAGCG	53.2	**226**	509–528	NC022598
	R:GTTTTTGGCTGCTTCTCTTG	55.3		715–734	
tst	F:ACCCCTGTTCCCTTATCATC	57.3	**326**	88–107	U93688
	R:TTTTCAGTATTTGTAACGCC	51.1		394–113	

Table (2): Amplification conditions of multiplex PCR

Steps	Temperature	Time	Cycles no.
Initial denaturation	94℃	5 min	1
Denaturation	94℃	1 min	35 cycles
Annealing	54℃	1 min	
Extension	72℃	1 min	
Final extension	72℃	7 min	1

RESULTS AND DISCUSSION

Identification of bacterial isolates

All selected clinical isolates had the ability to ferment the mannitol sugar and gave yellow colonies on mannitol salt agar. Nineteen of coagulase positive *staphylococci* were obtained from out of forty three food samples which appeared as black and shiny colonies surrounded by a double halo (opaque zone and clear zone) that indicates to lecithinase activity. All *Staphylococcus* isolates appeared as gram positive cocci, (+) catalase and (-) oxidase. From clinical samples, 48 (68.57%) of isolates were coagulase-positive and 22 (31.42%) of isolates were given negative results for coagulase test, whereas all 19 (100%) food isolates were gave positive results for coagulase tube test that explain that Baird parker agar is more specificity than mannitol salt agar in isolation and primary identification of *S. aureus.* API Staph system was applied only for 73 isolates, from all isolates of clinical and food samples, 62 (84.93%) isolates were confirmed as *S. aureus* and 11 (15.06%) isolates were given negative results for API test, this making the API Staph system an excellent test for diagnosis. 48 (68.57%) of clinical isolates and 19 (100%) of food isolates were appeared as pink to mauve colonies surrounded by a mistiness halo on CHROMagar *S. aureus* and characterized as *S. aureus,* whereas 22 (31.42%) of clinical isolates that diagnosed as coagulase-negative *Staphylococci* (CNS) were appeared in different colors of blue, white, or beige colonies. The composition of chromogenic mix is proprietary for the manufacturer company, but as noted that the isolates which not produce coagulase enzyme were given negative results on CHROMagar *S. aureus* (CSA), this meaning the CSA may be utilized coagulase substrate for detection of *S. aureus* as suggested by Manafi*et al*., 1991 (12) about using substrate to detect coagulase activity. According to the results of cultural on blood agar plates, 33 (69%) *S. aureus* isolates and 6 (27%) CNS isolates were showed β-hemolysis activity, while γ-hemolysis activity werefound in 15 (31%) *S. aureus* isolates and 16 (73%) CNS isolates, this indicates to CNS have an important role in the pathogenesis of infections and could be as dangerous as *S. aureus* for humans. Regarding *S. aureus* isolated from food exhibit the same percent that found in clinical *S. aureus* isolates (figure 1), *S. aureus* isolated from animal are mostly characterized β-hemolysin and *S. aureus* isolated from human is characterized α-hemolysin (13), the variable pattern of hemolysins of *S. aureus* could be used as important information to control staphylococcal food poisoning and determined for sources of these isolates that contaminated food (14).

Antibiotics susceptibility test

Results of antibiotic susceptibility test were explained in table (3). Results of antibiotic susceptibility obtained by this study confirmed that most *Staphylococci* are high resistant to beta-lactam antimicrobials such as oxacillin and Ceftriaxone, out of total 48 clinical *S. aureus* isolates, 43 (89.58%) isolates were oxacillin resistance, while out of total 22 isolates that identified as coagulase-negative (CNS), 21 (95.45%) isolates showed resistance to oxacillin. The results obtained out of total 19 *S. aureus* isolates that isolated from food, 11 (57.89%) isolates were oxacillin resistance and 8 (42.10%) isolates were oxacillin sensitive, and there was no intermediate resistance in all isolates of clinical and food. The antimicrobial sensitivity test showed that both *S. aureus* and CNS isolates were multidrug resistant (MDR) in addition to the oxacillin. Resistance to methicillin is encoded by *mecA* gene which is located on *Staphylococcal cassette chromosome (SCC),* the *SCC* can be transferred horizontally between various staphylococcal species which suggests that CNS acts as reservoir for the dissemination of resistance genes to *S. aureus* (15). The *Staphylococcal cassette chromosome mec (SCCmec)* also frequently carry resistance genes in addition to *mecA* gene for other antimicrobials, both *S. aureus* and MRSA can harbor various resistance genes on chromosome and/or plasmids (16, 17).Vancomycin and imipenem were showed high effective against clinical *S. aureus* isolates and CNS isolates, while just imipenem antibiotic showed effective against food *S. aureus* isolates.

Table (3): Antibiotic susceptibility results

Antimicrobial agents	Clinical *S. Aureus* isolates n= 48			Food *S. aureus* isolates n= 19			Clinical CNS isolates n= 22		
	S	I	R	S	I	R	S	I	R
Ciprofloxacin	32 (66.66%)	9 (18.75%)	7 (14.58%)	16 (84.21%)	3 (15.78%)	0 (0%)	18 (81.81%)	2 (9.09%)	2 (9.09%)
Clindamycin	27 (56.25%)	6 (12.5%)	15 (31.25%)	15 (78.94%)	2 (10.52%)	2 (10.52%)	14 (63.63%)	2 (9.09%)	6 (27.27%)
Erythromycin	20 (41.66%)	7 (14.58%)	21 (43.75%)	14 (73.68%)	1 (5.26%)	4 (21.05%)	10 (45.45%)	1 (4.54%)	11 (50%)
Oxacillin	5 (10.41%)	0 (0%)	43 (89.58%)	8 (42.10%)	0 (0%)	11 (57.89%)	1 (4.54%)	0 (0%)	21 (95.45%)
Tetracycline	21 (43.75%)	10 (20.83%)	17 (35.41%)	7 (36.84%)	1 (5.26%)	11 (57.89%)	5 (22.72%)	2 (9.09%)	15 (68.18%)
Vancomycin	45 (93.75%)	1 (2.08%)	2 (4.16%)	0 (0%)	15 (78.94%)	4 (21.05%)	21 (95.45%)	0 (0%)	1 (4.54%)
Gentamycin	30 (62.5%)	5 (10.41%)	13 (27.08%)	16 (84.21%)	0 (0%)	3 (15.78%)	18 (81.81%)	2 (9.09%)	2 (9.09%)
Imipenem	47 (97.91%)	0 (0%)	1 (2.08%)	19 (100%)	0 (0%)	0 (0%)	22 (100%)	0 (0%)	0 (0%)
Ceftriaxone	4 (8.33%)	3 (6.25%)	41 (85.41%)	2 (10.52%)	0 (0%)	17 (89.47%)	4 (18.18%)	1 (4.54%)	17 (77.27%)

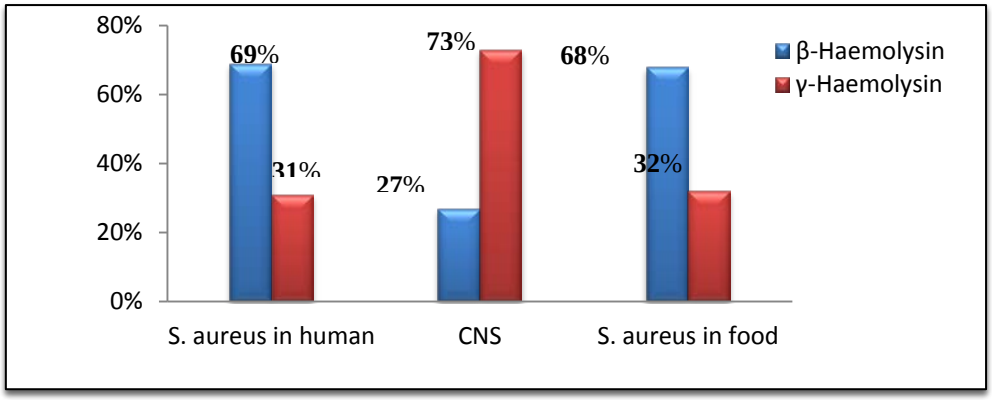

Figure (1): The percentage of distribution of β-hemolysin and γ- hemolysin toxins in *staphylococci* isolates.

Extraction of genomic DNA and estimation of its purity and concentration

Extraction results were very good for concentration and purity which determined by using nanodrop spectrophotometer at 260/280 nm. Concentration values of DNA ranged between 50-500 ng/µl while purity ranged from 1.8-2.0.

Detection of selected *Staphylococcus aureus* genes by using Multiplex PCR technique

All target genes were satisfactorily amplified to diagnostic pathogenicity of *S. aureus* isolated from patients and food, the results were shown in figures (2) and (3). Distribution of all selected *S. aureus* genes in this study were detected in various samples and showed high diversity in distribution between clinical and food samples as illustrated in the figure (4), and the results for the ten genes were *femA* (100%), *mecA* (100%), *tst* (86.51%), *eta* (5.61%), *etb* (2.24%), *sea* (48.31%), *seb* (44.94%), *sec* (6.74%), *sed* (3.37%) and *see* (16.85%). The variation of genes distribution may be due to origin of isolate, prevalence it in a certain geographic region and infection sites (18), (19) and (20). Results showed all 89 (100%) isolates were harboring *femA and mecA* gene (MRSA), this explain the trait of multidrug resistance (MDR), which is one of the major health problems worldwide, and indicates to that *mecA* gene has been horizontally transferred in *S. aureus* strains more frequently than may be acquired from other *Staphylococcus species*. Our study showed low rate of prevalence of exfoliative toxins genes which indicates that exfoliative toxins are not the main virulence factors in pathogenicity of local *S. aureus* isolates and may be not all diseases induced with exfoliative toxins are caused by this pathogen may be caused by *E. coli* (21), *tst* gene was the most prevalent genotype of toxins genes in local *S. aureus* isolates which consider a habitual feature in these isolates and can be used as diagnostic gene for detection toxigenic *S. aureus* strains. In our study, the results confirmed that 80% of clinical isolates and 63.15% of food isolates were enterotoxigenic and carried the five classical enterotoxins genes with different percent, the most frequently of enterotoxin genes were *sea* (48.31%) and *seb* (44.94%) genes, followed by *see* (16.85%), *sec* (6.74%) and *sed* (3.37%). All our sequenced genes (*sea, seb, sec, sed, see*) with related PCR product showed the same DNA sequences. All 89 isolates of *S. aureus* showed that toxins genes did not associated with specific source of specimens and distributed in different types of infections and food samples.

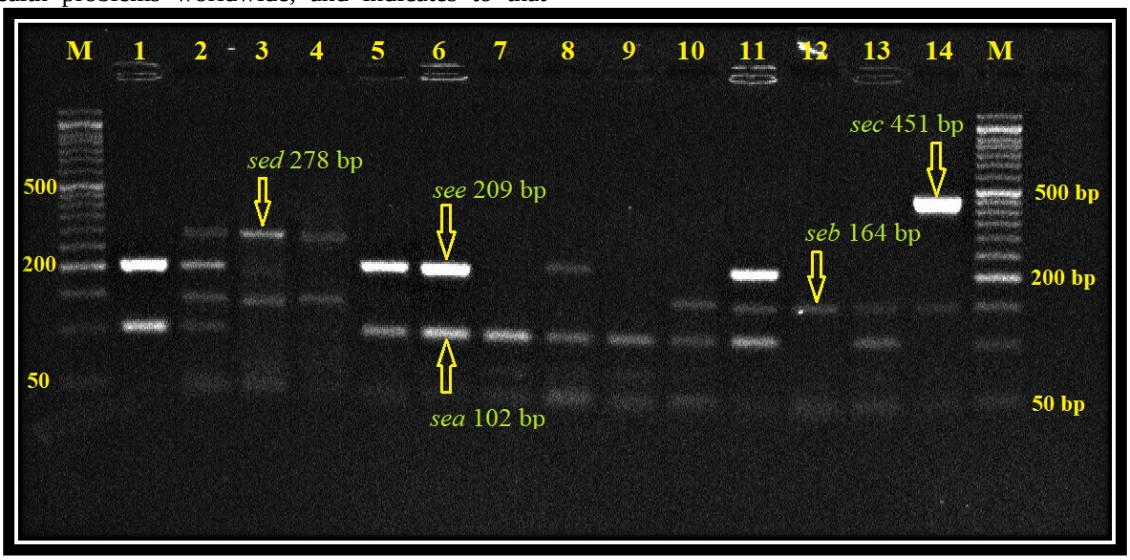

Figure (2): Gel electrophoresis of PCR products of staphylococcal enterotoxins genes (set A): *sea, seb,sec, sed* and *see* genes which amplified in multiplex PCR technique. M: DNA ladder (50bp), 2% agarose, TBE buffer (1x), 70 volt for 2hr.

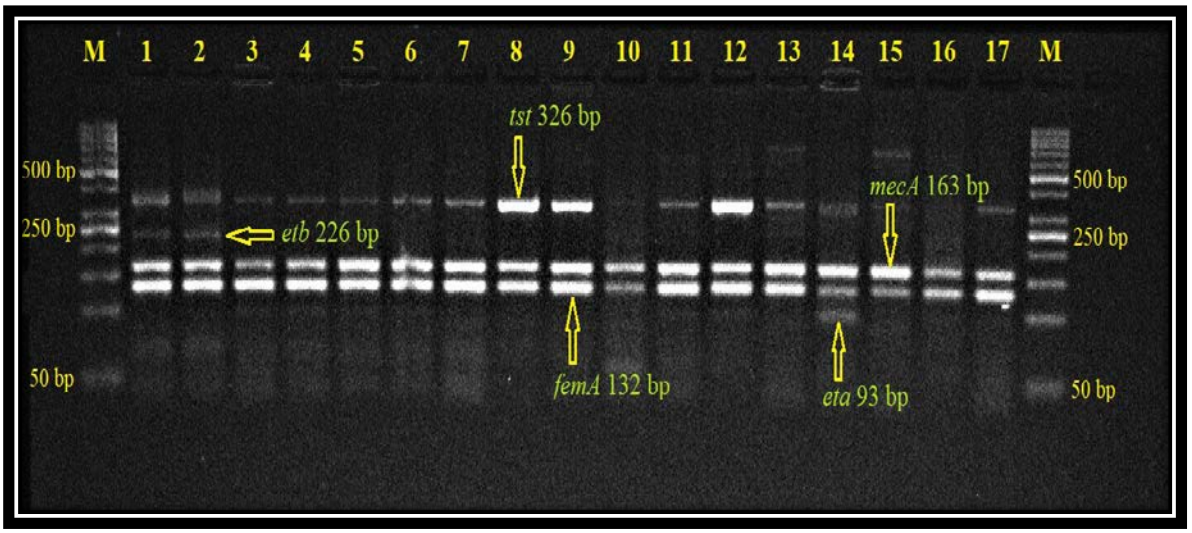

Figure (3): Gel electrophoresis of PCR products of set (B) genes that including: *femA, mecA, eta, etb* and *tst* gene which amplified in multiplex PCR technique. M: DNA ladder (50bp), 2% agarose, TBE buffer (1x), 70 volt for 2hr.

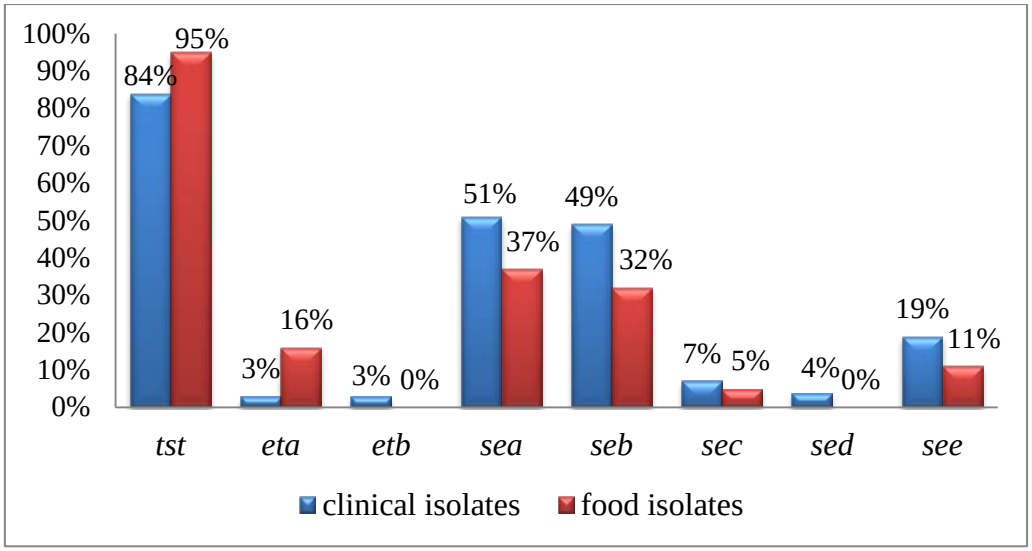

Figure (4):Distribution of the toxins genes of all clinical and food *S. aureus* isolates.

Also Adesiyun *et al*., 1992 (22) reported that the most of *S. aureus*-producing TssT-1 isolated from animals or animal product, this explains the high percent of *tst* gene distribution in food isolates more than clinical isolates (figure 4). The results from previous studies and present study indicates to that the high prevalence of each one of the enterotoxins genes depend on the origin of *S. aureus* strains and did not associated with specific samples, because it was observed by other researchers that *S. aureus* strains isolated from animals produce mainly SEC, whereas among strains isolated from humans, SEA was most frequently identified (23, 24), from that we can predict about the sources of human infections and contamination of foodstuff. Staphylococcal food poisoning (SFP) is one of the most common food-borne diseases caused by *S. aureus* strains which producing toxic shock syndrome toxin-1 (TSST-1) and enterotoxins (SEs), they also known as pyrogenic toxin superantigens (PTSAgs) (25). Usually, Staphylococcal food poisoning is associated with SE-producing *S. aureus* strains, more than TSST-1 which required certain conditions to producing it that mostly available in human such as animal protein, low levels of glucose, temperature of 37 to 40°C, a pH of 6.5 to 8, and oxygen (4), that meaning the production of this toxin in food refers to lack of maintaining cold chain throughout storage period.

Frequency of multiple genes occurring together in the same isolate

Out of 89 *S.aurues* isolates, 86 (96.62%) isolates were toxigenic and 66 (76.74%) isolates were harbored more than one gene of superantigenic toxins genesas shown in the table (5), this means that the local *S. aureus* isolates are high virulent and there is a large probability to infected with toxic shock syndrome disease by these isolates, while only three isolates carried *seb* gene, one isolate carried *sea* gene, three isolates detected did not harbored any of toxins genes and seventeen of isolates were carried just *tst* gene. The occurrence of multiple genes carried by the same isolate indicated the pathogenic potential of *S. aureus* and this is worrying especially if these isolates were MDR.

Table (5): High frequency of toxins genes occurring together in the same isolate

Gene combination	No. of isolates
tst + sea	15
tst + seb	14
tst + sec	1
tst + see	2
tst + eta	1
tst + sea + seb	12
tst + sea + see	6
tst + seb + sec	3
tst + etb + seb	1
tst + sea + seb + see	1
tst + eta + sea + see	1
tst + eta + sea + seb	1
tst + sea + seb + sec + sed + see	1
tst + eta + sea + seb + sec	1
sea + seb + see	3
etb + sed	1
seb + sed	1
eta + sea + see	1

Relationship of MRSA, VISA and VRSA isolates with toxins genes distribution in this study

Statistically, toxins genes showed significantly associated with MRSA isolates, but there is no association with VISA and VRSA isolates. Also, Wang *et al*., 2012 (26) demonstrated that toxins genes especially toxic shock syndrome toxin-1 gene and enterotoxins genes can be transferred together with *mecA* gene on *Staphylococcal cassette chromosome mec (SCCmec)* and there are five types of *SCCmec* found in *S. aureus* strains, that increases the rate of transmission of toxins genes between MRSA isolates. On the other hands, this characteristic can be used to predict the presence of enterotoxins or TSST-1 genes when detection the resistance of methicillin by *S. aureus* isolates.

Discrepancy between routine method and molecular method

Depending on molecular method, 22(31.42%) isolates of coagulase-negative *staphylococcus* were identified as *S. aureus* isolates, Młynarczyk *et al*., 1998 (27) reported that coagulase activity decreases or lack in *S. aureus* isolates when reduced the sensitivity for antimicrobial agents especially methicillin antibiotic, in additionally the temperature and time of incubation affected on coagulase production (28). Also, Dai *et al*., 2012 (29) indicated that *S. aureus* can be appeared changes in biological characteristics such as reduced coagulase activity, cell wall thickening, slow growth, smaller colonies, decreased pigment formation and less or no haemolysis, and that due to reduced vancomycin susceptibility. Concerning to API Staph system, the 11 (15.06%)of isolates from negative API test were given positive for *femA* gene, some *staphylococcus* species give the same results in API test such as *S. xylosus* and *S. sciuri*, the temperature and time of incubation, quantity of the inoculum and activity of isolate can be affect in results of identification of *S. aureus* (30, 31). In abbreviated, phenotypic changes of *S. aureus* isolates may create problems in diagnosis as coagulase-negative *Staphylococci* and thus lead to increased nosocomial infections of VRSA strains which may be extend in to society. For this purpose, the PCR method offers a reliable, rapid and highly specific tool for identification of *Staphylococcus aureus* especially coagulase-negative *S. aureus* that misidentification in routine methods.

In the present study, the results of 89 *S. aureus* isolates were given 75 (84.26%) isolates resistant to oxacillin while genotyping method illustrated that all 89 (100%) of *S. aureus* isolates was carried *mecA*gene. Phenotypic method is frequently show variations and therefore appears discrepancy with PCR method, heterogeneous resistance (32), culture conditions such as incubation temperature, pH, sodium chloride content in the medium and inoculum density (33), and the acquisition of vancomycin resistance may be led to inactivation of gene *mecA* caused complete loss of β-lactam resistance (34, 35) therefore, we found that 4 VRSA isolates and 4 VISA isolates were susceptible for oxacillin. All these factors complicate the detection of methicillin resistance, especially for heterogeneous strains, the PCR method has high sensitivity and specificity and are independent of the physical and chemical conditions of the culture (36).

CONCLUSIONS

This study demonstrated that 97% of *S. aureus*isolates were toxigenic and 66 (76.74%) isolates were harbored more than one gene of superantigenic toxins genes, the most frequently of toxins genes were *tst* gene then followed by genes of *sea* and *seb* in *S. aureus* isolates. All *S. aureus* isolates harbored *mecA* gene and there is significantly associated between MRSA isolates and toxins genes, that making local *S. aureus* isolates high virulent and difficult in therapy. The results showed that conventional bacteriological methods are non specific for identification and epidemiological study of *S. aureus* strains because of their phenotypic and genotypic variations. Finally, this study presented typical PCR primers, very specific, and can be used for an epidemiological study of the hazardous *S. aureus* in food poisoning outbreaks. Therefore a multiplex PCR procedure was used for simultaneous identification of toxins genes and provide the necessary information to identify effective treatment against these isolates.

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