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# Molecular Detection of Enterotoxin Genes of Multiresistant *Staphylococcus aureus* Isolates from Different Sources of Food

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**Abstract**

Foodborne diseases are a major risk for human health. Millions of people become sick as a result of eating contaminated food with microorganisms that cause diseases. *S. aureus* is considered as one of the most important pathogenic bacteria, having the ability to activate certain genes that encode for heat stable enterotoxins and cause Staphylococcal food poisoning. Thus, this study aimed to determine the prevalence of multi resistant *Staphylococcus aureus* that produce enterotoxins in different sources of food . Forty nine isolates were identified as *S.aureus,* according to morphological and biochemical tests. They were isolated from 387 different food samples from several randomly covered restaurants and supermarkets in different regions of Baghdad. Molecular diagnosis of *S. aureus* using specific primers for the 16S rRNA gene was carried out by Polymerase Chain Reaction (PCR ) technique . Susceptibility of 43 isolates of *S.aureus* was tested against 15 antimicrobial agents. The results revealed that all the isolates were resistant (100%) to mecillinam, highly resistant to vancomycine and meropenemin (74.4 %) and moderately resistant to Oxacillin, Erythromycin Cefotaxime, and Cefiximein (67.4, 60.4, 62.8, , 60.5 %, respectively), while they showed low resistance to Gentamicin (34.8%). In addition, all of these isolates were susceptible to Tigecycline and Amoxicillin/ clavulanic acid and Cefoxitin-Cloxacilin. High percentages of oxicillin resistant *S. aureus* were isolated from cooked food samples, followed by meat products, and with less percentage from pastry products. Molecular detection of enterotoxins A and B of *Staphylococcus aureus* isolates was performed using specific primers based on PCR. The results revealed that *S. aureus* isolated from cooked food had the highest percentage of the isolates producing the enterotoxins A and B. Type A enterotoxin gene showed a higher prevalence than type B gene among cooked food , dairy products and pastry. In conclusion, the results revealed a high prevalence of some classical enterotoxin genes in multi-drug resistant *S.aureus* isolated from different sources of food, which can cause food-poisoning and, consequently, a potential serious problem for public health.

**Keywords**: Multi resistant *Staphylococcus aureus,* foodborne poisoning, Staphyloccal Enterotoxins.

# التحري الجزيئي عن مورثات السموم المعوية في عزلات المكورات العنقودية الذهبية المعزولة من مصادر غذائية مختلفه

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**الخلاصة**

تشكل الأمراض التي تنتقل عن طريق الأغذية خطرا كبيرا على صحة الإنسان. ملايين من البشر يمرضون نتيجة تناول طعام ملوث بالكائنات الحية الدقيقة المسببة للأمراض ، وتعد المكورات العنقودية الذهبية من أهم الانواع البكتيرية المسببة للأمراض ، اذ تمتلك بعض المورثات المشفرة للسموم المعوية الثابتة بالحرارة والتي تسبب التسمم الغذائي بالمكورات العنقودية ، لذلك هدفت هذه الدراسة إلى تحديد مدى انتشار المكورات العنقودية الذهبية المتعددة المقاومة والمنتجة للسموم المعوية في مصادر مختلفة من الغذاء. شخصت تسعة وأربعون عزلة الى بكتريا المكورات العنقودية الذهبية *S.aureus* (اعتمادا على الاختبارات المظهرية والبايوكيميائية ) عزلت من 387 عينة غذائية مختلفة جمعت بشكل عشوائي من العديد من المطاعم ومحلات السوبر ماركت في مناطق مختلفة من بغداد. تم إجراء التشخيص الجزيئي لبكتريا المكورات العنقودية الذهبية باستخدام 16S rRNAباستخدام تقنية تفاعل البلمرة المتسلسل. اختبرت حساسية العزلات تجاه 15 مضاد حيوي واظهرت النتائج مقاومة جميع العزلات بنسبة 100 ٪ للميسيلينوم ، ومقاومة عالية لمضادي الفانكومايسين والميروبينيم بنسبة 74.4 ٪ ومقاومة معتدلة للأوكساسيلين ، والإيريثرومايسين، السيفوتاكسيم ، والسيفيكسيمين بنسبة (67.4 ، 60.4 ، 62.8 ، 60.5) ٪ على التوالي ، في حين اظهرت العزلات مقاومة منخفضة للجنتاميسين بنسبة 34.8 ٪ ، وجميع العزلات كانت ذات حساسية عالية لمضادات التيجيسيكلين و الأموكسيسيلين / كلافيولانيك والسيفوكسيتين – كلوكساسيلين .وكانت اعلى نسبة من العزلات المقاومة للاوكساسلين معزولة من عينات الطعام المطبوخ و بنسبة أقل من المعجنات . تم إجراء الكشف الجزيئي للسموم المعوية A و B لعزلات المكورات العنقودية الذهبية باستخدام بادئات متخصصة على تفاعل البلمرة المتسلسل ، وكشفت النتائج ارتفاع نسبة 31.3 ٪ لإنتاج نوعين من معوي السموم A و B من عزلات المكورات العنقودية الذهبية من الأغذية المطهية. تم الكشف عن مدى انتشار الجين المعوي من النوع A أعلى من جين seb الموزع بين الأطعمة المطبوخة ومنتجات الألبان والمعجنات. الأوكساسيلين ، الإريثروميسين سيفوتاكسيم ، والسيفيكسيمين بنسبة (67.4 ، 60.4 ، 62.8 ، 60.5) ٪ على التوالي ، في حين اظهرت العزلات مقاومة قليلة للجنتاميسين 34.8 ٪ ، في حين أن جميع هذه العزلات كانت حساسة لتيجيسكلين وأموكسيسيلين / كلافولانيك وحمض السيفوكسيلينزز تم عزل نسب عالية من عزلات المكورات العنقودية الذهبية المقاومة من عينات الطعام المطبوخ وتبعتها منتجات اللحوم وأقل نسبة من المعجنات. اجري الكشف الجزيئي عن السموم المعوية A و B في المكورات العنقودية الذهبية باستخدام بادئات متخصصة بتقنية تفاعل البلمرة المتسلسل ، واظهرت النتائج 31.3 ٪ من عزلات المكورات العنقودية الذهبية منتجة لنوعين من السموم A و B كانت النسبة الاعلى من الأغذية المطهوة. واظهرالجين المسوول عن السم المعوي A نسبة انتشار أعلى من B في الأطعمة المطبوخة يليه منتجات الألبان ومن ثم المعجنات .في الختام ، فإن الانتشار المتزايد لبعض جينات السموم المعوية الكلاسيكية في المكورات العنقودية الذهبية متعددة المقاومة للعوامل المضادة للميكروبات في مصادر مختلفة من الطعام والتي يمكن أن تسبب التسمم الغذائي قد يعتبر مشكلة خطيرة للصحة العامة.

## Introduction

Food safety is one of the most important areas of public health worldwide. Foodborne diseases, infections, and poisoning result from infections with viruses, bacteria or parasites [1]. Meat, plant surfaces, and dairy products are the main sources of these infections [2]. Among the involved pathogens, *S. aureus* is an opportunistic human pathogen that is globally considered as the third most common pathogen causing foodborne diseases [3] . Different food sources can provide a good medium for *S. aureus*, such as raw meat and meat products [4, 5], raw milk, dairy products [6, 7], and ready-to-eat foods [8] .

In addition, previous studies reported that food can be a reservoir of antibiotic resistant *S. aureus* strains, due to the widespread use of antimicrobials in public health and animal husbandry [9]. This caused dramatic increase in the antibiotic resistance of coagulase-positive staphylococci, especially *S. aureus* which has genes encoding antibiotic resistance that are usually located on mobile genetic elements, allowing their horizontal transfer to pathogenic staphylococci [10]. The risk of the transfer becomes more serious as some species, such as *S. xylosus*, *S. carnosus*, and *S. pasteuri*, are used at

high concentrations as components of starter cultures during the production of fermented food. *S. aureus* holds several virulence factors responsible for pathogenicity to the host, including Panton-Valentine leukocidin (PVL), γ-hemolysis , coagulase, and staphylococcal protein A. In addition, *S. aureus* possess toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxins (SEs), both belonging to the pyrogenic toxin superantigen (PTSAg) family [11]. These superantigens (SAg) can bind to the major histocompatibility complex (MHC) class II and form a complex with the Vβ chain of T-cell receptor, resulting in a nonspecific stimulation of T-cell proliferation. *S. aureus* is a leading cause of food poisoning, resulting from the consumption of contaminated food with staphylococcal enterotoxins, and the most prevalent cause of gastroenteritis worldwide.

Enterotoxins are highly thermostable; pasteurization and normal cooking cannot totally inactivate them, possibly leading to food poisoning [12]. The start of the symptoms depends on the amount of the enterotoxin ingested. The classic SEs antigens that have been identified are SEA, SEB, SEC1, SEC2, SEC3, SED and SEE [13]. The enterotoxin genes are accessory genetic elements in *S. aureus* that are encoded by mobile genes [14] .

The present study aimed to investigate the prevalence of *S.aureus* in different food sources, conduct molecular diagnosis to the species using 16SrRNA gene by PCR technique as a rapid and specific method, identify the susceptibility of *S.aureus* to different antimicrobial agents, and determine the presence of enterotoxin genes among these isolates using PCR.

## Materials and Methods

### Samples collection

A total of 387 samples of food were collected from several randomly selected restaurants and supermarkets in different regions of Baghdad (Al- Harithya, Al-Jadria, Al-Mansuer, Al-Karada, Palestien street, Al-Bayaa, New Baghdad city, Al-Mashtal), through eight months during the period between November 2017 and June 2018.

The samples were classified into four different categories that include cooked food (n = 186), meat and chicken products (n = 45), dairy products (n = 89), and other sources (n- 66) that included pastry (n = 23) and different types of salad and appetizers (n = 43).

All samples were collected in sterilized containers, transported under cold conditions to the laboratory, and screened for the presence of *Staphylococcus* species within an hour.

### Identification of *S. aureus*

*Staphylococcus* species isolated from different food samples were described according to the conventional procedure of Baird-Parker [15]. Serial dilutions of food samples in 0.85% sterile saline solution)were streaked onto Baird-Parker agar complemented with Egg Yolk emulsion (5%) and potassium tellurite saturation solution (0.3%). The plates were incubated under aerobic conditions at 37ºC for 24 to 48 h. From each plate, black colonies (from Baird-Parker agar) and yellow colonies (from mannitol salt agar) were chosen for further characterization of species by biochemical test and gene based methods. All isolates were morphologically and biochemically identified to the species level depending on Bergey's Manual of Systematic Bacteriology [16]. Identification of the isolates was performed after confirming the tests of Gram reaction, oxidase , catalase, coagulase and mannitol fermentation, where only the isolates that gave positive results were selected for further study.

### Antimicrobial Susceptibility Assay

All *S. aureus* isolates were subjected to antimicrobial susceptibility test by standard disc diffusion method on Muller-Hinton agar according to the Clinical and Laboratory Standards Institute's (CLSI) recommendations. Sensitivity patterns of the isolates to Gentamycin (GM) 10µg , Oxacillin (OX) 1µg, Ciprofloxacin (CIP) 5 µg, Erythromycin (E) 15µg , Tigecycline (TGC) 5 µg, Vancomycine (VA) 30µg, Meropenem (MEM)10 µg , Ceftriaxone (CTR)30 µg , Amikacin (AK) 30µg , Cefoxitin-Cloxacil (CXX) 30/200 µg , Imipenem (IPM) 10 µg , Mecillinam (MEC) 10µg, Amoxicillin (AUG) 30 µg , Cefotaxime (CTX) 30µg and Trimethoprim (SXT) 25 µg were determined. Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; susceptible, intermediate, and resistant, according to the standard guidelines recommended by the National Committee for Clinical Laboratory Standard. The diameters of inhibition zone were compared with those of the Performance Standards for Antimicrobial Susceptibility Testing [17]

### Extraction of DNA

Whole genomic DNA was extracted from *S.aureus* isolate using Genomic Bacterial DNA extraction kit (Promega, USA) with an addition of 30 µg/ml lysozyme enzymes according to the

manufacturer's instructions. The purity and concentration of DNA samples were estimated using Nano drop apparatus (ACT Gene/ Korea) .

**Detection of 16SrRNA , *SEA* and *SEB* genes using PCR technique**

**Detection of 16SrRNA gene**

The amplification of PCR reactions was performed in a thermal cycler machine (labnet- USA). The components were used as follows: 12.5 µl of Co Taq®Green Master Mix (Promega /USA ) that contained Taq DNA polymerase, MgCl2 deoxynucleosides (dNTP), buffer, and 1 µl of each primer (10pmol ). Also, 1µl of 100ng DNA was used as template , while 9.5 µl deionized water was added to achieve 25 µl total volume.

The sequence of oligonucleotide primers that were used in PCR to detect the presence of *Staphylococcus* specific genes were synthesized in Integrated DNA Technology (Canada). The primer sequences were -F 5'- GGCCGTGTTGAACGTGGTCAAATCA - 3' and -R5' - TIACCATTTCAGTACCTTCTGGTAA- 3' designed according to Martineau *et al.* [18] with a product yield of 370 bp fragment . The amplification of the *16s rRNA* gene was completed in 30 cycles : initial denaturation at 94 °C for 3min, second DNA denaturation at 94°C for 1 min, primer annealing at 55- 60 °C for 30 s, DNA extension at 72°C for 30 s and final extension at 72°C for 3 min . The PCR products were tested by agarose gel electrophoresis (1.5% w/v) at 70 voltage for 1.5 hr. 100 bp DNA ladder and DNA bands were visualized using UV transilluminator documentation system and photography.

**Detection of Staphylococcus Enterotoxines *( sea , seb) genes***

*The sea and seb* loci were determined among the extracted DNA of *S. aureus* isolates by polymerase chain reaction using forward primer 5'-GGTTATCAATGTGCGGGTGG-3' for *sea* and reverse primer 5'- CGGCACTTTTTTCTCTTCGG-3' , with a yield of 102bp fragment. While the forward primer for *seb* gene was '5'-GTATGGTGGTGTAACTGAGC-3' and the reverse primer was 5'-CCAAATAGTGACGAGTTAGG-3', with a yield of 164 bp fragment. All primers were designed according to Manisha *et al.* [19].

A volume of 25 µl of PCR solution contained master mix buffer (1X), forward and reverse primers (10 pmol/µl) , DNA (200 ng/µl) and deionized water. PCR was performed as follows: initial denaturation for 5 min at 94 °C, second denaturation for 2 min at 94 °C, annealing for 2min at 57 °C, extension at 72 °C for 1min and final extension at 72°C for 7 min. The amplification was applied at 35 cycles and the PCR product was analyzed by loading 10 µl of PCR mixture onto agarose 1.5% in the presence of 100 bp DNA ladder. After gel electrophoresis, the gel was exposed to U.V by using U.V Transilluminator.

**Statistical Analysis**

The Statistical Analysis System- SAS (2012) program was used to detect the effects of difference factors on study parameters. Chi-squared test was used to compare between percentages based on significant differences (at 0.05 and 0.01 probability).

The SPSS software (V 20.0) was applied to estimate the correlations amongst the samples , the source of isolates, and genes, using Spearman Correlation with a significance limit at $p< 0.05$. The SPSS software was also employed to estimate the correlations amongst the sources of isolates and genes using Pearson chi-squared (R) to test differences with significance of $p< 0.05$.

**Results and Discussion**

**Identification of *S. aureus* isolates from food samples**

A total of 387 different food samples were collected randomly from different regions in Baghdad governorate. 112 *Staphylococcus* isolates were identified depending on morphological examination , cultural media ( on Baird-Parker medium and ability to ferment mannitol aerobically on mannitol salt agar ). Only 49 isolates were identified as *S. aureus* according to biochemical tests (positive for catalase and coagulase and negative for oxidase) and molecular diagnosis for 16S rRNA (table -1).

From 49 isolates belonging to *S.aureus*, the highest percentage was obtained from samples of ready-to-eat food or cooked food (42.8%), followed by salad and appetizers (24.5%), meat products (14.3%), dairy products (10.2%) and cakes ( 8.2 %) , while no isolates were isolated from chicken products. The results in Figures-(1, 2) showed a high frequency of *S. aureus* isolates in the cooked food group compared with other main groups .

The highest percentages of *S. aureus* were represented in cooked food samples from rice (20.7%) and chicken (18.5%), while cooked food of vegetable samples reported the least percentage (5.6%)

which was close to the percentage of crisp (5.9%). In the dairy product samples, only two samples showed a positive result for *S.aureus* isolates, represented by cheese and raw cheese (11.1 and 9.1 %, respectively) while no isolates of *S.aureus* were found in raw cream , raw cheese, and cream cheese samples.

**Table 1**-The numbers of *S. aureus* isolates from different types of food samples

| No. | Type of sample | No. of samples | No. of Staphylococcus spp. | No. of *S.aureus* | %* |
|---|---|---|---|---|---|
| A | Cooked food | | | | |
| 1 | Meat with dough | 30 | 10 | 3 | 10 |
| 2 | Bread of erok | 38 | 11 | 3 | 7.9 |
| 3 | Crisp (cooked potatoes) | 17 | 3 | 1 | 5.9 |
| 4 | Rice | 29 | 10 | 6 | 20.7 |
| 5 | Grilled kebab | 18 | 4 | 2 | 11.1 |
| 6 | Cooked meat with vegetables | 18 | 5 | 1 | 5.6 |
| 7- | Chicken | 27 | 9 | 5 | 18.5 |
| 8- | Falafel | 10 | _ | _ | _ |
| B- 1- | Meat products (hamburger and martidela) | 34 | 9 | 7 | 20.5 |
| 2- | Chicken products | 11 | 1 | _ | _ |
| C -1- | Raw cream | 12 | 1 | _ | _ |
| 2- | Raw cheese | 22 | 7 | 2 | 9.1 |
| 3- | White cheese | 27 | 7 | 3 | 11.1 |
| 4- | Cream cheese | 12 | 4 | _ | _ |
| 5- | Soft cheese | 16 | 5 | _ | _ |
| D 1- | Salad and appetizers | 43 | 22 | 12 | 27.9 |
| 2- | Pastry (cakes) | 23 | 4 | 4 | 17.4 |
| Total | | 387 | 112 | 49 | |

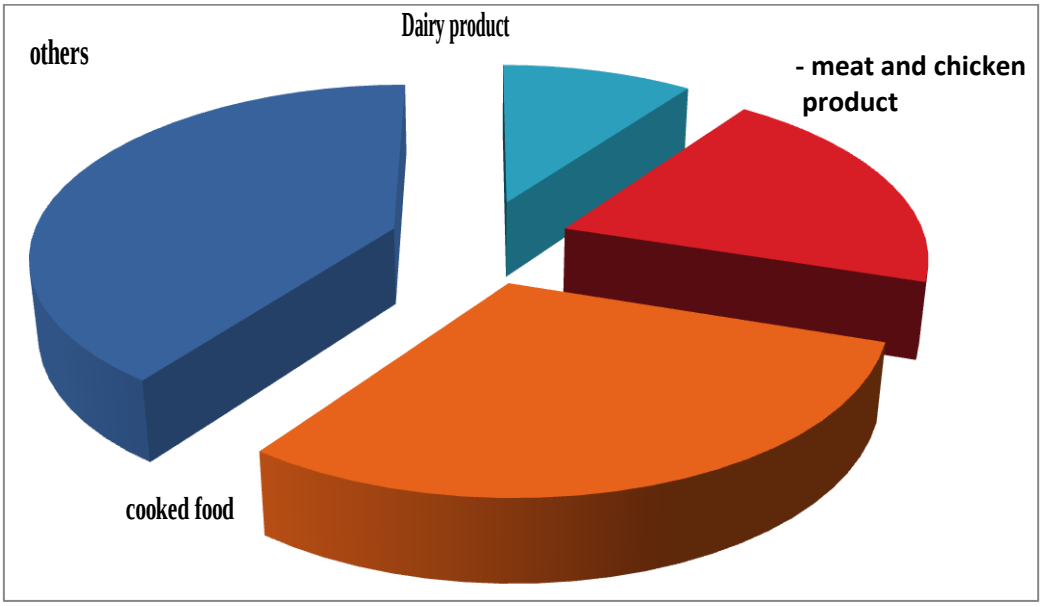


**Figure 1**-The frequency of *S.aureus* in main groups

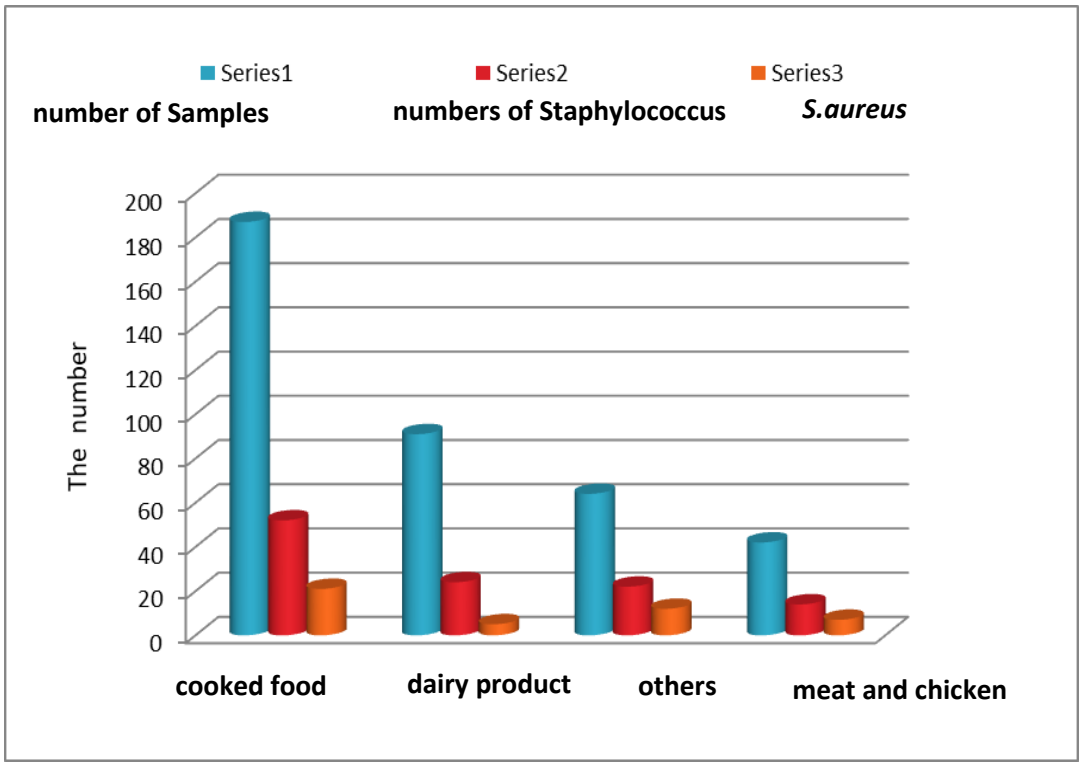


**Figure 2**-Comparison between the four groups of food samples depending on numbers of samples (blue) , Staphylococcus spp isolates (red), and *S. aureus* isolates (orange).

The results in Table- 2 show the statistically significant differences between numbers of positive samples for *S. aurues* in different types of food samples (P > 0.05).

**Table 2**-Statistical analysis of numbers of *Staphylococcus aureus* isolates in various types of food samples.

| | Type of food samples | No. of food samples | Staphylococcus spp | *S. aureus* | Chi-Squared ($\chi^2$) |
|---|---|---|---|---|---|
| A | Cooked food | 187 | 52 (27,8%) | 21(36.21 %) | 1.372 NS |
| B | Meat product+ Chicken product | 42 | 14(33,3%) | 7(50%) | 7.335 ** |
| C | Dairy product | 91 | 24(26.3%) | 5(20.8%) | 4.866 * |
| D | Salad and appetizer | 43 | 22(51.2%) | 12(54.5%) | 4.478 * |
| E | Pastry (cakes) | 21 | 4(17.4%) | 4(100%) | 6.952 ** |
| Total | | 387 | 112 | 49 | |
| Chi-Squared ($\chi^2$) | --- | 11.275 ** | 9.406 ** | --- | Chi-Square ($\chi^2$) |
| * P<0.05 : Significant , ** P<0.01: Highly Significant, NS: Non-Significant. | | | | | |

A previous study by Mahfoozi *et al*. (2019) reported that the most contaminated foods were found in samples of hamburger (47.3%) and kebab samples (61.5%), while variable rates of contamination for *S. aureus* were noticed in other food samples.

The variation in the numbers of *S. aureus* isolates between several studies might be due to different factors. Staphylococcal food poisoning is correlated with inappropriate handling of processed or cooked foods, rather than raw foods, followed by storage under conditions which allow *S. aureus*

growth and production of enterotoxins [20]. Foods that favor growth of coagulase positive staphylococci are characterized by high protein content. These include poultry, meat products and meat, dairy products and milk, egg products, salads, bakery products -particularly cream-filled pastries and cakes, and sandwich fillings, all have been frequently involved in Staphylococcal food poisoning (SFP) outbreaks. Besides, the production of enterotoxins from Staphylococci is affected by different factors, including water activity, pH, temperature, and other parameters [21, 22]. Milk products may cause severe health hazards to people due to high susceptibility to a variety of microorganisms, depending on their high nutritive value [23].Cheese types have complex microbial ecosystems identified by the presence of a large variety of molds, yeasts, bacteria , and other microorganisms including some species of lactococci or lactobacilli. The presence of certain microorganisms may lead to a higher health risk by contamination from the environment during handling and processing of raw milk. An important food-borne pathogen that is recognized worldwide is *S. aureus* which can produce different types of enterotoxins in food [24].

In this study, the different outcomes may be due to differences in production technologies, such as those used in cheese production, the number of samples, whether the used milk was raw or pasteurized, the adequacy of hygiene standards during manufacturing process, and the personnel involved in the production.

**Molecular diagnosis of *S. aureus* isolates**

To confirm the results of the biochemical tests, the molecular diagnosis of *S.aureus* isolates was performed using specific primers for *16S rRNA* gene as an indicator for the diagnosis of *S. aureus* isolates .The yield of the DNA extracted was in the range of 100-1500 ng/µl with a purity range of 1.8-2.1, as indicated by using nano drop apparatus. The results in Figure-1 show that 49 isolates of *S. aureus* gave positive results for 16S rRNA as a single DNA band of PCR product with a molecular base of 370 bp. This was shown by conventional PCR based on an annealing temperature of 55 °C, which was specified for *S.aureus* and considered as a critical feature for differentiation from other Staphylococci *spp*. [25]. Previous studies described the use of PCR for the detection of 16S rRNA as more reliable and rapid method. The results of the current study agree with those of Heidari *et al.* [26] who noticed that the amplification of 16S rRNA confirmed the identification of 126 Staphylococcal isolates as *S. aureus*. Several studies reported that 16S rDNA differentiated all tested isolates and identified 100% Of them as *S. aureus* [27, 28, 29].

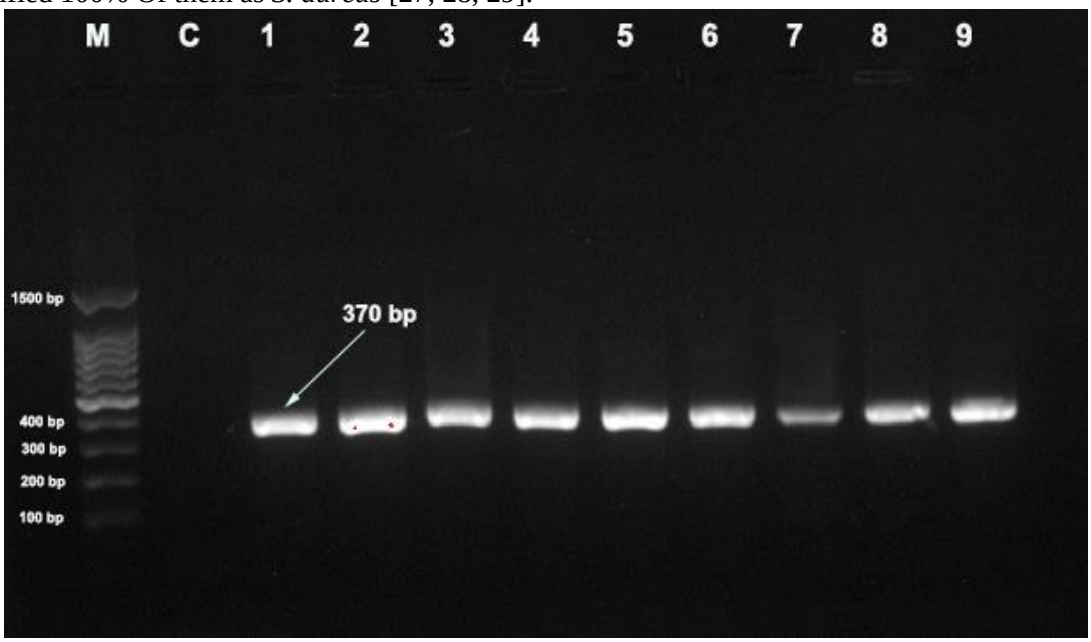


**Figure 1**-The amplification products of *Staphylococcus* isolates with specific primer gene (370bp) separated using 1.5% agarose gel electrophoresis (90 volt, TBE buffer 1X) for 1 hour after staining with ethidium bromide, conceived under UV light. M = Molecular size marker (100-1500bp). C = Negative control. (1-9) = *Staphylococcus* isolates.

**Antimicrobial susceptibility of *S. aureus* isolates**

All isolates of *S. aureus* were tested for resistance towards fifteen antimicrobial discs used in this study. The results in Figure-4 show that all isolates were relatively highly resistant to mecillinam (100%). Resistance to each of Vancomycine and Meropenemin was 74.4 %, to Oxacillin and was 62.8 %, to Erythromycin was 60.5 %,and to Gentamicin was 34.8%, whereas all of isolates were susceptible to Tigecycline and Amoxicillin/ clavulanic acid. In addition, the isolates showed resistance against the third generation of cephalosporin group which includes Cefotaxime and Cefiximein (67.4, 60.4 % respectively), while they were sensitive to Cefoxitin-Cloxacilin (9.3%). Food is the main source for the transfer of antibiotic resistance, possibly through the ingestion of resistant food-borne strains [30, 31]. The inhibition zone of mecillinam can be considered as an indicator for the critical qualification of producer/non-producer β lactamase *S.aureus* [32]. All the sensitive isolates within the mecillinam inhibition zone of less than 17mm could be considered as β lactamase producer *S.aureus* isolates. In this study, all the 49 isolates were resistant to mecillinam, possibly since it is used for the treatment of urinary tract infections caused by *E. coli* [33] and infections caused by gram positive *Staphylococcus saprophyticus* [34]. The high level of resistance toward β lactam group that was expressed within *S. aureus* isolates is mainly caused by the expression of *mec*A and its regulatory determinant which encode for the variant PBP called PBP2A and/or β lactamase encoded by *bla*Z, as well as the AbcA efflux pumps encoded by *abc*A. Also, a part of the efflux system in *S.aureus* is defined also as a resistant mechanism toward β-lactam antimicrobial agents [35, 36].

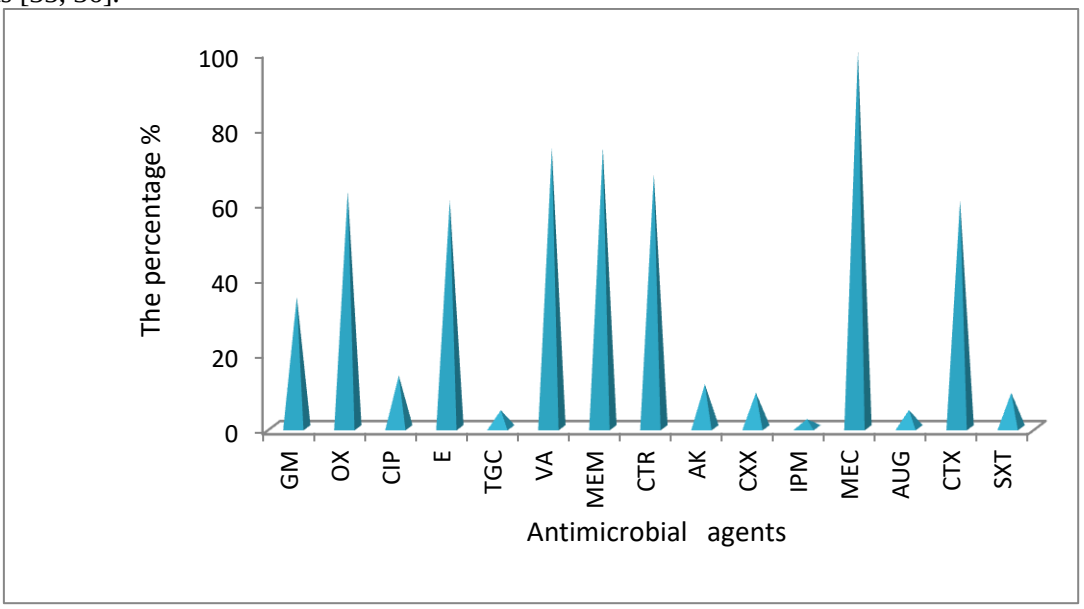


**Figure 4**-Susceptibility of *S.aureus* isolates to antimicrobials agents. Gentamicin (GM) , Oxacillin (OX) , Ciprofloxacin (CIP) , Erythromycin (E) , Tigecycline (TGC) , Vancomycine (VA) , Meropenem (MEM) , Ceftriaxone (CTR) , Amikacin (AK) , Cefoxitin-Cloxacil (CXX) , Imipenem (IPM) , Mecillinam (MEC) , Amoxicillin (AUG) , Cefotaxime (CTX) and Trimethoprim (SXT).

The present results showed high resistance to Oxicillin ,Vancomycine, and Meropenemas, which is in agreement with the results reported by Kader *et al.* [37], who showed that 88.24% of the isolates were resistant to methicillin and Oxacillin discs. Odonkora and Addob [38] noticed that the methicillin disc diffusion test detected 54 MRSA ( 21.6%) , with relatively moderate resistance to Vancomycin and Amikacin (60 and 62 %, respectively). Alibichewi *et al.* [39] found a low resistance of *S. aureus* isolates towards Vancomycin and Amikacin (9.7 and 10.0, %, respectively). For comparison purposes, the results of the statistical analysis based on isolation source and response to antibiotics are described in Table-3).

**Table 3**-The percentage of susceptibility of *S.aureus* to different types of antibiotics along with the results of Chi-Squared ($\chi^2$) analysis .

| No. | Type of antibiotic | Response type | No. and % of *S. aureus* in food samples | Chi-Squared ($\chi^2$) |
|---|---|---|---|---|
| 1- | Amikacin (AK) | R<br>S | 5 (11.63%)<br>38 (88.37%) | 13.74 ** |
| 2- | Amoxicillin (AUG) | R<br>S | 2 (4.65%)<br>41 (95.35%) | 14.68 ** |
| 3- | Cefotaxime (CTX) | R<br>S | 26 (60.46%)<br>17 (39.53%) | 7.62 ** |
| 4- | Cefoxitin-Cloxacil (CXX) | R<br>S | 4 (9.30%)<br>39 (90.69%) | 14.17 ** |
| 5- | Ceftriaxone (CTR) | R<br>S | 29 (67.44%)<br>14 (32.56%) | 9.35 ** |
| 6- | Ciprofloxacin (CIP) | R<br>S | 6 (13.95%)<br>37 (86.05%) | 13.71 ** |
| 7- | Erythromycin (E) | R<br>S | 26 (60.47%)<br>17 (39.53%) | 7.62 ** |
| 8- | Gentamicin (GM) | R<br>S | 15 (34.88%)<br>28 (65.12%) | 9.35 ** |
| 9- | Imipenem (IPM) | R<br>S | 1 (2.33 %)<br>42 (97.67%) | 14.82 ** |
| 10- | Mecillinam (MEC) | R<br>S | 43 (100%)<br>0 (0.00%) | 15.00 ** |
| 11- | Meropenem (MEM) | R<br>S | 28 (65.12%)<br>15 (34.88%) | 9.35 ** |
| 12- | Oxacillin (OX) | R<br>S | 27 (62.79%)<br>16 (37.21%) | 8.92 ** |
| 13- | Tigecycline (TGC) | R<br>S | 2 (4.65%)<br>41 (95.35%) | 15.00 ** |
| 14- | Trimethoprim (SXT) | R<br>S | 4 (9.30 %)<br>39 (90.69%) | 13.76 ** |
| 15- | Vancomycine (VA) | R<br>S | 32 (74.42%)<br>11 (25.58%) | 12.66 ** |
| | Chi-Square ($\chi^2$) | | 16.70 ** | --- |

** P<0.01: Highly Significant , NS: Non-Significant

The mechanisms of antimicrobial resistance in bacteria are due to several agents that include degradation of antibacterial drugs by enzymes, alteration of bacterial proteins that are antimicrobial targets, and changes in permeability of membrane to antibiotics. Antibiotic resistance can be mediated by either plasmid , integrons, or transposons. The hydrolysis reaction is mediated by the bacterial enzyme beta-lactamase, which is the most important mechanism for resistance to cephalosporins and penicillins. *S.aureus* becomes resistant to methicillin and other β-lactam antibiotics by acquiring a genomic island known as staphylococcal chromosome cassette (*mec*).

In the current study, the results showed an increasing prevalence of *S. aureus* and its emerging antibiotic resistance in different types of food. Especially, most of the isolates showed multi-resistance to more than one antibiotic (2-11). Also, one isolate from vegetable salad showed resistance to all the 15 antibiotics, which is considered as a serious problem for public health.

**Detection of *sea and seb* genes using PCR technique**

Nine among 49 isolates of *S.aureus* showed positive results for *sea* gene with single band (102 bp), while seven isolates showed a positive result for *seb* gene (164 bp), as shown in Table-4 and Figures-(5, 7). In this study, different isolates of *S. aureus* were shown to produce two types of enterotoxin. The prevalence of the type A enterotoxin gene was detected to be higher than that of the *seb* gene, distributed among cooked food , dairy products, and pastry.

*S. aureus* is considered as an important aetiological agent of food intoxications through the production of various staphylococcal enterotoxins (SEs), that might cause gastro-enteritis, which proved to have more tolerance to pasteurization and higher temperature [40]. Researchers have shown that SEs are highly resistant to heat treatment, as in the case of *sea* which retained its biological activity even after exposure to as high temperature as 121°C for 28 minutes [41].

The enterotoxins were reported as an important virulence agent involved in food poisoning [40]. A study by Mousa *et al.* [42] showed the 72 out of 103 isolates of *S. aureus* harbored at least one type of the SEs genes. Several studies reported a high proportion of bacterial isolates from outbreaks of staphylococcal food poisoning that occurred in the United Kingdom , South Korea , Japan and France, which could produce staphylococcus enterotoxin A, either alone or with another toxin [41].

Bystron *et al*. [43] tested food samples from half-baked chickens in Turkey, in order to the detect enterotoxin-producing *S. aureus*. Among 65 isolates, only 11 were identified to be *S. aureus* [44]. The production of enterotoxin from *S aureus* is affected by different environmental factors such as temperatures , water activity, pH and other parameters. The results of statistical analysis based on samples and enterotoxin A gene are listed in the Table-5, while those for enterotoxin B are described in Table-6.

**Table 4-**Results of the molecular detection of *sea* and *seb* genes from *S. aureus* isolates from different sources of food

| No. | Type of samples | No. of Staphylococcus aureus | %* | Enterotoxin A | Enterotoxin B | %* |
|---|---|---|---|---|---|---|
| A- | Cooked food | 21 | 42.8 | 3 | 2 | 31.3 |
| B- | Meat product | 7 | 14.3 | 1 | 2 | 18.7 |
| C- | Dairy product | 5 | 10.2 | 1 | _ | 6,3 |
| D-others | Salad and appetizers<br>Pastry(cakes) | 12<br>4 | 24.5<br>8.2 | 2<br>2 | 2<br>1 | 25<br>18.7 |
| | Total | 49 | 100% | 9 | 7 | 100% |

**Table 5-**Correlation between samples and enterotoxin A gene using Chi-Squared Test.

| | Value | D f | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 2.754[a] | 3 | .431 |
| Likelihood Ratio | 4.177 | 3 | .243 |
| Linear-by-Linear Association | 2.360 | 1 | .125 |

a: 4 cells (50.0%) have expected count less than 5. The minimum expected count is 61.

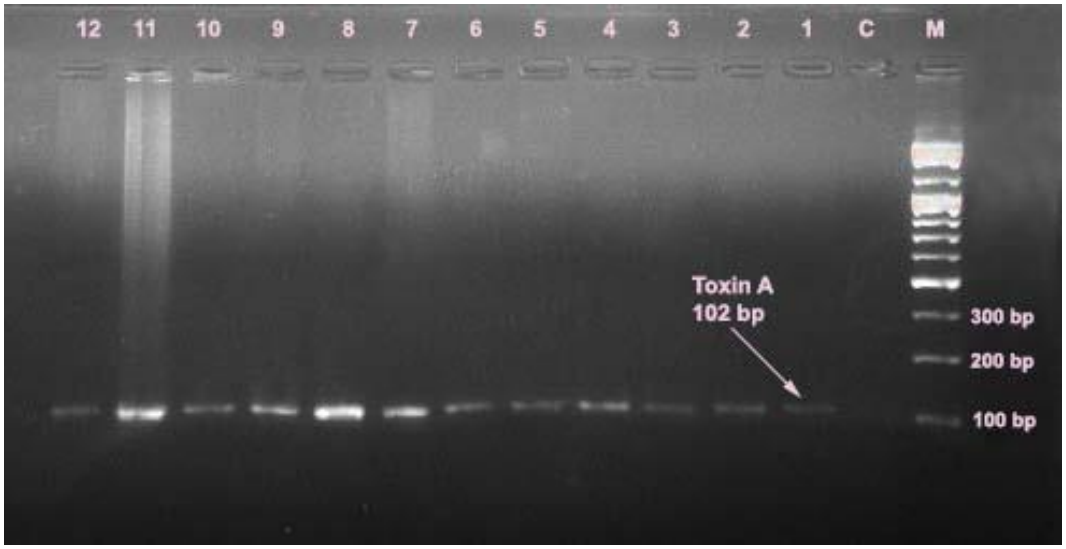


**Figure 5**-The PCR amplification products of *S. aureus* toxin A gene (102bp) separated on 1.5% agarose gel electrophoresis (90 volt , 1X TBE buffer) for 1 hour, stained with ethidium bromide , then conceived under UV light. M = Molecular size marker (100-1500bp). C = Negative control. 1-7 = *S. aureus* isolates.

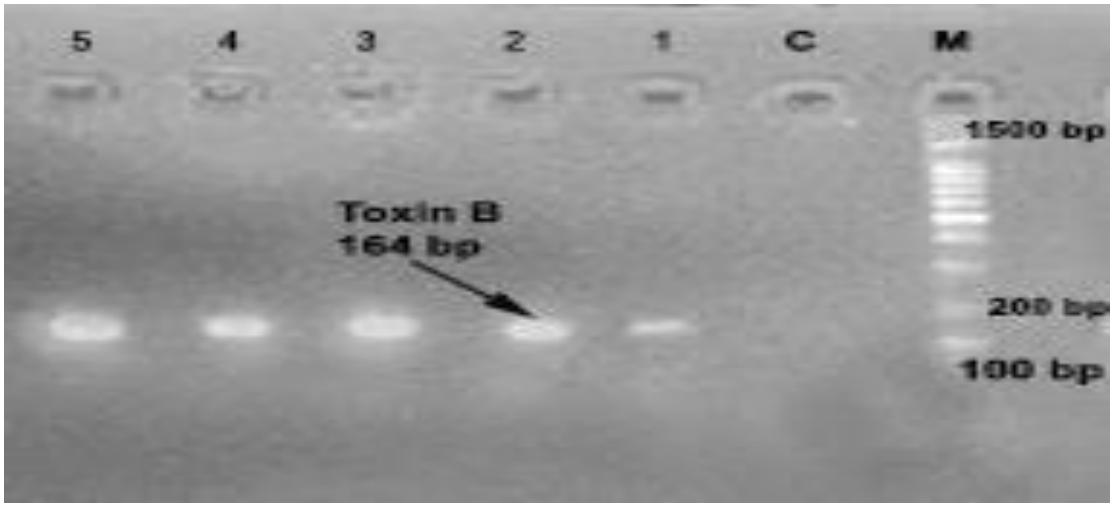


**Figure 7**-The PCR amplification products of *S. aureus* toxin B gene (164bp) separated on 1.5% agarose gel electrophoresis (90 volt , 1X TBE buffer) for 1 hour , stained with ethidium bromide then conceived under UV light . M = Molecular size marker (100-1500bp). C = Negative control. 1-5 = *S. aureus* isolates.

**Table 6**-Correlation between samples according to enterotoxin A gene expression using Chi-Squared Test.

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Squared | 1.744[a] | 3 | 0.627 |
| Likelihood Ratio | 2.492 | 3 | 0.477 |
| Linear-by-Linear Association | .171 | 1 | 0.680 |

4 cells (50.0%) have expected count less than 5. The minimum expected count is 0.76.

**Conclusions**

The increased emergence of antibiotic resistance in *S. aureus* isolated from different types of food is a part of a serious problem to public health. The relative prevalence of some classical enterotoxin genes (*sea* and *seb* genes) in the isolates revealed the potential of this bacterium to produce different kinds of enterotoxins, which can cause food-poisoning.

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**References**

**1.** Bunnoeng, N., Themphachana, M., Pewleang, T., Kongpheng, S., Singkhamanan, K., Saengsuwan, P. and Sukhumungoon, P. **2014**. High prevalence and molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from retailed meats, *South Thailand .International Food Research Journal*, **21**(2): 569-576.

**2.** Carfora V., Caprioli A., Marri N., Sagrafoli D., Boselli C., Giacinti G., et al. **2015**. Enterotoxin genes, enterotoxin production, and methicillin resistance in *Staphylococcus aureus* isolated from milk and dairy products in central Italy. *Int. Dairy J.* **42**: 12–15.

**3.** Witte,W.,Strommenger,B.,Stanek,C.,Cuny,C.**2007**. Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. *Emerging Infect. Dis*. **13**: 255–258.

**4.** Mahfoozi, A., Shirzad-Aski, H., Kaboosi, H. and Ghaemi, E. A. **2019**. Identification of the classical enterotoxin genes of *Staphylococcus aureus* in various foods by multiplex PCR assay. *Iranian Journal of Veterinary Research*, IJVR, **20**(3): 209-212.

**5.** Shi Wu, Jiahui Huang, Qingping Wu,1, Jumei Zhang, Feng Zhang, Xiaojuan Yang, Haoming Wu, HaiyanZeng, Moutong Chen, Yu Ding, Juan Wang, Tao Lei, Shuhong Zhang, and Liang Xue. *Staphylococcus aureus* Isolated From Retail Meat and Meat Products in China: Incidence, *Antibiotic Resistance and Genetic Diversity Front Microbiol*. 2018; **9**:(2767): 1-14

**6.** Toubar, S. M., Abdllah, A. E., Mahmoud, M,M and El-Shafey, A. S. **2018**. Prevalence of *Staphylococcus aureus* in Raw Milk and Some Dairy Products in Port Said Governorate. American Journal of Zoology .**1**(2): 40-46.

**7.** Scherrer D., Corti S., Muehlherr J.E., Zweifel C., Stephan R. Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from raw bulk-tank milk samples of goats and sheep. *Vet. Microbiol*. 2014; 101:101–107.

**8.** Podkowik, M., Bystroń, J. and Bania, J. **2012**. Prevalence of antibiotic resistance genes in staphylococci isolated from ready-to-eat meat products. *Polish Journal of Veterinary Sciences*. **15** (2): 233-237.

**9.** Simeoni, D. Rizzotti, L. Cocconcelli, P. Gazzola, S. Dellaglio, F and Torriani, S. **2008**. Antibiotic resistance genes and identification of staphylococci collected from the production chain of swine meat commodities. *Food Microbiol*, **25**: 196-201.

**10.** Resch, M., Nagel, V. and Hertel, C. **2008**. Antibiotic resistance of coagulase negative staphylococci associated with food and used in starter cultures.(2008). *Int. J. Food Microbiol*, **127**: 99-104.

**11.** Hwang ,S.Y., Kim ,S .H. and Jang, E.J. **2007**. Novel multiplex PCR for the detection of the Staphylococcus aureus super antigen and its application to raw meat isolates in Korea. (2007). *Int J Food Microbiol* . **117**: 99-105.

**12.** Hennekinne, J.A. De Buyser, M.L. and Dragacci S. **2012**. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbiology Reviews*, **36**: 815–836.

**13.** Riva, A., Borghi, E., Cirasola, D., Colmegna, S., Borgo, F., Amato, E., Pontello, M.M., Morace, G. **2015**. Methicillin-Resistant *Staphylococcus aureus* in Raw Milk: Prevalence, SCCmec Typing, Enterotoxin Characterization, and Antimicrobial Resistance Patterns. *J. Food Prot.*, **78**: 1142–1146.

**14.** Al-Ashmawy, M.A., Sallam, K.I., Abd-Elghany, S.M., Elhadidy, M., Tamura, T. **2016**. Prevalence, Molecular Characterization, and Antimicrobial Susceptibility of Methicillin-Resistant *Staphylococcus aureus* Isolated from Milk and Dairy Products. *Foodborne Pathog. Dis.*: **13**: 156–162.

**15.** Baird-Parker, A. C. **1962**. An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. *J. appl. Bmt*., **25**(I): 12-19.

**16.** William, B. W., Paul, D. V., George, M. G., Dorothy, J., Noel, R. K., Wolfgang, L., Fred, A. R. and Karl, S. **2009**. *Bargey's Manuel of systematic*. 2nd ed. Com.pp:392-433.

**17.** Cinical and Laboratory Standard Institute(CLST). **2016**. *Performance Standards for Antimicrobial Susceptibility Testing.*27th ed. Wayne, PA: USA.

**18.** Martineau, F., Picard, F.J., Ke, D., Paradis, S., Roy, P.H., Ouellette, M. and Bergeron, M.G. **2001**. Development of a PCR assay for identification of staphylococci at genus and species levels. *Journal of Clinical Microbiology*, **39**: 2541–2547.

**19.** Manisha, M., Gehua, WANG. and Wendy, M. J.**2000**. Multiplex PCR for Detection of Genes for *Staphylococcus aureus* Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance. **38**:1032-135.

**20.** Argudin, M.A., Mendoza, M.C. and Rodicio, M.R. **2010**. Food poisoning and Staphylococcus aureus enterotoxins. *Toxins*. **2**: 1751–1773.

**21.** Hait, J.M., Tallent, S.M. and Bennett, R.W. **2014**. Screening, detection, and serotyping methods for toxin genes and enterotoxins in Staphylococcus strains. *J. AOAC Int*. **97**: 1078–1083.

**22.** Denayer, S., Delbrassinne, L.Yacine, N. and Botteldoorn, N. **2017**. Food-Borne Outbreak Investigation and Molecular Typing: High Diversity of *Staphylococcus aureus* Strains and Importance of Toxin Detection .*Toxins*. **9**: 407- 419.

**23.** Soomro, A. H.; Arain, M. A.; Khaskheli, M. and Bhutto, B. **2013**. Isolation of *Staphylococcus aureus* from milk products sold at sweet meat shops of Hyderabad. *Online J. Biol. Sci*. **3**: 91-94.

**24.** Duquenne, M., Fleurot, I., Aigle, M. and Darrigo, C. **2010**. Tool for quantification of staphylococcal enterotoxin gene expression in cheese. *Appl Environ Microbiol*, **76**: 1367-1374.

**25.** Rajeh, M., Sabra, A., Kissoyan, K. and Fadlallah, S. **2015**. Molecular characterization of staphylococcal cassete chromosome mec an virulence encoding genes in methicillin resistant staphylococci at a medical center in Lebanon. *The International Journal of Antimicrobial Agents*. **5**(1): 1-12.

**26.** Heidari, M.; Momtaz, H. and Madani , M. **2011**. Detection of the antibiotic resistance genes in *Staphylococcus aureus* isolated from human infections and bovine mastitis. *African Journal of Microbiology Research*. **5**(28): 5132-5136.

**27.** Williams, L.L., Ajayi, O.A., Johnson, J.U., Carter., B. and Khatiwade, J. **2014**. Molecular typing and cytotoxicity testing of staphylococcus aureus isolated from milk ,meat and clinical sources. *African Journal of Microbiology research*. **8**(12): 1282-1291.

**28.** Al-Khafaji, M.H. and Flayyih, M.T. **2015**. Relationship between methicillin resistance and the presence of *femA* and *mec*A genes in coagulase positive and negative staphylococci isolated from milk and cheese *World J. Exp.Biosci*. **3**: 50-56.

**29.** Al-Alak ,S.K. and Qassim, D. K. **2016**. Molecular identification of 16s rRNA gene in Staphylococcus aureus isolated from wounds and burns by PCR technique and study resistant of fusidic acid. *Iraqi Journal of cancer and Medical Genetics*. **9**(1): 25-30.

**30.** Pereira V., Lopes C., Castro A., Silva J., Gibbs P. and Teixeira P. **2009**. characterization for enterotoxin production. Virulence factors, and antibiotic susceptibility of *staphylococcus aureus* isolates from various foods in Portugal. *Food Microbiol*, **26**: 278-282.

**31.** Pesavento G., Ducci B., Comodo N. and Nostro AL. **2007**. Antimicrobial resistance profile of *Staphylococcus aureus*isolated from raw meat: a research for methicillin resistant *Staphylococcus aureus*(MRSA). *Food Control*, **18**: 196-200.

**32.** Bruun, B and Gahn-Hansen. **2002**. Mecillinam susceptibility as an indicator of b-lactamase production in *Staphylococcus aureus*. *Clin Microbiol Infect*. **8**: 122-124.

**33.** Pallett A. and Hand K. **2010**. Complicated urinary tract infections: practical solutions for the treatment of multiresistant Gram-negative bacteria. *J. Antimicrob. Chemother*. **65**(3): 25– 33.

**34.** Nicole S . **2000**. Perlecan, the major proteoglycan of basement membranes, is altered in patients with Schwartz–Jampel syndrome (chondrodystrophicmyotonia). *Nature Genetics*, **26**: 480–483.

**35.** Jang S. **2016**. Multidrug efflux pumps in *Staphylococcus aureus* and their clinical implications. *J. Microbiol*. **5**(4): 1–8.

**36.** Souza, e. L., Meira, Q. G. S., Barbosa, I. M., Athayde, A. J. A. A., Conceição, M. L. and SiqueiraJúnior, J. P. **2014**. Biofilm formation by Staphylococcus aureus from food contact surfaces in a meat-based broth and sensitivity to sanitizers. *Brazilian Journal of Microbiology*, **45**(1): 67-75.

**37.** Kader,O.,Ebid,S.,Mostafa,N., El Sayed, S. H. and Ghazal, A. **2011**. Detection of community acquired methicillin resistance *Staphylococcus aureus* among *Staphylococcus aureus* isolates. *J. American Science*. **7**(1).

**38.** Odonkora,S.T. and Addob, K. K. **2011**. Evaluation of three methods for methicillin resistant S*taphylococcus aureus*( MREA). *Int, J, Bio. Med. Res*. **2**(4): 1031-1034.

**39.** Alebachew, T., Yasmaw,G., Derabe, A . and Sisay, Z. **2012**. Staphylococcus aureus burn wound infection among patients attending Yekait 12 Hospital burn unit ,Addis Ababa, Ethiopia. *J Health. Sci*. **22**(3).

**40.** Asao T., Kumeda Y. and Kawai T. **2003**. an extensive outbreak of Staphylococcal food poisoning due to low-fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. *Epidemiol Infect*, **130**: 33-40.

**41.** Orwin PM, Fitzgerald JR, Leung DY, Gutierrez JA. And Bohach GA, et al. 2003. Characterization of Staphylococcus aureus enterotoxin L. *Infect Immun*, **71**: 2916–2919.

**42.** Mousa, W.S ,. Abdeen, E., Hussein, H and Hadad, G. **2017**. Prevalence and Multiplex PCR for Enterotoxin Genes of Staphylococcus aureus Isolates from Subclinical Mastitis and Kareish Cheese. *J Infect Dis Preve Med*. **5**(4).

**43.** Bystron J., Molenda J., Bania J. and Czerw M. **2005**. Occurrence of enterotoxigenic strains of Staphylococcus aureus in raw poultry meat. *Pol J Vet Sci*, 2005; **8**(1): 37-40.

**44.** Ahani,N., Alipour, M. and Zahedan, E. **2014**. Detection of Enterotoxigenic Staphylococcus aureus in Schizothorax zarudnyi Using PCR Method. *Journal of Research in Medical Sciences*; **16**(4): 29-31.