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المستخلص:

الهدف من هذه الدراسة إنتاج صفائح من المواد الماصة للموجات الدقيقة باستخدام متراكبات مسحوق الكرافيت بتراكيز وزنية مختلفة(5wt.% - 40wt.%) محشوة داخل مادة البولي يوريثان الرغوي المرن، تم قياس الخواص الكهرومغناطيسية (النفاذية ، خسارة الرجوع ، القدرة الممتصة النسبية) لهذه المواد ولمدى من الترددات ضمن حزمة X (8.5-12.4GHz) باستخدام تقنية دليل الموجة المغلق.أظهرت النتائج إن اقل قيمة لخسارة الرجوع تقل إلى(-30dB) في التراكيز الوزنية التي هي اقل من (5wt.%) من حشوات الكرافيت وتصل قيم القدرة الممتصة النسبية إلى (5%) من القدرة المرسلة ولجميع الترددات.بينما أعلى قيمة لخسارة الرجوع تصل إلى (-18dB) في التركيز الوزني (30wt.%) من الكرافيت وتصل قيمة القدرة الممتصة النسبية إلى(25%) من القدرة المرسلة عند التردد 10GHz. إن هذا النوع المتراكبات مفيدة جدا لإنتاج الدروع الكهرومغناطيسية ، الصفائح الماصة والغرف المعزولة للموجات الدقيقة.

Detection of Toxic Shock Syndrome Toxin Genes in Enterotoxin Producing *Staphylococcus aureus* Isolated from Food and Food's Workers

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Abstract

One hundred fifty isolates of *Staphylococcus aureus* were isolated from foods and food worker s, distributed as: 42 isolates from milk, 22 from salads, 17 from meat, 14 from local cheese, 12 from chicken , 30 from nasal swabs and 13 from body swabs of food workers. The ability of *S. aureus* isolates for enterotoxin production was detected by SET-RPLA Kit Oxoid, and showed that 80.6% of the isolates were positive for enterotoxin production. Susceptibility of the isolates to eleven antibiotics was detected. The isolates showed high resistance against methicillin, ampicillin and amoxicillin. The resistance ranged between 60 to 66.6%for

cephalothin, ciprofloxacin, trimethoprim, cefotaxime, erythromycin and chloramphenicol. Resistance to vancomycin was 16%.

Polymerase chain reaction technique was used for detection the presence of toxic shock syndrome toxin genes(TSST-genes) in the DNA of 100 isolates of *S. aureus* which were methicillin resistance and enterotoxin producers. They appeared that only six isolates were carried TSST-genes, four isolates from food workers and two from milk samples.

الخلاصة

عزلت 150 عزلة لجرثومة *Staphylococcus aureus* من عينات الغذاء وعمال الأغذية, بواقع 42عزلة من عينات الحليب ، 22 عزلة من عينات السلطة ، 17عزلة من عينات اللحوم ،14عزلة من عينات الجبن المحلي،12عزلة من عينات الدجاج ،30 عزلة من مسحات المناخر للعمل و13عزلة من مسحات أجسام عمال الأغذية .كشف عن قابلية العزلات على إنتاج الذيفانات المعوية Enterotoxins، باستعمال عدة خاصة من شركة Oxoid ووجد أن 80.6% منها كانت منتجة للذيفانات المعوية .أختبرت حساسية العزلات لـ11 مضاداً حياتياً ، وأظهرت مقاومة عالية للمضادات مثسيلين، أمبيسلين وأموكسيسيلين ،وتراوحت مقاومتها بين 60%- 66.6% للمضادات الحياتية سيفالكسين، سبروفلوكساسين، ترايمثبريم، سيفوتاكسيم، آرثرومايسين وكلورامفينيكول ، فيما كانت مقاومة العزلات واطئة نسبيا للمضاد فانكومايسن وبنسبة 16% .

استعملت تقنية تفاعل السلسلة التضاعفية Polymerase Chain Reaction للكشف عن وجود جينات متلازمة الصدمة السمية(TSST-genes) في دنا 100 عزلة *S.aureus* مقاومة للمثسيلين ومنتجة للذيفانات المعوية من العزلات قيد الدراسة . ظهر أن 6 عزلات فقط كانت حاملة للجين المشفر لذيفان متلازمة الصدمة السمية وبواقع 4 عزلات من عمال الأغذية وعزلتين من عينات الحليب .

Introduction

Staphylococcus aureus is one of the normal flora in different parts of the human body such as skin and respiratory tract. These germs characterized by their ability to cause different infections in multiple parts of the body. Pathogenicity of these organisms depending on its ability to produce many of virulence factors, which include the production of toxins and extracellular enzymes and other factors, giving them the ability to multiply and spread within the tissues of the host, as well as the ability to withstand environmental conditions and high resistance to many antibiotics, making it spread in various environments

such as water, air, soil and human body, animalsand birds as well as its presence in food, especially milk and milk products [1,2].

The human is the most important vectors for these bacteria to the foods. About 40-50% of healthy people carrying these organisms without showing signs of infection. Colonization of these bacteria usually in the nose , throat ,hands and on the skin, especially in the cases of inflamed wounds, burns and boils. It is possible that contamination occurs by contaminated instruments used in the transport and preparation of foods. Contamination of animal foods can be occur by the animals themselves, which carry the bacteria naturally on their skin and mucous membranes or infected as mastitis. [3]

Enterotoxin producing by *S. aureus* is the main cause of the most common food borne diseases in the world [4]. The enterotoxin is a group of variable spherical single-chain protein, soluble in water with a molecular weight range 26-35 K.Da. The most common type of among them is Staphylococcal enterotoxin A (SEA) and molecular weight (27.1) k.Da. Enterotoxin type (SEB) is heat-stable and remains constant at 60 ° C for 16 hours. Its molecular weight (31.4) kDa, while the type (SEC) is the most stable genetically and isolated from strains colonize animal hosts. The type (SED) is considered the most second type cause food poisoning after (SEA) [5].

Some strains of *S.aureus* produce high antigenic activity toxin called toxic shock syndrome toxin (TSST-1) ,which is systemic toxin .The infection with these toxins lead to produce toxic shock syndrome represented by high fever, rash and redness of skin with hypotension for 1-2 weeks and may lead to death [6]. Toxic shock toxin is closely related to food and can enter the body through the digestive system by eating contaminated foods with these toxins [7]. The present study was aimed to detect the presence of TSST-genes and its relation with enterotoxins production and mithicillin resistance in *S. aureus* from foods and food workers.

Materials and Methods

1. Sampling

Food samples were collected from November 2010 to April 2011 . five types of foods (meat, chicken, milk, cheese and salad) were collected from the local markets of Al-Nasiriyah city. Samples placed in sterile bags were closed tightly, while milk samples placed in sterile bottles and then placed in a cool box and transported to the laboratory for bacterial examination at the moment they arrived.

Food samples (meat, chicken, cheese and salad) was prepared through weight 25 grams of the sample, mixed and squashed under sterile conditions. Milk was mixed, and 25 ml of each sample was taken. Samples were added to 225 ml of nutrient broth and incubated at 37 ° C for 24 hour, then transferred to soya tryptose broth plus 10% sodium chloride and incubated at 37 °C for 24 hours. Cultures were streaked on Baird- Parker agar supplemented with 5% emulsion of egg yolk and incubated at 37 °C for 24 hours [8].

Nasal and skin swabs were taken from food workers and cultured on mannitol salt agar and incubated at 37°C for 24hrs[9].

Black or gray, shiny, colonies surrounded by transparent zone on Baired Parker gar as well as golden colonies on mannitol salt agar were selected. Identification of the isolates based on Phenotypic and biochemical properties including :Gram stain, haemolysin, Catalase, Coagulase, phosphatase and oxidase. API-20S (bioMerieux) was used to confirm the identification [9].

2- Antibiotics Susceptibility Test

Susceptibility of the isolates toward 11 antibiotics was investigated [10] on Mueller by disc diffusion method according Bauer and Kirby Hinton agar. The antibiotics was amoxicillin, ampicillin, cefotaxime, Cephalexin, chloramphenicol, ciprofloxacin, erythromycin, gentamycin , methicillin, trimethoprim and vancomycin (Bioanalyse).Inhibition zone was messured according to NCCLs [11] .

3 – Detection of enterotoxin production

SET-RPLA Kit from Oxoid Company for the detection of viability of the isolates to produce enterotoxins was used according to the instructions of the company.

4 - Detection of toxic shock syndrome genes

Isolates were grown on luria-bertani broth. Specific DNA extraction kit (supplemented by Sacase Biotechnology, Italy) was used for the extraction and purification of DNA from 100 isolates of *S. aureus* in addition to one clinical isolates(positive control, supplemented from Public Health Lab.-Baghdad) . Polymerase chain reaction (PCR) was used (Faculty of Medicine - University of Al-Qadisiyah)for DNA amplification according to Sambrook *et al.*, [12] using PCR kit (**Promega-** The primers set used to detection of TSST- genes sequences as in **.USA)** table.1.

Table (1): primers set sequences used for TSST-genes amplification

No.	size of amplification products (bp)	Oligonucleotide sequence of Primer(5c'e-3')	The direction of the initiator	Location within gene
1	350	ATGGCAGCATCAGCTTGATA	Forward	251-270
2	350	TTTCCAATAACCACCCGTTT	Reverse	581-600

PCR was conducted according to the program of Johnson and Tyler [13] ,as in Table (2).

Table (2): Program steps for PCR amplification

Steps	Temperature °C	Time / min.	Number of cycles
Initial denaturation of DNA	96	4	1
DNA denaturation	94	2	30
Annealing	45	2	30
Elongation	72	1	30

Location of amplified DNA fragment (DNA bands) was detected by UV Transillumenator after migrated of PCR products by electrophoresis on 2% agarose. Molecular weight of DNA fragment was detected depending on DNA size marker.

Results

One hundred fifty isolates of *Staphylococcus aureus* were isolated from different food samples and swabs taken from nasal and bodies of food workers submitted for this study (Table 3).

Table (3). Percentages of *S. aureus* isolated from samples of food and workers.

sample	No. of examined Samples	Isolation of *S. aureus*	
		Number	Percentage%
Nasal swab	165	30	18.1
Worker bodies swabs	90	13	14.4
Raw milk	75	42	56
Salad	50	22	44
Meat	44	17	38.6
Local cheese	38	14	36.8
Chicken	33	12	36.3
Total	495	150	30.3

The results showed that enterotoxin production was detected from 121(80.6%) 0f 150 isolates of *S. aureus*. The highest percentage of production was from isolates of worker nasal swabs, while the lowest were from chicken (58.3%) (Table,4).

Table (4): Numbers and percentages of enterotoxin producing *S. aureus*

Sample	No. of isolates	Isolates producing enterotoxin	
		Number	Percentage%
Raw milk	42	37	88
Nasal swab	30	30	100
Salad	22	15	68.1
Meat	17	13	76.4
Local cheese	14	10	71.4
worker bodies swab	13	9	69.2
Chicken	12	7	58.3
Total	150	121	80.6

Most of the isolates showed resistance to the antibiotics used(Table,5). All the isolates were showed resistance to methicillin , while 90% of them were resistant to each of Ampicillin and amoxicillin. The lowest percentage of resistance was showed for Gentamycin(6.6%). Resistance against Vancomycin was 16% (Table,5).

Table (5) : Percentages of antibiotics resistance of *S.aureus* isolates.

Antibiotics	No. of resistant isolates	Percentage %
Methicillin	150	100
Ampicillin	135	90

Amoxicillin	135	90
Cephalexin	100	66.6
Ciprofloxacin	100	66.6
Trimethoprim	99	66
Cefotaxime	95	63.3
Erythromycin	93	62
Chloramphenicol	90	60
Vancomycin	24	16
Gentamycin	10	6.6

DNA from (100) methicillin resistant, enterotoxin producing *S. aureus* were extracted and detected for the presence of genes of toxic shock syndrome toxin using Polymerase Chain Reaction (PCR) and After migrated of PCR products on gel electrophoresis specific primers. and examined by UV-Transillumenator, six DNA bands in addition to one represent clinical positive isolate (positive control) were appeared. These bands is the DNA fragments amplified by PCR represent the sequences of toxic shock syndrome toxin genes ,while the results showed no bands appeared on the gel for the other 95 isolates in addition to negative control isolates.
Molecular weights of the amplified DNA fragment was detected according to their positions from DNA-Marker bands and estimated by 350 base pairs (bp) (Fig.1).

The results summarized that of 100 isolates of *S.aureus* ,only six carrier of the genes coding for the toxic shock syndrome toxin, four were isolated from worker nasal and two from raw milk (Table. 6).

Table (6) .Number of isolates carrying toxic shock syndrome toxin genes.

Type of sample	No. of tested isolates	No. of isolates carrying (TSST genes)
Raw milk	20	2
Nasal swab	20	4
Meat	15	-
Chicken	15	-
Local cheese	10	-
Worker body swab	10	-
Salad	10	-
Total	100	6

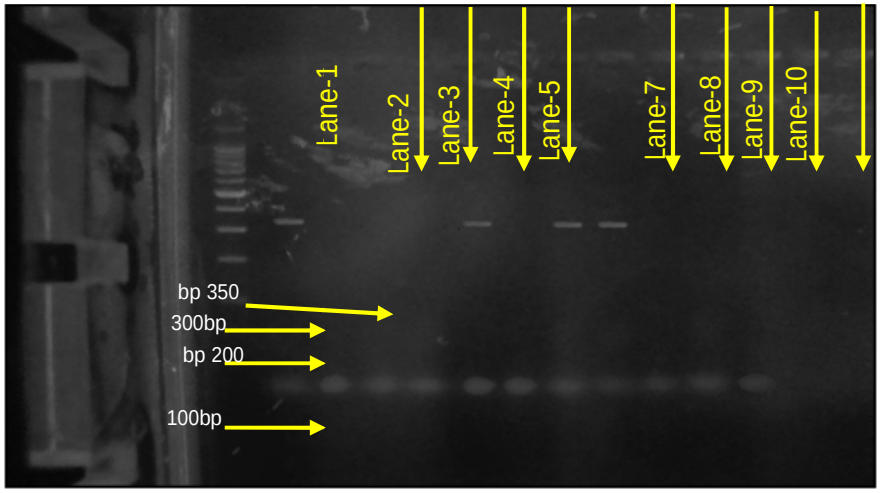

Fig. (1) . Agarose gel electrophoresis patterns showing typical amplification fragments in the PCR for the staphylococcal TSST-genes
Lane 1: DNA marker
Lane 2: Positive control (clinical isolate of *S. aureus*)
Lane 3: Negative control(*Salmonella typhymurium)*
Lanes 4,5,7,10, 11 and 12: negative isolates
Lanes 6, 8 and 9 positive isolates(carrying TSST-genes)

Discussion

The results of the present study are compatible with the findings of Jarraud *et al.* [14], were they found that 80% of *S. aureus* isolated from milk producing enterotoxins. Other studies have shown lower rates for the production of enterotoxins[15,16,17].

The results, as shown in table (4), there is a discrepancy in the rates of enterotoxin production of the isolates depending on the type of sample. The highest percentage of production (100%) from nasal isolates, while the lowest percentage (58.3%)of the production from chicken meat isolates.

The ability of *S.aureus* to produce enterotoxin return to genetic reasons, The producing isolates have one or more of genes encoding for enterotoxin production. Gene responsible for production of staphylococcal enterotoxin A (SEA) be carried by a family of temperate

bacteriophages [18]. While the gene responsible for production of enterotoxin (SEB) carried on the chromosome [19]. The gene responsible for production of enterotoxin(SEC) be carried on the pathogenicity islands, and so on for the rest types of enterotoxins [20].

The isolates showed complete(100%) resistance to methicillin . Resistance of *S. aureus* to methicillin known internal resistance, because it does not get due to

inhibition by ß-lactamase. Strains of MSRA have additional chromosomal fragment (40-60kb) called (*mec* gen), which is not found in susceptible strains, and consists of three genetic determinants: *(mec* A) responsible for the production of penicillin binding proteins with low affinity for beta-lactames, and *(mec* I) and *(mec* R) which are organized as regulatory genes controlling the transcription of *mec* A[21].

The percentage of resistance of the isolates to vancomycin was16%.Recently a major health problem was appeared, emergence of resistant of *S.aureus* to vancomycin [22].Resistance is due to acquired two plasmids one 4bp and the other 120 bp[23] .Chang *et al*.[24] mentioned that mobile genes ,exactly (Tn154) which is carrying *van*A gene encoded for enzyme cause modification in Peptidoglycan and inhibit vancomycin to bind with the active site.

The results showed high resistance (90%) toward Ampicillin and amoxicillin .Resistance of *S. aureus* to betalactams correlated with methicillin resistance, it mean cross resistance due to presence of penicillin binding proteins(PBP2a) with low affinity to bind with penicillins and cephalosporins[25].On the other hand, isolates appeared high sensitive to gentamycin(93.4%)and this agreement with the results of Srinivasan *et al*.[26].

S.aureus strains secreting kind of toxins (super antigens, SAgs) with a high antigenic activity including toxic shock syndrome toxin-1, which is a systemic toxins. It can stimulating T-Cell to produce large amounts of Cytokinase, which is the cause for the emergence of symptoms of toxic shock syndrome including high fever, rash and redness of skin with low blood pressure for 1-2 weeks (if not get death) . Super antigens would be linked directly with major histocompatibility complexes (MHC) ClassII [27].These toxins able to penetrate mucous membranes and enter the blood stream ,and also attach with the epithelial cells of tubules of kidney, leading to death these cells.[28].

Toxic shock toxins are closely linked to foods, as they can enter the body through the digestive system by eating foods containing these exotoxins secreted by *S.aureus.* The people of carrier for *S.aureus* which

produce these toxins is an important source for the arrival of these germs to the food[7].

Polymerase chain reaction technique was used for detection the presence of toxic shock syndrome toxin genes in the DNA of 100 isolates of methicillin resistant and enterotoxin producers *S. aureus* .It appeared that only six isolates were carried TSST-genes, four isolates from food workers and two from milk.

The viability of *S. aureus* on the production of toxic shock syndrome toxin is due to the acquisition of the genes encoding for these toxins. Genes encoding TSST-1 are often associated with mobile genetic elements such as pathogenicity islands, phages, and plasmids [29] . Jana *et al*. [17] found that 18 isolates out of 79 (22.7%) isolated from the bodies of goats and milk were carrier of the gene. In another study only 3 isolates out of 63 (4.7%) isolated from clinical samples and food were carrying the gene

encoding for TSST-1[30]. In Taiwan, 3 isolates out of 62 from clinical samples was carrying for (TSST genes) and no any of food isolates carrying this gene [31].

Many studies have confirmed the close link between ability of *S.aureus* to produce enterotoxins, especially STC and the production of toxic shock syndrome toxin [32.13]. El-Ghodban *et el*.[30] found that all the isolates carrying TSST genes are carrier for enterotoxin (STC) gene .This is consistent with our findings ,which improved that all the isolates carrier's TSST genes were produce enterotoxins.

The results of the present study showed that all the isolates carrying the genes of toxic shock syndrome was resistant to methicillin (MRSA). This is consistent with many studies that have shown a positive relationship between the viability of *S.aureus* resistant to methicillin and production of TSST, as it is believed that this relationship referred to that both genes responsible for those traits are regulating by accessory gen regulation[33]. Shimaokat *et al*.[34] found that 56% of MRSA producing TSST, while only 4% of methicillin sensitive *S. aureus* was produced of these toxins. Zahan *et al*.[35] found that 14% of MRSA was produced of TSST, while no in the sensitive isolates.

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