Molecular characterization of Staphylococcus aureus isolated from meat, milk and their products

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Abstract

This study was planned to throw the light on the incidence of S. aureus in 485 random samples of meat, milk and their products, The bacteriological examination of 200 meat and meat products revealed that the prevalence of S. aureus was 19 isolates (9.5%), The bacteriological examination of 285 milk and milk product revealed that the prevalence of S. aureus was16 isolates (18.8%).

The prevalence of **S. aureus** in examined milk collected from (farms, dairy shops and street vendors) were (16%, 16% and 22.8%) respectively, While milk products showed that, the prevalence of **S. aureus** was 8 isolates (4%). All isolated strains were identified as **S. aureus** by using specific culture media and biochemical tests.

Detection of enterotoxin in S**.** aureus isolates proved that, 27out of 43 of them were entrotoxignic , 15 isolates out of 27 were enterotoxin A producer,7 isolates were enterotoxin C producer and 5 isolates were harbored the type (A and C) .

PCR used for confirmation of **S. aureus** isolates, revealed that **S. aureus** had four DNA fragments patterns of 410, 740, 910 and 970 b.p. with primer (1) meanwhile with primer (2), the pattern of **S. aureus** had two bands of 562 and 928 b.p.

Studying the antibiotic sensitivity test of **S. aureus** for 15 different types of antibiotics in lab, the result revealed that most strains which isolated from meat, milk and their products were show highly degree of resistant to Methicillin followed by Oxacillin, Chloramphenicol, Amoxiciilin, Cephranin, Cephalothin, Amikacin, Gentamycin, Ciprofloxacin, doxycycline while they were sensitive to Cefaclor, Streptomycin, Norfloxacin, Erythromycin and trimethoprim- sulphamethoxazole. PCR also, confirmed the presence of mec A gene in 4 isolates (methicillin resistant **S. aureus**).

Introduction

Staphylococcus aureus is one of the most common agents in bacterial food poisoning outbreaks. It is also a major causative pathogen of clinical or subclinical mastitis of

dairy domestic ruminants. Meat and meat products as well as milk and milk products have been reported as common foods that may cause staphylococcal food poisoning ***(Le Loir et al, 2003).***

S. aureus strains produce a spectrum of protein toxins and virulence factors thought to contribute to the pathogenicity of this organism.

Staphylococcal food poisoning is caused by the ingestion of food containing pre-formed toxins secreted by the bacteria. These are known as staphylococcal enterotoxins, The staphylococcal enterotoxins (SEs) have been classified into many different types. These enterotoxins are heat-stable and resistant to the action of digestive enzymes ***(Brooks et al, 2001)***.

The staphylococcal enterotoxins (SEs) are responsible for the symptoms that associated with staphylococcal food poisoning ***(Llewelyn and Cohen, 2002)***. The disease is characterized by symptoms including nausea, vomiting, abdominal cramps and diarrhea lasting from 24 to 48 h and the complete recovery usually occurs within 1–3 days.

SEA is the most common enterotoxin recovered from food poisoning outbreaks ***(Balaban and Rasooly, 2000)*** and it is known that 59% of staphylococcal food poisoning outbreaks are caused by SEA to SEE ***(Bergdoll, 1989)***.

PCR-based techniques are used increasingly in food-microbiology research as they are well developed and when applied as culture confirmation tests, they are reliable, fast and sensitive. PCR methods offer a sensitive and specific detection of pathogens and can discriminate virulent bacteria from a virulent member of the same species as well ***(Olsen, 2000)***. In the last 10 years, many authors have proposed the use of PCR for the detection of food-borne pathogens to replace the time-consuming culture-based classical techniques ***(Gravet et al, 1999)***. So, the work was directed to investigate the prevalence of ***S. aureus*** in meat, milk and their products, examined biochemically, detection of the enterotoxin strains by Reverse Passive Latex agglutination technique (RPLA) and using PCR for confirmation of ***S. aureus*** and making Detection of the methicillin-resistant strains of ***S. aureus*** (MRSA).

Materials and Methods

Samples:

A total of 485 random samples of raw meat, meat products (sausage, hotdog, minced meat, burger and luncheon), raw milk and milk products (ice-cream, yoghurt, Kariesh cheese, Salted cheese) were collected from different markets, street vendors and farms in port-Said and Ismailia cities., The samples were represented as 50 samples from raw meat, 30 from

each of meat products (sausage, hotdog, minced meat, burger and luncheon) in addition to, raw milk and milk products (ice-cream, yoghurt, Kariesh cheese, Salted cheese) 85 samples from raw milk, 75 from each of (Ice cream and Yoghurt), 25 from each of cheese (Kariesh and salted cheese) .

1. Samples collection:

Twenty five grams/ ml from each sample were randomly collected from different retails in Port-Said city and Ismailia farms. Each sample was aseptically transported in ice-box to laboratory within 24 hours for bacteriological examinations.

2. Preparation of the samples:

The technique recommended by **APHA (2001)** was used for samples preparation. 25 gram/ ml of sample was aseptically added to 225 ml peptone saline and then homogenized in a stomacher for 2 min then incubated at 37°C for 24-48 hr.

3. Bacteriological isolation and identification of Staphylococcus *aureus*

According to ***Koneman et al (1996) and Quinn et al (2002)***; samples were cultured onto nutrient broth for 24 h at 37ºC and then a loopful was taken and cultured onto 5% sheep blood agar and then onto Baird parker medium. All inoculated plates were incubated at 37ºC for 24-48 hrs then colonies were identified.

Suspected colonies of ***S. aureus*** were examined morphologically, biochemically according to ***(FDA, 2001)*** and microscopically according to ***(Ryan and Ray, 2004)***.

4. Detection of Enterotoxins producing isolates by RPLA technique-: ***(Igarashi et al, 1986).***

Enterotoxin RPLA kits was used for detection of enterotoxin producing isolates, the isolated ***S. aureus*** were grown in 5 ml of brain heart infusion broth and incubated at 37ºC for 18hours, then the culture was centrifuged at 12000xg at 4ºC for 10 minutes, 25µl of each culture supernatant was diluted five folds and placed into microtitre plate wells. An equal volume of latex particle sensitized with specific anti enterotoxin of ***S. aureus*** (A, B, C, D, E) immunoglobulin was added to each well. Normal rabbit globulin sensitized latex particles were used as a control. After thorough mixing the plates were incubated at room temperature for 16 hours and the agglutination reactions with anti enterotoxin immunoglobulin sensitized latex particles were observed in the cultural supernatants of enterotoxin producing isolates of ***S. aureus***. **5. Polymerase chain reaction (Random amplified polymorphic DNA fingerprinting) (PCR) (RAPD)**

5.1. Preparation of genomic DNA of *S. aureus*: According to ***Sambrook et al., (1989).***

5.2. PCR reaction (RAPD): According to ***Van BelKum et al (1993)***

6. Methicillin-Resistant *Staphylococcus aureus* **(MRSA) detection and identification methods**

6.1. The antibiotic sensitivity test (disc diffusion Test) (Finegold and Martin, 1982).

6.2. PCR assay for Detection of *mec*A gene by using Multiplex PCR according to ***Stephens (2008).***

Primer used for detection of *(mecA)* gene: *mecA* duplex PCR:

Multiplex Polymerase Chain Reaction for detection of ***S. aureus*** species specific 16S rRNA and (SCCmec) type IV genes (responsible for methicillin resistance).

Two sets of primer pairs were used:

The first one was Staph756F (5`-AACTCTGTTATTAGGGAAGAA C-3 `) and Staph 750R(5 `-CCACCTTCCTCCGGTTTGTCAC C-3`) primers which could amplify 756 base pair fragments specific for 16S rRNA of ***S. aureus***.

The second one was SCC*mec* 4a1(5`-TTTAATGCCCATGAATAAAAT-3 `) and SCC*mec* 4a2(5 `-AGAAAAGATAGAAGTTCGAAAG A-3`) primers which could amplify450 base pair fragments specific for SCC*mec* subtype IVa gene according to ***Ryffel et al (1990).***

Table (1): *Primers used For coagulase and clumping factor genes : Oligonucleotide primers used*

Gene	Primer sequence 5` 3`	Product size bp	Referance
Coa	*Coa-F* CGA GAC CAA GAT TCAACAAG *Coa-R* AAA GAA AAC CAC TCACATCA	970,910,740,410 565 and 928	***Aslantas et al.,*** **(2007)**

Results

1-Isolation of *Staphylococcus aureus*:

The growing colonies of ***S. aureus*** on Baird parker medium characterized by circular, smooth, convex, moist, 2-3 mm in diameter, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone and the colonies have buttery to gummy consistency when touched with inoculating needle. ***S. aureus*** is Gram positive and appears as small round cocci and most commonly as grape-like clusters. It was subjected to several biochemical tests and it was positive for catalase, coagulase with (69.8%)**,** positive for oxidation fermentation test, Sugar fermentation test and all isolates showed clear zone of B-heamolysis around the colonies and showed DNase activity.

It was clear from table (2) presence of enterotoxigenic ***S. aureus*** with

higher incidence from milk specially dairy and street vendors milk samples (75%) followed by (64% and 60%) from meat products and raw meat respectively. The total incidence of enterotoxigenic ***S. aureus*** (62.8%).

2-Distribution of *S. aureus* in different samples and type of enterotoxins table 3.

3-Result of PCR assay for *S. aureus:*

The use of primer 1 and primer 2 for PCR amplification of 43 ***S. aureus*** isolates genomic DNAs resulted in reproducible DNA fragment patterns each unique to a particular strains as show in fig (1). Amplification with primer 1 resulted in ***S. aureus*** had four DNA fragments pattern of 410, 740, 910 and 970 base pair, while the amplification with primer 2 showed that the pattern of ***S. aureus*** had two bands 562 and 928 b.p (fig 2)

4-Result of Antibiotics sensitivity using disc diffusion Test

Antibiotic sensitivity of 43 ***S. aureus*** strains revealed that 28 (65.1%) were sensitive to trimethoprim- sulphamethoxazole , followed by 25(58.1%) for erythromycin , 24 (55.8%) for Norfloxacin and 22 (51.2%) for each of Cefaclor and Streptomycin, in the other hand 41 (95.3%) were resistant to Chloramphenicol, 34 (79.1%) were resulted at Amoxiciilin, 33(76.7%) were resistant to Cephradin, 32 (74.4%) resistant to Cephalothin, and 30(69.8%) were resistant to Amikacin.

5-Result of Methicillin-Resistant *Staphylococcus aureus* (MRSA) by PCR assay:

All previously identified phenotypically as ***S. aureus*** with bacteriological examination were used in PCR run accompanied with isolates identified as methicillin resistance strains plus four control strains. All strains are positive for amplification of 756 base fragments specific for 16S rRNA of ***S. aureus*** using Staph 756 F and Staph 750 R primers, While 4 strains (Known from antimicrobial sensitivity assay as methicillin positive) showed positive amplification of 450 base pair fragments specific for *SCCmec* subtype IVa genes using *SCCmec* 4a1 and SCCmec4a2 primers, as shown in Figure (3).

Table (2): *Number and percentage of enterotoxigenic* S. aureus *isolated from meat and meat products:*

Type of samples	No. of samples	Isolated Strains		Toxigenic strains	
		No.	%	No.	%
Meat Meat	50	5	10	3	60
Meat product	150	14	9.33	9	64.3
Milk: 1-Farm's milk	25	4	16	2	50
2-Dairy milk	25	4	16	3	75
3-Street vendors	35	8	22.8	6	75
Milk product	200	8	4	4	50
Total	485	43	8.8	27	62.8

Table (3) *the frequencies of* ***S. aureus*** *isolation from different sources in correlation to its type of enterotoxins:*

Type of examined samples	No. of samples	No. of *S. aureus*	Enterotoxigenic Strains		Type of enterotoxin		
			No	%	A	C	A&C
1- Raw meat	50	5	3	60	2	0	1
2- Sausage	30	4	2	50	1	1	0
3- Hotdog	30	1	0	0	0	0	0
4- Minced meat	30	5	4	80	1	1	2
5- Burger	30	1	1	100	1	0	0
6- Luncheon	30	3	2	66.6	0	1	1
7- Farm's milk	25	4	2	50	1	1	0
8- dairy shops	25	4	3	75	2	1	0
9- street vendors	35	8	6	75	4	1	1
10-Ice cream	75	3	2	66.6	1	1	0
11-Yoghurt	75	2	1	50	1	0	0
12- Kariesh cheese	25	2	1	50	1	0	0
13-Salted cheese	25	1	0	0	0	0	0
Total	485	43	27	62.8	15	7	5

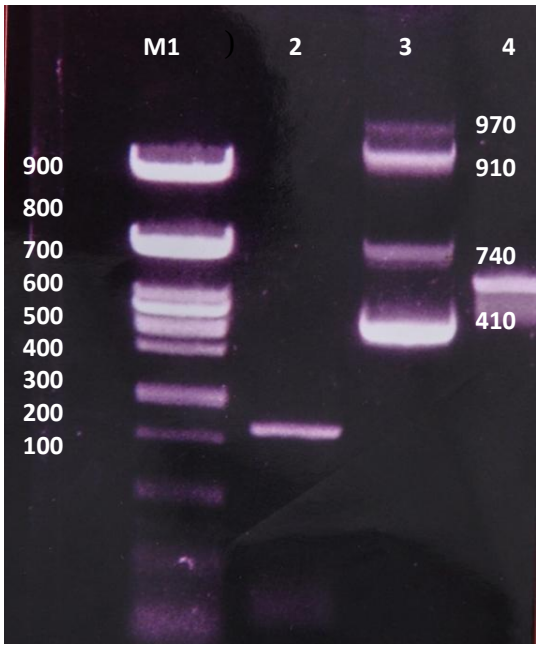

Fig (1): *DNA banding pattern following amplification with primer (1)*
Lane (1): Hi Lo DNA marker, Lane (2): Local strain other than ***S. aureus***
Lane (3): Locally isolated strain of ***S. aureus*** **,**Lane (4): Local strain other than ***S. aureus***

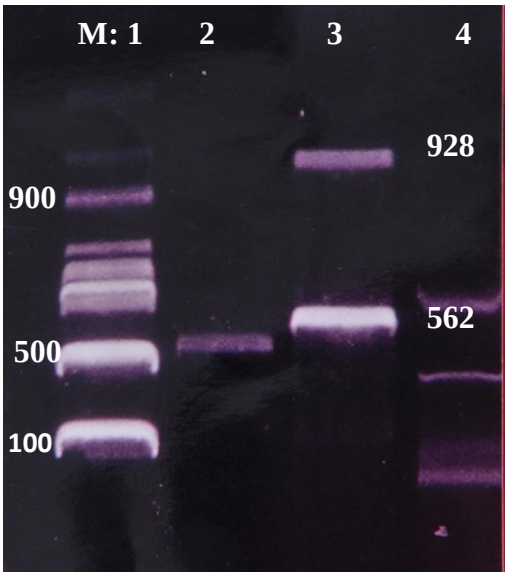

Fig (2): *DNA banding pattern following amplification with primer (2)*
Lane (1): Marker, Lane (2): Local strain other than ***S. aureus***
Lane (3): Locally isolated strain of ***S. aureus,*** Lane (4): Local strain other than ***S. aureus***

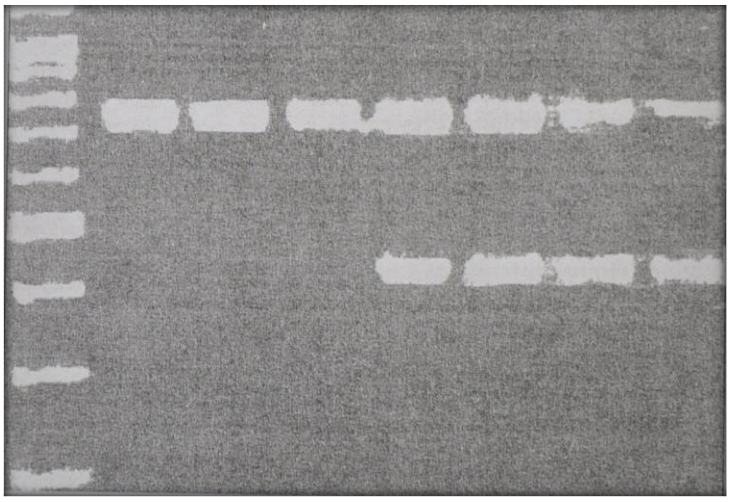

Figure 3. *Agrose gel electrophoresis showing lane1100 bp ladder.* Lanes 2,3,4,5,6,7 and 8 showing amplification of 756 bp fragments of 16S rRNA. While lanes 5, 6, 7 and 8 showing amplification of 450 bp fragments of SCC *mec*IVa gene.

Discussion

Staphylococcus aureus is considered to be one of the leading causes of food-borne illnesses. Milk, dairy products, meat and meat products are often contaminated with enterotoxigenic strains of this bacterium. Foodstuff contamination may occur directly from infected food-producing animals or may result from poor hygiene during production processes, or the retail and storage of foods, since humans may carry the microorganism. ***(Normanno et al, 2007)***.

A total of 200 samples of meat and meat products; sausage, hotdog, minced meat, luncheon and burger samples were examined bacteriologically to reveal the prevalence of pathogenic ***S. aureus***. The percentage of ***S. aureus*** was 9.5% (19 isolates) were identified as ***S. aureus*** by culturing using selective culture media (Blood agar and Baird parker media) for isolation. They were classified as 5 isolates from raw meat (10%), 5 isolates from minced meat (16.67%) , 4 isolates from sausage (13.33%), 3 isolates from luncheon (10%) and one isolate from Hotdog and burger (3.33% for each). The high percentage of ***S. aureus*** in meat and meat products is indication of poor hygiene. Also their contamination returned to unhygienic manner, processing, transportation and storage. These results were nearly similar to ***(Aseel et al, 2010 and Goja et al, 2013)*** who isolated ***S. aureus*** from fresh meat (beef) in a percent of 5.55% and 12% respectively,

On the other hand our results were less than the result of ***(Kanika et al, 2011)***; ***(Amal and Ola, 2009)*** who reported that incidence of ***S. aureus*** in meat samples from different markets were20.5% and 20% respectively. The higher incidence of ***S. aureus*** may be due to the insanitary condition of the butcher and absence of the health services in butcheries. The obtained results were less than ***(Soultos et al, 2003***, ***El-Khateib, 1997 and El-Sherbeeny, 1990)*** who reported higher incidence of ***S. aureus*** in sausage 19.4%, 29% and 43% respectively. While the results of minced meat were nearly agree with ***(Omar et al, 2009)*** who isolated ***S. aureus*** in a percentage of 14.6%, more than ***(Heredia et al, 2001)*** who detected ***S. aureus*** in Monterrey and Mexico in 2.3% of the ground meat samples, and less than ***(Vorster et al, 1994)*** they found ***S. aureus*** in 23.4% of minced beef in south Africa. On the other hand, our results of isolation from Luncheon were less than those reported by ***(Fatin, 2004 and Seham et al, 2013)*** who isolated ***S. aureus*** in 16% and 32% respectively. They mentioned that contamination may occure during the slicing and packaging of luncheon meat in supermarkets**.** The results of burger examination were less than ***(Zakaria, 2007***, ***Elshrek et al., 2008 and Fatin, 2004)*** they isolated ***S. aureus*** in 25%, 27.1% and 36% respectively.

In this study, a total of 85 milk samples and 200 milk products samples were examined bacteriologically to reveal the prevalence of pathogenic ***S. aureus***. Sixteen isolates of ***S. aureus*** were isolated from examined milk samples with incidence of (18.8%) and 8 isolates from 200 milk product samples were identified as ***S. aureus*** with incidence of (4%). Similar finding was recorded by **(*kader et al, 2002 , Haltia et al, 2006, Abd El Aal, 2008, and Tamminga et al, 2008)*** they isolated ***S. aureus*** from raw milk samples and from milk product samples with a percentage ranged from (15-18%). While ***(Devi et al, 1997)*** reported higher incidence of ***S. aureus*** in raw milk reached (75.3%) in India. Wide variation in the prevalence of ***S. aureus*** has also been reported elsewhere by ***(Rodostitis et al, 2000).*** As regards to the source of samples, 4 out of 25 milk samples (16%) were recovered from farms and 8 isolates out of 35 street vendors milk samples (22.8%) were isolated. On the other hand, bacteriological examination of raw milk 25 samples from dairy shops revealed the isolation of ***S. aureus*** with percent of 16% (4 isolates). These findings are in support with the observation of ***(Singh and Baxi, 1982, Prodhan et al, 1996, and Rahman et al, 1997)*** who reported that (17%), (21%) and (18.5%) were the incidence of ***S. aureus*** in raw milk samples, respectively.

Concerning the type of examined milk samples, the high incidence of ***S. aureus*** in street milk may be due to the interference of man hazard during handling and preparing of milk before marketing and the low incidence of company marketing milk was accepted due to the restricted measures applied in such companies before marketing. ***(Patrick et al, 2003).***

The bacteriological examination of milk products showed that, the prevalence of ***S. aureus*** from ice cream, yoghurt samples, Kariesh cheese and salted cheese samples were (4%) (0%), (2.6%) (0%), (8%) and (4%) from street vendors and marketing respectively. The results of isolation of ***S. aureus*** from ice cream were supported by ***(Ojokah 2006, Feng et al., 2007 and Tamminga et al., 2008,)*** who proved that the range of variation of ***S. aureus*** from ice cream ranged from (5-20%) due to man hazard effect like hand, skin , sneeze and cough which produce droplet infection during transportation, storage and retailing.

As regards to Kariesh cheese, our results were supported by ***(Klossaowska et al, 2005, Haltia et al, 2006 and Abd El Aal, 2008)*** who isolated and identified ***S. aureus*** from Kariesh cheese samples with a percentage ranged between 7-12%. While low percentage of ***S. aureus*** from yoghurt in our study was opposite to ***(Aman, 1994)*** who proved that the prevalence of ***S. aureus*** in yoghurt was (20%).

The lowest prevalence rate (2.6%) of ***S. aureus*** which was recorded in yoghurt might be attributed to the effect of heating and then freezing during its manufacture which inhibits the multiplication of this microorganism and kills the microorganism.

RPLA used to detect the presence of enterotoxins in meat and meat products out of 19 strains only 12 strains were enterotoxigenic and classified according to type of toxin into (5A, 3C, 4A&C). This result nearly similar to that recorded by ***(Mathieu et al, 1991)*** who found enterotoxin A in fresh beef. ***(Rosec et al, 1997)*** who found enterotoxin C the most frequently type in meat products.

Concerning the detection of enterotoxins in milk and milk products, out of 24 ***S. aureus*** strains recovered from milk and milk products only 15 strains were enterotoxigenic and classified into type (10A, 4C, 1A & C). These results were agreed with ***(Rafaels and Edgars, 2006 and Moon et al, 2007)*** who detected enterotoxins of ***S. aureus*** recovered from milk and milk products and classified as 2 types of enterotoxins mainly (SEA and SEC).

PCR used for Confirmation of ***S. aureus*** isolates, the result proved that ***S. aureus*** had four DNA fragments patterns of 410, 740, 910 and 970 b.p. with primer (1) meanwhile with primer (2), the

pattern of ***S. aureus*** had two bands of 562 and 928 b.p. The same results reported by ***(Belkum et al, 1992)*** and ***(Lipman et al, 1995)*** who found that the DNA fingerprinting technique using RAPD-PCR proved to be useful in differentiating isolates of ***S. aureus*** in rapid and accurate manner.

The results of antibiotics susceptibility revealed that, the most of ***S. aureus*** strains isolated in this work were resistant to Chloramphenicol (95.3%) followed by amoxiciilin, Cephradin, Cephalothin, Amikacin, Gentamycin, Ciprofloxacin, doxycycline, Cefaclor, Streptomycin, Norfloxacin, erythromycin and trimethoprim-sulphamethoxazole in a percentage of (79.1%,76.7%, 74.4%, 69.8%, 67.4%, 58.1%, 53.3%, 48.8%, 48.8%, 44.2%, 41.9%, 34.9%) respectively. These results were agreed with ***(Suleiman et al, 2012)*** who recorded that ***S. aureus*** were resistant to amoxicillin, amikacin and erythromycin. ***(Deresse et al, 2012)*** Who recorded that ***S. aureus*** were resistant to Ciprofloxacin, erythromycin trimethoprim-sulphamethoxazole.

The PCR assay confirmed the presence of *mec A* gene in 4 strains by PCR assay that is agree with ***(John, 2003, Rania et al, 2013, Riffon et al, 2001***) ***and (Sajith Khan et al, 2012)*** who found that PCR assay was rapid and accurate procedure for the detection of MRSA strains as compared to the conventional methods since the reporting time is less and can help efficiently in infection management.

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الملخص العربى

التصنيف الجزيئي للمكورات العنقودية الذهبية المعزولة من اللحوم والألبان ومنتجاتها

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قسم البكتريولوجي و المناعه و الفطريات - كلية الطب البيطري- جامعة قناة السويس- معهد بحوث صحة الحيوان الدقى -*** معهد بحوث صحة الحيوان ببورسعيد*

فى هذه الدراسة تم إلقاء الضوء على تواجد الميكروب العنقودى الذهبى في ٢٠٠عينة عشوائية من اللحوم النيئة ومنتجات اللحوم (سجق ، هوت دوج، اللحم المفروم، برجر ،اللانشون) ٣٠عينة لكل منها و ٥٠ عينة لحوم نيئة أظهرت نتائج الفحص البكتريولوجي والتعريف البيوكيميائي أن ١٩ عينه (٩,٥%) من اجمالي العينات كانت ايجابية للميكروب العنقودى الذهبى بنسبة (١٠% ، ١٣,٣٣% ، ٣,٣٣% ، ١٦,٦٧% ،٣,٣٣% ،١٠%) على التوالي. وتم إجراء خطوات الفحص البيكتيري عل ٢٨٥ عينة من اللبن الخام ومنتجات الألبان وتم عزل ١٦ من اللبن الخام نسبة ١٨,٨% بينما تم عزل ٨ عترات من ٢٠٠ من منتجات الألبان بنسبة ٤%. أما بالنسبة إلى عينات اللبن المفحوصة تم عزل ٤ عترات من ألبان المزارع بنسبة ١٦% وعدد ٤عترات من ٢٥ عينة من محلات الالبان بنسبة ١٦% ايضا وتم عزل ٨ عترات من ألبان الباعة الجائلين فى الشوارع بنسبة ٢٢,٨% . بالنسبة لمنتجات الألبان (آيس كريم، زبادى ، والجبن القريش، الجبن المملح) كانت ايجابية بنسبة (٤%، ٢,٦% ، ٨%، ٤%). وقد تم استخدام اختبار (RPLA) للكشف عن السم المعوى لميكروب المكورات العنقودية الذهبية، ووجد أن ٢٧ عترة من ٤٣عينة كانت لها القدرة على إفراز السموم ووجد منها عدد ١٥ عترة تحتوى عل السم من النوع (أ) وعدد٧عترة تحتوى عل السم من النوع (سي) وأيضاً عدده عترات تحمل النوعين(أ وسي) معاً . وتم استخدام جهاز تفاعل إنزيم البلمرة المتسلسل (PCR) للتأكيد على وجود الميكروب العنقودى الذهبى فى ١٠عينات وأثبت دقة وسرعة فى الكشف عن المكورات العنقودية الذهبية فى العينات.

ولقد تم دراسة حساسية لكل عترات الميكروب العنقودى الذهبى المعزولة من عينات اللحوم والالبان ومنتجاتها مستخدما المضادات الحيوية المختلفه فى المعمل وكانت النتائج كالتالى: أظهرت معظم العينات درجة مقاومة لكل من الكلورامفينكول والأموكسيسللين و السيفرادين والسيفالوسين و الأميكاسين والجنتاميسين و السيبروفلوكساسين و الدوكسي سيكلين . بينما العترات أظهرت حساسية لكل من السيفاكلور والإستربتومايسين والنورفلوكساسين و الإريثروميسين والسلفا ميثاكساسول/ ترايميثوبريم.

وتم استخدام جهاز تفاعل إنزيم البلمرة المتسلسل للتأكيد على وجود السلالات المقاومة للميثيسلين فى المكورات العنقودية الذهبية.