Phenotypic and Genotypic Detection of Virulence Factors of *Staphylococcus Aureus* Isolated from Meat and Meat Products

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Abstract

A total of 250 random samples of meat and meat products 50 of each meat, luncheon, burger, minced meat and sausage samples were collected from different butchers in Port-Said Governorate. All samples were subjected to bacteriological examination for isolation and identification of ***Staph. aureus***. The results revealed that the ***Staph. aureus*** was isolated in a percentage of 55 (22%). The prevalence of ***Staph. aureus*** isolates in the examined meat, luncheon, burger, minced meat and sausage samples were 14%, 20%, 30% ,34% and 12%, respectively. Coagulase positive ***Staph. aureus*** (CPS) was isolated in percentage of 33(60%) from the examined meat, luncheon, burger, minced meat and sausage samples. CPS isolates could be detected in meat, luncheon, burger, minced meat and sausage samples with a percentage of 57.15%, 600%, 60%, 58.82% and 66.66%, respectively. The antibiotic resistance of coagulase positive ***Staph. aureus*** using 14 antibacterial drugs by disk diffusion method were resistant to Cephalexin, Chloramphenicol, Cprofloxacin, Clindamycin, Erythromycin, Vancomycin, Trimethoprim + Sulphamethoxazole, Gentamicin and Streptomycin and found with a percentage of 87.88%, 84.85%, 75.76%, 72.73%, 72.73%, 69.70%, 63.64%, 60.60% and 57.58% respectively. The conventional PCR assays were developed with specific primers for the detection of different types of virulence genes in 20 strong CPS strains as protein A in ***Staphyloccocus*** (*spa*), coagulase (*coa*), haemolysin (*hlg*) and intacellular adhesion (*icaA*) while multiplex PCR assay were developed to detect of two types of Staphylococcal enterotoxins (*sea* and *seb*). The PCR amplification of the spa gene was 3/4, 2/4, 1/4, 1/4 and 2/4 coagulase positive ***Staph. aureus*** at 226 bp from meat, luncheon, burger, minced meat and sausage respectively,

While the all 20 confirmed coagulase positive ***Staph. aureus*** was examined for coagulase (coa) gene using specific gene were positive at 360 bp. 0/4, 2/4, 0/4, 0/4 and 2/4. Identified coagulase positive ***Staph. aureus*** isolates were amplified the diagnostic DNA band successfully at 937 bp for *hlg* gene from meat, luncheon, burger, minced meat and sausage were 4/4, 3/4, 3/4, 3/4 and 2/4 respectively. Amplification of the *icaA* gene was 4/4, 3/4, 3/4, 3/4 and 2/4 at 1315 bp from meat, luncheon, burger, minced meat and sausage respectively. Multiplex PCR could successfully amplify the diagnostic DNA bands of 102bp and 164 bp, of genes for staphylococcal enterotoxins *sea* and *seb*, respectively. The results showed that 1/4, 1/4, 2/4, 1/4 and 0/4 isolates of ***Staph. aureus*** was positive for seb at 164 bp from meat, luncheon, burger, minced meat and sausage respectively. While none of the samples were positive for sea. The public health hazards of this microorganism, as well as improve quality status of meat and meat products were discussed.

Keywords: Coagulase positive *Staph. aureus*, meat and meat products, virulence genes, PCR, Public health

Introduction

Meat and meat products have an important role in human nutrition as they are desirable foodstuffs. They are important sources for protein, fat, essential amino acids, minerals, vitamins and other nutrients essential for human ***(Biesalski, 2005)***. On the other hand, they are considered an ideal culture medium for growth of many organisms due to their high content of moisture, nitrogenous compounds of various degree of complexity, plentiful supply of minerals, accessory growth factors and some fermentable carbohydrates of a favorable pH as glycogen ***(Mohammed, 2011)***.

The contamination of meat and meat products with microorganisms from meat handlers, which may have carried the pathogenic microorganism during the processes of manufacturing, packing and marketing. Improper cooking, poor hygiene during production processes, refrigeration or the retail and storage of foods may lead to meat borne illness and food poisoning causing death in developing countries costing billions of dollars in medical care, medical and social costs ***(Fratmico et al., 2005 and FDA, 2012)***.

Foods that require considerable handling during preparation such as meat and meat products are frequently involved in *Staphylococcal* food poisoning because Staphylococci exist in air, dust, sewage, water, milk, and food or on food equipment,

environmental surfaces, humans, and animals. *Staphylococci* are present in the nasal passages, throats, on the hair and skin of 50 percent or more of healthy individuals. ***(Scott, 2003)***.

Staph. aureus is the predominant species involved in staphylococcal food-poisoning outbreaks ***(Aragon-Alegro et al., 2007)***. ***Staph. aureus***is gram positive, cocci, facultative anaerobic, non-sporulating bacteria in which most of them are recognized on the skin, mucous membranes of humans and animals and also as environmental contaminants ***(Feizi et al., 2012)***. 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., 2000)***. ***Staph. aureus*** is considered as one of the most dangerous pathogenic bacteria due to the production of a variety of extracellular protein toxins such as toxic shock syndrome toxin 1 (TSST-1), exfoliative toxin (ET), coagulase, hemolysins, and at least 15 types of enterotoxins ***(Mehrotra and Johnson 2000)***.

Polymerase chain reactions (PCR) is one of the molecular biology-based detection methods commonly used for detecting the main pathogens in food samples as a method provides a promising option for the rapid identification of pathogen and it can be used as a rapid, reliable method, high sensitivity, high specificity and can improve the level of detection within few hours ***(Tamparapu et al., 2001)***. PCR methods offer a sensitive and specific detection of pathogens and can discriminate virulent bacteria thus PCR-based techniques are used increasingly in food microbiology ***(Olsen, 2000)***.

Due to the rising incidence of food-borne infections, there is an urgent need for control and/or prophylaxis for food poisoning outbreaks associated with meat and meat products. It depends greatly on the investigating of the causative agents in meat and meat products, eliminating them to ensure food safety and to protect public health from microbial contamination of food. In the current study, we evaluated the prevalence and antibiotic susceptibility profiles of coagulase positive ***Staph. aureus*** (CPS) in meat and meat products including luncheon, burger, minced meat and sausage obtained from different butchers in Port-Said Governorate and to detect the presence of some of the virulence genes (*spa*, *coa*, *hlg* and *icaA*) responsible for initiation of pathogenesis of CPS using conventional PCR while multiplex PCR for detection of classical staphylococcal enterotoxins A and

B (*sea* and *seb*) as rapid, sensitive, powerful and accurate method.

Materials and methods

1- Collection of Samples:

A total of 250 samples of meat and meat products 50 of each meat, luncheon, burger, minced meat and sausage were collected from different butcher's shop in Port-Said Governorate for isolation and identification of coagulase positive ***Staph. aureus***. All the collected samples were put in a sterile polyethylene bag, labeled and transferred in an ice box directly to the laboratory as soon as possible.

2-Bacteriological examination:

2-1 Food homogenates preparation and isolation of the *Staph. aureus*.

Twenty-five gram of each of the examined samples were taken under aseptic condition into sterile blender jar to which 225 ml peptone saline (0.1%) was added. Then blend the mixture at 3000 rpm for 2 minute. ***(ICMSF, 1978).*** A loopful of the food homogenates was taken and cultured onto Baird parker medium, mannitol salt agar, 5% sheep blood agar and then onto Nutrient agar. All inoculated plates were incubated at 37ºC for 24-48h then colonies were identified ***(Koneman et al., (1996) and Quinn et al. (2002)***. Suspected colonies of ***S. aureus*** were examined morphologically and biochemically according to ***(FDA, 2001)*** and microscopically according to ***(Ryan and Ray 2004).***

2-2 Morphological characteristics:

The smear was prepared from the isolated culture and stained with Gram's stain. The stained smear revealed Gram positive, spherical cells arranged in irregular clusters resembling to bunch of grapes according to ***Qunin et al. (2002)***.

2-3 Biochemical examination:

The biochemical tests were performed to confirm CPS using Catalase test, Coagulase test, Indole production, methyl red, Voges-Proskauer, nitrate reduction, oxidase test and D-mannitol fermentation according to ***Thaker et al. (2013)***.

3- Antibiotic susceptibility testing:

The antimicrobial susceptibility test was performed using agar disc diffusion assay as described by Clinical and Laboratory Standards Institute ***(NCCLS, 2004)***. The isolates were tested for their susceptibility to 14 different antimicrobials drugs (Himedia, India) using Kirby-bauer disk diffusion method. Antimicrobials used were Amikacin (Ak) 30 mcg; cephaloxin (CL) 30 mg; Cefaclor (CEC) 30 mcg; Chloramphenicol (C) 30 ug; Clindamycin (DA) 2 mg; Ciprofloxacin (CIP) 5 mcg; Erythromycin (E) 15mcg; Gentamicin (CN) (10mg) ; Nalidexic acid (NA) 30 ug; Neomycin (N) 30 ug; Streptomycin (S) 10 mg; Tetracycline (TE) 30ug; Trimethoprim + Sulphamexazole (STX) (1.25 +23.75) and

Vancomycin (VA) 30 mg .Pure colonies of isolated ***Staph. aureus***. were emulsified in normal saline and turbidity was matched with 0.5 McFarland turbidity standards. Selected antimicrobial discs were placed on Mueller Hinton Agar plates seeded with bacteria. These plates were incubated at 37°C for 24 hours. The organisms were observed for antimicrobial sensitivity based on diameters of zones of inhibition on petri-dishes. Susceptible and resistant isolates were defined according to the criteria suggested by the ***NCCLS (2004)***.

4- Molecular examination of (CPS)

4.1- DNA extraction:

DNA extraction from CPS was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH). Briefly, 200 µl of the bacterial culture suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. Then 200 µl of 100% ethanol was added to the lysate. The bacterial culture was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

4.2- Oligonucleotide Primer *staph. aureus*: Primers used were supplied from **Metabion (Germany) are listed in table (1)**.

4.3- PCR amplification:

Multiplex PCR of enterotoxins *sea, seb, sec,* and *see*, Primers were utilized in a 50 µl reaction containing 25 µl of Emerald Amp Max PCR Master Mix **(Takara, Japan)**, 1 µl of each primer of 20 pmol concentrations, 7 µl of water, and 10 µl of DNA template**.** The reaction was performed in an applied biosystem 2720 thermal cycler.

4.4- Analysis of the PCR Products:

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 40 µl of the multiplex PCR products were loaded in each gel slot and generuler 100 bp ladders (Qiagen, Germany, GmbH) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1): *Primers sequences, target genes, amplicon sizes and cycling conditions:*

Gene	Primary denaturan	Secondary denaturation	Annealing	Extension	No. of cycles	Final extensi on
Coa	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
spa	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
Sea and *seb*	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
hlg	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
icaA	94˚C 5 min.	94˚C 30 sec.	49˚C 40 sec.	72˚C 1 min.	35	72˚C 12 min.

Table (2): *Oligonucleotide primers sequences.*

Gene	Primer Sequence	Amplified product	Reference
Coa	ATA GAG ATG CTG GTA CAG G	360 bp	**Iyer and Kumosani, (2011)**
	GCT TCC GAT TGT TCG ATG C		
Spa	TCA ACA AAG AAC AAC AAA ATG C	226 bp	**Wada *et al*., (2010)**
	GCT TTC GGT GCT TGA GAT TC		
Sea	GGTTATCAATGTGCGGGTGG	102 bp	**Mehrotra *et al*., (2000)**
	CGGCACTTTTTTCTCTTCGG		
Seb	GTATGGTGGTGTAACTGAGC	164 bp	
	CCAAATAGTGACGAGTTAGG		
Hlg	GCCAATCCGTTATTAGAAAATGC	937 bp	**Kumar *et al*., (2009)**
	CCATAGACGTAGCAACGGAT		
icaA	CCT AAC TAA CGA AAG GTA G	1315 bp	**Ciftci *et al*., (2009)**
	AAG ATA TAG CGA TAA GTG C		

Results

Table (3): *Prevalence of Staph. aureus from the examined samples of meat and meat products*

Types of samples	Examined samples	*Staph. aureus* positive samples		*Staph. aureus* negative samples	
	No.	No.	%	No.	%
Meat	50	7	14	43	86
Luncheon	50	10	20	40	80
Burger	50	15	30	35	70
Minced meat	50	17	34	33	66
Sausage	50	6	12	44	88
Total	250	55	22	195	78

Table (4): *Prevalence of coagulase positive and negative Staph. aureus from the examined samples of meat and meat products*

Types of samples	Examined strain	Coagulase +ve strain of *Staph. aureus*		Coagulase -ve strain of *Staph .aureus*	
	No.	No.	%	No.	%
Meat	7	4	57.15	3	42.85
Luncheon	10	6	60	4	40
Burger	15	9	60	6	40
Minced meat	17	10	58.82	7	41.18
Sausage	6	4	66.66	2	33.33
Total	55	33	60	22	40

Table (5): *Results of coagulase ++++ve test of Staph. aureus from examined samples of meat and meat products*

Types of samples	Examined strain	Coagulase ++++ve strain of *Staph. aureus*
	No.	No.
Meat	4	4
Luncheon	6	4
Burger	9	4
Minced meat	10	4
Sausage	4	4
Total	33	20

Table (6): *Antibiotic resistance of coagulase positive Staph. aureus (33 isolates) isolated from the examined meat and meat products to different antimicrobial agents.*

Antibacterial agents	Resistant		Sensitive	
	No. of strain	%	No. of strain	%
Cephaloxin (CL)	29	87.88	4	12.12
Chloramphenicol (C)	28	84.85	5	15.15
Ciprofloxacin (CIP)	25	75.76	8	24.24
Clindamycin (DA)	24	72.73	9	27.27
Erythromycin (E)	24	72.73	9	27.27
Trimethoprim - Sulphamethoxazole (SXT)	21	63.64	12	36.36
Vancomycin (VA)	23	69.70	10	30.30
Gentamicin (CN)	20	60.60	13	39.40
Streptomycin (S)	19	57.58	14	42.42
Amikacin (Ak)	14	42.42	19	57.58
Neomycin (N)	1	3.03	32	96.97
Cefaclor (CEC)	7	21.21	26	78.79
Tetracyclin (TE)	3	9.10%	30	90.90
Nalidixic acid (NA)	4	12.12%	29	87.88

Table (7): *Distribution of the* ***virulence genes of Staph. aureus isolated from the examined samples of meat and meat products***

Gene	Meat				Luncheon				Burger				Minced meat				Sausage			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
spa	+	+	+	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	+	+
coa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
hlg	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	+
icaA	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-	+	-	-	+	+
Sea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seb	-	-	-	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-	-

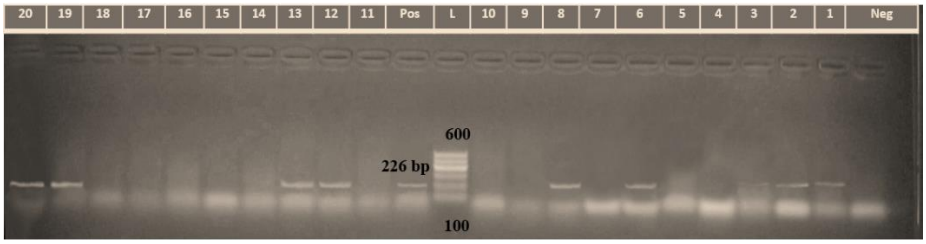
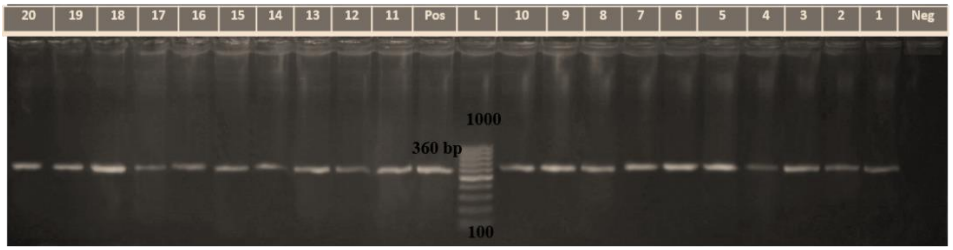


Figure 1: Agarose gel electrophoresis of PCR products after amplification of:
1- MWM-molecular weight marker (100 – 600 bp DNA ladder), + control (Positive, Negative) + different strains of *S. aureus.*

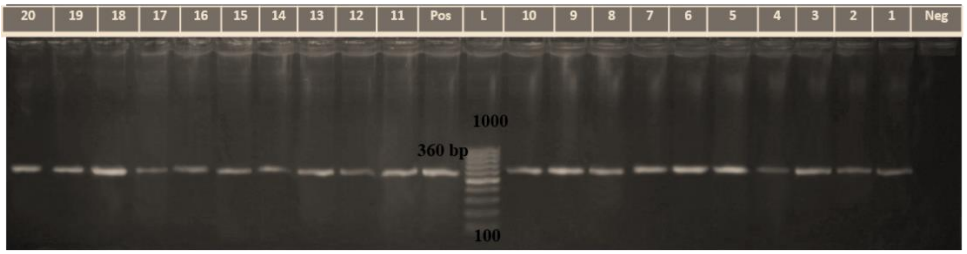
2- *spa* gene (*spa* gene products at 226bp).



Figures (2): Agarose gel electrophoresis of PCR products after amplification of:

1- MWM-molecular weight marker (100 – 1000 bp DNA ladder), + control (Positive, Negative) + different strains of *Staph. aureus.*

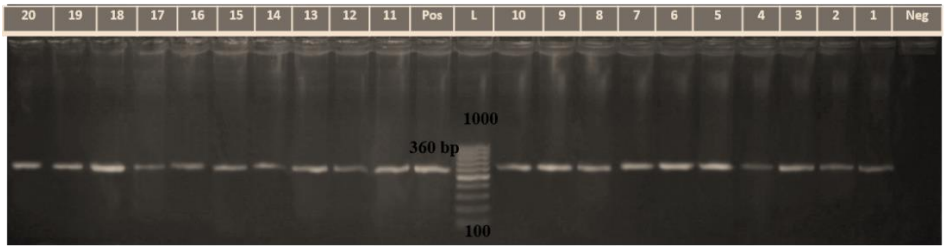
2- *coa* gene (*coa* gene products at 360bp).



Figures (3): Agarose gel electrophoresis of PCR products after amplification of:

1- MWM-molecular weight marker (100 – 1000 bp DNA ladder), + control (Positive, Negative) + different strains of *Staph. aureus.*

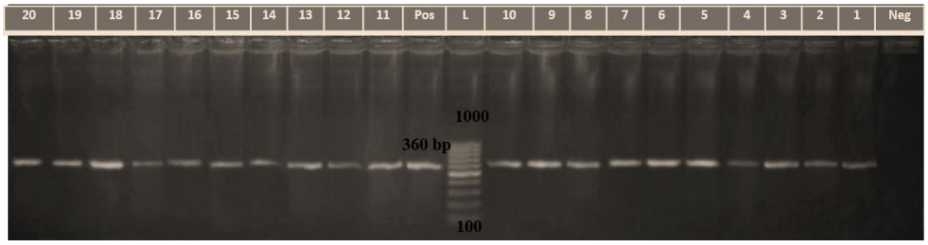
2- *hlg* gene (*hlg* gene products at 937bp).



figures (4): Agarose gel electrophoresis of PCR products after amplification of:

1- MWM-molecular weight marker (100 – 1500 bp DNA ladder), + control (Positive, Negative) + different strains of *Staph. aureus.*

2- *icaA* gene (*icaA* gene products at 1315 bp).



Figures (5): Agarose gel electrophoresis of PCR products after amplification of:
1- MWM-molecular weight marker (100 – 1500 bp DNA ladder), + control (Positive, Negative) + different strains of *Staph. aureus.*
2- *sea* gene (*sea* gene products at 102 bp).
3- *seb* gene (*seb* gene products at 164 bp

Discussion

Meat and meat products are considered the most important sources of food for people in everywhere as they supply the consumers with the required nutritive elements such as high quality proteins, essential amino acids, B-complex vitamins and certain minerals especially iron and phosphorous and beside that the meat has high calories value *(Ogilvie, 2015).* On the other hand meat and meat products are recognized as a major source of food borne pathogens that cause food poisoning in humans. *(le Loir et al., 2003).* ***Staph. Aureus*** is one of the most common causes of food-borne infections in most of the countries of the world *(Bhatia and Zahoor, 2007).* Coagulase positive ***Staph. aureus*** (CPS) is an important food borne pathogen and a major cause of food poisoning worldwide. Foods that require extensive handling during preparation and are kept above refrigeration temperature (4°C) for extended periods after preparation are often involved in staphylococcal food poisoning *(Argudín et al., 2010).*

In the present study a total of 250 samples 50 of each meat, luncheon, burger, minced meat and sausage were collected from different butcher's shops in Port-Said Governorate and investigated bacteriological to detect the prevalence of ***Staph. aureus*** among the examined meat and meat products samples (Table 3). The prevalence of ***Staph. aureus*** was 55 (22 %). These results were more than that recorded by *Gihan et al., (2015)* and *Gihan et al., (2016)* who isolated CPS from meat and some meat products in proportion of 17.6% and 14.6% respectively. And also more than that recorded by *Goja et al., (2013)* and *Ezzat et al., (2014)* who isolated CPS from meat in proportion of 12.0 % and 10%, respectively. On other hand the obtained results were less than that recorded by *Normanno et al., (2005); Hassan et al., (2010); Gousia et al., (2011); Hanson et al., (2011; Datta et al., (2012);*

Ezzat et al., (2014) and Fan et al., (2015) who showed lower prevalence of ***Staph. aureus*** ranged from 9.5% to 35%. Another studies showed higher prevalence of ***Staph. aureus*** was obtained by *Gundogan et al., (2005) and Ahmad et al., (2013)* 53.3% and 51%, respectively.

CPS isolates was detected in meat, luncheon, burger, minced meat and sausage samples with a percentage of 14%, 20%, 30%, 34% and 12%, respectively as mentioned in (Table 3). These results were less than those reported by *Watres et al., (2011)* who isolated CPS from meat, sausage, Kofta, burger, meat and luncheon samples with a percentage of 64%, 56%, 52%, 40% and 24%, respectively. On the other hand, recoded results were less than those recorded by *Azeez et al., (2016) ; Mansour and Basha (2009) ; Kanika et al., (2011) and Gihan et al., (2015)* who reported that incidence of CPS in meat samples from different markets were 30.2 %, 20% , 20.5% and 16% respectively. The obtained results were higher than *Hamzah et al., (2010)* who reported lower incidence of CPS in meat 5.53%. While the results of burger samples were less than *Reyad and Naser, (2016) ; Gihan et al., (2015); **Zakaria**, (2007); Elshrek et al., (2008) and Hassanien, (2004)* who isolated CPS in a percentage of 22%, 24 %, 25.0% and 27.1% and 36% respectively. While the obtained results of burger samples were higher than *Abass, (2014)* who isolated CPS in a percentage of 3.33%. The obtained results from sausage samples were less than *El-Sherbeeny, (1990); Reyad and Naser (2016); El-Khateib, (1997); Mohammed et al., (2015); Soultos et al., (2003); Azeez et al., (2016) and Abass, (2014)* who reported higher incidence of CPS in sausage 43%, 30%, 29%, 24%, 19.4% , 18.7% and 13.33%, respectively. Also our results were more than *Gihan et al., (2016)* who isolated CPS from meat, meat, burger, sausage and kofta in proportion of 13.33%, 20%, 6.66% and 16.66% respectively. The difference between the obtained results and the previous studies may be attributed to the total number of samples, types of samples, sampling techniques, sources and handling of samples and types of media. The high percentage of CPS in meat and meat products is indication of poor hygiene. Also their contamination returned to unhygienic manner of processing, transportation, storage and due to the insanitary condition of the butcher and absence of the health services in butcheries.

The antibiotic resistance of ***Staph. aureus*** strains isolated from animal products such as meat, luncheon, burger, minced meat and sausage of the examined samples were shown in (Table 6). The results of antibiotic sensitivity, revealed that ***Staph. aureus*** isolates were resist to Cephaoxin (86.88%) followed by

Chloramphenicol (84.85%), Ciprofloxacin (75.76%), Clindamycin (72.73%), Erythromycin (72.73%), trimethoprim- sulphamethoxazole (63.64%), Vancomycin (69.70%),Gentamycin (60.60%), Streptomycin (57.58%), Amikacin (42.42%), Cefalocor (21.21%), Nalidixic acid (12.12%), Tetracyclin (9.10%) and Neomycin (3.03%). The staphylococcal isolates from the meat and meat products were found to be resistant to many of the antibiotics tested. The obtained results come within those studies by *Normanno et al. (2007)* who found remarkable level of resistance to several antibiotics such as Tetracycline, Erythromycin and Trimethoprim – Sulfametoxazole**.** ***Gousia et al. (2011)*** stated that ***Staph. aureus*** isolates from beef, pork, and lamb and goat meat was resistant to one or more antimicrobials from the classes of Penicillins (narrow and broad spectrum), Macrolides (Erythromycin), Lincosamides (clindamycin), Tetracycline and Cephalosporins (Ciprofloxacin). ***Sharma et al. (2011)*** observed that ***Staph. aureus*** strains were resistant to Nalidixic acid, Amoxycillin + Sulbactam, Cloxacillin, Erythromycin, Kanamycin and Vancomycin. On the other hand several isolates were found susceptible to the Ofloxacin, Ampicillin, Tetracycline, Oxacillin, Streptomycin, Sulphafurazole and Ciprofloxacin; ***Daka et al., 2012)*** recorded that ***Staph. aureus*** were resistant to Erythromycin. ***Ezzat et al. (2014)*** observed that ***Staph. aureus*** strains isolated in their work were resistant to Chloramphenicol (95.3%) followed by Amoxiciilin, Cephranin, Cephalothin, Amikacin, Gentamycin, Doxycycline, Cefaclor, Norfloxacin and Erythromycin. Our results disagree with ***Eid et al. (2015)*** who detected the highest resistance rates were against Oxytetracyclin and Trimethoprim Sulphamethazole with resistance rates of 90% and 86.7%, respectively.

Conventional PCR assay were developed with specific primers for confirmation and detection of different type of virulence genes PCR assays were developed with specific primers for detection of different types of virulence genes as Protein A in ***Staphyloccocus*** (*spa*) ,coagulase (*coa*), haemolysin (*hlg*) and intacellular adhesion (*icaA*).Multiplex PCR assay were developed with specific primers for detection of different type of *Staphylococcal* enterotoxins (*sea* and *seb*) as mentioned in Tables (7) and Figures (1, 2, 3, 4, and 5).

Conventional PCR assays could successfully amplify the diagnostic DNA bands for Protein A (*spa*) ***(Foster, 2005 and Algammal et al., 2013)*** which is a component of ***Staph. aureus*** cell wall and is covalently bound to the peptidoglycan. The PCR amplification of the *spa* gene 3/4,

2/4, 1/4, 1/4 and 2/4 coagulase positive ***Staph. aureus*** at 226 bp from meat, luncheon, burger, minced meat and sausage respectively, these results varied with those obtained by ***(Enany et al., 2013****; Mehndiratta et al., 2009 and* ***Bekhit et al., 2010)*** 100%, 94.6% and 32.4% respectively, While the all 20 confirmed coagulase positive ***Staph. aureus*** was examined for coagulase (*coa*) gene using specific gene were positive at 360 bp. 0/4, 2/4, 0/4, 0/4 and 2/4 previously. The most important phonotypical features used in the identification of ***Staph. aureus*** its ability to produce coagulase, an enzyme that causes clotting of the blood plasma, the production of coagulase enzyme could be confirmed by the presence of coagulase gene which detected by PCR ***(Reinoso, 2004*** *and* ***Qing et al., 2012)***.

Identified coagulase positive ***Staph. aureus*** isolates were amplified the diagnostic DNA band successfully at 937 bp for *hlg* gene from meat, luncheon, burger, minced meat and sausage respectively. The results showed that the 4/4, 3/4, 3/4, 3/4 and 2/4 respectively. Amplification of the *icaA* gene 4/4, 3/4, 3/4, 3/4 and 2/4 were positive at 1315 bp from meat, luncheon, burger, minced meat and sausage respectively. These (*hlg, icaA*) were chosen because they have been determined to be more common among invasive isolates.11 Amplification of the genes revealed these genes were present among the different ***Staph. aureus*** isolates ***Jothi et al., (2009)***

Multiplex PCR assays were developed with specific primers for the detection of two different enterotoxins genes (*sea* and *seb*), which may be considered a significant in food safety threat. ***Staph. aureus*** produces a variety of enterotoxins types that may contribute to its pathogenicity. These results showed that from 1/4, 1/4, 2/4, 1/4 and 0/4 isolates of ***Staph. aureus*** positive for *seb* at 164 bp from meat, luncheon, burger, minced meat and sausage respectively. None of the samples were positive for *sea* in Table (7). The predominant classical SE gene varied from country to country – Germany, *sea, sec*; ***(Becker et al., 2001)****;* Japan, *seb***; *(Omoe et al., 2005)***; New Zealand, *seb*; ***(Boerema, et al., 2006)*** Poland, *sec*; ***(Bania et al., 2006)***; Bulgaria, *sea*; ***(Nashev et al., 2007)*** and Ireland, *seb*; ***(Collery et al., 2008)***. The differences in toxin types depend upon the origins of *staphylococcal* food poisoning which differed widely among countries. This may be due to differences in the consumption and food habits in each of the countries ***(Bhatia and Zahoor, 2007)***. Also the predominant classical SE gene varied from authors to another as ***Marcia et al. (2009)*** found staphylococcal enterotoxins (*sea* and *see*) in colonial sausage, and (*sed* and *see*) genes in colonial and

American cheese, while ***Nashwa et al. (2015)*** detected *sea* genes only in raw meat. So the differences in the distributions of enterotoxins types in the populations strengthen the probability that some pathogenic ***Staph. aureus*** strains may returned to the different environmental, types of examined samples and geographical distributions. From the obtained results, can conclude that ***S. aureus*** was isolated from different and randomly samples of animal products such as meat and some meat products as luncheon, burger, minced meat and sausage samples from different butchers in port-said city. Meat and meat Products can be a source of toxigenic ***S. aureus*** which could potentially be spread to community through the food and represents a potential health risk for consumers. Surfaces, equipment, disinfection of animal slaughter houses and good personal hygiene can reduce spreading of ***S. aureus*** and its virulence genes.

Thus from the our results we recommended that a hygienic awareness should be applied for personnel whom involved on handling and preparing of food which included proper hand washing, careful and safe handling and through cooking and processing of raw meat and meat products and also for consumers. Hazard Analysis Critical Control Point (HACCP) procedures should be adopted during all steps of manufacture, handling and storage of meat and meat products to produce safe and high quality products. Also a good hygienic practice (GHP) during meat and meat products processing is essential to control the hazards of Staphylococcal food poisoning. Quality control and routine microbiological examination should be adopted in meat butchers shops and other food rendering outlet as restaurants with a consequent certificate of nil presence food borne bacteria.

The conventional & Multiplex PCR assay can be used as an accurate, safe and fast technique for the detection of virulence genes of ***S. aureus*** in of animal products such as meat and some meat products. Those assay can be done within few hours so it act as rapid and specific diagnostic tool used for characterization of ***S. aureus*** isolates.

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الملخص العربي

الفحص الظاهري و الجيني لعوامل الضراوة في ميكروب المكور العنقودي الذهبي المعزول من اللحوم ومنتجاتها

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تم جمع 250 من عينات اللحوم و بعض منتجاتها (اللانشون ، البرجر ، اللحم المفروم والسجق) بواقع 50 عينة لكل منهما والتي تم جمعها عشوائيا من محلات الجزارة المختلفة في محافظة بورسعيد و ذلك لدراسة مدي تواجد الميكروب العنقودي الذهبي في العينات موضع الدراسة .

وقد خضعت هذه العينات للفحص البكتريولوجي للعزل والتصنيف باستخدام الاختبارات البيوكميائية على المعزولان من بكتريا المكور العنقودى الذهبي وقد أظهرت النتائج أنه تم عزل 33 عترة من نوع المكور العنقودى الذهبي من 250 عينة بنسبة إجمالية 22 ٪ و بلغت نسبة المكور العنقودي الذهبي المعزول من اللحم ، اللانشون ، البرجر ، اللحم المفروم والسجق 14٪ ، 20٪ ، 30٪ ، 34٪ و 12٪ على التوالي. تم تحديد جميع السلالات المعزولة باسم المكور العنقودى الذهبي باستخدام ميديا خاصة واختبارات كيميائية حيوية .

كانت النسبة الإجمالية للمعزول الايجابي للتجلط من المكورالعنقودي الذهبي بنسبة 60٪. بينما كانت نسبة المكورالعنقودي الذهبي المعزول من اللحم ، اللانشون ، البرجر ، اللحم المفروم والسجق 57.15٪ ، 60٪ ، 60٪ ، 58.82٪ و 66.66٪ على التوالي.

و بتحديد بعض جينات الضراوة لميكروب المكور العنقودى الذهبي المعزول من اللحوم ومنتجات اللحوم (لانشون ، برجر ، اللحم المفروم والسجق) حيث انه العامل المسبب المتكرر للتسمم الغذائي كما فى جين التجلط و والبروتين أ(جين spa) في المكور العنقودى الذهبي ، جين التحلل hlg و عامل الالتصاق بين الخلاياicaA. تم تطوير تقنية تفاعل انزيم البلمرة المتعددة باستخدام بادئات محددة للكشف عن نوعين من السموم المعوية العنقودية (sea, seb).

باستخدام تقنية تفاعل انزيم البلمرة المتسلسل التقليدية ، تم تحديد الجين sPa في كل من اللحوم ، اللانشون ، البرجر ، اللحم المفروم والسجق كالتالي 4/3 ، 4/2 ، 4/1 ، 4/1 و 4/2 عند 226 قاعدة زوجية على التوالى .