Detection of Virulence Genes of *Staphylococcus Aureus* Isolated from Chicken Using Polymerase Chain Reaction.

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Abstract

A total of 300 chicken samples (100 samples for breast muscles, 100 samples for thigh muscles and 100 samples for joints) were collected randomly from different markets in Port-said city during the period of work from July 2015 till August 2016. Overall percentage of coagulase positive *S. aureus* which isolated from chicken samples were 37.2% (49/132) while coagulase positive *S. aureus* of breast muscles, thigh muscles and joints were 15.1%, 14.5% and 7.6% respectively. PCR revealed that 15 tested isolates were *S. aureus* using 16SrRNA at 791 bp. The study conducted to detect virulence genes, *coa* 15 isolates were positive at 570 bp. (100%), *clfA*15 isolates were positive at 638 bp. (100%), *See* gene is the highest SE, 5 isolates were positive at 209 bp. (33.33%), followed by *Seb* gene 1isolate were positive at164 bp. (6.67%). None of 15 isolates were positive for *Sea, Sec* and *Sed*.

Introduction

Poultry meat was a common vehicle of food borne illness, with *S. aureus* which usually being one of the causes of outbreaks involving large numbers of people. ***(Losito et al., 2005)*** *S. aureus* is the only staphylococcal species in poultry considered to be pathogenic. Typical pathogenic *S. aureus* strains are Gram-positive, coccoid in shape, found in clusters, aerobic, facultative anaerobic**,** non-spore forming and non-motile belong to the family *Micrococcaceae*.***(Willett, 1992)*** *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. 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)***

This study was designed to detect *S. aureus* isolated from chicken meat and joints which collected randomly from the Port-said city with respect

to its virulence genes using conventional method of PCR.

Material and Methods

1. Samples:

A total of 300 chicken samples were collected randomly from different markets in Port-said city during the period of work from July 2015 till August 2016.The samples were represented as 100 samples from breast muscles, 100 samples from thigh muscles and 100 from joints of chickens.

2.Bacteriological isolation and identification of *Staphylococcus aureus*:

Isolation and identification of *S. aureus* were determined according to ***Koneman et al. (1996) and Quinn et al. (2002)***

3. Biochemical reactions of important Staphylococci *(Quinn et al., 1994)&(FDA, 2001)*.

4. Molecular Identification of Isolates:

4.1. Extraction of DNA: It was done according to QIAamp DNA mini kit instructions

4.2. Preparation of PCR Master Mix used for cPCR : It was done according to **Emerald Amp GT PCR mastermix (Takara)** Code No. **RR310A**kit **2.4.3.**

Table (1): *Oligonucleotide primers used in cPCR*

Gene	Primer	Primer sequence (5'-3')	Length of amplified product	Reference
16S rRNA	16S Rrna-F	CCTATAAGACTGGGATAACTTCGGG	791bp	Mason *et al.*(2001)
	16S Rrna-R	CTTTGAGTTTCAACCTTGCGGTCG		
Coa	Coa-FP	ATA GAG ATG CTG GTA CAG G	570 bp	*Iyer and Kumosani, (2011)*
	Coa-RP	GCT TCC GAT TGT TCG ATG C		
clfA	ClfA.F	GCAAAATCCAGCACAACAGGAAACGA	638 bp	Mason *et al.*, 2001
	ClfA.R	CTTGATCTCCAGCCATAATTGGTGG		
Sea	GSEAF-1	GGTTATCAATGTGCGGGTGG	102 bp	*Mehrotra et al.,(2000)*
	GSEAR-2	CGGCACTTTTTTCTCTTCGG		
Seb	GSEBF-1	GTATGGTGGTGTAACTGAGC	164 bp	
	GSEBR-2	CCAAATAGTGACGAGTTAGG		
Sec	GSECF-1	AGATGAAGTAGTTGATGTGTATGG	451bp	
	GSECR-2	CACACTTTTAGAATCAACCG		
Seb	GSEDF-1	CCAATAATAGGAGAAAATAAAAG	278 bp	
	GSEDR-2	ATTGGTATTTTTTTTCGTTC		
See	GSEEF-1	AGGTTTTTTCACAGGTCATCC	209 bp	
	GSEER-2	CTTTTTTTTCTTCGGTCAATC		

4. 4. Table (2): *Cycling conditions of cPCR for detection of different genes of S. aureus*

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension	Reference
Coa	94˚C 5 min.	94˚C 30 sec.	55˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.	*Iyer & Kumosani, (2011)*
clfA	94˚C 5 min.	94˚C 30 sec.	55˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.	Mason *et al. (* 2001)
Sea, Seb , Sec, Sed*and *See	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 40 sec.	35	72˚C 10 min.	*Mehrotra et al. (2000)*

2.4. 5. DNA Molecular weight marker (100-1500bp.)

2.4. 6. Agarose gel electrophoresis: *(Sambrook et al., 1989)*

Results & Discussion

1. Table (3): *Prevalence of staphylococci in chicken samples:*

Types of samples	Number of examined samples	Bacteriological finding		
		No. of Staphylococcus isolate	%	
1-Breast muscle	100	64	64	48.5
2-Thigh muscle	100	58	58	43.9
3-Joints	100	10	10	7.6
Total	300	132	44	

*The percentage was calculated according to total number of Staphylococci & total samples isolated from chickens.

2. Table (4): *Prevalence of coagulase positive Staphylococcus aureus in chickens:*

Types of samples	No. of examined samples	No. of *S. aureus* isolate	Tube Coagulase test			
			Positive		Negative	
			No.	%	No.	%
1-Breast muscle	**100**	**64**	**20**	**15.10**	**44**	**33.3**
2-Thigh muscle	**100**	**58**	**19**	**14.5**	**39**	**29.5**
3-Joints	**100**	**10**	**10**	**7.60**	**–**	**–**
Total	**300**	**132**	**49**	**37.2**	**83**	**62.8**

* The percentage was calculated according to total number of *S. aureus* isolated from chickens.

3. Prevalence of virulence genes of *Staphylococcus aureus* in chickens:

Table (5): *Results of virulence genes of S. aureusin chickens.*

Type of sample	Breast Ms.	Thigh Ms.	Joints	Total	%
No. of coagulase positive *S. aureus* isolate	20	19	10	49	37.2
No. of examined sample for virulence genes	5	5	5	15	
Virulence genes	5	5	5	15	100
16srRNA					
coa	5	5	5	15	100
*clf*A	5	5	5	15	100
Enterotoxins	0	0	0	0	0
Sea					
Seb	0	0	1	1	6.67
Sec	0	0	0	0	0
Sed	0	0	0	0	0
See	3	2	0	5	33.33

* The percentage was calculated according to total number of examined sample for virulence genes.

4. Molecular confirmation of *Staphylococcus aureus* isolated from chicken:

4.1. Result of Polymerase Chain Reaction of *Staphylococcus aureus*:

As shown in **Photo (1)** all tested isolates gave electrophoresis with a specific band at 791 base pair and identified as *Staphylococcus aureus* using 16SrRNA.

4.2. Result of Polymerase Chain Reaction for detection of *coa* gene of *Staphylococcus aureus*:

As shown in **Photo (2)** all tested isolates gave positive electrophoresis of coagulase of *S. aureus* with a specific band at 570 base pair.

4.3. Result of Polymerase Chain Reaction of Clumping factor A (*clf*A) of *Staphylococcus aureus*:

As shown in **Photo (3)** all tested isolates gave positive electrophoresis of clumping factor A of *S. aureus* with a specific band at 638 base pair.

4.4. Result of Polymerase Chain Reaction for detection of Staphylococcal Enterotoxin (SE) of *Staphylococcus aureus*:

As shown in Photo (4) Lane 2, 3, 4, 6 and 10 isolates gave positive electrophoresis *(See)* of *S. aureus* with a specific band at 209 base

pair. Lane 11 isolates gave positive electrophoresis of *(Seb)* of *S. aureus* with a specific band at 164 base pair.

A total of 300 samples of chickens (100 samples from breast muscle, 100 samples from thigh muscle and 100 samples from joint) were examined bacteriologically to show prevalence of pathogenic *S. aureus*. The percentage of overall coagulase positive *S. aureus* which isolated from chicken samples were 37.2% (49 isolates from 132 samples) as mentioned in Table (4).These results were nearly agreed with those recorded by ***(Mulders et al., 2010)*** who isolated *S. aureus* from broiler flock in The Netherlands with percentage 35%.

Many studies reported higher percentage of *S. aureus* from raw chicken samples as reported by ***(Ashraf et al., 2014)*** who isolated *S. aureus* from raw chicken samples with percentage 51.6%. On the other hand ***(Momtaz et al., 2013)*** who isolated *S. aureus* from raw chicken samples with percentage 22.7%.

Concerning to breast muscle samples, out of 100 samples were collected from breast muscles of chicken 20 isolates of *S. aureus* with percentage 15.1% (20/132) as mentioned in Table (4).These results were nearly similar with ***(Hanson et al., 2011)*** who recorded that out of 45 samples collected from chicken breasts 8 isolates of *S. aureus* with percentage 17.8% were isolated, (***El-Enean et al., 2008)*** who recorded that out of 180 samples collected from chicken breasts 33 isolates of *S. aureus* were isolated with percentage 18.3% and (***Khalafalla et al., 2015)*** who isolated *S. aureus* from breasts chicken samples with percentage 20%. On the other hand many studies disagreed with these results as ***(Kozacinski et al., 2006)*** who recorded that *S. aureus* was 46.15% in chicken breasts without skin – "fillet" and 28.75% from chicken breasts with skin and ***(Kitai et al., 2005)*** who reported that, out of 51 samples were collected from chicken breasts 19 isolates of *S. aureus* with percentage 37.3% were isolated.

Regarding to thigh muscle samples, out of 100 samples collected from thigh muscles of chicken 19 isolates of *S. aureus* were isolated with percentage 14.5% (19/132) as mentioned in Table (4). Many studies disagreed with these results as recorded by(***Khalafalla et al., 2015)*** who reported that out of 15 samples collected from thigh muscles of chicken 4 isolates of *S. aureus* were isolated with percentage 26.6% (4/15), ***(Kitai et al., 2005)*** who reported that, out of 114 samples collected from thigh of chicken 47 isolates of *S. aureus* were isolated with percentage 41.2% and (***El-Enean et al., 2008)*** recoded that, out of 140 samples collected from thigh muscle of chicken 48 isolates of *S. aureus* were isolated with percentage 34.3%.

Concerning to joint samples, out of 100 samples collected from joint of chicken 10 isolates of *S. aureus* with percentage 7.6% (10/132) were isolated as mentioned in Table (4). The obtained results were nearly agreed with ***(Enany et al., 2013)*** who reported that, out of 33 samples collected from joint of chicken 3 isolates of *S. aureus* were isolated with percentage 9.1%.On the other hand many studies disagree with results as recorded by ***(Heba et al., 2012)*** who isolated *S. aureus* from joint of chicken samples with percentage 35.7%.

Conventional PCR assay were developed with specific primers for confirmation and detection of different types of virulence genes as mentioned in Table (1). Results of agarose gel electrophoresis the using of 16SrRNA revealed that all 15 presumptive samples as mentioned in **Table (5)** indicated that all tested strains were *Staphylococcus aureus* at 791 bp.

PCR assays were developed with specific primers for detection of different types of virulence genes as *coagulase (coa.), clumping factor A (clfA)*and *Staphylococcal enterotoxins (Se.) as (Sea, Seb, Sec, Sed, See).*

The fifteen isolated strains of *S. aureus* were positive for *coagulase* gene *(coa)* with percentage 100% (15/15)at 570 bp as mentioned in **Table (5)** These results were nearly agreed with ***(Momtaz et al., 2013)*** who reported that presence of *coagulase* gene in chicken meat in Isfahan province, Iran were isolated with percentage 63.41% (52/82).

The fifteen isolated strains of *S. aureus* were positive for *clf*A gene with percentage 100% (15/15) at 638 bp as mentioned in Table (5).These results were nearly agreed with ***(Momtaz et al., 2013)*** who reported that presence of *Clumping factor* A (*clf*A) gene in chicken meat in Isfahan province, Iran with percentage 76.82 % (63/82).

Staphylococcal food poisoning, one of the most common food-borne diseases, results from ingestion of one or more staphylococcal enterotoxins (SEs) produced by *S. aureus* (*SEA* to *SEE*) have been reported to cause 95% of staphylococcal food poisoning. *See* gene is the highest staphylococcal enterotoxins (SEs) followed by *Seb* gene. None of the samples were positive for *Sea, Sec* and *Sed* as mentioned in Table (5).

See gene was detected in 5 isolates from 15 isolates of *S. aureus* from chicken meat with percentage 33.3% at 209 bp as mentioned in Table (5).These results were nearly agreed with ***(Gihan et al., 2015)*** who recorded that *See* gene in 3 isolates from 11 isolates of *S. aureus* from chicken meat with percentage 27.2% . On the other hand, there were studies disagreed with these results as (***Abdalrahman et al., 2015)*** who recorded *See* toxin gene in 168 isolates of *S. aureus* from poultry with percentage 1.2%. In addition, the results revealed presence of *Seb* gene in one isolate

from 15 isolates of *S. aureus* from chicken meat with percentage 6.67% at 164 bp as mentioned in Table (5).These results were nearly agreed with ***(Madahi et al., 2014)*** who recorded *Seb* gene from *S. aureus* isolated from chicken nugget in Iran with percentage 4.16% .

The obtained results, revealed that *S. aureus* is an important pathogen. Chicken meat can be a source of toxigenic *S. aureus* which could potentially be spread to community through the food which may create a health risk for consumers. The presence of these isolates in chickens represents a potential health hazard for consumers and deserves further attention for proper handling of raw chicken meat, adequate cleaning of hands, surfaces, equipments, disinfection of poultry slaughter houses, good personal hygiene and all steps of manufacture, handling and storage of chicken meat should be under control to produce safe and high quality products and to reduce spreading of *S. aureus* and its virulence genes.

Photo (1): Agarose gel (1%) electrophoresis showing of PCR for detection of *S. aureus* using 16SrRNA

Lane(1: 15) ⟶ positive for *S. aureus* with 791bp band.

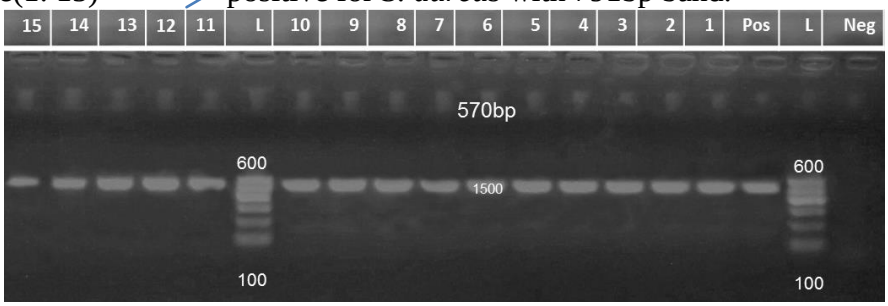

Photo (2): Agarose gel (1%) electrophoresis showing result of PCR for detection of *Coa* gene of *S. aureus*

Lane(1: 15) ⟶ positive for *coa* with 570bp band.

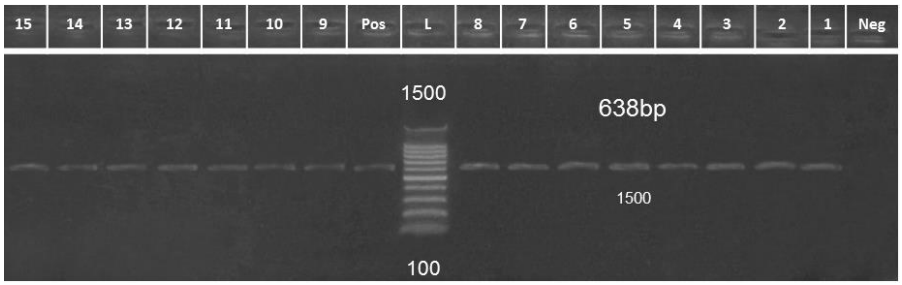

Photo (3): Agarose gel (1%) electrophoresis showing result of PCR for detection of Clumping factor A gene (*clf*A gene) of *S. aureus*
Lane(1: 15) → positive for *clf*A with 638bp band.

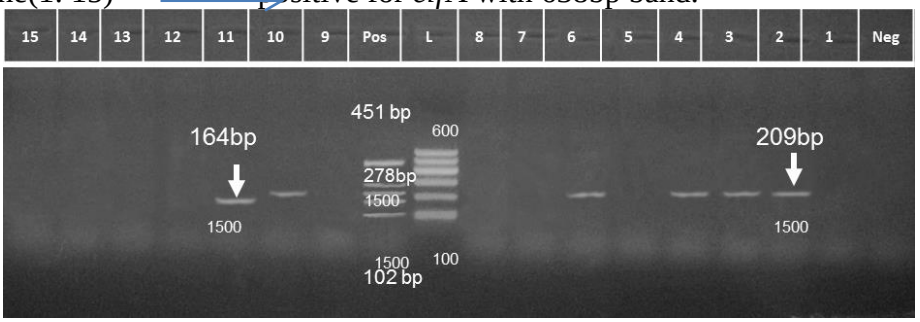

Photo (4): Agarose gel (1%) electrophoresis showing result of PCR for detection of Staphylococcal Enterotoxin of *S. aureus*
Sea gene (Sea gene products at 102bp). Seb gene (Seb gene products at 164 bp). Sec gene (Sec gene products at 451 bp). Sed gene (Sed gene products at 278 bp).
See gene (Sed gene products at 209 bp).
Lane (2, 3, 4, 6 and 10) → Positive for See with 209 bp band.
Lane (11) → Positive for Seb with 164 bp band.

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الملخص العربي

اكتشاف جينات الضراوة للمكور العنقودي الذهبي المعزول من الدجاج باستخدام تفاعل إنزيم البلمرة المتسلسل

حمزة محمد عيد **أماني محمود شلبيميرا محمد شتيوي**

**قسم البكتريولوجي و المناعة و الفطريات - كلية الطب البيطري- جامعة قناة السويس*

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تم جمع 300 عينة عشوائية من الدجاج (100 عينة من عضلات صدور الدجاج – 100 عينة من عضلات فخذ الدجاج و 100 عينة من مفاصل الدجاج) من أسواق مختلفة ببورسعيد أثناء فترة العمل من يوليو 2015 حتى أغسطس 2016لعزل وتصنيف المكور العنقودي الذهبي ايجابي التجلط الذي وجد بنسبة كلية 37.2% (132/49) بينما المكور العنقودي الذهبي ايجابي التجلط في عضلات الصدور, الفخذ و المفاصل 15.1%,14.5%,7.6% على التوالي. اظهر تفاعل إنزيم البلمرة المتسلسل أن 15 عترة المعزولين من الدجاج يوجد بهم المكور عنقودي ذهبي عند 791 قاعدة زوجية. هذه الدراسة أجريت لاكتشاف جينات الضراوة للمكور العنقودي الذهبي مثل جين التجلط حيث أن 15 عترة كانت ايجابية بنسبة 100% عند 570 قاعدة زوجية, جين التكتل لعامل أ حيث أن 15 عترة كانت ايجابية بنسبة 100% عند 638 قاعدة زوجية وجين المكور العنقودي المعوي (هـ) هو أعلى نسبة في جينات المكور العنقودي المعوي حيث أن 5 عترات ايجابية بنسبة 33.33% عند 209 قاعدة زوجية يليها جين المكور العنقودي المعوي (ب) حيث أن عترة واحدة ايجابية بنسبة 6.67%عند 164قاعدة زوجية. لا يوجد اي عترة من 15 عترة معزولة من المكور العنقودي الذهبي ايجابي التجلط ,ايجابية لجينات المكور العنقودي المعوي(أ), (ج) ,(د).