Incidence and characterization of *S. aureus* in broiler carcasses

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A B S T R A C T

One hundred random samples and swabs taken from freshly broiler chicken were collected from one of the largest half automated poultry slaughterhouse in Al-kalubiah governorate. The samples were classified as follow : 40 samples of chicken breast and thigh (20 of each), and 60 swabs from knives after slaughtering of these chickens and from chicken carcasses surfaces after defeathering from the region under the right wings and after evisceration from the region under the left wings (20 of each).

The obtained results showed that the mean values of *Staphylococcus* counts/ cm^2 were $2.62\times10^8 \pm 9.04\times10^7$, $2.42\times10^7 \pm 8.01\times10^6$ and $3.63\times10^7 \pm 2.74\times10^7$ swabs from knives, swabs after defeathering and swabs after evisceration, respectively. While the mean values/g were $1.42\times10^6 \pm 8.19\times10^5$ and $1.24\times10^6 \pm 5.88\times10^5$ for breast and thigh samples. The average counts were of *S. aureus* were $1.29\times10^8 \pm 4.75\times10^7$, $1.24\times10^7 \pm 4.22\times10^6$ and $1.68\times10^7 \pm 1.24\times10^7$CFU/$cm^2$from knives, swabs after defeathering and swabs after evisceration, and $2.92\times 10^5 \pm 2.10\times 10^5$ and $1.91 \times 10^5 \pm 1.54\times 10^5$ for breast and thigh, respectively. Application of PCR technique declared that the occurrence of one or more virulence genes in *S. aureus* isolated from the examined chicken meat samples. The enterotoxin type A was produced by 2 strains of *S. aureus*, while enterotoxin type C, A+B and B+D were produced by single strain of *S. aureus*. On the other hand, there were 11 strains of *S. aureus* were not capable of producing enterotoxin. Finally, the significance of isolated *S. aureus* toxins in the examined samples of various raw chicken meats, possible sources of their contamination and their antimicrobial resistance against certain antibiotics as well as some recommendations to improve the quality of such food items were discussed.

Keywords: *S. aureus*, broiler, carcasses.

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1. INTRODUCTION

Broiler carcass is an important food items in most countries due to its contribution in solving the problem of animal meat shortage. It comprises about two thirds of the total production of animal protein in the world (Ruban et al., 2010). Broiler carcass is very popular food throughout the world since it is delicious, nutritious, characterized by good flavor, easily digested, low fat content and high in poly unsaturated and saturated fatty acids ratio, good protein source of high biological value as well as better palatability

due to less connective tissue, shorter cooking time and superior in water holding capacity (Smith, 2001).

Microbial food safety and food borne infections are important public health concern worldwide. There have been a number of food-borne illnesses resulting from the ingestion of contaminated foods such as chicken meat. Most of the pathogens that play a role in food borne diseases have a zoonotic origin (Busani et al., 2006).

Staphylococcus aureus is often found closely associated with the human body and may be also found in many parts of environment (dust, water, air, faeces and on clothes or utensils) (Bremer et al., 2004). The technique used for opening the abdomen and the technique of hand evisceration predominantly practiced in the traditional shops led to high prevalence of *S. aureus* in broiler carcass (Yves et al., 2002). After ingestion of food containing *S. aureus* toxins, they will be absorbed from the gastrointestinal tract into the blood causing nausea, emesis, abdominal cramps and diarrhea (Chapval et al., 2006). It depends on individual susceptibility and the amount of enterotoxin ingested (Do carmo et al., 2004).Sometimes, severe symptoms arises which require hospitalization of patients, in rare cases deaths may occur (Martin et al., 2004).

S*taphylococcus aureus* produces wide variety of toxins including staphylococcal enterotoxins (*SEs: SEA* to *SEE.SEG* to *SEI, SER* to *SET*) with demonstrated emetic activity, and staphylococcal-like (*SEI*) proteins, which are not emetic in primate model (*SEIL* and *SEIQ*) or have yet to be tested (*SEEIJ, SEIK, SEIM* to *SEIP, SEIU, SEIU2,* and *SEIV*). *SEs* and *SEIs* have been traditionally subdivided into classical (*SEA* to *SEE*) and new (*SEG* to *SEIU2*) types (Argudin et al., 2010).

The concentration of *S. aureus* necessary to cause food poisoning ranges from 10^6 to 10^8 CFU/g in food samples, and for sensitive persons even 10^5 CFU/g of staphylococcal bacteria are capable of producing enough *SEs* (around 1µ /g) to generate symptoms (Alarcon et al., 2006). *SE* actually is highly thermostable; heat treatment, such as normal cooking cannot totally inactivate them, and thus they survive thermal treatment, leading to food poisoning (Nagarajappa et al., 2012). Indeed, enterotoxin in food has caused outbreaks where the incriminated food had already undergone heat treatment (Asao et al., 2003).SEs cause food poisoning has super antigenic activity; they stimulate T- cell proliferation, enhance endotoxic shock, suppress immunoglobulin production and are pyrogenic (Le loir et al., 2003).

The enterotoxin genes are accessory genetic elements in *S. aureus*, meaning that not all strains of this organism is enterotoxin-producing. They are encoded by mobile genetic elements including phages, plasmid and pathogenicity islands (Holeckova et al., 2002 and Martin et al., 2004).

2. Materials and methods

2.1. Samples:

A total number of 100 samples were collected from 20freshly broiler chickens (about 2 kg in weight) immediately after slaughtering and de-feathering from one of the largest half automated poultry slaughterhouse in Al-Qalubiah governorate. The samples were classified as follow : 40 samples of chicken breast and thigh (20 of each), and 60 swabs from knives after slaughtering of these chickens and from chicken carcasses surfaces after de-feathering from the region under the right wings and after evisceration from the region under the left wings (20 of each).

2.2. Preparation of samples (APHA 2001):

Tissue samples:

Twenty five grams of the examined samples from breast and thigh of the broiler chicken were aseptically transferred to polyethylene bags, to which 225 ml of 0.1% of sterilized buffered peptone water (0.1%) were aseptically added to the content of the bag. Each sample was then homogenized for 2 minutes at 2500 r.p.m using a sterile homogenizer to provide a homogenate of 1/10 dilution. The mixture was allowed to stand for 15 minutes at room temperature then one ml from the original dilution was transferred by means of sterile pipette to another sterile tube containing 9 ml of sterile peptone water (1%) from which further serial decimal dilution were prepared.

Swabs:

The collected swabs were separately mixed in 10 ml of sterile buffered peptone water 0.1% to give 1/10 dilution 1ml from the original dilution was transferred with sterile pipette to another sterile test tube containing nine millimeter of buffered peptone water and mixed well to make the next dilution from which further decimal serial dilution were prepared. The prepared samples and swabs were subjected to the following bacteriological examination.

2.3. Determination of Staphylococci counts ICMSF (1996):

Accurately, 0.1 ml from each of previously prepared serial dilutions was spread over duplicated plates of Baired- parker agar using a sterile glass spreader. The inoculated and control plates were incubated at 37^{o}c for 48 hours. The developed (black) colonies were enumerated and the total *Staphylococci* count was calculated, also the colonies were picked up and purified on nutrient agar slops for further identification**.**

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2.4. Identification of suspected Staph. aureus:

A loopful from colonies grows on mannitol a dry surface of Baired parker agar plates in duplicate. The inoculated and control plates were inverted and incubated at 37^{o}C for 48 hrs. black and shiny convex colonies, 1-1.5 mm in diameter with narrow white margin and surrounded by clear halo zone extended into opaque medium were picked up and cultured on nutrient slope agar for further morphological and biochemical identification:

- Grams staining: according to (Cruickshank et al., 1975)

-Detection of hemolysis: according to (Bailey and Scott, 1978)

- Motility test: according to (ICMSF, 1996)
- Biochemical identification: according to Quinn et al., (2002).

2.5. Detection of Toxin producing genes in isolated Staph. aureus strains using Multiplex PCR:

Using material for PCR which contains reagents as agarose powder, Trisacetate EDTA and Ethedium bromide solution. It also contains Gel loading buffer, DNA ladder, 5X *Taq* master and Primer sequences of *S.aureus.*

DNA Extraction using QIA amp kit (Shah et al., 2009):

DNA amplification:

Amplification of enterotoxin genes of Staph. aureus (Mehrotra et al., 2000):

Ten µl of DNA sample was diluted in 990 µl of nuclease free water for PCR. The genomic DNA samples were amplified by PCR in a reaction mixture (25µl) containing 13.25 sterile dH_2O, 2.5ml 10 x buffer, 0.63ml 10mMNTPs, 1ml 25Mm $Mgcl_2$, 1.25 µl primer F(20pmol/ml) , 1.25 µl primer R (20pmol/ml) and fill up to 25 µl PCR grade water. Concerning the primers used for demonstration of S. aureus enterotoxins (sea, seb, sec, sed&see), the amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany).

DNA amplification was performed using the following conditions:

Initial denaturation for 5 min at 95°C followed by 30 cycles of denaturation (94°C for 2 min), annealing (55°C for 1 min), and extension (72°C for 2 min).A final extension step (72 °C for 5 min) was performed after the completion of the cycles. Amplified products were analyzed by 3% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBEbuffer stained with Ethedium bromide and captured as well as visualized on UV transilluminator at 254 nm. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes.

2.6. Statistical Analysis:

The obtained results were statically evaluated by application of analysis of variance (ANOVA) test according to Feldman *e*t al. (2003).

3. RESULTS

Prevalence of Staphylococcus and S.aureus in examined samples:

In table (2) *Staphylococcal* count (cfu/cm^2) of the tested swabs samples obtained from knives ranged from 9.50×10^5 to $1.05\times10^9$with percentage of 80% but for swabs from carcasses after de-feathering it ranged from $7.1\times10^5$to 1.02×10^8 with percentage of 85%while for swabs from carcasses surfaces after evisceration, the count ranged from 8.5×10^4 to $5.50\times10^8$with percentage of 85%. The count/g ranged from 6.40×10^3 to $1.45\times10^7$for breast samples with percentage of 70% while for thigh samples; the count was ranged from 5.60×10^3 to 8.90×10^6 with percentage of80% with highly significant differences ($p<0.01$).

Table (3) indicated that *S. aureus* count (CFU/cm^2) of the tested swabs samples obtained from knives ranged from $3.9\times10^5$to 6.30×10^8 with percentage of 80% but for swabs from carcasses after de-feathering it ranged from 3.5×10^5 to 5.40×10^7 with percentage of85% while for swabs from carcasses surfaces after evisceration the count ranged from 4.3×10^4 to 2.50×10^8 with percentage of 85%. The count/granged from 2.5×10^3 to 4.20×10^6 with percentage of 55% for breast samples while for thigh samples; the count/g ranged from 2.5×10^3 to 3.10×10^6 with percentage of 55% having highly significant differences ($p<0.01$).

S.aureus enterotoxins in examined samples:

Staph. aureus enterotoxins in chicken samples of type A, C, A+B and B+D. Type A **(***sea***)** is found with percentage of 12.5% of examined samples (two samples) but type c **(***sec***)** found with percentage of 6.25% of examined samples (one sample) also, type A+B **(***sea) + (seb)* found with percentage of 6.25% (One sample) and in finally type B+D (*seb) + (sed)* found with percentage of 6.25% (One sample). On the other hand there are about 11 samples with percentage of 68.75% without any enterotoxins secretion as in table (4) and photo. (1).

Table 1: Primer sequences of *S. aureus.*

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size	Reference
sea (F)	5′ TTGGAAACGGTTAAAACGAA′3	120	*Rall et al. (2008)*
sea (R)	5′ GAACCTTCCCATCAAAAACA ′3		
seb (F)	5′ TCGCATCAAACTGACAAACG ′3	478	
seb (R)	5′ GCGGTACTCTATAAGTGCC ′3		
sec (F)	5′ GACATAAAAGCTAGGAATTT ′3	257	
sec (R)	5′ AAATCGGATTAACATTATCC ′3		
sed (F)	5′ CTAGTTTGGTAATATCTCCT ′3	317	
sed (R)	5′ TAATGCTATATCTTATAGGG ′3		

Table 2: Statistical analytical results of total Staphylococcus counts in different samples (n=20).

Sample	+ve samples		Min.	Max.	Mean ± S.E.M
	NO.	%			
Swab from knives/cm^2	17	85	9.50×10^5	1.05×10^9	$2.62\times10^{8\,**} \pm 9.04\times10^7$
Swab after defeathering/cm^2	17	85	7.1×10^5	1.02×10^8	$2.42\times10^{7\,**} \pm 8.01\times10^6$
Swab after evisceration/cm^2	17	85	8.5×10^4	5.50×10^8	$3.63\times10^{7**} \pm 2.74\times10^7$
Breast/g	14	70	6.40×10^3	1.45×10^7	$1.42\times10^{6\,**} \pm 8.19\times10^5$
Thigh/g	16	80	5.60×10^3	8.90×10^6	$1.24\times10^{6\,**} \pm 5.88\times10^5$

No. of samples = 20, S.E.M. = standard error of mean. ** High significant difference ($p < 0.01$)

Table 3: Statistical analytical results of *Staph. aureus* counts in different samples (n=20).

Sample	+ve samples		Min.	Max.	Mean ± S.E.M
	NO.	%			
Swab from knives/cm^2	16	80	3.9×10^5	6.30×10^8	$1.29\times10^{8**} \pm 4.75\times10^7$
Swab after defeathering/cm^2	17	85	3.5×10^5	5.40×10^7	$1.24\times10^{7**} \pm 4.22\times10^6$
Swab after evisceration/cm^2	17	85	4.3×10^4	2.50×10^8	$1.68\times10^{7**} \pm 1.24\times10^7$
Breast/g	11	55	2.5×10^3	4.20×10^6	$2.92\times10^{5**} \pm 2.10\times10^5$
Thigh/g	11	55	2.5×10^3	3.10×10^6	$1.91\times10^{5**} \pm 1.54\times10^5$

Table 4: Occurrence of enterotoxin genes of *Staph. aureus* strains isolated from examined samples.

Staph.aureus enterotoxins	Number	Percentage
A	2	12.50%
C	1	6.25%
A + B	1	6.25%
B + D	1	6.25%
− ve	11	68.75%
Total	16	100%

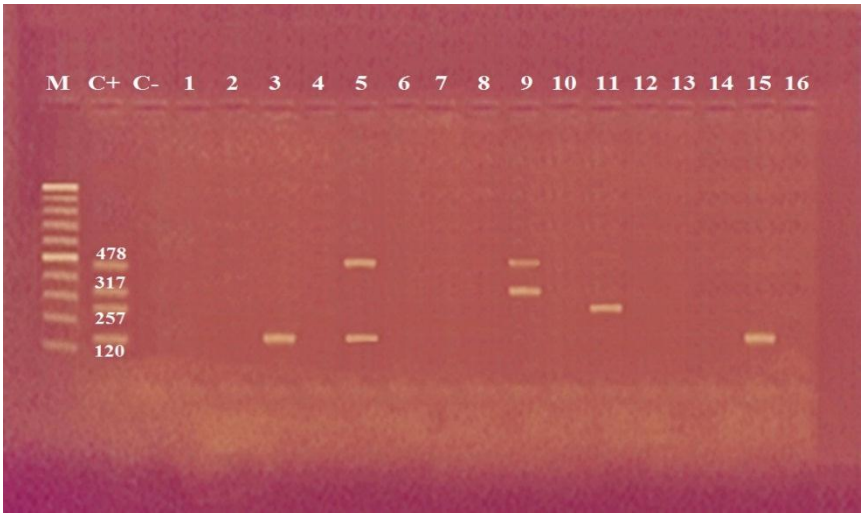

Fig.1. Agarose gel electrophoresis of multiplex PCR of sea (120 bp), seb (478 bp), sec (257 bp) and sed (317 bp) enterotoxin genes for characterization of S.aureus.

Lane M: 100 bp ladder as molecular size DNA marker.

Lane C+: Control positive for for sea, seb, sec and sed genes.

Lane C-: Control negative.

Lanes 3 & 15: Positive S.aureus strains for sea gene.

Lane 11: Positive S.aureus strain for sec gene.

Lane 5: Positive S.aureus strain for sea and seb genes.

Lane 9: Positive S.aureus strain for seb and sed genes.

Lanes 1, 2, 4, 6, 7, 8, 10, 12, 13, 14 & 16: Negative *S. aureus* for enterotoxins.

4. DISCUSSION

Mean value of total staphylococcal count (CFU/cm^2) of the tested swab samples obtained from knives was 2.62×10^8 but for swabs from carcasses surfaces after de-feathering it was 2.42×10^7 while for swabs from carcasses after evisceration it was 3.63×10^7.The mean count/g was $1.42\times 10^6$for breast samples while for thigh samples it was 1.24×10^6CFU/gas these results is higher than results obtained by Anower *e*t al. (2004) who

isolated staph in mean count of 2.09×10^3 CFU/g for breast and 4.57×10^3CFU/g for thigh. Vaidya *e*t al. (2008) recorded count of 2×10^4CFU/cm^{2}after evisceration. Also Goksoy *e*t al. (2004) found that *Staphylococci* can be isolated from two processing plants in mean count of 6.03×10^4CFU/cm^2 after de-feathering and 5.1×10^4CFU/cm^{2}after evisceration in first plant and 5.9×10^4 CFU/cm^{2}after de-feathering and 3.6×10^4CFU/cm^{2}after evisceration in second plant but Ruban and nadeem (2011) isolated it with mean count of 4.59×10^3 CFU/g in breast and 7.73×10^3CFU/gin thigh.

For percentage of *Staphylococcus,* it were 85% in swabs from knives, after de-feathering and after evisceration and 70%, 80% for samples of breast and thigh respectively. Anower *e*t al. (2004) isolated *Staphylococcus* in 96% of samples. Ruban *e*t al. (2012) found *Staphylococcus* in 88.57% in breast 91.43% in thigh. Results were more than that recorded by Helmy-salwa *e*t al. (2015).

Staphylococcus aureus mean count (CFU/cm^2) of the tested swabs samples obtained from knives was 1.29×10^8 but for swabs from carcasses after de-feathering, it was 1.24×10^7 while for swabs from carcasses after evisceration 1.68×10^7. It was 2.92×10^5 CFU/g or breast samples while for thigh samples it was1.91×10^5CFU/g. The result is higher than that recorded by Mead *e*t al. (1992) who found that *S. aureus* after de-feathering in five slaughterhouse with mean count of 0.6×10^3CFU/cm^2, 0.4×10^3CFU/cm^2, 0.2×10^3CFU/cm^2, 0.3×10^3CFU/cm^2 and 0.8×10^3CFU/cm^{2}alsoAbu-ruwaida *e*t al. (1994) isolated *S. aureus* in 2 abattoirs after de-feathering with mean of $.8\times10^3$CFU/cm^{2}for the first abattoir and1.6×10^4CFU/cm^{2}for the second one. Holder *e*t al. (1997) isolated *S. aureus* from thigh samples in mean of 1.5×10^3 CFU/g and 0.5×10^3CFU/g for breast samples. Khalafalla *e*t al. (2015) recorded that *S. aureus* count was 2×10^5CFU/g for fresh breast and 3×10^5CFU/g for thigh.

Staphylococcus was found in all tested samples in percentage of 80%, 85%, and 85% in swabs from knives, after de-feathering and after evisceration and it was 55% for samples of breast and thigh. These results are more than that isolated by Mohamed-eman (1998) who isolated *Staph. aureus* from swabs after de-feathering and after evisceration with a percentage of 62.85%.Guerguebet al. (2014) isolated *Staph. aureus* in 46.66 %. Helmy-salwa *e*t al. (2015) isolated *S. aureus* in percentage of 63.04%, but these results were less than those obtained by Owuna et al. (2015) which were100%.

Table (4) discussed the *Staph. aureus* enterotoxins in chicken samples of type A, C, A+B and B+D. Type A was (12.5%), type cwas (6.25%) also, type A+B(6.25%) and in finally type B+D (6.25%). On the other hand there are about 68.75% without any enterotoxins secretion. The results were differ than that obtained by Harvey *e*t al. (1982) who isolated *Staph. aureus* enterotoxins and found that enterotoxin type A was 2.74%, enterotoxin type D was 14.81% and *Staphylococcal* enterotoxin C+D was 1.23%. Britta *e*t al. (2016) isolated *Staph. aureus* enterotoxins and found that enterotoxin type A was 3.1%, enterotoxin type G was 25%, enterotoxin type I was 25%, enterotoxin type M was 25%, enterotoxin type N was 25%, enterotoxin type O was 25%, enterotoxin type U was 25%.

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