

Studies on bacteriological profile of chicken meat cuts in Kaliobia governorate

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A B S T R A C T

The study was performed on 120 random samples of fresh chicken meat cuts of Drumstick, thigh and breast (40 of each) purchased from different supermarkets and retail chicken butchers in Kaliobia governorate to evaluate their bacterial contamination. The bacteriological examination revealed that the mean values of APC, *Staphylococci aureus* counts (cuf/g) in drumstick samples were $7.47 \times 10^{4} \pm 0.31 \times 10^{4}$; $2.31 \times 10^{3} \pm 0.11 \times 10^{3} \& 0.26 \times 10^{3} \pm 0.07 \times 10^{3}$, $6.51 \times 10^{4} \pm 0.30 \times 10^{4}$; $2.21 \times 10^{3} \pm 0.11 \times 10^{3}$ in thigh $\& 0.22 \times 10^{3} \pm 0.07 \times 10^{3}$, and $6.13 \times 10^{4} \pm 0.32 \times 10^{4}$; $1.99 \times 10^{3} \pm 0.09 \times 10^{3} \& 0.19 \times 10^{3} \pm 0.06 \times 10^{3}$ in breast samples, respectively. Coagulase positive *aureus* strains were isolated from the examined fresh chicken meat cuts represented as 32.5% in drumstick 27.5% in thigh and 22.5% in breast samples. The isolated strains were highly resistant for methicillin followed by oxacillin; ampicillin; cefotaxime and amoxicillin. Meanwhile, they were highly sensitive to ciprofloxacin followed by enrofloxacin and gentamycin.

Keywords: Chicken meat, Bacteriological evaluation, S. aureus

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1. INTRODUCTION

Chicken meat is a nutritious, healthy food that is low in fat and cholesterol compared to other meats as well as it is an excellent source of protein. On the other hand, it is an ideal medium for bacterial growth because of high moisture content, richness in nitrogenous compounds (essential amino acids, proteins), good source of minerals, vitamins and other growth factors. Furthermore, its pH is favorable for the growth of microorganisms.

Both poultry muscle and skin are excellent substrates for supporting the growth of a wide variety of microorganisms, especially, Staphylococci mainly *S. aureus* that considered as one of the most important causes of foodborne outbreaks in people (Prange et al., 2005 and Bhaisare et al., 2014). Chicken meat may be contaminated at any stage of the production process, from feather plucking, evisceration and washing until storage by chilling or freezing. Microorganisms from the environment, equipment and operator's hands can also contaminate poultry meat possible at any stage of the production process, from feather plucking, evisceration and washing until storage by cooling or freezing. Microorganisms from the environment, equipment and operator's hands can contaminate poultry meat (Živković, 2001).

S. aureus is considered an excellent indicator of thermal processing inefficiency, inadequate hygienic conditions during food production/ preparation or inadequate cooling after food preparation (Malheiros et al., 2010;

Alexandra et al., 2011 and Sasidharan et al., 2011). S. aureus produces a wide variety of enterotoxins (SEs; SEA to SEE, SEG, SEI, SER, SET) with demonstrated emetic activity (María et al., 2010). The Staphylococcal enterotoxins (SEs) are responsible for the symptoms that associated with Staphylococcal food poisoning (Llewelyn and Cohen, 2002). Such poisoning is characterized by rapid onset symptoms including nausea, violent vomiting, abdominal cramps and diarrhea lasting from 24 to 48 h and the complete recovery usually occurs within 1-3 days. The illness is usually self-limiting and only occasionally it is severe enough to warrant hospitalization. Moreover, sea is the most common enterotoxin recovered from food poisoning outbreaks (Pinchuk et al., 2010; María et al., 2010 and Shijia et al., 2016). Therefore, this study was performed for detection the prevalence of Staphylococci with special reference to coagulase positive S. aureus beside the phenotypic characterization of the isolated aureus strains and detection S. of sensitivity against various antibiotics.

2. MATERIAL AND METHODS

2.1. Samples collection

A total of 120 random samples (about 250 g for each) of fresh chicken meat cuts represented by Drumstick, thigh and breast (40 of each) purchased from different supermarkets and retail chicken butchers in Kaliobia governorate for bacteriological examination.

2.2. Bacteriological examination

1. Preparation of samples (APHA, 2001)

Twenty five grams of the sample were taken under aseptic condition to sterile Stomacher bag then 225 ml sterile 0.1% peptone water were added. The contents were homogenized at Stomacher (M A 106402France,450 to 640 strokes per minute) for 2 minutes and the mixture was allowed to stand for 5 minutes at room temperature. The contents were transferred into sterile flask and thoroughly mixed by shaking and 1 ml was transferred into separate tube each containing 9 ml sterile 0.1% peptone water, from which tenth- fold serial dilutions were prepared. The prepared samples were subjected to the following bacteriological examination:

2. Determination of Aerobic Plate Count (APC) using the standard plate count following (FDA, 2001).

3. Determination of Staphylococcus and *S. aureus* counts (FDA, 2001)

4. Isolation and identification of suspected *S. aureus*:

Isolation of *S. aureus* was done using Baird-Parker Agar Plates. Suspected colonies were picked up onto slants of nutrient agar for further purification then identified morphologically by Gram-stain; biochemically and coagulase activities according to ICMSF (1996) and Quinn et al., (2002).

5. In-Vitro anti-microbial sensitivity test:

The isolated *S. aureus* strains were subjected to the sensitivity test against different antibiotics, using the disc and agar diffusion method (Koneman et al., 1997).

3. RESULTS

It is evident from the result recorded in table (1) that the mean values of APC (cfu/g) in the examined samples of fresh chicken meat cuts (drumstick, thigh and breast) were $7.47 \times 10^{-4} \pm 0.31 \times 10^{4}$; $6.51 \times 10^{4} \pm 0.30 \times 10^{4}$ and $6.13 \times 10^{4} \pm 0.32 \times 10^{4}$ respectively.

However, All samples were acceptable, as these counts were lower than those suggested by EOS (4178 /2005)(10 cfu/g). Moreover, the statistical results revealed that, fresh drumstick samples showed a significant (P \leq 0.05) increase of APC when compared with other ones. Moreover, there were no significant difference (P>0.05) of APC between fresh thigh and breast samples.

The obtained results in Table (2) revealed that, the average Staphylococcus count(cfu/g) in the drumstick, thigh and breast samples were $2.31 \times 10^{-3} \pm 0.11 \times 10^{-3}$;

 $2.21 \times 10^3 \pm 0.11 \times 10^3$ and $1.99 \times 10^3 \pm 0.09 \times 10^3$, respectively. Moreover, the statistical results revealed that, fresh drumstick samples showed a significant (P ≤ 0.05) increase of Staphylococci counts when compared with other ones. However, there were no significant difference (P>0.05) of Staphylococci counts between fresh breast and fresh thigh samples.

The recorded results in Table (3)declared that the mean value of *S.aureus* count (cfu/g) in the examined fresh drumstick, thigh and breast samples were $0.26 \times 10^3 \pm 0.07 \times 10^3$; $0.22 \times 10^3 \pm 0.07 \times 10^3$ and $0.19 \times 10^3 \pm 0.06 \times 10^3$, respectively. The incidence of *S.aureus* were 13(32.5%) in drumstick, 11(27.5%) in thigh and 9(22.5%) in breast samples.

According to safe permissible limits stipulated by EOS (1090/2005) for the *S. aureus* count (free). All samples were unaccepted. Moreover, there was no significant difference of *S. aureus* counts between examined samples of fresh drumstick, thigh and breast samples.

The results obtained in Table (4) illustrated that. 33 isolates of Coagulase positive S.aureus were isolated from examined fresh chicken meat cuts (drumstick, thigh breast samples represented and as 13(32.5%) from drumstick samples; 11(27.5%) from thigh samples and 9(22.5%) from breast samples. Moreover, 72.5% of samples were accepted (Fig. 5), as they were free from Coagulase Positive S.aureus isolates according to EOS (1090. 2005).

Results in (Table, 6) showed that, the isolated S.aureus were highly resistant for methicillin (87.9%) followed by oxacillin (84.9%); ampicillin (66.7%); cefotaxime (63.6%)and amoxicillin (60.6%). Meanwhile, the isolated S. aureus strains were highly sensitive to ciprofloxacin (84.9%) followed by enrofloxacin (81.8%) and gentamycin (78.8%). Moreover, they were intermediate sensitive to sulfatrimethoprim (69.7%); neomycin (66.7%); erythromycin (60.6%) and streptomycin (57.6%).

Table (1): Mean values of Aerobic plate counts. (cfu/g) in the examined samples of fresh chicken meat cuts (n=40)

Sample	Min.	Max.	Mean ±SEM**		
Drumstick	2.2×10^4	1.05×10^{5}	$7.47 \times 10^{4} \pm 0.31 \times 10^{4a}$		
Thigh	2.4×10^4	9.5×10^{4}	$6.51{\times}10^4 \pm 0.30{\times}10^{4b}$		
Breast	1.6×10^4	9.4×10^4	$6.13 {\times} 10^4 {\pm} 0.32 {\times} 10^{4b}$		

* Percentage in relation to total number of sample in each row.**Standard error of mean Significant difference of fresh drumstick samples ($P \le 0.05$). No significant difference between fresh thigh and breast samples (P > 0.05).

Table (2): Mean values of Staphylococci counts(cfu/g) in the examined samples of fresh chicken meat cuts (n=40)

Samples	Min.	Max.	Mean ±SEM**
Drumstick	1.5×10^{3}	4.1×10^{3}	2.31×10 ³ ±0.11×10 ^{3a}
Thigh	1.0×10^{3}	4.1×10^{3}	$2.21 \times 10^3 \pm 0.11 \times 10^{3ab}$
Breast	0.9×10^{3}	3.6×10^3	$1.99 \times 10^3 \pm 0.09 \times 10^{3b}$

* Percentage in relation to total number of sample in each row.**Standard error of mean Significant difference of fresh drumstick samples ($P \le 0.05$). No significant difference between fresh thigh and breast samples (P > 0.05).

Samples	Negative		Positive		Min.	Max.	Mean ±SEM**
	No.	%*	No.	%*			
Drumstick	27	67.5	13	32.5	$< 1 \times 10^{2}$	1.5×10^{3}	$0.26 \times 10^3 \pm 0.07 \times 10^{3a}$
Thigh	29	72.5	11	27.5	$< 1 \times 10^{2}$	1.5×10^{3}	$0.22{\times}10^3{\pm}0.07{\times}10^{3a}$
Breast	31	77.5	9	22.5	$< 1 \times 10^{2}$	1.3×10^{3}	$0.19{\times}10^3{\pm}0.06{\times}10^{3a}$

Table (3): Mean values of *S. aureus* counts (cfu/g) in the examined samples of fresh chicken meat cuts (n=40)

* Percentage in relation to total number of sample in each row.**Standard error of mean Significant difference of fresh drumstick samples ($P \le 0.05$). No significant difference between fresh thigh and breast samples (P > 0.05).

Table (4): Acceptability of Coagulase Positive *S. aureus* examined samples of fresh chicken meat cuts (n=40)

	Positive		No. of accepted			
Samples	No.	%*	samples**	% of accepted samples**		
Drumstick	13	32.5	27	32.5		
Thigh	11	27.5	29	27.5		
Breast	9	22.5	31	22.5		
Total	33	27.5	87	27.5		

* Percentage in relation to total number of sample in each row, EOS (4178, 2005).

Table (5): Anti-microbial Sensitivity for isolated *S.aureus* strains

Antimicrobial agents	Disk	Sensitive		Intermediate		Resistant		AA	
Antimicrobial agents	Concentrations	No.	%	No.	%	No.	%	ΠΠ	
Amoxicillin	25µg	5	15.2	8	24.2	20	60.6	R	
Ampicillin	20µg	11	33.3	0	0.0	22	66.7	R	
Cefotaxime	30µg	3	9.1	9	27.3	21	63.6	R	
Ciprofloxacin	5 µg	28	84.9	4	12.1	1	3.0	S	
Enrofloxacin	5 µg	27	81.8	4	12.1	2	6.1	S	
Erythromycin	15 µg	5	15.2	20	60.6	8	24.2	IS	
Gentamicin	10 µg	26	78.8	4	12.1	3	9.1	S	
Methicillin	5 µg	1	3.0	3	9.1	29	87.9	R	
Neomycin	30 µg	4	12.1	22	66.7	7	21.2	IS	
Oxacillin	1µg	1	3.0	4	12.1	28	84.9	R	
Streptomycin	S/10	4	12.1	19	57.6	10	30.3	IS	
Sulfa-trimethoprim	TMP5	6	18.2	23	69.7	4	12.1	IS	

No.: Number of isolates %: Percentage in relation to total number of isolates (33).AA: Antibiogram activity, R: Resistant, S: Sensitive, I: Intermediate.

4. DISCUSSION

It is evident from the result recorded in table (1) that the total APC in examined samples nearly similar to that obtained by Marwan- Heba (2016) who revealed that, the mean value of APC/gfor fresh chicken meat were $(7.50 \times 10^{4} \pm 0.22 \times 10^{4})$ While, the results disagreed with those of Mohamed (2016) who recorded higher

count $(3.78 \times 10^6 \pm 0.93 \times 10^6)$ Also, disagreed with Daoud (2012) who obtained lower counts were (2.1×10^3) .

The higher aerobic plate count in chicken meat was due to slaughtering and sale of chicken meat in the same place, which provokes the cross contamination of the carcasses. Moreover, the carcasses are kept at ambient temperature, which allows the multiplication of mesophilic microorganisms Moreover, the chopping tables which manufactured from wood were found to be same every day without proper cleanliness. This enhanced the chance of cross contamination for uninfected carcass. As well as the processing of carcass into parts lead to further spread of contamination by exposing more carcass surface and susceptible fleshy parts to the contaminants if the same cutting tables and knives are used (Satin, 2002).

Table (2) indicated that the total Staphylococcus count were nearly similar to results that obtained by Marwan-Heba (2016) who found that the mean value of total Staphylococcus is $(1.73 \times 10^4 \pm 0.09)$ $\times 10^4$). Meanwhile, the results disagreed with those of Saif-Marwa (2015) and Mohamed(2016)who reported higher Staphylococcus counts in examined 2.50×10^4 + samples (6.88±0.01 and 0.93×10^3) cfu/g respectively. The increase of total Staphylococcal count indicates poor hygienic condition. In addition, it can occur at multiple steps along the food chain. which includes production, processing, distribution, retail marketing, handling and preparation.

The presence of *S. aureus* in foods commonly indicates direct contamination from worker's hands with abrasion and wounds or inadequately cleaned equipment resulting in *S.aureus* intoxication. Accordingly, *S.aureus* count can be taken as an index of sanitary conditions under which the meat and its products are manufactured and handled (potter, 2001).

It is obvious from results obtained in table (4) that total *S.aureus* counts were nearly similar to that recorded by Shareef et al. (2012) who reported that, *S.aureus* count was (1.61±0.16 log₁₀cfu/cm²) Meanwhile, the results disagreed with those of Saif-Marwa (2015) and Mohamed (2016) who reported higher *S. aureus* counts in examined samples of thigh and breast were $(1.14 \times 10^4 \pm 0.85 \times 10^3 \text{cfu/g} \text{ and } 1.12 \times 10^4 \pm 0.83 \times 10^3 \text{cfu/g})$ respectively.

Most previous studies were interested with coagulase Positive *S.aureus* strains of Staphylococci species as it is still a major cause of food poisoning due to ingestion of enterotoxin (Le Loir, 2003) and the ability to produce such enterotoxin in food is more likely when competing microorganisms were absent, resulting in symptoms of intoxication, not an infection common symptoms and appear approximately 3-8 hr. after ingestion as nausea, vomiting, abdominal cramps and diarrhea. Generally, symptoms are short in duration, "approximately 24-48hrs (Sandle and Mckillip, 2004).

It declared that results in table (4) of coagulase positive S.aureus came in accordance with those obtained by Shareef et al. (2012); and Marwan- Heba (2016). Meanwhile, these results were disagreed Abo-Samra with those of (2013): Abdalrahman et al., (2015); Ahmed (2015) and Moustafa et al. (2016) who isolated S. aureus from chicken meat samples with higher incidence and also, disagreed with Abd El- Fattah- Shereen (2014) who isolated S. aureus from chicken meat samples with lower incidence.

The presence of coagulase Positive *S. aureus* in poultry meat and its products indicates poor hygiene of meat handlers as well as lack of sterilization of utensils and they grow without pronounced change in odour or taste in the products and producing heat stable enterotoxins which lead to food poisoning with severe diarrhoea and gastroenteritis among consumers (Le Loir, 2003and Bakr et al 2004).

The widespread use of antibiotics has undoubtedly accelerated the virulence of *S. aureus*, by acquiring multiple resistance genes, has become able to survive almost all antibiotic families (Stefani and Goglio, 2010). Several workers have reported the occurrence of multidrug resistant *S.aureus* in poultry (Waters et al., 2011).

As shown in table (5) the antimicrobial sensitivity for isolated *S.aureus* strains were agreed with Akbar and Anal (2013); Abd El- Salam- Azza (2014); Ezzat et al. (2014); Abdalrahman et al.(2015) and Afifi-Dina (2016). In addition, the results

proved that multiple antibiotic resistances are widely spread among isolated strains of S.aureus. Meanwhile, the isolated S. aureus strains were highly sensitive to (84.9%) ciprofloxacin followed bv enrofloxacin (81.8%) and gentamycin (78.8%). Moreover, they were intermediate sensitive to sulfa-trimethoprim (69.7%); neomycin (66.7%); erythromycin (60.6%) and streptomycin (57.6%). These results were agreed with Hansons et al.(2011); Waters et al.(2011); Al-Ghamdi (2012); Enany et al.(2013);; Abdalrahman et al.(2015) and Afifi-Dina (2016).

Finally, the results proved that multiple antibiotic resistances are widely spread among S.aureus isolated strains and decided the fact of Shalini and Rameshwar (2005) that the food chain can be considered the main as route of transmission of antibiotic resistant bacteria between the animal and human populations. Moreover, the present study proved that fresh chicken meat cuts are considered public health hazard and the of presence aerobic bacteria and Staphylococci mainly coagulase Positive S. aureus may be due to mishandling and the negligence of hygienic aspects.

CONCLUSION

we found that drum stick samples were more contaminated part than thigh and breast samples due to direct contamination from worker's hands with abrasion and wounds or inadequately cleaned equipment resulting in *S.aureus* intoxication .Accordingly, *S.aureus* count can be taken as an index of sanitary conditions under which the meat and its products are manufactured and handled.

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