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Assessment of some pathogens in retailed poultry meat

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ABSTRACT

The present study was performed on 150 random samples of Fresh, half cooked (nuggets) and cooked (grilled) chicken meat (50 of each) were purchased from retail chicken butchers, different supermarkets and restaurants in Kaliobia Governorate, Egypt, to evaluate their bacterial quality and safety. Bacteriological examination of chicken meat samples revealed that the mean values of APC, Enterobacteriaceae, Coliform, Staphylococcus and *Staph. aureus* (cfu/g) were $9.88\times10^5\pm0.61\times10^5$, $3.36\times10^4\pm0.26\times10^4$, $1.43\times10^4\pm0.16\times10^4$, $2.71\times10^3\pm0.17\times10^3$ and $2.05\times10^3\pm0.24\times10^3$ in fresh, $5.48\times10^4\pm0.23\times10^4$, $3.15\times10^2\pm0.15\times10^2$, $1.00\times10^2\pm0.08\times10^2$, $1.73\times10^2\pm0.11\times10^2$ and $1.26\times10^2\pm0.19\times10^2$ in half cooked and $4.50\times10^4\pm0.22\times10^4$, $2.06\times10^2\pm0.13\times10^2$, $1.09\times10^2\pm0.10\times10^2$, $1.05\times10^2\pm0.09\times10^2$ and $1.02\times10^2\pm0.15\times10^2$ cooked chicken meat samples, respectively. Moreover, the incidence of *E. coli* was 14% in fresh O55: H7(6%), O125: H21(4%), O111: H2 (2%) and O146: H21(2%), 6% in half cooked O55: H7(2%), O125: H21(2%) and O111: H2(2%) and 6% in cooked chicken meat samples O55: H7(4%) and O111: H2(2%). Meanwhile, the incidence of Coagulase positive *Staph. aureus* were 38%, 26% and 20% in fresh, half cooked and cooked chicken meat samples. According to SET- RPLA test, seven strains out of twelve examined strains were enterotoxigenic. In addition, PCR showed that *Staph. aureus* enterotoxins *sea, seb, sec, sed, see* were detected in ten *S. aureus* isolate from samples.

1. INTRODUCTION

Poultry meat, mainly chicken broilers is one of the most popular food products worldwide because of its easy digestibility; acceptance by the majority of people and solve the problem of the shortage in fresh meat of high price that is not within the reach of large numbers of families with limited income (Biesalski, 2005; Sousa, 2008). Chicken meat often get contamination from different sources starting from defeathering, evisceration and subsequent processing plant (Houf *et al.,* 2002). The bacterial contamination and hygienic measures during poultry meat production can be measured using the aerobic plate count and three Gram - negative indicator groups of Enterobacteriaceae, Coliforms and *Escherichia coli* biotype 1, which is the most important indicator for faecal contamination (Paulsen *et al*., 2006). The most important bacterial pathogens in chicken meat that is responsible for food-borne infections include *E. coli, Salmonellae* and coagulase positive *S. aureus* (Bhaisare *et al*., 2014; Noori and Alwan, 2016). *E. coli* has become recognized as a serious food borne pathogen and has been associated with numerous outbreaks of disease resulting from contaminated meat products (Gi *et al*., 2009). Moreover, avian strains of *E. coli* show many similarities with human extra intestinal pathogenic *E. coli* (ExPEC) strains, in that most of the virulence genes they possess and it can be transferred to humans through consumption of contaminated food or food products causing a variety of infections, including bacteremia, urinary tract infections, neonatal meningitis, pneumonia, deep surgical wound infections, endovascular infections, vertebral osteomyelitis, and septicemia (Ewers *et al.*, 2007). *Staphylococcus aureus* is considered the third-most important cause of food-borne disease in the world (Normanno *et al*., 2007) and the isolates from chicken carcasses showed proteolytic and lipolytic activity at+20 °C, causing meat spoilage (Gundogan and Devren, 2005). This pathogen is considered an excellent indicator of thermal processing inefficiency, inadequate hygienic conditions during food production/preparation or inadequate cooling after food preparation and determined the origin of food poisoning (Alexandra *et al*., 2011; Sasidharan *et al*., 2011). They produce disease when the bacteria contaminate food, produce some enzymes which are implicated with Staphylococcus invasiveness and many extracellular substances some of which are heat stable enterotoxins that renders the food dangerous even though it appears normal and extensive cooking can be killed the bacteria but the toxins may not be destroyed because most of them are gene based i.e. they can be carried on the plasmid (Prescott *et al.,* 2005). Moreover, most Salmonella strains found on poultry meat are non-host-specific and are considered capable of causing human food poisoning. Salmonellosis (gastroenteritis) is the most common disease in human (Muth, 2009). Therefore, the present study was conducted to throw the light over the assessment of some

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pathogens and sanitary status of chicken meat (raw; half cooked and cooked) in Kaliobia Governorate, beside the phenotypic characterization of the isolate; determination of enterotoxin virulent genes in some isolated *S. aureus* strains by using PCR.

2. MATERIAL AND METHODS

2.1. Samples

Total of 150 random samples (about 250 g for each) of chicken meat of Fresh, half cooked (nuggets) and cooked (grilled) chicken meat (50 of each) were collected from retail chicken butchers, different supermarkets and restaurants in Kaliobia Governorate.

2.2. Preparation of samples (APHA,2001):

Twenty five grams of each sample under examination 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 for 2 minutes, the mixture was allowed to stand for 5 minutes at room temperature the contents were transferred into sterile flask and thoroughly mixed, 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:

2.3. Aerobic Plate Count (APC)/g, using the standard plate count following FDA (2001).

2.4. Enterobacteriaceae count by the surface plating method of ICMSF (1996) using Violet Red Bile Glucose agar medium (VRBG).

2.5. Coliform count by the surface plating method of ICMSF (1996) using Violet Red Bile agar medium.

2.6. Isolation and identification of E. coli following (ISO 2001).

2.7. Staphylococcus and S. aureus counts (FDA, 2001).

2.8. Isolation and identification of S. aureus (Quinn et al., 2002)

2.9. Isolation and identification of Salmonella (ISO, 2002)

2.10. Detection of Enterotoxins producing S. aureus isolates

2.10.1. Detection by Reversed Passive Latex agglutination kit (SET-RPLA) test (Igarashi *et al.*, 1986).

2.10.2. Molecular detection of enterotoxins of some isolated S. aureus strains:

Genotyping detection of enterotoxins A (*sea*) gene, enterotoxins B (*seb*) gene, enterotoxins C (*sec*) gene enterotoxins D (*sed*) gene and enterotoxins E (*see*) gene in 10 random *S. aureus* strains using uniplex polymerase chain reaction, following QIAamp® DNA Mini Kit instructions (Qiagen, Germany, GmbH), Emerald Amp GT PCR mastermix (Takara, Japan) and 1.5% agarose gel electrophoreses (Sambrook *et al.,* 1989) using the Primers sequences, target genes, amplicons sizes and cycling conditions.

2.3. Statistical analysis

The obtained data were analyzed using the computer software program (SPSS, 2001).

3. RESULTS

It is evident from the result recorded in table (1) that the APC and Enterobacteriaceae counts (cfu/g) in the examined samples were $9.88\times10^5 \pm0.61\times10^5$ and $3.36\times10^4\pm0.26\times10^4$ in fresh chicken meat samples, $5.48\times10^4\pm0.23\times10^4$ and $3.15\times10^2\pm0.15\times10^2$ in half cooked and $4.50\times10^4\pm0.22\times10^4$ and $2.06\times10^2 \pm0.13\times10^2$ in cooked samples. While Coliforms, Staphylococci and *S. aureus* counts (cfu/g) were $1.43\times10^4\pm0.16\times10^4$, $2.71\times10^3\pm0.17\times10^3$ and $2.05\times10^3\pm 0.24\times10^3$ in fresh samples, $1.00\times10^2\pm0.08\times10^2$, $1.73\times10^2\pm 0.11\times10^2$ and $1.26\times10^2\pm0.19\times10^2$ in half cooked and $1.09\times10^2\pm0.10\times10^2$, $1.05\times10^2\pm0.09\times10^2$ and $1.02\times10^2\pm 0.15\times10^2$ in cooked samples, respectively.

From the previous results it's revealed that fresh samples showed a significant increase when compared with half cooked and fried ($P\leq0.05$). Moreover, half cooked showed a significant increase of Enterobacteriaceae and Staphylococci counts when compared with cooked samples. While there is no significant difference of APC, Coliforms and *S. aureus* counts between half cooked and cooked samples ($P>0.05$).

The results in table (1) also showed that the incidence of Enterobacteriaceae, Coliform, *E.coli*, Staphylococci and *S. aureus* were 100, 82, 14, 100, 38 %, respectively in fresh samples , 84, 72, 6, 78, 26 % in half cooked and 82, 32, 6, 72, 20 %, respectively in cooked samples.

Table (2) declared that the incidence and serotyping of Enteropathogenic *E. coli* isolated from fresh; half cooked and cooked chicken meat samples were O55: H7 (3, 1 and 2) respectively, O125: H21 (2 from fresh and 1 from each samples half cooked and cooked), O111: H2 (1 from each samples of fresh and half cooked samples only) and O146: H21 (1 from fresh samples only).

Results in table (3) revealed that the percentage of accepted samples for APC, Enterobacteriaceae, Coliform, *E.coli*, and Staphylococci counts were 100%, 0%, 18%, 86% and 0% in fresh samples, 100%, 100%, 100%, 94% and 100% in half cooked and cooked samples, respectively, according to the safe permissible limits stipulated by EOS (2005).

The results of SET -RPLA test in table (4) showed that, the incidence of *s. aureus* enterotoxigenic strains in 12 isolates were 25% of both of (A), (B),(C), and (A&C) in fresh, 25% of (A) and (D) in half cooked and 25% of (A) in cooked. 7 (58.3%) were enterotoxigenic strain.

As shown in table (5) PCR results showed that the incidences of *S. aureus* enterotoxins *sea, seb, sec, sed* and *see* were (25%, 25%, 25%, 25% and 50%) in fresh samples, (33,3%, 0%, 33,3%, 33,3% and 33,3%) in half cooked samples and (33,3%, 0%, 0%, 0% and 0%) in cooked samples respectively, With total no (3, 1, 2, 2 and 3) and incidence (30%, 10%, 20%, 20% and 30%) respectively. Salmonella serovars were failed to be detected in all examined samples of chicken meat.

4. DISCUSSION

Poultry meat mainly chicken is a common vehicle for pathogenic microorganisms. These pathogens are transmitted mainly through consumption of contaminated food and the presence of these organisms in meat has relevant public health implications (Bhaisare *et al*., 2014; Darshana *et al.,* 2014).

The data shown in table (1) revealed that, APC was higher in fresh samples than half cooked and cooked chicken meat samples. It may be due to insufficient heating or post-cooking contamination that may be due to lack of hygienic measure in handling of chicken meat.

According to our results of APC, nearly similar results in fresh samples were obtained by Mohamed (2016)

($3.78\times10^6 \pm 0.93\times10^6$), but higher than that reported by Daoud (2012) (2.1×10^3), lower than that reported by Vural *et al*. (2006) (1.48×10^7). The results in half cooked and cooked chicken meat samples were nearly similar to that recorded by Arab (2010) ($6.3\times10^4 \pm 0.35 \times10^4$), but higher than that reported by El-Taher (2009) ($9.05\times10^3 \pm 2.51 \times10^3$), meanwhile, they were lower than that recorded by Kirralla (2007) ($2.20\times10^6 \pm 2.12 \times10^5$).

Moreover, table (1) showed that Enterobacteriaceae was higher in fresh samples than half cooked and cooked samples. this may explain the fact that the GIT is common habitat of Enterobacteriacae and is considered the main source of contamination during slaughtering, dressing, evisceration, handling and transportation to butcher shops.

Enterobacteriaceae count in fresh samples nearly similar to that obtained by Marwan (2016) ($3.37\times10^4 \pm 0.23 \times10^4$), but higher than that reported by Ibrahim (2005) (6.72 ±1.09 x 10). Meanwhile, the results of half cooked and cooked chicken meat samples were agreed with those of Marwan (2016) ($0.92\times10^2 \pm 0.08 \times10^2$) but disagree with those of Abd El-Aal (2015) ($8.73\times10^3 \pm 1.96\times10^3$).

Results achieved in table (1) illustrated that Coliform count was higher in fresh samples than half cooked and cooked samples. This may be attributed to higher contamination from the ground and fecal matter.

Table 1 Mean values of microbial counts (cfu/g) and their Incidence in the examined chicken meat samples (n -50).

Isolates	Fresh samples			Half cooked samples			Cooked samples		
	+ve samples		Mean ±SE**	+ve samples		Mean ±SE**	+ve samples		Mean ±SE**
	No.	%*		No.	%*		No.	%*	
APC	__	__	$9.88\times10^5 \pm 0.61\times10^5$	__	__	$5.48\times10^4 \pm 0.23\times10^4$	__	__	$4.50\times10^4 \pm 0.22\times10^4$
Enterobacteriaceae	50	100	$3.36\times10^4 \pm 0.26 \times10^4$	42	84	$3.15\times10^2 \pm 0.15 \times10^2$	41	82	$2.06\times10^2 \pm 0.13\times10^2$
Coliform	41	82	$1.43\times10^4 \pm 0.16 \times10^4$	36	72	$1.00\times10^2 \pm 0.08 \times10^2$	16	32	$1.09\times10^2 \pm 0.10 \times10^2$
Staphylococci	50	100	$2.71\times10^3 \pm 0.17 \times10^3$	39	78	$1.73\times10^2 \pm 0.11 \times10^2$	36	72	$1.05\times10^2 \pm 0.09 \times10^2$
Staph. Aureus	19	38	$2.05\times10^3 \pm 0.24 \times10^3$	13	26	$1.26\times10^2 \pm 0.19 \times10^2$	10	20	$1.02\times10^2 \pm 0.15 \times10^2$
Coagulase Positive *S. aureus*	19	38	__	13	26	__	10	20	__

* Percentage in relation to total number of samples in each row (50). **Standard error

Table 2 Incidence and serotyping of *E. coli* isolated from positive samples of chicken meat (n=50 of each)

E. coli serotype	Fresh chicken meat		Half cooked chicken meat		Cooked chicken meat		Strain characteristic
	No.	%*	No.	%*	No.	%*	
O55 : H7	3	6.0	1	2.0	2	4.0	EPEC
O111 : H2	1	2.0	1	2.0	0	0.0	EHEC
O125 : H21	2	4.0	1	2.0	1	2.0	ETEC
O146 : H21	1	2.0	0	0.0	0	0.0	EPEC
Total	7	14.0	3	6.0	3	6.0	-

* % was calculated in relation to total number of each sample (50). EPEC: Enteropathogenic *E. coli.* ETEC: Enterotoxigenic *E. coli.* EHEC= Enterohaemorrhagic *E. coli*

Table 3 Acceptability of examined chicken meat samples according to EOS (2005). (n=50) for each.

	Accepted Samples								
	Fresh chicken meat			Half cooked chicken meat			Cooked chicken meat		
	P.L/g*	No.	%*	P.L/g*	No.	%*	P.L/g*	No.	%*
APC	$\geq 10^5$	50	100	$\geq 10^4$	50	100	$\geq 10^4$	50	100
Enterobacteriaceae	$\leq 10^2$	0	0	$\leq 10^2$	50	100	$\leq 10^2$	50	100
Coliform	$\leq 10^2$	9	18	$\leq 10^2$	50	100	$\leq 10^2$	50	100
e. coli	$\leq 10^2$	43	86	$\leq 10^2$	47	94	$\leq 10^2$	47	94
Staphylococci	$\leq 10^2$	0	0	$\leq 10^2$	50	100	$\leq 10^2$	50	100

* EOS (2005)

Table 4 The incidence of enterotoxins production from (12) *S. aureus* isolates 4 from each according to SET -RPLA test

Samples	A		B		C		D		A&C	
	No.	%	No.	%	No.	%	No.	%	No.	%
Fresh	1	25	1	25	1	25	__	__	1	25
Half cooked	1	25	__	__	__	__	1	25	__	__
Cooked	1	25	__	__	__	__	__	__	__	__
Total no.	3		1		1		1		1	
%	25		8.3		8.3		8.3		8.3	

Table 5 The results of PCR amplifications of different used enterotoxins genes of *S. aureus.*

Samples	No.	*Sea*		*Seb*		*Sec*		*Sed*		*See*	
		No.	%	No.	%	No.	%	No.	%	No.	%
Fresh	4	1	25	1	25	1	25	1	25	2	50
Half cooked	3	1	33.3	__	__	1	33.3	1	33.3	1	33.3
Cooked	3	1	33.3	__	__	__	__	__	__	__	__
Total no.		3		1		2		2		3	
%		30		10		20		20		30	

sea (enterotoxin A). *seb* (enterotoxin B). *sec* (enterotoxin C). *sed* (enterotoxin D. *see* (enterotoxin E)

Coliform counts were low in half cooked and fried chicken meat, this may be due to the hygienic preparation and the attained temperature for both cooking and frying was sufficient to kill vegetative bacteria on the surface of meat.

Coliforms count in fresh samples came in parallel with those of Marwan (2016) ($1.35\times10^4\pm0.08\times10^4$) but disagree with that reported by Hassan (2015) ($1.7x10^3\pm1.5 \times 10^2$) and Mohamed (2016) ($2.07\times10^3\pm0.60\times10^3$). Meanwhile, the results in half cooked and cooked chicken meat samples were nearly similar to that recorded by Marwan (2016) ($0.25\times10^2 \pm0.04 \times10^2$), but disagree with that reported by Ibrahim *et al*. (2014) ($1.18x10^3\pm0.26x10^3$) and Abd El-Aal (2015) ($6.40\times10^3\pm 1.23\times10^3$).

Table (1) showed also that, Staphylococci count was higher in fresh than half cooked and cooked samples, It may be due to it considered as a part of normal flora that found on the skin and upper respiratory tract of animal and man, because their ubiquitous occurrence in nature, they were found in various raw foods.

According to Staphylococci count was nearly similar to the results obtained by Ruban and Fairoze (2011) ($5.5\times10^3\pm 0.12\times10^3$) but disagree with that reported by Hassan (2015) ($2.5\times10^5\pm4.2\times10^4$) and Marwan (2016) ($1.73\times10^4 \pm0.09\times10^4$). Meanwhile, the results in half cooked and cooked chicken meat samples were similar to that recorded by Marwan (2016) ($0.80\times10^2\pm0.04\times10^2$) but disagree with that reported by Abd El-Aal- (2015) ($2.10\times10^3\pm0.32\times10^3$).

In addition, table (1) revealed that, *S. aureus* count was higher in fresh than half cooked and cooked samples. It is commonly indicate a direct contamination from worker's hands with abrasion and wounds or inadequately cleaned equipment resulting in *S. aureus* intoxication. Staphylococcal food poisoning is the result of performed enterotoxins that are produced by certain strains of *S. aureus* resulting in symptoms of intoxication.

The results of *S. aureus* count in fresh samples agree with those of Mahmoud and Hamouda (2006) ($8.9\times10^3\pm 0.3\times10^3$) but disagree with that reported by Mohamed (2016) ($1.12\times10^4\pm0.83\times10^3$). Meanwhile, the results in half cooked and cooked chicken meat samples was disagreed with that reported by Ibrahim (2005), who reported that no detectable level of *S. aureus* was obtained.

While incidence of Coagulase Positive *S. aureus* in table (1) go in parallel with this obtained by Abd Allah (2017). but, disagreed with those of Ahmed (2015) and Olukemi *et al*. (2015), who isolated *S. aureus* with higher incidence. Also, disagreed with Osman *et al*. (2016), who isolated *S. aureus* with lower incidence and with Ibrahim (2005) who failed to isolated *S. aureus* from cooked chicken meat samples.

The recorded results in table (2) showed that, the incidence of *E. coli* was higher in fresh samples than half cooked and cooked. Moreover, it may be due to mishandling during production, processing and distribution Kagambega *et al*. (2012). The pathogenic strains of *E. coli* can cause distinct disease syndrome as different diarrheal diseases, wound infections, meningitis, septicemia, atherosclerosis, hemolytic uremic syndrome and immunological diseases such as reactive and rheumatoid arthritis.

These results and the same serotypes of *E. coli* were previously isolated from both fresh and cooked chicken meat, came in accordance with those obtained by Marwan (2016) and Abd El–Alim (2017). Meanwhile, disagree with those of Abbass (2011), who failed to isolate E. *coli* form chicken meat samples.

Moreover, results in table (3) revealed that, the acceptable samples of fresh samples were lower than that of half cooked and cooked ones. This may be due to that chicken meat may be contaminated by infected handlers during slaughter and processing of livestock or by cross-contamination during food preparation.

The results of SET -RPLA test recorded in table (4) were nearly similar to that recorded by Abdalrahman *et al.* (2015) and El-sayed (2015).

Regarding to the results of PCR listed in table (5) the incidence of sea and see genes were higher than incidence of sec and Sed genes, followed by seb.

Nearly similar results of sea gene obtained by Abdalrahman *et al*. (2015) and Abd Allah (2017). Meanwhile, the results were disagreed with FeBler *et al.* (2011), who failed to detect *sea* gene in *S. aureus* strains isolated from poultry products. Also, the results of *seb* gene, nearly similar to those obtained by Abdalrahman *et al.* (2015) and Abd Allah (2017). In addition, the results of *sec* gene, nearly similar to those obtained by Madahi *et al.* (2014). Meanwhile, the results of *sed* gene, agree with those obtained by Abdalrahman *et al.* (2015) and Abd Allah (2017). Moreover, the results of *see* gene come in parallel with those obtained by Mostafa (2014).

5. CONCULSION

The present study concluded that, chicken meat (fresh, half cooked and cooked) were subjected to various degree of bacterial contamination. Consequently, fresh samples were the most contaminated ones. Therefore, a concerted effort should be made to maintain sanitary condition and strict maintenance of good practices of hygiene in all processing to ensure meat quality and safety.

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