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Incidence of *Staphylococcus aureus* and its Enterotoxins in Chicken Meat and its Products

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ABSTRACT

Chicken meat are being widely consumed as they contain high protein and a healthier unsaturated fat type. Chicken burger represent a consumer palatable chicken product. Both chicken and its products are liable to different types of contamination during their preparation and processing. Contamination by *Staphylococcus aureus* and its enterotoxins poses a major public health hazard to chicken meat consumes. During this study 100 different samples of chicken fillet, deboned thigh, wing, mechanically deboned meat (MDM) and chicken burger (20 each) were collected from market and investigated for their *S. aureus* count and ability of the isolated strains to produce enterotoxins using conventional plating and isolation technique as well as using SET-RPLA toxin detection kit. Results revealed that mean values of *S. aureus* count in all samples exceeded the permissible limits and hence being unacceptable. MDM isolated exhibited staphylococcal enterotoxins (SEs) production of three different types SEA, SEC and SED. Meanwhile chicken burger *S. aureus* isolates produced only SEA and SEC enterotoxins. While isolated *S. aureus* from chicken fillet and deboned thigh didn't exhibit any enterotoxin production activity. It's recommended to follow the hygienic practices during different processing stages to avoid the risk of *S. aureus* and its enterotoxins.

Key words: Chicken, Staph, MDM, Enterotoxin, Burger, SEA, SEC, SEC.

INTRODUCTION

Among different types of edible meat, chicken meat has special preference to consumers due to its unique characteristics. Beside its excellent nutritional value, chicken meat considered a healthier protein source which has a lower fat and cholesterol content when compared to other types of meat. Chicken meat are more likely to be contaminated with different foodborne pathogens during chicken preparation and processing steps. This microbial contamination represents a public health issue which significantly affect the healthcare as well as product production cost leading to high economic losses to related industries and personnel (Cavitte, 2003). In 2015, a report by WHO stated that nearly 420,000 people die out of 600,000 infected patients due to foodborne pathogens, mainly due to *Salmonella* sp., *Listeria* sp., *Campylobacter* sp., *Vibrio cholera*, and *S. aureus* (WHO, 2015; Haque *et al*., 2020). Majority of these pathogens could be found in chicken samples (Gonçalves-Tenório *et al*., 2018) specially that chicken meat has a high moisture percentage, nitrogen rich compounds (protein and essential amino acids), good mineral and vitamin content which makes chicken meat the ideal medium for bacterial growth (Prange *et al*., 2005). MDM defined as the chicken leftovers and wastes such as skin, bones, and unusable parts which transferred to meat processing plants in an unhealthy and unsanitary condition and the adherent meat to bone is mechanically separated into MDM (Mechanically Deboned Poultry Meat). Due to these unhygienic conditions and preparations MDM are more liable to contain *S. aureus* (Khorram *et al.*, 2012). Staphylococci mainly *S. aureus* are more liable to be found in chicken meat owing to its adhesion and chlorine resistance in final rinse water (Pepe *et al.*, 2006). Also *S. aureus* is considered the 3rd worldwide cause of food poisoning reported cases leading to major foodborne outbreaks (Losito *et al.*, 2005). The crucial risk of *S. aureus* is the ability to produce variety of staphylococcal enterotoxins (SEs) (A, B, C, D, E, G, H, I, J, K, L, M, N,

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O, P, Q, R and U) but only SEA, SEB, SEC and SED are of major significance which cause 95% of enterotoxins food poisoning (Letertre *et al.,* 2003; Abdelghany *et al.,* 2020; El Jalil *et al.,* 2020; Mehmood *et al.,* 2020). Furthermore, SEs are heat stable toxins which means that they are able to withstand high temperatures and normal cooking cannot destruct them. Moreover, those toxins are difficult to be notable in food as they have neither specific taste nor distinct appearance in food (Aycicek *et al.,* 2005). Ingestion of SEs contaminated food result in food poisoning occurs shortly after 30 min (Argudín *et al.,* 2010). The aim of the present study was to investigate level of contamination with *S. aureus* and its enterotoxins in chicken meat (Fillet, deboned thigh, wings) and chicken meat products (MDM and burger) and their relevance to public health importance.

MATERIALS AND METHODS

Samples collection

A total of 100 random samples of frozen chicken meat cuts {Fillet, deboned thigh, wings (20 of each)} and frozen chicken meat products {MDM and burger (20 of each)} were collected from different markets of Cairo governorate, Egypt. Each sample was kept in a separate sterile bag, placed in an insulated ice container and immediately transferred to the laboratory of food hygiene department, Animal Health Research Institute, ARC, Dokki, Giza, Egypt for further bacteriological examination.

Samples preparation

For each sample, a food homogenate was prepared using 25g which were cut by sterilized scissor and placed under aseptic condition to a sterile stomacher bag then 225ml of 0.1% sterilized buffer peptone water were added. The contents were homogenized at stomacher (Lab blender 400, Sweard Lab. Model No. AB 6021) for 2 minutes and the mixture was let to stand for 5 minutes at room temperature then transferred into sterile glass flask and mixed thoroughly by shaking then 1ml was transferred into separate tube each containing 9ml sterile diluent of 0.1% peptone water (Silva *et al.,* 2019).

Determination of *Staphylococcus aureus* count

From each previously prepared tenth- fold serial dilutions of sample homogenate, 100µl were aseptically spread using sterile bent glass spreader onto the dry surface of double set of Baird-Parker agar plates (Oxoid CM 275, SR 54). Inoculated plates were incubated in an inverted position at 37°C for 48 hours. All typical colonies (black shining convex, 1-1.5mm with narrow white margin and surrounded by a clear extending into an opaque medium) were enumerated and recorded as presumptive *S. aureus* count then picked up and cultivated in nutrient agar slope for further identification (Silva *et al.,* 2019).

Identification of *Staphylococcus aureus*

Morphological examination and Gram staining of *S. aureus* showed Gram positive grapes like cocci and arranged in clusters under light microscope (Cruickshank *et al.,* 1975). While biochemical identification was performed according to Silva *et al.* (2019) where *S. aureus* was confirmed throw coagulase activity, catalase test, anaerobic utilization of glucose and mannitol, lysostaphin sensitivity and thermostable nuclease production.

Serology confirmation of *Staphylococcus aureus*

Serology confirmation of *S. aureus* was done using a reliable latex slide agglutination test kit (Dry Spot Staphytect Plus Kit Oxoid DR0100M) for differentiation of *S. aureus* by detection of clumping factor, Protein A and certain polysaccharides where agglutination of the latex particles occurs within 20 seconds, which indicates the presence of *S. aureus*.

Detection and typing of *Staphylococcus aureus* enterotoxins

Serologically confirmed positive *S. aureus* strains were examined for their ability to produce enterotoxins. First the Sac culture method was performed according to method described by Donnelly *et al.* (1967) to obtain a final clear culture supernatant fluid then detection and typing of enterotoxin was carried out using the clear culture supernatant fluid which tested serologically by RPLA technique using SET-RPLA KIT TOXIN DETECTION KIT (Oxoid TD0900, Japan LTD) a kit for the detection of staphylococcal enterotoxins A, B, C and D (Oda *et al.,* 1979; Shingaki *et al.,* 1981).

RESULTS AND DISCUSSION

Chicken meat and its products are more likely to be contaminated with many types of microorganisms. Such contaminants may pose a public health hazard to consumers. *S. aureus* is considered one of the major causes of foodborne diseases which result from different sources starting from defeathering, evisceration and subsequent processing steps (Levin *et al.,* 2001; Houf *et al.,* 2002). *S. aureus* is the major public health significant bacteria and Staphylococcal foodborne disease (SFD) is one of the most common foodborne diseases worldwide resulting from contamination of food by pre-formed *S. aureus* enterotoxins (Bordoloi *et al.,* 2014; Kadariya *et al.,* 2014). Results in Table 1 revealed that the maximum counts of *S. aureus* isolated from raw frozen chicken meat products represented by fillet, deboned thigh, wings, MDM and burger were 3×10^3, 1.1×10^4, 6×10^3, 8×10^3 and 8×10^3, respectively. This exceeds the maximum permissible limit (10^2 CFU/g) as recommended by ESS (2005a, b, c). Findings also showed that the lowest *S. aureus* recovery percentage (35%) as well as lowest mean value of *S. aureus* count were obtained from fillet 5.1×10^2 CFU/g. Although, the highest *S. aureus* recovery percentage (100%) was recorded from MDM, the highest mean count was obtained from burger 1.4×10^3 CFU/g, this may be linked to the variety of initial raw materials used in product processing primary chicken skin as fat source which contains a high microbial load. Meanwhile the obtained count of deboned thigh, wings, MDM and burger was 1.1×10^3, 6.5×10^2, 9.8×10^2 CFU/g, respectively which also exceeds the maximum permissible limit (10^2 CFU/g) as recommended by ESS (2005a,b,c). Regarding fillet samples, nearly the same results were recorded by Amin *et al.* (2016), who detected *S. aureus* in fillet samples with mean value of 5.10×10^2 CFU/g and Hassan-Aisha (2007) with mean count value of 5×10^2 CFU/g.

Table 1: *S. aureus* count (CFU/g) in examined samples (n=20 each)

	Fillet	Thigh	Wings	MDM	Burger
No (%)*	7 (35)	9 (45)	10 (50)	20 (100)	12 (60)
Min.	$<10^2$	$<10^2$	$<10^2$	1×10^2	$<10^2$
Max.	3×10^3	1.1×10^4	6×10^3	8×10^3	8×10^3
Mean ±SE	$5.1\times10^2 \pm1.9\times10^2$	$1.1\times10^3 \pm0.6\times10^3$	$6.5\times10^2 \pm2.1\times10^2$	$9.8\times10^2 \pm2.8\times10^2$	$1.4\times10^3 \pm0.51\times10^3$

* Number and percentage of samples which *Staphylococcus aureus* count could be detected out of the total examined samples for each category i.e. (n=20).

Table 2: Incidence of coagulase positive *S. aureus* and type of toxin production

		Fillet	Thigh	Wings	MDM	Burger
Samples containing coagulase positive *S. aureus*						
No.		3	4	2	5	6
Incidence*		15%	20%	10%	25%	30%
Enterotoxin production of isolated *S. aureus* strains						
No.		ND**	ND	1	4	3
Incidence***		-	-	50%	80%	50%
Toxin type	A	-	-	ND	1	2
	B	-	-	1	ND	ND
	C	-	-	ND	2	1
	D	-	-	ND	1	ND

*Incidence was calculated as the percentage of samples containing coagulase positive *S. aureus* out of total number of examined samples for each category i.e. (n=20): ** ND = not detected: *** Incidence was calculated as the percentage of enterotoxin producing strains out of the isolated coagulase positive *S. aureus* strains

Lower results were obtained by Al-Dughaym and Al-tabari (2010) with mean count value of 10^2 CFU/g. While higher results were obtained by Abubakr (2012) who detected *S. aureus* in chicken fillet samples with mean value of 6.50×10^2 CFU/g and by Elbagory *et al.* (2005) who recorded *S. aureus* in fillet samples with mean value of 1.25×10^3 CFU/g and by Saad *et al.* (2018) who recorded 1.42×10^6 CFU/g. On the other hand, higher incidences were reported by Ahmed (2004), Gad (2004), Essa *et al.* (2004), Mahmoud and Hamouda-Seham (2006) and Mira-Enshrah and Eskandar (2007) with mean count values of 3.8×10^3, 3.8×10^3, 3.8×10^3, 2.7×10^3 CFU/g and 3.4 log CFU/g count. In chicken burger samples, higher results were recorded by Abubakr (2012) who detected *S. aureus* mean count of 3.40×10^3 CFU/g, while lower results were obtained by Gad (2004) and Al-Dughaym and Al-Tabari (2010) with *S. aureus* mean count of 2.5×10^2 CFU/g. and 10^2 respectively, also by Elbagory *et al.* (2005) who detected mean value of 2.5×10^2 CFU/g. However, higher results of 4.6×10^3 CFU/g were obtained by Hafez *et al.* (1987). For deboned thigh samples higher incidence of 1.24×10^6 CFU/g was recorded by Saad *et al.* (2018), on the other hand lower values of 10^2, 9.7×10^2 CFU/g and 2.15 log CFU/g were reported by Al-Dughaym and Altabari (2010), Elbagory *et al.* (2005) and Abdel-Rahman *et al.* (2008). For wings samples nearly the same results obtained by Malpass *et al.* (2010) and Abdel-Rahman *et al.* (2008) with count of 1.75 log CFU/g and 1.93 log CFU/g, respectively. Furthermore, a higher *S. aureus* mean count values of 10^4 CFU/g was addressed by Al-Dughaym and Al-Tabari (2010) for MDM samples. These high counts may indicate bacterial contamination during handling, storage, mechanical separation and packaging.

Results shown in Table 2 revealed that coagulase positive *S. aureus* could be isolated by 15, 20, 10, 25 and 30% from fillet, thigh, wings, MDM and burger respectively. As shown the highest values were also from chicken burger samples, meanwhile, lower percentage of 12 and 25% was detected by Bkheet *et al.* (2007) and Abubakr (2012) respectively, 25% and a higher result (48.6%) was reported by Khalifa and Abd El-Shaheed (2005). However, the lowest coagulase positive *S. aureus* detection percentage was in wings samples while higher incidence was reported by Kitai *et al.* (2005) 39.9%. Regarding fillet chicken samples, a lower incidence was reported by Abubakr (2012), Hassan-Aisha (2007) and Isis (2002) who detected coagulase positive *S. aureus* in 10, 5 and 7.5% in the examined chicken fillet samples respectively. On the other hand, higher incidence was reported by Hassanen *et al.* (2017), Mahmoud and Hamouda-Seham (2006) and Elshraway *et al.* (2018) with 22.5, 51.5 and 53% respectively of the examined chicken fillet samples. Moreover, Saad *et al.* (2018), Hassanen *et al.* (2017) and Elshraway *et al.* (2018) reported a percentage of 80, 27.5 and 45% respectively. In MDM samples nearly the same results were obtained by Kozačinski *et al.* (2006) who detected coagulase positive *S. aureus* in 30.3% of the examined MDM samples.

Staphylococcal enterotoxins are serologically grouped into four major classical types which are SEA, SEB, SEC and SED detected by reversed passive latex agglutination kit (RPLA) (Jorgensen *et al.*, 2005; Zouharova and Rysanek, 2008). In addition, Bendahou *et al.* (2009) reported that SET-RPLA is an immunological technique used for typing of classical enterotoxins produced by *S. aureus* (SE: A, B, C and D).

The present study detected toxigenic strains in *S. aureus* isolates using available kits, (RPLA). As shown in Table 2, the results illustrated that strains of *S. aureus* were isolated from raw chicken meat products (3 fillet, 4 deboned thigh, 2 wings, 5 MDM and 6 burger). Testing those isolates for enterotoxin production revealed that the previously obtained coagulase positive *S. aureus* isolates were not enterotoxigenic in fillet and thigh samples (0%). These results are in accordance with Zaki-Eman and Shehata-Amal (2008) who found that enterotoxins were not detected in fillet samples. On the other hand, Gad (2004) and Hassan-Aisha (2007) stated that *S. aureus* isolates from chicken fillet produced enterotoxin type A. While Elshraway *et al.* (2018) recorded that *S. aureus* isolates from neither chicken fillet nor thigh produced enterotoxin type B. While types B, C and D were produced by strains isolated from chicken thigh in a percentage (14.2%) was recorded by Elbagory *et al.* (2005). Moreover, only 3 burger samples out of 6 (50%) were enterotoxigenic, where only 2 strains produced SEA and 1 strain produced SEC, these results came in accordance with Abubakr (2012) who found that 40% from *S. aureus* isolates were enterotoxigenic, 20%

produced SEC and 20% produced SED. On the other hand, Elbagory *et al.* (2005) found no enterotoxigenic strains in the examined chicken burger samples. For wings samples, the current study revealed that only 1 out of 2 strain (50%) produced SEB however, Kozačinski *et al.* (2006) detected SEA, SEB and SEC produced by strains isolated from chicken wings in a different percentage (16.3%). Regarding MDM samples, the most variable enterotoxins types were observed with 4 samples out of 5 (80%) of the isolates were enterotoxigenic, where 1 strain produced SEA, 2 strains produced SEC and 1 strain produced SED but no SEB was detected, on the other hand Azevedo *et al.* (2009) recorded that 30% of the examined strains were enterotoxigenic and produced SEA and SEB.

Conclusion

Chicken meat and its products are considered a potential source for *S. aureus* due to various manipulations during processing steps and bad personnel hygiene of the food handlers. Many of *S. aureus* strains isolated from chicken meat and its products have the ability to produce various staphylococcal enterotoxins (SEs) as SEA, SEC and SED which represent a public health hazard as those toxins are heat stable even after cooking. Chicken meat products are more liable to contamination due to various processing and manipulation techniques during processing. So strict application of good hygienic practices and good personnel hygiene is recommended to avoid *S. aureus* and its enterotoxins (SEs) hazards.

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