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PREVALENCE OF *Staphylococcus aureus* AND *Salmonella* SPECIES IN CHICKEN MEAT PRODUCTS RETAILED IN EGYPT

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Abstract: Chicken meat products represent an important source of animal derived proteins, vitamins, and minerals. However, chicken meat products might act as potential sources of human exposure to foodborne pathogens such as *Staphylococcus aureus (S. aureus)* and *Salmonella* species. The objectives of the present study were first to investigate the prevalence rates of *S. aureus* and *Salmonella* species in the retailed chicken meat products at Zagazig city, Egypt. Second, serological identification of the isolated bacteria was followed. Third, screening of *S. aureus* enterotoxin coding genes (*sea seb, and sed*) as well as *Salmonella* virulence associated genes including *invA*, *Salmonella hyper-invasive locus* (*hil*A), and *Salmonella* enterotoxin (*stn*) was done using PCR. The obtained results revealed isolation of *S. aureus* from the examined chicken meat products at 22%, and *Salmonella* spp., at 6.66%. Where *S. aureus* was isolated at 33.33%, 36.66%, 13.33%, 6.66%, and 20% from the examined chicken burger, fillet, luncheon, nuggets, and panne, respectively. *Salmonella* spp. could be isolated only from chicken burger and fillet at 10%, and 23.33%, respectively. The recovered *S. aureus* isolates harbored enterotoxin coding genes (*sea,* and *seb*). Likely the recovered *Salmonella* spp. isolates harbored virulence associated genes such as *invA, hilA,* and *stn*. Moreover, antimicrobial sensitivity testing of the recovered isolates showed multidrug resistance profiling. In conclusion, chicken meat products retailed in Egypt might be potential sources for the spread of multidrug resistant *S. aureus* and *Salmonella* spp. Therefore, strict hygienic measures should be adopted during manufacturing of such meat products.

Key words: chicken meat products; *Staphylococcus aureus*; *Salmonella* species; Egypt

Introduction

Chicken meat products make a substantial contribution as a reasonably affordable substitute for red meat, which is severely undersupplied in Egypt. Such meat source is rich in high-quality animal protein, essential amino acids, and trace elements. Additionally, a number of chicken meat products, including chicken burgers, chicken fillets, chicken luncheons, chicken nuggets, and chicken panne, were produced and released into the chicken meat markets as a result of the quick advancements in food processing and technology. Such important products are distinguished by their distinct flavor and aroma, which draws the attention of customers, especially kids (1, 2).

Chicken meat products may get contaminated by microbes at any stage of production, including the preparation of the raw materials, the manufacturing process, distribution, and storage. As a result, products made from chicken are thought to be a possible source for the spread of bacteria that cause food poisoning, including

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Staphylococcus aureus (*S. aureus*) and *Salmonella* spp., (3, 4).

The major cause of food foodborne intoxication cases is consuming foods contaminated with *S. aureus*-enterotoxins, which are known for their quick onset (between one and six hours after ingesting contaminated foods), nausea, vomiting, stomach cramps, and diarrhea (5). *S. aureus* and staphylococcal enterotoxins (SEs) were identified and found in chicken meat and giblets sold in Egypt (3, 6), and US markets (7), and chicken breast and thigh sold in Cambodian markets (8).

Non-typhoidal salmonellosis is a major foodborne infection caused by consumption of foods contaminated with *Salmonella* spp. The disease is characterized by 12 to 36 hours incubation period post ingestion of contaminated foods, nausea, vomiting, fever, severe diarrhea, abdominal cramps, and malaise (9). Several studies had reported isolation of *Salmonella* spp. from chicken meat and meat products as reported in Vietnam (10), Japan (11), and Egypt (3).

Antimicrobials are extensively used in poultry farms for the purposes of prevention and control of bacterial diseases, as feed additives for improving the feed conversion ratio, and as preservatives in meat products' industry (12). However, the uncontrolled usage of such antimicrobials had led to development of drug resistance among foodborne pathogens (13).

Taken the previous notes, this study was conducted to investigate the prevalence rates of *S. aureus*, and *Salmonella* spp. in retailed chicken meat products including chicken burgers, chicken fillets, chicken luncheons, chicken nuggets, and chicken panne retailed in Egypt. Furthermore, detection of enterotoxin coding genes and virulence associated genes was done using PCR. Antimicrobial susceptibility of the recovered *S. aureus*, and *Salmonella* spp. isolates was carried out using the disk diffusion method.

Material and ethods

Sampling and samples preparation:

A hundred and fifty chicken meat product samples including thirty each of chicken burger, fillet, luncheon, nuggets, and panne were collected from retail markets and grocery stores in Zagazig city, Egypt. The samples were transferred cooled without delay to Animal Health Research Institute, Zagazig branch for bacteriological examination.

The method of APHA (14) was followed during preparation of samples for bacteriological examination. In short, 10 g from each sample was a-septically homogenized in 90 mL of sterile peptone water 1% (Oxoid CM9, UK) and blended for 3 min at 3000 rpm. Serial dilution from each sample was also prepared.

Isolation and identification of S. aureus

The method of APHA (14) was used for isolation and identification of *S. aureus*. In brief, the surface spreading method was used to inoculate 0.1 mL of each prepared serial dilution to a Baird Parker agar (Oxoid, UK) plate supplemented with egg yolk emulsion using. The plates were left in the incubator at 37°C for 48 h on inverted positions. The colonies of *S. aureus* appeared as circular black, shiny with narrow white margin and surrounded by a clear zone extending into the opaque medium. From each cultured plate, five suspected *S. aureus* colonies were picked up and purified on nutrient agar slopes. Identification of *S. aureus* colonies was done using morphological, biochemical, and serological characteristics (14).

Isolation and identification of Salmonella spp.

The method of ISO 6579 (15) was followed during the isolation and identification procedures of *Salmonella* spp. Ten mL from each of the previously prepared meat homogenate were incubated at 37°C for 18 ± 2 h as pre-enrichment. Rappaport Vassiliadis soya broth (Oxoid, UK) was used as a selective enrichment medium. Enriched cultures were incubated at 41.5°C for 24 ± 2 h. The surface streaking method was used to inoculate a loopful from that enriched culture on the surface of xylose lysine desoxycholate (XLD) agar (Oxoid, UK) plate and incubated 37°C for 24 ± 2 h. Suspected colonies (red with or without black centers) were purified and sub-cultured onto nutrient agar slopes and incubated at 37°C for 24 h. The purified colonies were subjected to morphological, biochemical, and serological identification (15).

Molecular identification of Staphylococcal enterotoxins and Salmonella-associated virulence genes:

A Genomic DNA extraction kit (Alliance Global, Dubai, UAE) was used for DNA isolation from the cultured and identified bacteria according to the instructions of the manufacturer. Oligonucleotide pairs for *S. aureus*-specific enterotoxin genes (*Sea, Seb,* and *Sed)*, and *Salmonella-specific hilA, invA,* and *stn* virulence associated genes were bought from Metabion International, Gmbh, Germany, and presented in Table 1.

PCR amplifications were done according to the previously published method (18) on a Thermal Cycler (Master cycler, Eppendorf, Germany) using PCR technique. The cycling conditions started with an initial denaturation at 95°C for 2 min, followed by 35 amplification cycles that consisted of, for each cycle, denaturation for 15 sec at 95°C, annealing for 30 sec at 50°C for *S. aureus* targets, and 60°C for Salmonella targets, and an extension for 1 min at 72°C. A final extension was followed for 7 min at 72°C and ended by holding at 4°C. The resultant PCR products were electrophoresed on agarose gel 1.5% (AppliChem, GmbH, Germany) in 1x Tris Borate EDTA (TBE) buffer stained with ethidium bromide. PCR products were visualized on a UV transilluminator.

Statistical analysis:

All microbial counts were transferred into log 10 of CFU/g. Data were expressed as means ± SD. Data were analyzed using a one-way ANOVA procedure, followed by Tukey's multiple comparison tests (SPSS Inc., Chicago, Illinois, The USA), with a *P*-value of 0.05.

Table 1: Oligonucleotide primer sequences used in the study

Target	Primer sequence (5'-3')	Product size (bp)	Reference
***S. aureus* genes**			
sea	F- GGTTATCAATGTGCGGGTGG	102 bp	**(16)**
	R- CGGCACTTTTTTCTCTTCGG		
seb	F- GTATGGTGGTGTAACTGAGC	164 bp	
	R- CCAAATAGTGACGAGTTAGG		
sed	F- CCAATAATAGGAGAAAATAAAAG	278 bp	
	R- ATTGGTATTTTTTTTCGTTC		
***Salmonella* genes**			
invA	F- GTGAAATTATCGCCACGTTCGGGCAA	284 bp	**(17)**
	R- TCATCGCACCGTCAAAGGAACC		
stn	F- TTGTGTCGCTATCACTGGCAACC	617 bp	
	R- ATTCGTAACCCGCTCTCGTCC		
hilA	F- CGGAAGCTTATTTGCGCCATGCTGAGGTAG	854 bp	**(18)**
	R- GCATGGATCCCCGCCGGCGAGATTGTG		

Results

The obtained results revealed isolation of *S. aureus* from the examined chicken meat products at 22% (33 out of 150 samples), and *Salmonella* spp., at 6.66% (10 out of 150 samples). Where *S. aureus* was isolated at 33.33%, 36.66%, 13.33%, 6.66%, and 20% from the examined chicken burger, fillet, luncheon, nuggets, and panne, respectively. Salmonella spp. was not isolated from chicken luncheon, nuggets and panne but could be isolated from chicken burger and fillet at 10%, and 23.33%, respectively (Table 2).

Total *S. aureus* counts were 3.80±0.22, 3.58±0.20, 3.71±0.20, 3.39±0.19, and 3.60±0.16 log 10 CFU/g from the examined chicken burger, fillet, luncheon, nuggets, and panne, respectively (Table 3).

Three *Salmonella* species were recovered from the examined chicken fillet namely, *S.* Typhimurium, *S.* Infantis, and *S.* Kentucky. *S.* Typhimurium and *S.* Kentucky were isolated both at 28.57%, and *S.* Infantis at 42.86%. *S.* Infantis was the only recovered serotype from chicken burger at 100% (Table 4).

PCR testing of 15 randomly selected *S. aureus* isolates for harboring *Staphylococcal* enterotoxins revealed detection of *sea* in 7 out of 15 of the tested isolates and *seb* was detected in 10 out of 15 of the tested isolates, while *sed* was not detected at any isolate. Similarly, PCR testing of four selected *S.* Typhimurium (2), *S.* Infantis (1), and *S.* Kentucky (1) isolates for harboring *invA*, *hilA*, and *stn* virulence associated genes revealed detection of *invA* in all tested isolates, *hilA* in the two tested *S.* Typhimurium isolates, and one *S.* Infantis isolate, while *stn* was detected in only one each of *S.* Typhimurium and *S.* Infantis isolate (data are not shown).

Antimicrobial sensitivity testing of the recovered *S. aureus* isolates towards the most commonly used antimicrobials for treatment of *S. aureus* infections revealed resistance of the recovered isolates to methicillin (100%), erythromycin (75.75%), cefotaxime (72.72%), chloramphenicol (57.57%), norfloxacin (9.09%), doxycycline (3.03%), ciprofloxacin (3.03%), and ofloxacin (3.03%), respectively (Table 5).

Regarding antimicrobial sensitivity of the recovered Salmonella isolates, they showed resistance to ampicillin (100%), cefoxitin (100%), trimethoprim/sulfamethoxazole (80%), tetracycline (80%), ciprofloxacin (70%), chloramphenicol (70%), gentamicin (60%), azithromycin (50%), and amoxicillin/clavulanic acid (40%) (Table 6).

Table 2: Prevalence of *S. aureus* and *Salmonella spp.* in the examined chicken meat products (n=30, for each).

Bacterial types / Sample Types	***S. aureus***		***Salmonella* spp.**	
	No.	**%**	**No.**	**%**
Chicken burger	10	33.33	3	10
Chicken Fillet	11	36.66	7	23.33
Chicken luncheon	4	13.33	0	0
Chicken nuggets	2	6.66	0	0
Chicken Panne	6	20	0	0
Total	33	22	10	6.66

Table 3: *S. aureus* count (log 10 CFU/g) in the positive examined samples

Sample type	**Min**	**Max**	**Mean± SE**
Chicken burger	3	4.48	3.80±0.22[a]
Chicken Fillet	3	4.20	3.58±0.20[ab]
Chicken luncheon	3.30	4.26	3.71±0.20[ab]
Chicken nuggets	3	3.70	3.39±0.19[b]
Chicken Panne	3.30	4	3.60±0.16[ab]

Values carrying different superscript letter within the same column are significantly different at $P < 0.05$

Table 4: Serological identification of *Salmonella* spp. isolated from the examined samples

***Salmonella* serotypes**	**Chicken burger (n=3)**		**Chicken fillet (n=7)**		**Total (n=10)**		**Group**	**Antigenic Structure**	
	No.	**%**	**No.**	**%**	**No.**	**%**		**O**	**H**
***S.* Typhimurium**	-	-	2	28.57	2	20	B	1,4,5,12	i : 1,2
***S.* Infantis**	3	100	3	42.86	6	60	C1	6,7,14	r:1,5
***S.* Kentucky**	-	-	2	28.57	2	20	E1	8,20	i:Z_6
Total	3	100	7	100	10	100			

Table 5: Antimicrobial resistance profiling of the recovered *S. aureus* isolates

Antimicrobial agents (AMA)	Resistant (R)		Intermediate (I)		Sensitive (S)	
	NO.	%	NO.	%	NO.	%
Methicillin (ME)	33	100	-	-	-	-
Nitrofurantoin (F)	-	-	-	-	33	100
Norfloxacin (NOR)	3	9.09	2	6.06	28	84.84
Erythromycin (E)	25	75.75	2	6.06	6	18.18
Doxycycline (DO)	1	3.03	1	3.03	31	93.93
Ciprofloxacin (CIP)	1	3.03	3	9.09	29	87.87
Chloramphenicol (C)	19	57.57	-	-	14	42.42
Ofloxacin (OFX)	1	3.03	1	3.03	31	93.93
Gatifloxacin (GAT)	-	-	-	-	33	100
Cefotaxime (CTX)	24	72.72	7	21.21	2	6.06

Table 6: Antimicrobial resistance profiling of the recovered Salmonella spp., isolates

Antimicrobial agents (AMA)	Resistant(R)		Intermediate(I)		Sensitive(S)	
	NO.	%	NO.	%	NO.	%
Trimethoprim/ Sulfamethoxazole(SXT)	8	80	-	-	2	20
Ampicillin (Am)	10	100	-	-	-	-
Cefoxitin (FOX)	10	100	-	-	-	-
Azithromycin (AZM)	5	50	-	-	5	50
Amoxicillin/clavulanic acid (AMC)	4	40	3	30	3	30
Tetracycline (TE)	8	80	1	10	1	10
Ciprofloxacin (CIP)	7	70	3	30	-	-
Gentamicin (CN)	6	60	2	20	2	20
Meropenem (MEM)	-	-	-	-	10	100
Chloramphenicol (C)	7	70	1	10	2	20

Discussion

Products made from chicken meat can become contaminated by various pathogens from a variety of sources starting with pre-processing, processing processes, and post-processing during packing, marketing, and storage. The chicken products are rendered unsafe for consumption by humans due to these pathogenic bacteria (19). Contamination of chicken meat products with *S. aureus* was confirmed in the present study, which reflects the poor hygienic measures adopted during processing of such products. Contamination of chicken meat and its products with *S. aureus* was also reported in several studies around the world as in Iowa, USA (20), Germany (21), Thailand (22), Iran (23), and Egypt (19). *S. aureus* is implicated in many cases of foodborne intoxications (24, 25). Such intoxication cases occur via production of enterotoxins such as *Sea, Seb,* and *Sed* (26, 27). Such enterotoxins were clearly detected in *S. aureus* isolates recovered from the current study. Likely, enterotoxins were detected in *S. aureus* recovered from chicken meat products sampled in Italy (28), Iran (29), and Egypt (3).

The recovered *S. aureus* isolates in the present study showed clear multiple drug resistance profiling. Such drug resistance was developed possibly via the uncontrolled usage of antimicrobials in poultry farming. Similar observation was reported in *S. aureus* isolated from chicken meat in Iran (23), chicken giblets sampled from Egypt (3), and poultry and poultry products retailed in South Africa (30).

Similar to *S. aureus*, some examined chicken burgers and chicken fillet in the present study were found contaminated with *Salmonella* spp. This agrees with other reports which showed contamination of retailed chicken meat products with *Salmonella* spp. as reported in chicken fillet in Egypt (4), in retail chicken meat in Hanoi, Vietnam (10), Japan (11), and Malaysia (31).

Salmonella was also implicated in many cases of foodborne infections which are characterized by high fever, abdominal pain, diarrhea. Foodborne outbreaks of *Salmonella* were reported in the United States (32), Switzerland, during May-June 2008 (33), and in the United Kingdom during 2010 (34). In addition, three Australian states and territories reported seven *S.* Typhimurium outbreaks (35). Salmonella enterotoxin (*Stn*) and hyper-invasive locus (*hilA*) coding were shown to be two virulence factors in the isolated *S.* Typhimurium. *Salmonella* serovars Typhi, Typhimurium, and Enteritidis have been found to contain *Salmonella* enterotoxin (*Stn*), which contributes to the pathogenicity process of Salmonella and predominantly causes diarrhea (36). A regulator that triggers the expression of invasion genes in response to both environmental and genetic regulatory cues is encoded by the *hilA* gene (37). Moreover, the recovered *Salmonella* spp. were found to have multidrug resistance profiling. This could be attributed to the massive uncontrolled usage of antimicrobials in poultry farms (13, 38).

Conclusion

This study indicated contamination of chicken meat products retailed in Egypt with two important foodborne pathogens, namely, *S. aureus* and *Salmonella* spp. Therefore, strict hygienic precautions should be adopted during processing of such products to avoid cross contamination with pathogens of public health significances.

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