

Incidence of *Staphylococcus aureus* and *Salmonella* in Poultry Meat

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Abstract: A total of 110 random poultry meat samples was collected from different markets in Kafr El-Shiekh city. The samples were transferred to the laboratory under possible complete aseptic condition, where they were examined bacteriologically. The results revealed that *S.aureus* could be isolated from examined poultry meat samples with an incidence of 41.8%. According to PCR, from 10 examined samples, 90% of samples were positive for *Staphylococcus aureus*. By ELISA, three types of staphylococcal enterotoxins were detected (SEA, SEB and SED) by a percentage of 4.3, 2.2 and 6.5%, respectively. Twelve samples were exposed to PCR and the results were as follow: three isolates harbored 1(sea) gene, 1 isolate harbored 1(seb) gene, 1 isolate harbored 1(sed) gene and 1 isolate harbored mixed 1(sea) and 1(sed). Genes encoding sea represented by a percent of (33.3%), seb (8.3%) while sed (16.7%). On the other hand, *Salmonellae* could be isolated with an incidence of 6.4% and by PCR 100% of the examined samples were positive. The isolated *Salmonella* were *Salmonella enteritidis* (1.8%), *Salmonella typhimurium* (2.7%) and *Salmonella Kentucky* (1.8%).

Key words: Poultry meat • *Salmonella* • *Staphylococcus aureus* • Staphylococcal enterotoxins.

INTRODUCTION

Production and consumption of poultry meat and meat products show upward trend. This, of course requires adequate control and inspection both during poultry rearing and in slaughter houses, processing and shops. Consumers are also a link in the chain of food-borne human diseases, because of the way they store and cook poultry meat and meat products [1]. During the slaughter of poultry birds, there can be fecal contamination of the carcasses from the gut of these birds which means bacteria present in the spilled gut content is passed on as contaminants [2].

The presence of *S.aureus* in meat is often attributed to inadequate hygiene during handling by the individuals involved in the production of meat [3]. Contamination with *S.aureus* is important in the evaluation of the safety and hygienic quality of chicken meat and also in determining the origin of food poisoning [4]. *S.aureus* concentrations of 10⁵ CFU per gram of food are considered critical for the possible accumulation of enterotoxin with the potential to cause illness in the consumer [5]. Food poisoning by *S.aureus* affects hundreds of thousands of people each year. *S. aureus* also causes invasive diseases such as arthritis (in poultry)

and septicemia (in poultry and humans) [6]. *S.aureus* produces a wide variety of toxins including staphylococcal enterotoxins (SEA to SEE, SEG to SEI, SER to SET) with demonstrated emetic activity [7]. These staphylococcal enterotoxin proteins have a remarkable ability to resist heat and acid. Therefore, they may not be completely denatured by mild cooking of contaminated food. They are pyrogenic and share some other important properties that include the ability to induce emesis and gastroenteritis as well as their noted superantigenicity. They are resistant to inactivation by gastrointestinal proteases including pepsin, trypsin, rennin and papain [8]. *Salmonella* is of increasing public health concern because they are the most incriminated pathogenic microorganisms of bacterial food poisoning especially present in poultry meat with infection being through the handling of raw poultry carcasses and products, together with the consumption of undercooked poultry meat [2]. Because *Salmonella* typically is found in poultry, this type of meat has been an important vehicle in food borne diseases rendering salmonellosis is one of the most frequently reported food borne diseases worldwide [9]. Therefore, the aim of this work was planned for evaluation of the sanitary condition of poultry meat through isolation and identification of *S.aureus* and *Salmonella* and

detection of staphylococcal enterotoxins by ELISA and staphylococcal enterotoxin genes by PCR.

MATERIALS AND METHODS

Collection of Samples: A total of (110) poultry meat samples was randomly collected from different shops in Kafr-El-Sheikh city. The collected samples were transferred to the laboratory and examined bacteriologically immediately after arrival to the laboratory.

Methods

Preparation of Samples According to Apha[10]: Bacteriological examination: Isolation and identification of *S. aureus* according to ICMSF [11].

Polymerase Chain Reaction Confirmation Concerning *S. aureus*: DNA Extraction using QIA amp kit according to Shah *et al.* [12].
DNA amplification:

Amplification Reaction for Nuc Gene of *S. Aureus* According to Chu *et Al.* [13]: The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany) using 25 µl of PCR mixture containing 3 µl of boiled cell lysate, 200 M of deoxynucleotide triphosphate (dNTP mixture), 1.4 U of Taq DNA polymerase (Biotools, Madrid, Spain), buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl and 3 mM MgCl₂, Biotools) and 20 M of each primer (nuc). PCR condition was denaturation for 5 min at 94°C, followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 45 sec and then a final extension at 72°C for ten minutes. PCR amplified products were analyzed by 1.5% of agarose gel electrophoresis stained with ethidium bromide and visualized and captured on UV transilluminator.

Amplification of Enterotoxin Genes of *S. Aureus* According to Mehrotra *et Al.* [14]: 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 dH₂O, 2.5ml 10 x buffer, 0.63ml 10mM NTPs, 1ml 25Mm Mgcl₂, 1.25 µl primer F(20pmol/ml), 1.25 µl primer R(20pmol/ml) and filled up to 25 µl PCR grade water. Concerning the primers used for demonstration of *S. aureus* enterotoxins (sea, seb, sec & sed), DNA amplification was performed using the following conditions: initial denaturation for 5 min at 94°C followed by 30 cycles of denaturation (94°C for 2 min), annealing (50°C for 1 min) and extension (72°C for 1 min). A final extension step (72 °C for 5 min) was performed after the completion of the cycles. The PCR products were electrophoresed in 1% agarose gel and stained with ethidium bromide.

Detection of *S. aureus* enterotoxins by ELISA according to Ewalid [15].

Isolation and identification of salmonellae according to ISO [16].

Serological identification of salmonellae according to Kauffman [17].

Polymerase chain reaction confirmation concerning Salmonella:

DNA Extraction using QIA amp kit according to Shah *et al.* [12]:

As mentioned before.

DNA amplification:

Amplification Reaction for Salmonella-Specific Gene (INVA) According to Shanmugasamy *et Al.* [18]: The PCR for amplification of *invA* gene was performed in a total reaction volume of 50 µl using solution containing 25 microliters of PCR Master mix (Genei, Bangalore), 2 µl of each primer, 19 µl of molecular grade water and 2 µl of extraction for each isolate were used in the reaction. The PCR cycling protocol was applied as following: An initial denaturation at 94°C for 60 sec, followed by 35 cycles of denaturation at 94°C for 60 second, annealing at 64°C for 30 second and extension at 72°C for 30 sec, followed by a final extension at 72°C for 7 min. Finally, 5 µl of each amplicon was electrophoresed in 1.5 % agarose gel (Sigma -USA) and visualized under UV transilluminator. A 100 bp DNA ladder was used as a marker for PCR products.

RESULTS

Table 1: Incidence of *S. aureus* in examined chicken meat samples.

Type of samples	No. of examined samples	Positive samples according to colonial characters on Baird parker agar medium		Positive samples according to biochemical tests	
		No.	%	No.	%
Chicken meat	110	104	94.5	46	41.8

Table 2: PCR confirmation of *S. aureus* isolated from chicken meat samples.

Type of samples	No. of examined samples	Positive samples	
		No.	%
Chicken meat	10	9	90

Table 3: Detection of staphylococcal enterotoxins and enterotoxin genes.

Type of samples	Staphylococcal enterotoxins detected by ELISA				Staphylococcal enterotoxin genes detected by PCR					
	No. of examined samples	No. of positive enterotoxin producing samples	Types of produced staphylococcal enterotoxins		No. of examined samples	No. of positive samples	%	Enterotoxin genes for characterization of <i>S. aureus</i>		
			Types	%				Types	%	
Chicken meat	46	4	SEA	4.3	12	7	58.3	Sea	33.3	
			SEB	2.2				Seb	8.3	
			SED	6.5				Sed	16.7	

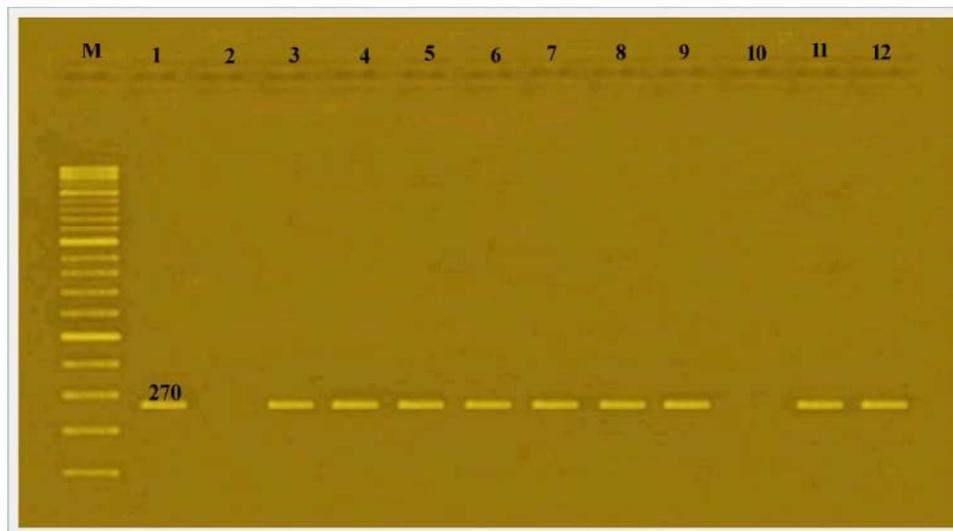


Fig. 1: Agarose gel electrophoresis of PCR products of nuc gene (270 bp) specific for demonstration and characterization of *S. aureus*.

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

Lane 1: Control positive *S. aureus* for nuc gene.

Lane 2: Control negative.

Lanes 3, 4, 5, 6, 7, 8, 9, 11 & 12: Positive *S. aureus* strains for nuc gene.

Lane 10: Negative *S. aureus* for nuc gene.

Table 4: Incidence of *Salmonella* in examined poultry meat samples.

Type of samples	No. of examined samples	Positive samples according to colonial characters		Positive samples according to biochemical tests	
		No.	%	No.	%
Chicken meat	110	21	19.1	7	6.4

Table 5: PCR confirmation of *Salmonella* isolated from examined chicken meat samples:

Type of samples	No. of examined samples	Positive samples	
		No.	%
Chicken meat	7	7	100

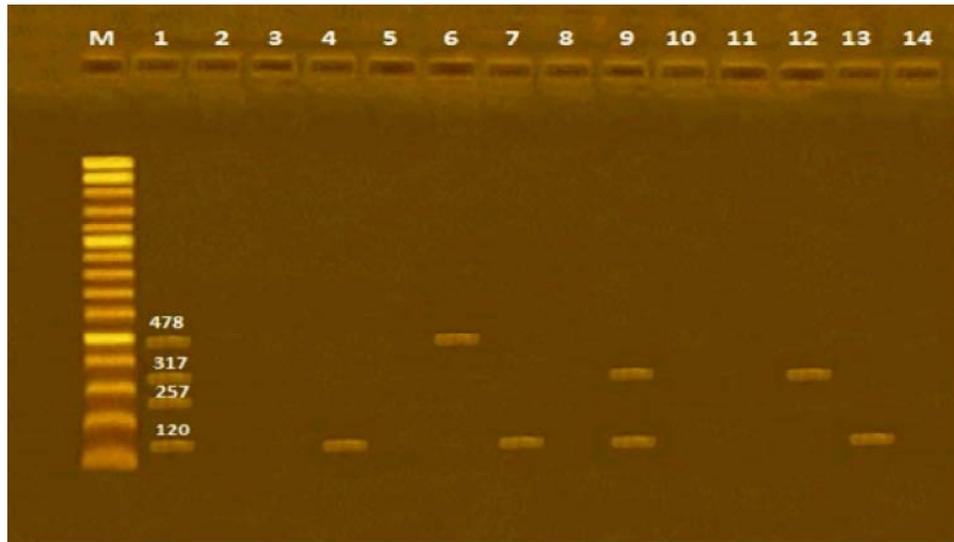


Fig. 2: Agarose gel electrophoresis of multiplex PCR of sea (120 bp), seb (478 bp), sec (257 bp) and sed (317 bp) enterotoxin genes for characterization of *S.aureus*.

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

Lane 1: Control positive for for sea, seb, sec and sed genes.

Lane 2: Control negative.

Lanes 4, 7 & 13: Positive *Staphylococcus aureus* strains for sea gene.

Lane 6: Positive *Staphylococcus aureus* strain for seb gene.

Lane 12: Positive *Staphylococcus aureus* strain for sed gene.

Lane 9: Positive *Staphylococcus aureus* strain for sea & sed genes.

Lanes 3, 5, 8, 10, 11 & 14: Negative *Staphylococcus aureus* strains for enterotoxins.

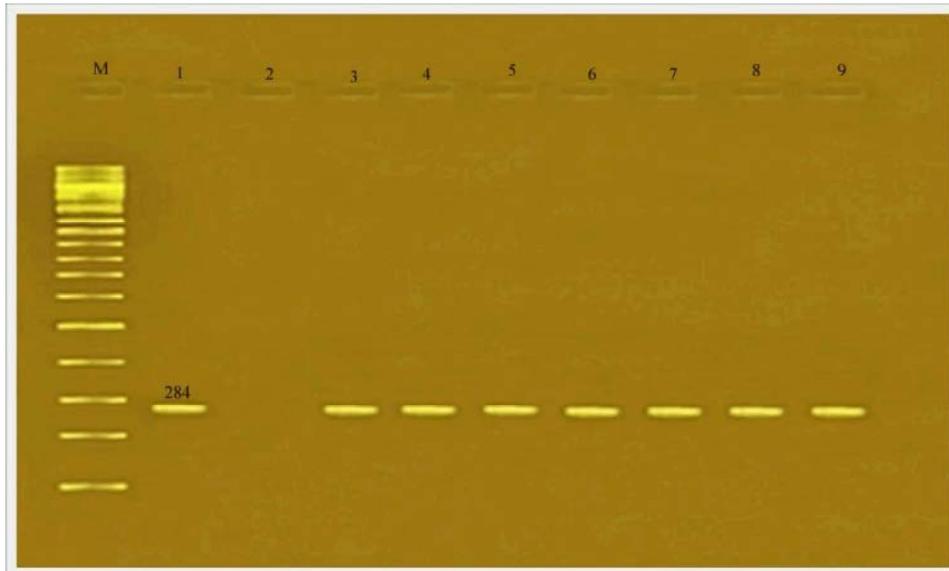


Fig. 3: Agarose gel electrophoresis of PCR of invA gene (284 bp) Specific for characterization of all *Salmonella* species.

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

Lane 1: Control positive *Salmonella* strain for invA gene.

Lane 2: Control negative.

Lanes from 3 to 9: Positive *Salmonella* species for invA gene.

Table 6: Incidence distribution of *Salmonella* serotypes isolated from chicken meat samples (n=110):

Identified strains	Incidence of <i>Salmonella</i> serotypes		Antigenic structure	
	No.	%	O	H
<i>Salmonella</i> Enteritidis group D ₁	2	1.8	1,9,12	g,m: -
<i>Salmonella</i> Typhimurium group B	3	2.7	1,4,5,12	i: 1,2
<i>Salmonella</i> Kentucky group C ₃	2	1.8	8,20	i: Z ₆

DISCUSSIONS AND CONCLUSIONS

Poultry is a major fast growing source of meat in the world today [19]. Because the poultry meat and its products are cheaper than other meat, they are widely accepted and consumed in all parts of the world [20]. Staphylococci can be carried on human hands and nasal passages or throats. Most food borne illness outbreaks are resulted from food handler contamination and production of heat stable toxins in the food. Sanitary food handling, proper cooking and refrigeration could prevent Staphylococcus food borne illness [21].

Table (1) showed that 104 (94.5%) of the examined chicken meat samples were positive for the suspected *S.aureus* according to colonial characters on Baird Parker agar medium. While according to biochemical identification 46 (41.8%) of samples presumed to be contaminated with *S.aureus*. The lower results were obtained by Pesavanto *et al.* [22], Bhargava *et al.* [23] and Irene *et al.* [24] by a percent of 12, 25 and 11.2%, respectively while the higher results were obtained by Waters *et al.* [25] and Karmi [26] by a percent of 92 and 81.18, respectively. The high isolation rate of *S.aureus* indicate poor hygiene and working practices of the meat handlers during the processing stage as well as lack of sterilization of utensils and working surfaces [27]. The variation of *S. aureus* prevalence in retail chicken among different countries could be attributed to differences in a number of factors in each study, including sample size, type of chicken samples (whole chicken versus parts), sampling seasons and the isolation method, etc. [28]. Staphylococci can be introduced into foods during processing from unclean hands, unsanitary utensils and equipment. Both humans and animals may carry staphylococci in nostrils and throats [29]. Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxins preformed in the food [8].

Table (2) and Fig. (1) show that 10 samples of the positive samples were subjected to molecular identification by using PCR and the results revealed that 9 (90%) of samples were positive for *S.aureus*. Some reports indicated that the nuc gene was encoded to

enzyme thermonuclease and the length fragment of nuc gene was equal to 270 pb [30].

Table (3) showed that three types of enterotoxins were detected by ELISA named SEA (4.3%), SEB (2.2%) and SED (6.5%). Contaminated food is a real threat to human welfare. Food-borne diseases are mainly caused by pathogenic bacteria which are either transmitted to humans from the animal reservoir or which contaminate the food process line. *S.aureus* currently attracted increasing attention due to its capability of producing a range of enterotoxins and tissue degrading enzymes [31], the toxins of *S.aureus* are known as enterotoxins because they are able to promote water loss from the small intestinal mucosa resulting in vomiting and diarrhea [32]. It was clear that 12 isolates of the examined chicken meat samples were subjected to multiplex PCR for detection of *S.aureus* classic enterotoxin genes (Table 3 and Fig. 2) and the results revealed seven isolates harbored SE genes as follow: three isolates harbored 1(sea) gene, 1 isolate harbored 1(seb) gene, 1 isolate harbored 1(sed) gene and 1 isolate harbored mixed 1(sea) and 1(sed). Genes encoding sea were represented by a percent of (33.3%), seb (8.3%) while sed (16.7%). Lower result for gene encoding sea was obtained by Zargar *et al.* [33] by a percent of 14.2. The detection of staphylococcal enterotoxin genes by PCR allow the determination of potentially enterotoxigenic *S.aureus* irrespective of whether the strain produces the toxin or not, the inability to detect the enterotoxin by immunological methods may occur due to either low level of production of enterotoxin or to mutation in the coding region or in a regulatory region [34]. For this reason PCR may be considered more sensitive than methods that determine SEs production as immunological methods [35]. The mechanisms of SEs causing food poisoning are not clearly known. However, it is believed that SEs directly affects intestinal epithelium and vagus nerve causing stimulation of the emetic center [36]. Staphylococcal enterotoxin A is one the most important gastroenteritis causing agents. In some areas, more than 50% of FP is caused by staphylococcal enterotoxin A (SEA) [37]. SEA and SEB are two of the most important gastroenteritis causing agents. In some

areas, more than 50% of SFP are caused by SEA. SEA and SEB are the most food poisoning agents (>60%) in USA and England [38]. Infection with *Salmonella* is the most frequently reported cause of bacterial food-borne illness worldwide. Poultry are a common source and, in recent years, much attention has been focused in determining the prevalence of *Salmonella* during the different stages in the poultry production chain [39].

Table (4) showed that 21 (19.1%) of chicken meat samples were positive for *Salmonella* according to colonial characters while according to biochemical characters 7 (6.4%) of samples were contaminated by *Salmonella*. The lower results were obtained by Jakabiet *et al.* [40] and Kiran *et al.* [41] by a percent of 4.6 and 2.42 respectively while the higher results were obtained by Huang *et al.* [39], Van *et al.* [42] and Angélica *et al.* [43] by a percent of 48.9, 53.3 and 9.6 respectively. Nearly similar results were detected by Akbar *et al.* [44] by a percent of 5.26. The low prevalence of *Salmonella* spp. could be due to the fact that pre-enrichment step and selective media required for optimal isolation of *Salmonella* was not used because of inadequate funds [45]. The high prevalence of *Salmonella* in chicken meat may be a result of cross-contamination from intestines during processing and cutting or from cages, floor and workers during retailing or marketing [46].

Table (5) and Fig. (3) show that the seven positive isolates of *Salmonella* were subjected to multiplex PCR and the results showed that 100% of samples were positive. Lower results were obtained by Ramya *et al.* [47] by a percent of 64%. *InvA* gene is involved in the invasion of the cells of the intestinal epithelium and is present in pathogenic *Salmonella*. Therefore for salmonellosis to occur it is important that a gene responsible for invasion must be present. This gene is essential for full virulence in *Salmonella* and is thought to trigger the internalization required for invasion of deeper tissue [48]. PCR assay using the *invA* primers specific for *Salmonella* spp. considerably decreases the number of false-negative results which commonly occur in diagnostic laboratories [49]. Table (6) showed that *Salmonella* serovars identified from chicken meat samples were 2 strains as *Salmonella enteritidis* (1.8%), 3 strains as *Salmonella typhimurium* (2.7%) and 2 strains as *Salmonella Kentucky* (1.8%). Lower results of *Salmonella enteritidis* were obtained by Kaushik *et al.* [50] by a percent of 0.4% while the higher results were obtained by Kim *et al.* [51] and Soomoro *et al.* [52] by a percent of 57.4 and 42.10 respectively. The higher results of *Salmonella typhimurium* were detected by Ibrahim *et al.* [53] and

Kaushik *et al.* [50] by a percent of 41.7 and 6.1% respectively while higher results of *Salmonella Kentucky* were obtained by Khallaf *et al.* [54] by a percent of 23.7%. The high incidence of *Salmonella* species and *Salmonella enteritidis* in chicken might be due to not following hygienic methods in rearing, slaughtering and marketing [47]. The presence of *Salmonella typhimurium* in poultry is of considerable importance from the standpoint of public health [46]. The differences in *Salmonella* prevalence among studies could be attributed to differences in sampling scheme or design, sample type (whole chicken versus chicken parts and chilled versus frozen chickens), the *Salmonella* detection protocol and chicken production systems and companies (conventional versus organic and/or free-range raising practices and integrated versus nonintegrated companies) [55]. The obtained results in the present study revealed that raw poultry meat constitute a certain hazard to consumers as most of the samples were exceeded the permissible limits issued by Commission Regulation (EU) where *Salmonella* must be absent in 25 gm of raw poultry meat. The bacteriological examination of poultry meat including *Salmonella* and *S.aureus* revealed that samples were contaminated. This may be attributed to loss of correct hygienic measures during slaughter, storage and consumption.

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