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DETECTION OF SOME PATHOGENIC BACTERIA IN CHICKEN MEAT SOLD IN POULTRY SHOPS

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ABSTRACT

The current study was done to evaluate the microbiological status of some of the retailed chicken meat. A total of 50 random samples of chicken meat were collected from different poultry shops at Kafrelshiekh Governorate. The samples were evaluated bacteriologically for the detection of some food poisoning pathogens (*Salmonella, E.coli and Staphylococcus aureus*). The obtained results revealed that *Salmonella* spp. were isolated with a percentage of 18%, furthermore, the serological identification of the obtained isolates revealed the presence of *S.* Typhimurium (6%), *S.* Enteritidis (4%), *S.* Kentucky (4%), *S.* Molade (2%) and *S.* Infants (2%). On the other hand, *E.coli* was detected in 12% of the examined sample, and the serological identification of the obtained isolates revealed the presence of the following serotypes O_{78}, O_{103}:H_2, O_1:H_7 and O_{125}:H_{21}. About 20% (10 isolates) of the examined samples were belonging to *Staph. aureus*. Detection of *Staph. aureus* enterotoxin by using multiplex PCR indicates that only 4 strains from the 10 isolates were positive for enterotoxin production One isolate produce Sea, Sec; One isolate produce Sea; one isolate Sec; and the last isolate produce Sea, Seb, Sed enterotoxin. Based on the obtained results, it was concluded that chicken meat poses high risk for public heath, so strict hygienic measures should be taken during slaughtering and processing to prevent cross contamination.

Key words: *Chicken meat, Salmonellae, E.coli, Staphylococcus aureus, Staphylococal enterotoxins*

INTRODUCTION

Meat of chicken broilers are more popular to the consumers because of its easy digestibility and acceptance by the majority of people, although it could be contaminated with a variety of potentially pathogenic food borne pathogens that may cause human illness such as *Salmonella*, *staph. aureus*, and *E.coli* (Mulder and Schlundt, 1999).

Outbreaks of food borne illness occur following ingestion of undercooked meat, handling of raw meat, cross contamination of ready-to-eat products with microbial contaminants from the raw poultry or others introduced during preparation of food (Anon, 1996). Poultry and poultry products are frequently contaminated with salmonellae that can be transmitted to humans either through the handling of raw poultry carcasses and products (Kimura *et al.*, 2004). Because salmonella typically is found in poultry, this type of meat has been an important vehicle in food borne diseases rendering salmonellosis as one of the most frequently reported food borne diseases worldwide (WHO/FAO, 2002).

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Escherichia coli is a major component of the normal intestinal flora of humans and other mammals. Some *E.coli* strains represent primary pathogens with an enhanced potential to cause disease after acquiring specific virulence attributes. These virulence attributes are normally encoded on genetic elements that can be exchanged between different strains, the presence of these virulence genes can magnitude the severity of infection caused by these strains (Li *et al.*, 2005).

Escherichia coli is known to be an indicator of fecal contamination, and its presence in food indicates the possible presence of other enteric pathogen. Some of the *E.coli* strains itself are highly pathogenic in human and animal. People with low immunity are the prime target of the pathogenic strains of *E.coli* (Akbar and Anal, 2011).

Staphylococcus aureus is one of the most important amongst staphylococci species, it is considered the third worldwide cause amongst the food-borne illnesses reported cases (Tamarapu *et al.*, 2001).

In human this bacterium is a major cause of food poisoning, pneumonia, postoperative wound infections, and nosocomial bacteremia, (Sidhu *et al.*, 2007). Staphylococcal enterotoxins are resistant to environmental conditions (freezing, drying, heat

treatment and low pH) that easily destroy the enterotoxin-producing strain. They are also resistant to proteolytic enzymes retaining their activity in the digestive tract after ingestion (Bergdoll, 1989).

Therefore, the present study was planned out to throw the light on some pathogenic bacteria as *Salmonellae, E. coli* and *Staph. aureus* isolated from chicken meat and their public health importance.

MATERIALS AND METHODS

Collection of samples:
A total of 50 samples of deboned poultry meat (25 chicken breast and 25 chicken thigh) were randomly collected from different retail poultry shops at Kafrelsheikh Governorate. The samples were transferred aseptically with minimum of delay to the laboratory in an insulating container where about 10 grams of chicken meat were transferred to 90 ml of 0.1% sterile buffered peptone water then stomached for 2 min. the homogenate then used for bacteriological examination.

1. Isolation and identification of Salmonellae:
The procedures for isolation of Salmonellae according to the techniques recommended by ISO 6579 (2002), morphological and biochemical identification were carried out according to Cruickshank *et al.* (1975). Serological identification of salmonellae isolates was carried out according to (Kauffman, 1974) for the determination of Somatic (O) and Flagellar (H) antigens using Salmonellae antiserum (DENKA SEIKEN Co., Japan). In the food analysis center, faculty of veterinary medicine Benha University.

2. Isolation and identification *E. coli*:
Isolation of *E. coli* was done according to (Feng *et al.,* 2002). Further biochemical assays, as per Bergey's manual of determinative bacteriology (Holt *et al.*, 1994). The serological identification of isolates was carried out according to Kok *et al.* (1996) by using rapid diagnostic *E. coli* antisera kits (Denka Seiken Co., Japan) for diagnosis of the Enteropathogenic types.

3. Isolation and identification of *Staph. aureus*:
It was carried out according to per Bergey's manual of determinative bacteriology (Holt *et al.,* 1994). Screening for pathogenic *Staph. aureus* was done by performing various biochemical assays, including Coagulase test, DNase test (Baird, 1996), and Thermostable nuclease test (TNase) (Lachica *et al.*, 1971).

4. Multiplex PCR for demonstration of staphylococcus enterotoxins genes:-
4.1. Primer sequences of *Staph. aureus* used for PCR identification system:
Application of PCR for demonstration of enterotoxins A, B, C and D (sea, seb, sec &sed) genes as virulence factors of *Staph. aureus* was performed essentially by using primers (Pharmacia Biotech) as shown in the following table:

Table 1: Target genes used in multiplex PCR assay:

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
Sea (F)	**5′** TTGGAAACGGTTAAAACGAA**′3**	120	**Rall *et al.* (2008)**
Sea (R)	**5′** GAACCTTCCCATCAAAAACA **′3**		
Seb (F)	**5′** TCGCATCAAACTGACAAACG **′3**	478	
Seb (R)	**5′** GCGGTACTCTATAAGTGCC **′3**		
Sec (F)	**5′** GACATAAAAGCTAGGAATTT **′3**	257	
Sec (R)	**5′** AAATCGGATTAACATTATCC **′3**		
Sed (F)	**5′** CTAGTTTGGTAATATCTCCT **′3**	317	
Sed (R)	**5′** TAATGCTATATCTTATAGGG **′3**		

4.2. DNA Extraction using QIA amp kit (Shah *et al.*, 2009):
After overnight culture on nutrient agar plates, one or two colonies were suspended in 20 ml of sterile distilled water, then heated at 100ºC for 20 minutes. Accurately, 50-200 µl of the culture were placed in Eppendorf tube and then equal volume from the lysate (50-200 µl) was added, addition of 20-50µl of proteinase K, then incubation at 56 ºC for 20-30 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The solution was added to the column and centrifuged at 8000 rpm for 1 min. then the

filtrate was discarded. The sediment was washed using AW1 buffer (200 µl), the column was centrifuged at 8000 rpm / 1 min, and the filtrate was discarded. Washing was applied by using the AW2 buffer (200µl), the column was centrifuged at 8000 rpm / 1 min. and the filtrate was discarded. The column was placed in a new clean tube then, 25-50 µl from the Elution buffer was added, centrifuged at 8000 rpm/1min. Then the column was discarded. The filtrate was put in clean tube containing the pure genomic DNA. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

4.3. Amplification of enterotoxin genes of *Staph. aureus* (Mehrotra *et al.*, 2000):

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). DNA amplification was performed using the following conditions: initial denaturation for 5 min at 95°C followed by 30 cycles of denaturation (94°C for 2 min), annealing (55°C for 1 min), and extension (72°C for 2 min). A final extension step (72 °C for 5 min) was done after the completion of the cycles.

Amplified products were analyzed by 3% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer stained with ethidium bromide and captured as well as visualized on UV transilluminator at 254 nm. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes.

RESULTS

Table 2: Incidence of bacteria isolated from examined chicken meat samples.

Type of the samples	No of the samples	*Salmonella*		*E. coli*		*Staph. aureus*	
		No	%	No	%	No	%
Chicken breast	25	3	12	0	0	7	28
Chicken thigh	25	6	24	6	24	3	12
Total	50	9	18	6	12	10	20

Table 3: Incidence and serotypes of Salmonellae isolated from examined chicken meat samples. (no. =50)

No. of total positive samples	**Percentage%**	**Identified serotypes**	**No. of isolates**	**Percentage%**	**Antigenic structure**	
					O	**H**
9	18%	***S*.Typhmurium**	3	6%	i = 1, 2	1, 4, 5, 12
		***S*. Enteritidis**	2	4%	g, m: 1, 7	1, 9, 12
		***S*. Kentucky**	2	4%	i: Z_6	8, 20
		***S*. Molade**	1	2%	Z_{10}:Z_6	8,20
		***S*. Infantis**	1	2%	r:1.5	6,7

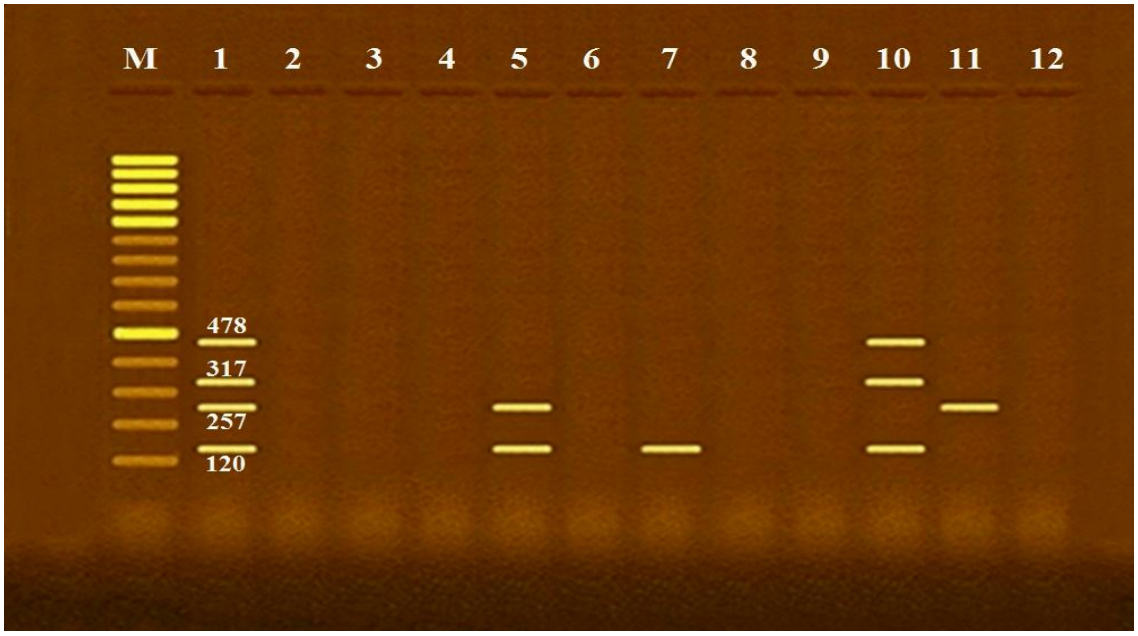
Table 4: Incidence and serotypes of *E.coli* isolated from examined chicken meat samples. (no. =50)

No. of total positive samples	Percentage%	*E. coli* serotype	No. of isolates	%	Strain characterization
6	12%	O_{78}	3	6	EPEC*
		$O_{103} : H_2$	1	2	EHEC*
		$O_1 : H_7$	1	2	EPEC*
		$O_{125} : H_{21}$	1	2	ETEC*
		Total	6	12%	

EPEC*(enteropathogenic E.coli).
EHEC*(enteroheamorrgic E.coli).
ETEC*(enterotoxogenic E. coli).

Table 5: Incidence of *Staph. aureus* from examined chicken meat samples. (no.=50)

No. of isolates	%
10	20%



Photograph 1: 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 sea, seb, sec and sed genes.
Lane 2: Control negative.
Lane 5: Positive *S. aureus* strain for sea and sec genes.
Lane 7: Positive *S. aureus* strain for sea gene.
Lane 10: Positive *S. aureus* strain for sea, seb and sed genes.
Lane 11: Positive *S. aureus* strain for sec gene.
Lanes 3, 4, 6, 8, 9 & 12: Negative *S. aureus* strains for enterotoxins

DISCUSSION

Although chicken meat is rightly regarded as a wholesome nutritious and cheap form of dietary protein, it is associated with some of food-borne illness due to microbial contamination and improper handling. Mass production of chicken meat and its rapid distribution pose a particular risk for wide spread food-borne outbreak infection with enteropathogens (Letellier, 1999).

The muscle tissue and body fluids of healthy living birds are usually free from bacteria, but during slaughtering and processing contamination occurs and cannot be avoided leading to introduction of pathogens into the meat. The source of these pathogens may be endogenous from the gastrointestinal tract or from surrounding environment in farm or slaughterhouse. Poultry are the most common food vehicle of human infection with enteropathogens throughout the world (Abd El-Aziz *et al.*, 2001).

Salmonellae, a genus within Enterobacteriaceae remains as an important human pathogen and it has been reported to be the most common foodborne bacterial disease in the world (Coburn *et al.*, 2007).

Poultry is one of the most important reservoirs of Salmonellae that can be transmitted to humans through the dealing with chicken meat and/or consumption of uncooked meat and eggs (Wales and Davies, 2011; Nawar and Khedr 2014).

In the present study, incidence of Salmonellae as shown in Table (3) revealed the isolation of 9 Salmonellae strains with a percentage of (18%); including 3 (6%) *S.* Typhimurium and 2(4%) for each of *S.* Enteritidis and *S.*Kentucky, and 1 (2%) for each of *S.* Molade and *S.* Infantis. Nearly similar percentage of isolation were recorded by Geilhausen *et al.* (1996), Uyttendaele *et al.* (1998) and Salehi *et al.* (2005) who isolated Salmonellae at percentage of 20%, 19% and 16%, respectively.

Higher results were shown by Change (2000), Whyte *et al.* (2002), Zhao *et al.* (2006) and Mohammed (2012) who isolated Salmonellae at percentage of 25.9%, 23%, 39% and 56% respectively. While on the other hand lower results were shown by Jamshidi *et al.* (2009), Rabie *et al.* (2012) and Amin and Abd el- Rahman (2015) who isolated Salmonellae at percentage of 8.3%, 4% and 3.5% respectively.

The isolated Salmonellae in chicken meat may be attributed to contamination during slaughtering and/or processing which nearly similar to results achieved by Carraminana *et al.* (1997) who reported that the prevalence of Salmonellae in environmental samples ranged from 30% in faeces to 75% in scale water samples. The incidence rate for Salmonellae organisms on carcasses at the post-spray wash site.

It is noticed that, *S.*Typhimurium was the most prevalent isolated Salmonellae serotypes from broiler carcasses and many authors also recorded the isolation of *S.* Typhimurium from poultry meat as Moury *et al.* (1998), Tibaijuka *et al.* (2003), Hosam (2005), Amin and Abd el- Rahman (2015) who mentioned that *S.* Typhimurium was the predominant serotypes recovered from poultry meat.

The antigenic structure of isolated Salmonellae from broiler carcasses was: *S.* Enteritidis, 1, 9, 12 (O.strain), g, m: 1, 7 (H.strain);*S.* Typhmurium, 1, 4, 5, 12 (O.strain), i = 1, 2 (H.strain); *S.* Infantis, 6, 7(O.strain), r : 1.5 (H.strain); *S.* Molade, 8, 20 (O.strain), Z_{10}:Z_6 (H.strain); *S.* Kentucky 8, 20 (O.strain), i: Z_6 (H.strain).

E. coli predominate among aerobic commensal flora present in the gut of man, animal and poultry. So, their presence in meat and poultry meat is indicative of faecal contamination and reflects the influence of handling practices of these products including preparation and faulty dealing by hands (Echeverria *et al.*, 1987).

In many raw foods of animal origin, small number of *E. coli* can be expected because of the close association of these foods with animal environment and the likelihood of contamination of poultry carcasses from fecal material during processing (National Academy of science, 1985).

Data from this work revealed that E. *coli* strains were isolated from broiler carcasses at a percentage of 12% as shown in Table (4), nearly similar results were obtained by Wehab and Aideia (2002) and Vaidya *et al.* (2005) who isolated *E. coli* at percentage of 10% and 14.57% respectively. Higher percentages of isolation were reported by Bhattacharjee *et al.* (1996), Zhao *et al.* (2001), Hossain *et al.* (2008) who isolated *E. coli* at rates of 40.82% , 38.7%, % and 63.6% respectively. Lower percentages of isolation were reported by Suthienkul *et al.* (1990) and Hossam (2012) who isolated *E. coli* at rates of 9%, and 8%, respectively.

Generally, the presence of *E. coli* in examined chicken meat is considered as an indicator for improper handling or unhygienic conditions (Frazier and Westhoff, 1983 and Hashim, 2003).

Out of the 6 strain isolated from chicken meat and the serovars were: O_{78}, O_1:H_7 These isolated serovars belonged to enteropathogenic *E.coli* (EPEC); O_{103}:H_2 belonged to enteroheamorrgic *E.coli* (EHEC); O_{125}:H_{21} belonged to enterotoxogenic *E. coli* (ETEC).

Presence of pathogenic strains of *E. coli* in poultry meat is not only a potential threat of cross contamination but can also lead to become an infectious dose for handlers and consumers. *E. coli* presence in food materials are considered to be an indicator for the presence of other pathogenic bacteria in the respective food items (Shar *et al.,* 2010).

Staph.aureus is the most concern to food microbiologists. Staphylococcal food poisoning is a syndrome characterized by nausea, vomiting, diarrhea, general malaise and weakness beginning one to six hours (usually 2 to 4 hrs.) after ingestion although the illness is seldom fatal and the complications including dehydration shock and may be accompanied with severe attacks. Recovery usually occurs after about 24 hours but may take several days (Eley, 1992 and Ward *et al.*, 1997).

Results in Table (5) revealed that the incidence of *staph.aureus* was 20% in examined Broiler carcasses. Nearly similar results were detected by Momtaz *et al.* (2013) who isolated *staph.aureus* at a rate of 22.77% higher incidence were obtained by Kozacinski *et al.* (2006), Amin (2008), and Mohamed (2013) by incidence of 30.03%, 37%, and 32% respectively.

Staphylococcal food poisoning (SFP) is one of the most common food-borne diseases and results from the ingestion of staphylococcal enterotoxins (SEs) preformed in food by enterotoxigenic strains of *Staph. aureus* more than 20 SEs have been described from SEA to SElV. All of them have super antigenic activity whereas half of them have been proved to be emetic, representing a potential hazard for consumers Hennekinne *et al.* (2012).

The five major serological groups of enterotoxins (SEA, SEB, SEC, SED, and SEE) have been proven to induce gastro-enteric syndrome Klotz *et al.* (2003).

As shown in photopgraph (1), only four strains from the positive 10 strains of *Staph.aureus* were positive for enterotoxins detection and SEA was superior to other toxic genes as it detected in 3 isolates. One isolate produce Sea, Sec; One isolate produce Sea; one isolate Sec; and one isolate Sea, Seb, Sed enterotoxin.

Multiplex PCR technique has been recently used for rapid detection and discrimination of enterotoxins genes. Regarding the enterotoxin genotype, previous studies on *Staph.aureus* proved that enterotoxin PCR determinations are in a high agreement (97–100%) with the toxin production as defined by immunoassays (Letertre *et al.,* 2003). Pinto *et al.* (2005) found a total of 40 (30%) *Staph.aureus* food isolates positive for se genes. Among them, the sec genotype was the most frequent (22 strains, 20% of total se positive strains) and sea the second more frequent (14 strains, 13%), which is in accordance with the results obtained by (Fueyo *et al.,* 2001). Meanwhile Ikeda *et al.* (2004) did not detect any of Seb, Sec, Sed genes in any of the skim milk samples but could detect Sea and Seh in 10 samples and Seg and Sei genes were detect in 7 samples.

CONCLUSION

The microbiological examination of the present study revealed that high incidence of different kinds of pathogenic microorganisms that collectively constitutes public health hazard to consumers as *Salmonellae, E.coli* and *Staph.aureus* which are a true indicator of poor sanitary condition, cross contamination, fecal pollution, and bad personal hygiene conditions during handling, packing and selling. So, new strategies such as control of raw materials, proper handling, cleaning and disinfection of equipment from farm to fork must be adopted. Also, using of multiplex PCR technique for detection of *Staph.aureus* enterotoxins based on genotypic basis allowed rapid, reliable, effeicient and less costly compared with routine laboratory diagnosis. It could be implemented as an alternative to phynotype and immunology- based tests in the routine food microbiological analysis.

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الكشف عن بعض البكتريا الممرضة فى لحوم الدجاج المباعة في محلات الدواجن

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تم في هذه الدراسة تجميع عدد ٥٠عينة من لحوم الدجاج (٢٥ من الصدور و٢٥ من الأوراك) من المحلات الموجودة في محافظة كفر الشيخ لاستبيان تواجد بعض الميكروبات االممرضة مثل ميكروب السالمونيلا ، الايشيريكية القولونية والمكور العنقودى الذهبى وقد أظهرت النتائج أن ميكروب السالمونيلا تم عزله بنسبة ١٨ % (٩ عترات) وتم تصنيفهم سيرولوجيا إلى ٣ (٦%) *S.* Typhimurium و٢ (٤%) *S.* Enteritidis و٢ (٤%) *S.*Kentucky و١ (٢%) *S.* Molade و ١ (٢%) *S.* Infants وتم عزل ميكروب الإيشيريكية القولونية بنسبة ١٢ % (٦عترات) وتم تصنيفهم سيرولوجيا الي: *E.coli* O_{78}, O_{103}:H_2, O_1:H_7, O_{125}:H_{21} أما بالنسبة لميكروب المكور العنقودى الذهبى فقد تم عزله بنسبة 20% (10عترات) من لحوم الدجاج وقد تم الكشف عن جينات السموم المعوية A, B, C, D لهذا الميكروب في بعض العينات الإيجابية للعزل وقد أظهرت النتائج احتواء ٤عترات من العترات الإيجابية لميكروب المكور العنقودي علي نسب مختلفة من هذه السموم (عترة تحتوي علي A,C ،عترة تحتوي علي A ،عترة تحتوي علي C وعترة تحتوي علي A,b,d) وقد تمت مناقشة تاثير الميكروبات محل الدراسة علي الصحة العامة ووضع التوصيات اللازمة للحد من إنتشار هذه الميكروبات والحصول علي منتج صحي آمن للمستهلك.