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Enteropathogenic *Escherichia coli* contaminating chicken meat cuts

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ABSTRACT

Although chicken meat cuts are of high nutritious, economic and consumers' demanded meats, but it may be a serious microbial food poisoning cause referring to low hygienic procedures during production. Therefore, the current study was established to isolate and identify enteropathogenic *Escherichia coli* in one hundred random samples of raw chicken meat cuts represented by chicken breast and thigh meat samples (50 of each) that were collected from different poultry shops in Qalubiya Governorate. The obtained results revealed that the incidence of *E. coli* was (24%) in all the examined samples, where it in thigh samples (28%) were more than in breast ones (20%). Serotyping of the isolated *E. coli* strains revealed that they belonged to both enteropathogenic (EPEC) and enterotoxigenic *E. coli* (ETEC) serotypes. Moreover, out of six isolates that were molecularly investigated for Shiga toxins producing genes, only one isolate revealed presence of Shiga toxin-2 producing gene (*stx*-2) with prevalence of 16.67%. So, it was concluded that, breast and thigh chicken meat cuts may harbor pathogenic *E. coli* that possess public health hazards affecting consumers' health.

1. INTRODUCTION

Chicken trade has been progressively raised a magnificent development rate, that referred to that chicken meats have reliable reasonable prices, highly nutritious, rapid production cycle, and a flexible good sort of advanced-processed products (Barbut, 2015).

Chicken meat cuts are continuously reported to be an incriminated common source of foodborne pathogens like salmonella *spp*. and *E. coli* (Yulistiani*et al.*, 2019) which can be loaded to chicken meat across the production cycle beginning with scalding, defeathering and evisceration; also, cross contamination from contaminated close carcasses and equipment.

In addition, Mpundu *et al.* (2019) indicated that the importance of the used water sources in chicken carcass's dressing which represents a major source of high contamination levels. They found *E. coli* in 70% of the selected dressed chickens; where the number of total coliforms and *E. coli* were significantly higher in washed carcasses than pre-washed carcasses (65 and 35%, respectively). Furthermore, intestinal contents may contaminate carcass's meat regarding to improper evisceration (Abd Elzaher et al., 2018).

The most of virulence genes of *E. coli*, especially Shiga toxins producing genes (*stx*-1 and *stx*-2), can infect consumers by consumption of the inadequately heat-treated contaminated meats causing significant troubles, including digestive, hematological, urinary, and respiratory affections (Makvanaand Krilov, 2015). Moreover, their presence in poultry meat and its products indicates lack of proper sanitation and possible fecal contamination (Nagi, 2020).

Also, Shiga-toxin producing *E. coli* (STEC) in patients especially young children develop watery diarrhea accompanied with abdominal pain (Brzuszkiewicz *et al.*, 2011) and after which bloody diarrhea may appear within 2-4 days in about 80% of cases. In other cases, urinary tract infection, sepsis and other extra intestinal infections may occur (Griffin *et al.*, 2012).

Other than *E. coli* O_{157} STEC strains, O26:H11/H^-, O_{91}:H_{21}/H^-, O_{103}:H_2, O_{111}:H^-, O_{113}:H_{21}, O_{121}:H_{19}, O_{128}:H_2/H^-, and O_{145}:H_{28}/H^- were among the most common causes of foodborne and HUS diseases (Ursula *et al.*, 2012).

Large outbreak with more than 800 hemolytic uremic syndrome (HUS) and 50 deaths in 2011 caused by STEC strains were reported (Tozzoli*et al.*, 2014); this pathogenic *E. coli* cause diarrhea, hemorrhagic colitis (HC) and HUS as it has the capability of attaching and effacing (A/E) lesion to the enterocyte sharing with another *E. coli* group as entero-aggregative *E. coli*.

Therefore, the current study aimed to define the prevalence of enteropathogenic *E. coli* in chicken meat cuts.

2. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 100 random samples of different raw chilled chicken meat cuts (breast and thigh) was collected from different chicken shops (50 of each), at Qalubiya Governorate, Egypt, for detection the prevalence of enteropathogenic *E. coli* strains in these samples, serotyping, and molecular typing of toxinogenic *E. coli.*

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2.2. Preparation of samples (ISO, 2017):
Under complete aseptic condition, twenty-five grams of the examined meat samples with 225ml 0.1% sterile peptone water were aseptically mixed, and homogenized, then ten–fold serial dilutions were prepared.
2.3. Enumeration and isolation of E. coli (ISO, 2001):
One ml from the previously prepared serial dilution was cultured in TBX agar by pour-plate technique and incubated at 44ºC/24hrs. Suspected colonies (greenish to blue colonies) were counted and isolated for more identification.
2.4. Identification of E. coli isolates:
It was operated based on the morphological and biochemical characters as was reported by (MacFaddin, 2000); additionally, serological identification was performed according to Kok*et al.* (1996).
2.5. Statistical Analysis:
the obtained results were statistically described using SPSS software according to Feldman *et al.* (2003).
2.6. Molecular detection of Shiga toxins 1 and 2 (stx-1 and stx-2):
2.6.1. Oligonucleotide primers sequences were prepared according to Dipineto *et al.*, 2006 as mentioned in Table (1).
2.6.2. DNA extraction, and amplification processes were performed following the commercial ready QIAamp DNA mini kit instructions.

Table 1 Oligonucleotide primers sequences

Gene	Primer sequence (5'-3')	Length of amplified product	Reference
Stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614 bp	Dipineto*et al.*, 2006
Stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779 bp	

3. RESULTS

The bacteriological examinations of the examined chicken breast and thigh samples as mentioned in Table (2), revealed detection of *E. coli* in (24%) of the total examined samples, which were considered rejected based on the Egyptian standards, where thigh samples were more contaminated than breast ones. In details, 10 (20%) and 14 (28%) of breast and thigh samples appeared to harbor *E. coli*, respectively.

Table 2 Statistical analytical results of E. coli count (log10) in the examined fresh breast and thigh chicken samples (n = 50 for each)

Meat samples	+ve samples		Min.	Max.	Mean ± S.E.M*	EOS standards	Accepted		Rejected	
	No.	%					No.	%	No.	%
Breast	10	20	3.00	5.14	4.21±0.01	Free	40	80	10	20
Thigh	14	28	4.54	5.34	4.94±0.02	Free	36	72	14	28
Total	24	24					76	76	24	24

*S. E.M= Standard error of mean.

EOS legislation referring to the Egyptian standard no. (1651/ 2005).
Escherichia coli isolates were serotyped which belonged to enteropathogenic (EPEC) as O_{55}:H_7 andO_{114}:H_{21} serotypes with total incidence of 54.2 and 25.0%, respectively, and enterotoxigenic *E. coli* (ETEC) as O_{125}:H_{18} serotype with total incidence of 20.8%, as mentioned in Table (3).

Table 3 Serotyping of E. coli isolated from the examined fresh chicken meat samples (n= 24 isolates)

E. coli strains	Samples: Breast No.	Breast %*	Thigh No.	Thigh %*	Total No.	Total %**	Strain characteristics
O_{55}:H_7	5	50	8	57.1	13	54.2	EPEC
O_{114}:H_{21}	1	10	5	35.7	6	25.0	EPEC
O_{125}:H_{18}	4	40	1	7.14	5	20.8	ETEC

EPEC = Enter pathogenic E. coli ETEC = Enter toxigenic E. coli
%*: Incidence of E. coli serotypes in relation to number of isolates (10 for breast, 14 for thigh samples).
%**: Incidence of total *E. coli* serotypes in relation to total number of isolates (24).

Moreover, out of six isolates that were molecularly investigated for Shiga toxins producing genes, only one isolate revealed presence of Shiga toxin-2 producing gene with prevalence of 16.67% as mentioned in Tables (4) and Fig. (1).

Table 4 Occurrence of virulence genes of Shiga toxin-producing E. coli isolated from the examined samples of chicken cuts (n=6 isolates)

E. coli virulence genes	*stx1* No.	*stx1* %	*stx2* No.	*stx2* %
	0	0	1	16.67

E. coli ID	Results: *Stx1*	*Stx2*
1	-	-
2	-	-
3	-	+
4	-	-
5	-	-
6	-	-

STX1: Shiga- toxin 1 gene
STX2: Shiga- toxin 2 gene

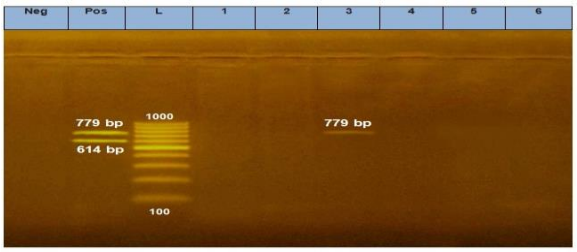

Figure 1 Agarose gel electrophoresis of multiplex PCR of *stx1* (614 bp), and *stx2* (779 bp) virulence genes of Enteropathogenic *E. coli*. Lane L: 100 bp ladder as molecular size DNA marker. Lane Pos: Control positive *E. coli* for *stx1*, and *stx2* genes. Lane Neg: Control negative. Lane 3: Positive *E. coli* for *stx2* gene, while negative for *stx1*.Lanes 1, 2, 4, 5, and 6: Negative *E. coli* for *stx1* and *stx2* genes.

4. DISCUSSION

Bacterial foodborne illness has been reported as international problem causing decline in economic growth (WHO, 2005), as the bacterial contamination of food with

different foodborne pathogens and its multiplication, growth and/or toxin production has public health importance (Mensah *et al.*, 2002).

Detection of various foodborne pathogens in fresh chicken meat cuts throw-lights on the poorly hygiene and personal conditions performed during different stages of slaughtering, storage, transportation and handling processes, such as contaminated water (Mpundu *et al.*, 2019), intestinal contamination (Kamal, 2017), dusty air currents, sewage, and used equipment, and surrounding environmental surfaces (USFDA, 2012).

Escherichia coli is one of the most frequently isolated bacterial contaminations of chicken meat samples. So, referring to the documented results in Tables (1 to 4) and Fig. (1), they can be compared with the previously recorded results by Arakeeb (2020) (42.8% and 62.5% in breast and thigh samples, respectively); and Nagi (2020) who detected *E. coli* in 20 and 26.67% of the examined breast and thigh samples, respectively. Moreover, Elsabagh-Rasha (2010), Edris-Shima (2012), Hassanin *et al.* (2017), and Elsisy (2019) who recorded isolation of different *E. coli* serotypes included O_{55}:H_7, O114:H21, andO_{125}:H_{18}that belonged to EPEC and ETEC groups.

Furthermore, khattab (2020) and Nagi (2020) recorded detection of Shiga-toxin producing genes in their *E. coli* strains that were isolated from raw chicken meat cuts.

Variations between the current results and different authors may be referred to variations in sanitary and hygienic conditions of the collected samples, groceries variations, surrounded environment, and location of collection.

In addition, incidence of thigh contamination more than breast ones may be attributed to that the high fat content of thigh and the evisceration faults leading to fecal contamination which has been usually more prevalence in thigh than breast meats.

5. CONCLUSION

Finally, the obtained results proved that *E. coli* is a seriously, significant widespread foodborne bacterium, which represents public health hazards threatening meat safety and consumer's health. In addition, fresh thigh samples showed higher contamination rate with *E. coli* than breast samples with isolation of EPEC and ETEC strains from the examined samples. Therefore, addition of safe, hygienic sanitizers in washing water, followed by thorough cooking and hygienic handling is strongly recommended.

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