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Study on Enterotoxigenic *Escherichia coli* Producing Extended Spectrum Beta Lactamase (ESBL) from Chicken Meat and its Products

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ABSTRACT

This study aimed to identify the prevalence of enterotoxigenic *E. coli* producing ESBL in some chicken meat and chicken meat products. A total of 300 samples, including 120 chicken meat samples, 180 nuggets, frankfurter and luncheon (60 each) were taken from different supermarkets of different sanitation levels. These samples were subjected to bacteriological examination. The results showed that the prevalence of *E. coli* in chicken meat, nuggets, frankfurter and luncheon was 36.7, 16.6, 20 and 26.7%, respectively. In addition, the mean values of Aerobic plate count in chicken meat, nuggets, frankfurter and luncheon were $2.05\times10^5\pm0.43\times10^4$, $3.4\times10^3\pm0.22\times10^3$, $4.7\times10^3\pm0.11\times10^3$ and $6.5\times10^3\pm0.15\times10^3$cfu/g; and the mean values of Coliform count were $2.4\times10^3\pm0.11\times10^3$, $6.3\times10^2\pm0.36\times10^2$, $8.24\times10^2\pm0.38\times10^2$ and $8.5\times10^2\pm 0.34\times10^2$cfu/g, respectively. *E. coli* isolates were serogrouped into O_{86}:k_{61}, O_{119}:k_{69}, O_{55}:k_{59}, O_{125}:k_{70}, O_{25}:k_{11}, O_{128}:k_{67}, O_{78}:k_{80} and untyped serogroups. Antimicrobial susceptibility testing of most isolates revealed the presence of multidrug-resistant strains. In addition, virulence genes (*Sta*, *Lt* and *Stx2* genes) were detected in 2, 1 and 4 isolates; and antibiotic resistance genes (*bla TEM*, *Bla CTX-M* and *AmpC* genes) were detected in 100% of isolates indicating high resistance to beta-lactamase antibiotics.

Key words: *E. coli*, ESBL, Chicken Meat, Chicken meat Products, Virulence Genes, Antibiotic resistance.

INTRODUCTION

People of all ages get nutritional benefits from chicken and chicken products because it contains all the essential amino acids, high level of unsaturated fatty acids and low cholesterol level. Furthermore, vitamins, including niacin, riboflavin, thiamine, and ascorbic acid are abundant in chicken meat (Cartoni et al. 2022; Raheel et al. 2022). On the other hand, chicken meat is a common source of many food-borne bacteria such as *Escherichia coli*, *Salmonellae* and coagulase-positive *Staph. aureus* (Gonçalves-Tenório et al. 2018). The carcass could be contaminated at several points throughout the processing operation; during scalding, de-feathering, evisceration, processing equipment, improper cooking, refrigeration or storage (Gaafar et al. 2019).

Escherichia coli is regularly recovered from the digestive tracts of domestic and wild animals as well as humans. It is one of the most important foods borne infections of public health significance incriminated in poultry meat and meat products worldwide (Samy et al. 2022). Enterotoxigenic *E. coli* infections cause abdominal cramps, low-grade fever, watery diarrhea, and nausea (Yang et al. 2017). It produces two types of enterotoxins: heat-stable (ST) and heat-labile (LT) each has a different function in pathogenesis.

Antibiotic usage in food-producing animals is a public health concern because antibiotic-resistant zoonotic infections could be transmitted to humans through the consumption and/or handling of animal-derived food (Adzitey et al. 2021; Ijaz et al. 2022; Mickdam et al. 2022).

Misuse of antimicrobial medications and poor infection control methods hastened the spread of antimicrobial resistance (El Jalil et al. 2020; WHO 2021). Infections with extended-spectrum Beta-lactamase (ESBL)-producing *Enterobacteriaceae* become a significant problem Worldwide Badr et al. (2022). The existence of ESBL genes in food-producing animals

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indicates a potential hazard for animals (Moawad et al. 2018) and humans (Rahman et al. 2020) which highlights the importance of continuous monitoring.

This study was planned to investigate the prevalence of enterotoxigenic *E. coli* producing ESBL in chicken meat and its products. Moreover, monitoring the sensitivity to various antimicrobial agents. Also, the presence of virulence and antibiotic resistance genes were determined using PCR.

MATERIALS AND METHODS

Ethical approval

The study was conducted at Bacteriology Department, Animal Health Research Institute, Dokki, Giza, Egypt. The experimental protocol was approved by the Institutional Animal Care and Use Committee, Agriculture Research Center, Giza, Egypt.

Sampling

A total of Three hundred samples including 120 chicken meat and 180 chicken nuggets, frankfurter and luncheon samples (60 each) were randomly collected from different supermarkets and retailers of different sanitation levels in different cities in El-Behera governorate, Egypt. Samples were collected aseptically, packed, identified and transferred in cooling iceboxes to the laboratory for bacteriological examination.

Preparation of the samples according to (APHA 2001): Twenty-five grams of each examined samples were transferred to a septic blender jar, and 225 ml of 0.1% sterile peptone water was aseptically added to the jar's contents. Each sample was then homogenized in the stomacher at 2000rpm for 1-2min to provide a homogenate from which ten-fold serial dilutions were prepared.

Determination of Aerobic plate count according to APHA (2001): It was conducted using standard plate count agar media.

Determination of Coliform count according to ISO 4832 (2006): Violet red bile agar medium has been used.

Isolation and identification of *E. coli* according to Quinn et al. (2011): Samples were inoculated in buffered peptone water and incubated at 37^{O}C for 18 to 24h under aerobic conditions. A loopful from the enriched broth was streaked onto MacConkey's agar and eosin methylene blue agar (EMB) and incubated at 37^{O}C for 24h. The metallic green colonies were picked up and identified biochemically and serologically. Media and biochemical test reagents were purchased from Hi-media®.

Serogrouping of *E. coli* isolate according to Quinn et al. (2011): *E. coli* isolates were serogrouped by slide agglutination test using polyvalent and monovalent antisera (Denka Seiken Co. LTD, Tokyo, Japan for antisera).

Antibiotic sensitivity of *E. coli* isolates according to CLSI (2022): Antibiogram of *E. coli* isolates was performed by disc diffusion method using available antibiotic discs purchased from MAST® Diagnostics. As the antibiotic discs used in the study were Levofloxacin (5µ), Amikacin (30µg), Gentamycin (5µg), Amoxicillin (25µg), Oxytetracycline (30µg), Vancomycin (30µg), Cefotaxime (30µg), Ampicillin (30µg), Ciprofloxacin (10µg) and Penicillin G (10µg). Antibiotic sensitivity in relation to zone of inhibition was interpreted by the Manufacturing Company.

Molecular characterization of *E. coli* isolates

DNA extraction: The QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was used to extract DNA from samples with slight modifications to the manufacturer's recommendations. Briefly, 200µL of the sample suspension was incubated with 10µL of proteinase K and 200µL of lysis buffer at 56°C for 10min. After incubation, 200µL of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100µL of elution buffer provided in the kit.

Table 1: Primers sequences, target genes, amplicon sizes and cycling conditions for conventional PCR

Target Gene	Primers sequences	Product size(bp)	Primary Denaturation	Amplification (35 cycles) Secondary denaturation	Annealing	Extension	Final extension	References
phoA	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	720bp	94˚C 5min	94˚C 30S.	55˚C 40S	72˚C 45S	72˚C 10min	Hu et al. (2011)
blaTEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516bp	94˚C 5min.	94˚C 30S	54˚C 40S	72˚C 45S	72˚C 10min	Colom et al. (2003)
ampC	TTCTATCAAMACTGGCARCC CCYTTTTATGTACCCAYGA	550bp	94˚C 5S	94˚C 30S	50˚C 40S	72˚C 45S	72˚C 10min.	Srinivasan et al. (2005)
blaCTX-M	ATG TGC AGY ACC AGT AAR GTK ATG GC TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593bp	94˚C 5min	94˚C 30S	54˚C 40S	72˚C 45S	72˚C. 10min	Archambault et al. (2006)
Stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTT TG	779bp	94˚C 5min	94˚C 30S	58˚C 40S	72˚C 45S	72˚C 10min	Dipineto et al. (2006)
Sta/St1	GAAACAACATGACGGGAGGT GCACAGGCAGGATTACAACA	229bp	94˚C 5min	94˚C 30S	57˚C 30S	72˚C 30S	72˚C 7min	Lee et al. (2008)
Lt	GGTTTCTGCGTTAGGTGGAA GGGACTTCGACCTGAAATGT	606bp	94˚C 5min	94˚C 30S	57˚C 40S	72˚C 45S	72˚C 10min	

phoA=Alkaline Phosphatase (Fig.1), Stx2= Shiga toxins 2 (Fig. 2), LT=heat-labile toxin (Fig. 3), Sta/St1=heat-stable toxin 1 (Fig.4), bLaCTX-M=Beta-LactamaseCTX-M (Fig. 5), blaTEM=Beta-LactamaseTEM (Fig. 6), and ampC=AmpC Beta-Lactamase (Fig. 7).

Oligonucleotide Primer: The primers used were provided by Metabion (Germany), and they are listed in Table 1.

Statistical Analysis

All the obtained results were statistically evaluated according to Feldman (2003).

RESULTS AND DISCUSSION

Contamination of poultry meat with food-borne pathogens is still a significant public health concern. Chicken meat may contain many microorganisms that cause food poisoning. in the last two decades *E. coli*-producing ESBLS have been widely reported in animals and humans posing a severe public health threat (Koju et al. 2022). Moreover, the presence of *E. coli* in chicken meat implies fecal pollution which could be caused by poor slaughtering practices, contaminated surfaces and/or meat handling (Samy et al. 2022).

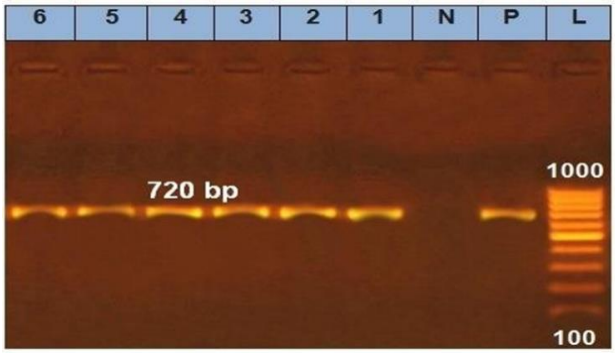

Fig. 1: Agarose gel electrophoresis of PCR products showing amplification of *E. coli phoA* gene products at 720bp. MWM-molecular weight marker (100-1000bp DNA ladder), + control (positive, negative) + six isolates of *E. coli as* isolates No. (2, 4 and 6) chicken meat and isolate No. (1) nuggets, No. (3) frankfurter and isolate No. (5) from luncheon. All isolates were positive *E. coli* gene.

Results relating to the prevalence of *E. coli* in chicken meat and chicken meat products shown in Table 2 appear to be similar to Eman and Sherifa (2012) as 25% in luncheon. Lower results (6.66%) in nuggets were detected by Gaafar et al. (2019). Abdel Tawab et al. (2015) and Al-Jobori et al. (2016) recorded higher results in chicken meat as 40 and 45%, respectively and results from nuggets as 33.3 and 30%, respectively. However, El-Kewaiey (2012) didn't detect *E. coli* in nuggets.

Results regarding aerobic plate count (APC) shown in Table 3 indicated the highest level of contamination in chicken luncheon which could be due to insufficient cooking, post-processing contamination, cross-contamination via slicing machines or cutting knives used in food service facilities, raw materials and spices introduced during manufacturing. In contrast, chicken nuggets were the lowest product, which may be due to the freezing effect that minimizes the total count of *E. coli* (Abdel Fattah 2014). Findings were like the findings reported by Ibrahim et al. (2018) and Khalafalla et al. (2019) in chicken meat and nuggets as $3.04\times10^5\pm0.39\times10^5$ and $3\times10^3\pm5\times10^1$, respectively. In addition, Eman and Sherifa (2012) recorded 8.5×10^3 in chicken luncheon. Higher results have also been reported by Hassanin et al. (2016) and Ibrahim et al. (2018) in chicken meat and nuggets as 3.78×10^6cfu/g and $4.78\times10^5\pm0.51\times10^5$, respectively. Furthermore, Nabil et al. (2014) recorded the mean APC in luncheon and frankfurter as $7.4\times10^6\pm3.1\times10^6$ and $5.8\times10^7\pm2.5\times10^7$cfu/g, respectively. Lower results were obtained by Daoud (2012) and Khalafalla et al. (2019) in chicken meat and frankfurter as 2.1×10^3 and $2\times10^3\pm 3\times10^3$cfu/g, respectively.

Table 2: Prevalence of *E. coli* in examined chicken meat and chicken meat products samples

Sample Type	Samples examined	*E. coli* positive samples	
		No.	%
Chicken meat	120	44	36.7
Nuggets	60	10	16.6
Frankfurter	60	12	20
Luncheon	60	16	26.7
Total	300	82	27.3

Table 3: Total aerobic plate count (APC) of examined chicken meat and chicken meat samples.

Samples	Minimum	Maximum	Mean±SEM
Chicken meat	13.3×10^4	2.7×10^5	$2.05\times10^5\pm0.43\times10^4$
Nuggets	1.2×10^3	6.3×10^3	$3.4\times10^3\pm0.22\times10^3$
Frankfurter	3.6×10^3	5.8×10^3	$4.7\times10^3\pm0.11\times10^3$
Luncheon	5.8×10^3	8.4×10^3	$6.5\times10^3\pm0.15\times10^3$

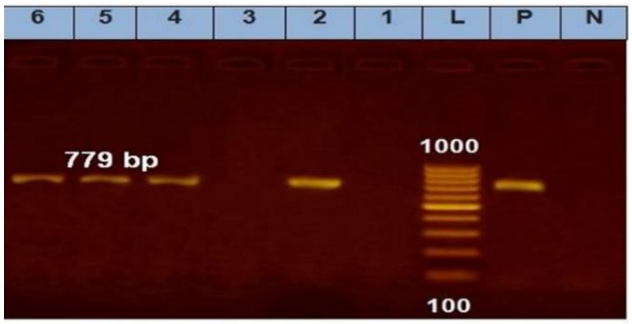

Fig. 2. Agarose gel electrophoresis of PCR products showing amplification of *Stx2* gene products at 779bp. MWM-molecular weight marker (100-1000bp DNA ladder), + control (positive, negative) + six isolates of *E. coli*. The isolates (2,4,5 and 6) were positive, while isolates (1,3) were negative.

Results regarding coliform count (Table 4) are similar to 2.07×10^3 reported by Hassanin et al. (2016). The source of coliform contamination could be due to chopping, dressing of carcasses, unclean hands, shopping blocks, knives used for handling and cutting as well as contaminated water (Elsaid et al. 2019). Hamada et al. (2008) and Javadi and Safarmashaei (2011) recorded higher values of coliform count in luncheon and chicken meat as 5.46×10^3 and 1.4×10^4, respectively. In addition, higher values in luncheon and frankfurter were reported by Bkheet et al. (2007) as 2.3×10^4 and 6.4×10^3cfu/g, respectively. Lower counts were reported by Daoud (2012) and Hassan (2015) as 5.1×10^3 and 1.7×10^3, respectively. Lower counts in luncheon and frankfurter were reported by Nabil et al. (2014) as $3.0\times10^2\pm0.9\times10^2$ and $5.3\times10^2\pm5.0\times10^2$, respectively.

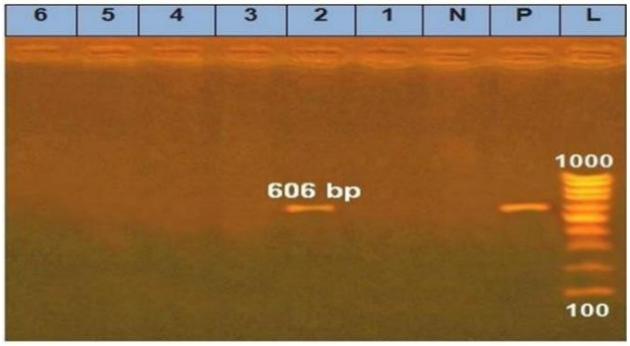

Fig. 3. Agarose gel electrophoresis of PCR products showing amplification of *Lt* gene products at 606bp. MWM-molecular weight marker (100-1000bp DNA ladder), + control (positive, negative) + six isolates of *E. coli*. Isolate No (2) was positive, while other isolates (1,3,4,5, 6) were negative.

Table 4: Total coliform count of examined chicken meat and chicken meat samples

Samples	Minimum	Maximum	Mean±SEM
Chicken meat	1.0×10^3	4.2×10^3	$2.4\times10^3\pm0.11\times10^3$
Nuggets	4.0×10^2	1.2×10^3	$6.3\times10^2\pm0.36\times10^2$
Frankfurter	6.0×10^2	1.3×10^3	$8.24\times10^2\pm0.38\times10^2$
Luncheon	3.0×10^2	1.1×10^3	$8.5\times10^2\pm0.34\times10^2$

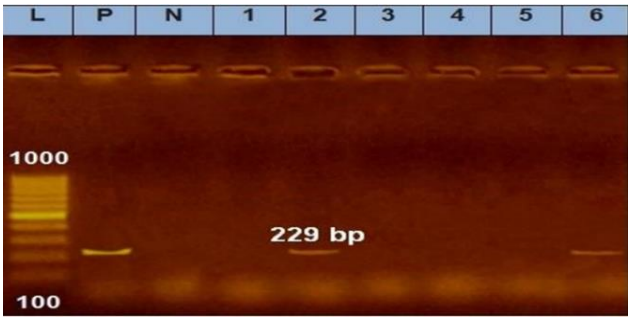

Fig. 4. Agarose gel electrophoresis of PCR products showing amplification of *Sta/St1* gene products at 229bp. MWM-molecular weight marker (100-1000bp DNA ladder), + control (positive, negative) + six isolates of *E. coli*. The isolates No (2 and 6) were positive while isolates (1,3,4,5) were negative.

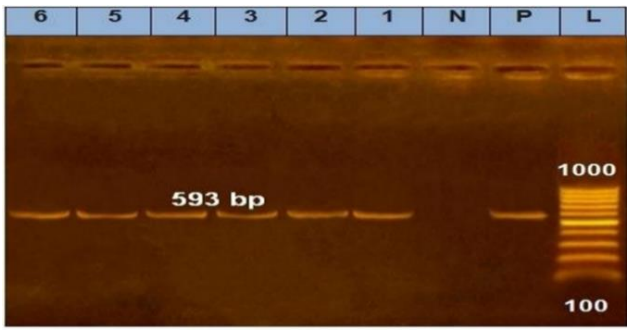

Fig. 5. Agarose gel electrophoresis of PCR products showing amplification of *blaCTX-M* gene products at 593bp. MWM-molecular weight marker (100-1000bp DNA ladder), + control (positive, negative), all six isolates were positive for *blaCTX- M* gene.

Similar to the results of the present study (Table 5), *E. coli* serogroups O_{55}: K_{59} and O_{119}: K_{69} were detected in poultry cuts (thigh and breast) by Saad et al. (2011). In addition, Samah and Erfan (2013) detected O_{125}:K_{70},

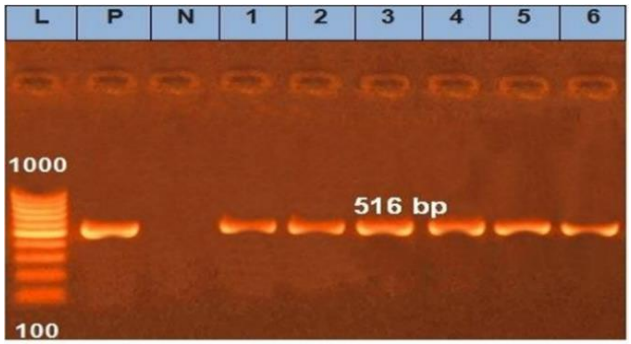

Fig. 6. Agarose gel electrophoresis of PCR products showing amplification of *blaTEM* gene products at 516bp. MWM-molecular weight marker (100-1000bp DNA ladder), + control (positive, negative), all six isolates were positive for *blaTEM* gene.

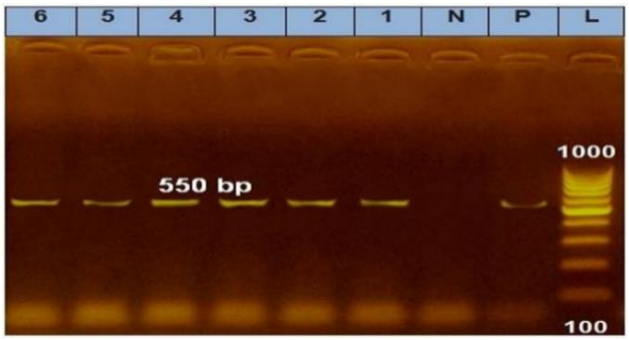

Fig. 7. Agarose gel electrophoresis of PCR products showing amplification of *ampC* gene products at 516bp**.** MWM-molecular weight marker (100-1000bp DNA ladder), + control (Positive, negative), all six isolates were positive for *ampC* gene.

O_{55}:K_{59}, O_{111}:K_{58} and O_{25}:K_{11} serogroups from broiler flocks showed high mortality rates in Sharkia Governorate, Egypt. However, Afify et al. (2020) detected *E. coli* O_{157}:H_7, O_{114}:H_{21} and O_{12} and O_{26} from raw chicken meat. In addition, Abdel Tawab et al. (2015) detected different serotypes of *E. coli* O55:H7, O78, O119:H4, O125:H18 and 10 untyped strains from chicken meat and meat products.

Results regarding to susceptibility of *E. coli* shown in Table 6 are similar to those of Badau (2021) and Badr et al. (2022), who reported a significant antibiotic resistance among *E. coli* strains, that could be due to the unregulated use of antibiotics in chickens, resulting in antimicrobial-resistant *E. coli* strains in chicken meat and its products.

Results of detection of some virulence genes and antibiotic resistance genes shown in Table 7 are in accordance with those of Pehlivanlar et al. (2015), who detected *blaTEM, blaCTX-M, blaSHV, blaOXA and blaAmpC* genes from 95.4% of *E. coli* isolates. In addition, Beutin et al. (2007) detected *Stx2* gene in 67.44% of *E. coli* isolates. On the contrary, Kagambèga et al. (2012) detected *Stx2* genes in only 8% of isolates. In addition, Zende et al. (2013) detected Sta gene in 18.18% of the *E. coli* isolates and Lt gene was not detected in any isolates. Lower detection rate for *the ampC* gene obtained by Rasmussen et al. (2015) was 9%.

Table 5: Serogrouping of *E. coli* isolates from examined samples (n=20)

E. coli serogroups	Chicken meat		Nuggets		Frankfurter		Luncheon		Characterization
	No	%	No	%	No	%	No	%	
O_{86}:k_{61}	1	5	-	-	-	-	1	5	Enteropathogenic
O_{55}:k_{59}	2	10	-	-	-	-	1	5	Enteropathogenic
O_{128}:k_{67}	1	5	1	5	-	-	1	5	Enterotoxigenic
O_{119}:k_{69}	1	5	-	-	-	-	-	-	Enteropathogenic
O_{125}:k_{70}	2	10	-	-	-	-	1	5	Enterotoxigenic
O_{78}:k_{80}	1	5	-	-	1	5	-	-	Enteropathogenic
O_{25}:k_{11}	2	10	-	-	-	-	-	-	Enterotoxigenic
Untyped serogroups	2	10	1	5	1	5	-	-	-
Total	12	60	2	10	2	10	4	20	20 serotypes

Table 6: Antimicrobial susceptibility of some isolated *E. coli.*

Antimicrobial Discs	N=10	
	Resistant	Sensitive
Amoxicillin	9	1
Amikacin	4	6
Ampicillin	9	1
Ciprofloxacin	2	8
Cefotaxime	7	3
Gentamycin	4	6
Levofloxacin	1	9
Oxytetracyclin	9	1
Penicillin G	10	0
Vancomycin	8	2

Table 7: Detection of some virulence genes and antibiotic resistant genes in some selected *E. coli* isolates

E. coli Sample	phoA	blaTEM	blaCTX-M	ampC	Sta/St1	Lt	Stx2
1	+	+	+	+	-	-	-
2	+	+	+	+	+	+	+
3	+	+	+	+	-	-	-
4	+	+	+	+	-	-	+
5	+	+	+	+	-	-	+
6	+	+	+	+	+	-	+

phoA=Alkaline Phosphatase, blaTEM=Beta-LactamaseTEM, bLaCTX-M=Beta-LactamaseCTX-M, ampC=AmpC Beta-Lactamase, Sta/St1=heat-stable toxin 1, LT=heat-labile toxin, and Stx2=Shiga toxins 2.

Conclusion

ESBL-producing *E. coli* was detected in most of examined chicken meat and chicken meat products. Antimicrobial susceptibility testing revealed the presence of Multidrug resistant strains. PCR assay was used for confirmation and molecular characterization of some virulence genes and antibiotic resistance genes in *E. coli* isolates.

Author Contributions

MAH, SAS and EM jointly developed the hypothesis and concept of the study and contributed to the manuscript's chemistry, materials, and technique. For this research and scientific paper, MAH, SAS, GEB and EM are all involved in the experimental procedures and analyses for this study and scientific paper. All authors participated in the experimental analysis and helped rewrite the manuscript. The final manuscript was read and approved by all authors.

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