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Contamination of chicken meat and edible offal commercialized at retail in Sharkia Governorate, Egypt by *Enterobacteriaceae*

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ABSTRACT

Chicken meat and its edible offal have a high biological value and act as a good substrate for different types of bacteria implicated in foodborne disease outbreaks. Therefore, a total of 150 random samples of chicken (Breast, thigh and edible offal, 50 of each) were collected from different outlets, Sharkia Governorate, Egypt to be examined bacteriologically. The obtained results revealed that the mean *Enterobacteriaceae* count was 3.73±0.07, 4.02±0.10 and 4.34±0.12 $\log_{10}$ CFU/g in breast, thigh and edible offal samples, respectively. *E. coli* was isolated from 12(24%), 15(30%) and 20(40%) of breast, thigh and edible offal samples, respectively, five different serotypes were identified (O157:H7, O158:H19, O128:H2, O26:H11and O55:H7) and the isolated *E. coli* strains were resistant to penicillin (100%), while the resistance was 72.3%, 65.9%, 51.1% and 51.1% to sulfamethoxazole-trimethoprim, oxytetracycline, chloramphenicol and kanamycin, respectively, meanwhile, all strains were sensitive to amoxicillin-clavulanic acid. *Salmonella* spp. were isolated from 22(14.67%) of the examined samples with a prevalence of 5(10%), 7(14%) and 10(20%) in breast, thigh and edible offal, respectively, serological identification revealed five different serotypes (*S.* Typhimurium, *S.* Entritidis, *S.* Lindenberg , *S.* Infantis and *S.* Kentucky), and the isolated *Salmonella* spp. were resistant to penicillin and sulfamethoxazole-trimethoprim (100%), meanwhile, the sensitivity was 100% to amoxicillin-clavulanic acid and ampicillin.

Introduction

Recently, chicken meat and its products are one of the most popular foods all over the world due to its high nutritive value and palatability. It is a good supply of protein, essential amino acids, vitamins and minerals which supply our bodies with many health benefits and promote growth (Morshdy *et al*., 2023). In spite of this biological value, chicken meat is an ideal medium for growth and multiplication of different types of microorganisms (Shaltout *et al*., 2020) including food spoilage and food poisoning microorganism. There are many sources contaminating chicken meat by different types of bacteria starting from slaughtering, de-feathering, evisceration till processing in meat plants (Yar *et al*., 2020), in addition to unhygienic practices and use of contaminated equipment (Rahman *et al*., 2020) causing food spoilage and foodborne illness to consumers, resulting in many health problems and economic losses.

Family *Enterobacteriaceae* is a group of many organisms that infect the gastrointestinal tract (Fox, 2016). *Escherichia coli* (*E. coli*) is a member of family *Enterobacteriaceae*, it is a gram-negative facultative anaerobic, rod-shaped, flagellated and none sporulating bacteria. *E. coli* is a major component of the normal intestinal micro-flora of mammals and can cause diseases when it breaches the gastrointestinal barriers in the immune-compromised hosts (Ibrahim *et al*., 2018). It is classified into six subgroups including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli*, enterohemorrhagic *E. coli* (EHEC), enteroadherent *E. coli*, and diffusely adherent *E. coli* (Holko *et al.,* 2006). These hazardous subgroups can cause many health hazards all over the world. *Salmonella* is another member of family *Enterobacteriaceae*, it is a gram-negative non-spore forming, bacilli. It is one of the most important food poisoning bacteria (Pesewu *et al.,* 2018) associated with poultry meat resulting in worldwide public health significance (Pavelquesi *et al.,* 2023) particularly foodborne diseases (CDC, 2020). In poultry production chain transmission of *Salmonella* spp. can occur either directly or indirectly from farms, feed, slaughterhouses or packing plants, and during manufacturing and processing of chicken meat (Antunes *et al.,* 2016; Borges *et al*., 2019). Therefore, the present study was conducted to evaluate to what extent chicken meat and edible offal in Sharkia Governorate, Egypt was contaminated by food poisoning micro-organisms which belong to family *Enterobacteriaceae*.

Materials and methods

Collection of samples

A total of 150 chicken samples (Breast, thigh and edible offal, 50 of each) were collected randomly from various outlets, Sharkia governorate, Egypt. As soon as possible, the samples were transferred aseptically in an ice box to the Microbiology Laboratory, Zagazig University, Egypt.

Preparation of samples

In a sterile homogenizer, twenty five grams were homogenized with 225 ml buffered peptone water (BPW) 1% at 2500 round per minute for three minutes to obtain a homogenate of 10-1 initial dilution. One ml from the initial dilution was transferred into another tube containing 9 ml BPW 0.1% in subsequent manners to make ten-fold serial dilution (APHA, 2001).

Determination of Enterobacteriaceae count

One ml was poured into the surface of Violet Red Bile Glucose agar

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(OXOID CM1082), then mixed and left to solidify, then incubated in an inverted position at 37°C for 24 hour. Suspected colonies (purplish to red colonies surrounded by a red zone) were enumerated (ISO, 2004).

Isolation and identification of E. coli

A loopful from previously prepared homogenate was streaked on MacConkey agar (OXOID CM0115) as a pre-enrichment step then incubated at 37°C for 24 hours. Suspected colonies (bright pink to red surrounded with pink halo zone) were streaked into Eosin Methylene Blue agar (OXOID CM0069), incubated at 37°C for 24-48 hours (ICMSF, 1996). Typical colonies of *E. coli* (shiny green metallic) were picked up and kept in nutrient broth for further morphological and biochemical identification (APHA, 1992) as well as serological identification (Kok *et al.,* 1996) by rapid diagnostic *E. coli* antisera.

Isolation and identification of Salmonella spp.

Pre-enrichment was done to the homogenate then incubated at 37°C for18 hour, then, 1 ml was transferred into sterile test tubes containing 10 ml of Rappaport Vassiliadis with soya for enrichment and incubated at 41.5°C for 24 hours (Vassiliadis *et al.,* 1978), then, a loopful was streaked on Xylose Lysine Deoxycholate ager (OXOID CM0469) and incubated at 37°C for 24 hour. Typical colonies (pink to red colonies with black center) were picked up and kept in nutrient broth for further morphological and biochemical identification (APHA, 1992), in addition to serological identification according to Kauffman-White scheme (Kauffman, 1974).

Molecular identification of E. coli and Salmonella virulence genes:

DNA extraction was conducted by QIA amp Kit according to the manufacturing instructions. Oligonucleotide primer sequences for Shiga toxin (Stx) one were CGATGTTACGGTTTGTTACTGTGACAGC and AATGCCACGCTTCCCAGAATTG (244 bp) according to Muller *et al.* (2006), while, GTTTTGACCATCTTCGTCTGATTATTGAG and AGCGTAAGGCTTCTGCTGTGAC for *Stx2* (324 bp) according to Muller *et al.* (2007) and GTG AAA TTA TCG CCA CGT TCG GGC AA and TCA TCG CAC CGT CAA AGG AAC C (248bp) for *inv*A gene of *Salmonella* spp. (Kumar *et al.,* 2008). PCR assay was done according to Sambrook *et al.* (1989).

Antibiogram of the isolated E. coli and Salmonella spp.

Antimicrobial susceptibility was tested by the single diffusion assay (Kirby-Bauer) according to the guidelines stipulated by NCCLS (2001), using ten different antibiotics discs. The disc contents and interpretation of results were illustrated in Table 4 and 5. The tested strains were evaluated as susceptible, intermediate and resistant according to the inhibition zones diameter.

Statistical analysis

Results were expressed as mean ±standard error (S.E). Statistical analysis was done by one-way analysis of variance using SPSS-21.; Chicago, IL, USA. Differences among individual means were compared by Duncan Multiple Range test, at 95% level of confidence.

Results

Different types of pathogenic micro-organisms from various sources can contaminate chicken meat and its products resulting in many health risks to consumers. Results illustrated in Table 1 declared that *Enterobacteriaceae* count ranged from 2.06 to 4.91, 2.14 to 5.38 and 2.99 to 5.99 $\log_{10}$ CFU/g, with mean counts of 3.73±0.07, 4.02±0.10 and 4.34±0.12 $\log_{10}$ CFU/g in the examined breast, thigh and edible offal, respectively. There was a significance difference between the examined samples ($P<0.05$). According to EOS (2005), the examined breast, thigh and edible offal were 45(90%), 39(78%) and 33(66%) accepted for *Enterobacteriaceae* count, respectively (Table 1).

As recorded in Table 2, the prevalence of *E. coli* was 47(31.33%), it was detected in 12(24%), 15(30%) and 20(40%) of the examined breast, thigh and edible offal, respectively. Serological identification of the isolated *E. coli* strains revealed five different serotypes (enteropathogenic *E. coli* O157:H7, O158:H19 and O55:H7 as well as enterotoxigenic *E. coli* O128:H2 and enterohemorrhagic *E. coli* O125:H18), with a prevalence of 2(4.3%), 9(19.1%), 14(29.8%), 9(19.1%), and 13(27.7%), respectively. Only

Table 1. *Enterobacteriaceae* counts ($\log_{10}$ CFU/g) of chicken meat and edible offal.

Samples	Range	Mean ± S.E	MPL	Accepted samples	Unaccepted samples
Breast	2.06-4.91	3.73^{c}±0.07		45(90%)	5(10%)
Thigh	2.14-5.38	4.02^{b}±0.10	4 $\log_{10}$ CFU/g	39(78%)	11(22%)
Giblets	2.99-5.99	4.34^{a}±0.12		33(66%)	17(34%)

CFU/g: Colony forming unit per gram; S.E.: Standard error; MPL: maximum permissible limit does not exceed 4 $\log_{10}$ CFU/g according to EOS (2005) for raw chicken meat and products. Means carrying different superscript letters are significantly different ($P<0.05$).

Table 2. Prevalence and serotyping of *E. coli* in chicken meat and edible offal.

Samples	Prevalence	Serotypes				
		O157:H7	O158:H19	O128: H2	O26: H11	O55:H7
Breast	12(24%)	0	2	2	4	4
Thigh	15(20%)	1	3	3	3	5
Giblets	20(40%)	1	4	4	6	5
Total	47(31.33%)	2(4.3%) EPEC	9(19.1%) EPEC	9(19.1%) ETEC	13(27.7%) EHEC	14(29.8%) EPEC

Table 3. Prevalence and serotyping of *Salmonella* spp. in chicken meat and edible offal.

Samples	Prevalence	*S. Typhimurium*	*S.* Entritidis	*S.* Lindenberg	*S.* Infantis	*S.* Kentucky
Breast	5(10%)	1	1	2	1	0
Thigh	7(14%)	1	2	1	2	1
Giblets	10(20%)	2	2	3	1	2
Total	22(14.67)	4(18.2%)	5(22.7%)	6(27.3%)	4(18.2%)	3(13.6%)

EPEC O157:H7 harbored both *Stx1*, and *Stx2*; while EPEC O55:H7 harbored *Stx1*only (Fig. 1). All the isolated *E. coli* strains were resistant to penicillin (100%), while the resistance was 72.3%, 65.9%, 51.1% and 51.1% to sulfamethoxazole-trimethoprim, oxytetracycline, chloramphenicol and kanamycin, respectively, moreover, all the isolated strains were sensitive to amoxicillin-clavulanic acid (100%), meanwhile, the sensitivity was 95.7%, 72.4%, 63.8% and 57.5% to ampicillin, gentamicin, neomycin and cefadroxil, respectively (Table 4).

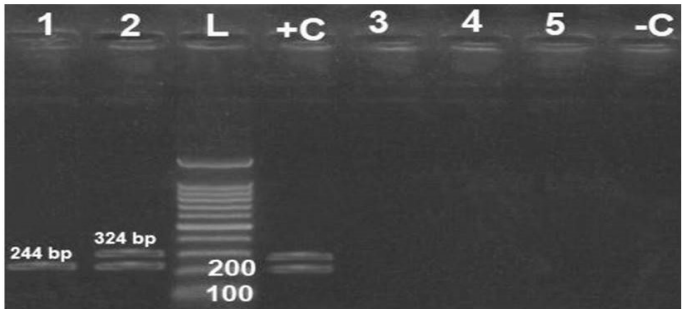

Fig. 1. Agarose gel electrophoresis of *Stx1* (244 bp), *Stx2* (324 bp) genes for characterization of *E. coli*. O157:H7 harbored both *Stx1*, and *Stx2*; while O55:H7 harbored *Stx1*only.

Salmonella spp. were isolated from 22(14.67%) of the examined samples, its prevalence was 5(10%), 7(14%) and 10(20%) in the breast, thigh and edible offal, respectively (Table 3). Serological identification of the isolated strains revealed five different serotypes including *S.* Typhimurium, *S.* Entritidis, *S.* Lindenberg, *S.* Infantis and *S.* Kentucky with a prevalence of 4(18.2%), 5(22.7%), 6(27.3%), 4(18.2%) and 3(13.6%), respectively (Table 3). As shown in Fig. 2, the isolated *S.* Typhimurium, *S.* Entritidis, *S.* Lindenberg and *S.* Infantis harbored invasive (*inv*A) gene while, this gene wasn't detected in *S.* Kentucky. The isolated *Salmonella* spp. were resistant to penicillin and sulfamethoxazole-trimethoprim (100%), while the resistance was 81.8% and 86.2% to chloramphenicol and oxytetracycline, meanwhile, the sensitivity was 100% to amoxicillin-clavulanic acid and ampicillin, but it was 81.8% and 90.9% to gentamicin and neomycin (Table 5).

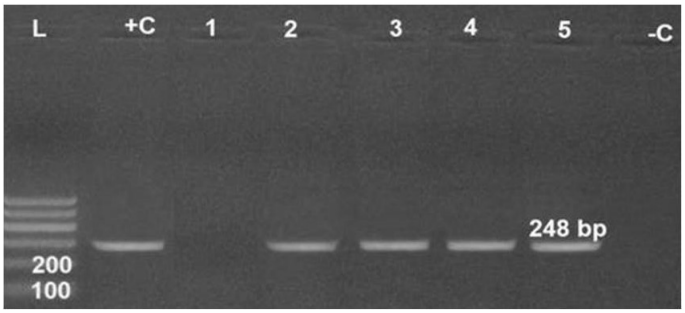

Fig. 2. Agarose gel electrophoresis of *inv*A (248 bp) genes for characterization of *Salmonella*. *S.* Typhimurium, *S.* Entritidis, *S.* Lindenberg and *S.* Infantis harbored *inv*A gene, while it wasn't detected in S. Kentucky.

Discussion

Contamination of chicken meat with many food pathogens is due to improper hygiene as well as personal faults during slaughtering, storage, transportation and handling processes, in addition to gastrointestinal contamination, contaminated water, air, equipment and environmental surfaces (USFDA, 2012). These contaminations make chicken meat unfit for human consumption or even harmful to consumers. *Enterobacteriaceae* is a useful indicator to evaluate the hygienic status during chicken slaughtering and processing. High count of *Enterobacteriaceae* gives an indication on enteric contamination with the intestinal contents because the gastrointestinal tract is a common habitat of the enteric bacteria and it is considered as a main source of contamination with these organisms from slaughtering, dressing, evisceration, handling till transportation to butcher shops (Hassanin *et al.,* 2013; Shaltout *et al.,* 2020).

In the present study, *Enterobacteriaceae* counts were nearly similar to what had been reported by Saikia and Joshi (2010); El-Deeb *et al.* (2011) and Moustafa *et al.* (2016), meanwhile, higher counts (5.08, 4.86, 6.8, 6.7 and 4.7, 4.8 $\log_{10}$ CFU/g for breast and thigh) were recorded by Ibrahim *et al.* (2014); Shaltout *et al.* (2019) and Shaltout *et al.* (2020). Generally, presence of *Enterobacteriaceae* in chicken meat and its products is an indicator for improper handling and unhygienic conditions after slaughtering. This result agreed with Moustafa *et al.* (2016)

Isolation of *E. coli* from chicken meat gives an indication on the fecal contamination during the production process resulting in severe diarrhea, especially in infants and young, as well as gastroenteritis and food poisoning among the adults (Liu *et al.,* 2015; Helali and Abdelghani, 2020). Chicken edible offal had the highest incidence of *E. coli*, while the lowest incidence was in breast samples. This result agreed with Al-Dughaym

Table 4. Disc content, inhibition zone diameter and antimicrobial susceptibility of the isolated *E. coli*.

Antimicrobial agent and disc content	Sensitive		Intermediate		Resistant	
	No	%	No	%	No	%
Penicillin (10 IU)	0	0	0	0	47	100
	29 or more mm		21-28 mm		20 or less mm	
Sulfamethoxazole, Trimethoprim (25 ug)	10	21.3	3	6.4	34	72.3
	16 or more mm		11-15 mm		10 or less mm	
Oxytetracycline (30 ug)	10	21.3	6	12.8	31	65.9
	19 or more mm		15-18 mm		14 or less mm	
Chloramphenicol (30 ug)	18	38.3	5	10.6	24	51.1
	18 or more mm		13-17 mm		12 or less mm	
Kanamycin (30 ug)	23	48.9	0	0	24	51.1
	18 or more mm		14-17 mm		13 or less mm	
Cefadroxil (30 ug)	27	57.5	8	17	12	25.5
	17 or more mm		13-16 mm		12 or less mm	
Neomycin (30 ug)	30	63.8	6	12.8	11	23.4
	17 or more mm		13-16 mm		12 or less mm	
Gentamicin (10 ug)	34	72.4	5	10.6	8	17
	15 or more mm		13-14 mm		12 or less mm	
Ampicillin (10 ug)	45	95.7	2	4.3	0	0
	18 or more mm		14-17 mm		13 or less mm	
Amoxycillin, Clavulanic acid (5 ug)	47	100	0	0	0	0
	13 or more		12		11 or less	

Table 5. Disc content, inhibition zone diameter and antimicrobial susceptibility of the isolated *Salmonella* spp.

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Penicillin (10 IU)	0	0	0	0	22	100
	29 or more mm		21-28 mm		20 or less mm	
Sulfamethoxazole-Trimethoprim (25 ug)	0	0	0	0	22	100
	16 or more mm		11-15 mm		10 or less mm	
Chloramphenicol (30 ug)	0	0	4	18.2	18	81.8
	18 or more mm		13-17 mm		12 or less mm	
Oxytetracycline (30 ug)	5	22.7	2	9.1	15	68.2
	19 or more mm		15-18 mm		14 or less mm	
Kanamycin (30 ug)	8	36.4	4	18.2	10	45.4
	18 or more mm		14-17 mm		13 or less mm	
Cefadroxil (30 ug)	8	36.4	6	27.2	8	36.4
	17 or more mm		13-16 mm		12 or less mm	
Gentamicin (10 ug)	18	81.8	0	0	4	18.2
	15 or more mm		13-14 mm		12 or less mm	
Neomycin (30 ug)	16	72.8	6	27.2	0	0
	17 or more mm		13-16 mm		12 or less mm	
Ampicillin (10 ug)	22	100	0	0	0	0
	18 or more mm		14-17 mm		13 or less mm	
Amoxycillin, Clavulanic acid (5 ug)	22	100	0	0	0	0
	13 or more		12		11 or less	

and Altabari (2010); Sharaf and Sabra (2012) and Awadallah *et al.* (2014) who isolated *E. coli* from chicken meat and its products. On the other hand, lower incidence of *E. coli* was reported by Ibrahim *et al.* (2015), Shaltout *et al.* (2019) and Shaltout *et al.* (2020) (13.33%, 10% and 12%). In contrast to this study, Abdel-Haffeiz (1999) didn't find *E. coli* in chicken meat products. Serological identification of the isolated strains agreed with Shaltout *et al.* (2020), while differed from Ibrahim *et al.* (2014) who identified different serotypes. The variation of results could be attributed to the differences in handling and hygienic practices applied during the chicken preparation. Presence of *E. coli* in examined samples indicated faecal contamination and bad sanitary measures during production. The ability of pathogenic *E. coli* to colonize and cause extra-intestinal diseases is mainly due to presence of many virulence factors including toxins, adhesins, invasins, capsules and iron uptake systems (Dale and Woodford, 2015). Our study was directed to identify shiga toxin which is one of these virulence genes that play an important role in *E. coli* virulence by using PCR; EPEC O157:H7 harbored both *Stx1*, and *Stx2*; while EPEC O55:H7 harbored *Stx1* only. Shiga toxin 1 and 2 are the main genes of pathogenicity and virulence properties. Shiga-toxin producing *E. coli* (STEC) and EPEC are able to produce A/E lesion by *eae*A chromosomal gene, while shiga toxin encoding gene (*Stx1*, *Stx2*) is present only in STEC (Sirous *et al.,* 2020). Antibiotics are used widely in poultry farms for various purposes (growth promoters, prophylaxis and therapeutics); however, misuse of these antibiotics is the main cause of increased bacterial resistance (Abdellah *et al.,* 2009). In the present study, disk diffusion method was performed to evaluate the antibiotic resistance of the isolated bacteria using different types of antibiotics. It was found that the isolated *E. coli* strains were resistant to many of the tested antibiotics, while all strains were sensitive to amoxicillin-clavulanic acid. The resistant rate of *E. coli* was in line with Adzitey *et al.* (2020) and Ekli *et al.* (2020).

Salmonella spp. is an important microorganism frequently associated with many food-born outbreaks. Generally, chicken meat is the most common sources of food poisoning by *Salmonella* spp. and the main cause of gastroenteritis all over the world (Rasschaert *et al.,* 2005; Ibrahim *et al.,* 2021). The obtained results revealed that the highest incidence of *Salmonella* spp. was in chicken edible offal followed by thigh and breast. Prevalence of *Salmonella* in the examined samples agreed with Kallaf *et al.* (2014) and Ibrahim *et al.* (2015) who detected *Salmonella* spp. in 12.7% and 12% of raw chicken meat. Meanwhile, higher prevalence of *Salmonella* spp. was found by Hassanin *et al.* (2017) who reported a prevalence of 24% and 36% of the examined breast and thigh samples, respectively. Moreover, low prevalence of *Salmonella* spp. was reported by Colmegna *et al.* (2009) and Anju *et al.* (2014) who reported that 4.7% and 4.4% of examined chicken meat samples were contaminated by *Salmonella* spp. Meanwhile, this result disagreed with many previous studies which failed to detect *Salmonella* spp. in chicken meat samples (Killinger *et al.,* 2010; Javadi and Safarmashaei, 2011). In addition, serological identification of the isolated strains was in accordance with Abd El-Aziz (2013) and Ibrahim *et al.* (2014) who identified these serotypes. Isolation of *Salmonella* spp. from the examined samples attributes to the improper hygienic measures during slaughtering, processing and from workers' hands (Saad *et al.,* 2018). In all *Salmonella* spp., there is a genetic element on the chromosome contains the virulence genes responsible for invasion of the epithelial cells called *Salmonella* Pathogenicity Island 1 (Hensel, 2004) in addition to adhesions, intracellular survival, antimicrobial resistance, systemic infections and toxin production (Aydin *et al.,* 2011). Furthermore, the *inv*A gene detected in the isolated *Salmonella* spp. strains consists of two additional invasion genes responsible for *Salmonella* invasion to phagocytic and non- phagocytic cells (Elemfareji and Thong, 2013). Results of the disc diffusion technique illustrated that all the isolated *Salmonella* strains were resistant to penicillin and sulfamethoxazole-trimethoprim, while it was sensitive to amoxicillin-clavulanic acid and ampicillin (100%). Similar resistance was reported by Almashhadany (2019) and Ibrahim *et al.* (2021). Antibiotic resistant *Salmonella* is associated with the improper use of antibiotics in animals and poultry farms; resistant bacteria can be transmitted to consumers through foods, mainly of animal origin (Nygard *et al.,* 2008; Suleiman *et al.,* 2013) resulting in public health hazards because it affects the efficacy of drug treatment in humans (Abdellah *et al.,* 2009).

Conclusion

The current study indicated that chicken meat and its edible offal could be considered as a potential source of food poisoning microorganisms which affect its quality and human health Therefore, strict hygienic measures during handling till consumer consumption should be adopted adopt.

Conflict of interest

The authors declare that they have no conflict of interest.

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