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Article Review

Prevalence of *Escherichia coli* in chicken meat and meat products in Egypt

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ABSTRACT

Meat from chicken source is considered one of the most common foods all over the world being easily digested, easily prepared, tasty nutritious food in addition for being economically reasonable. However, it constitutes one of the highly nutritious media for many micro-organisms, especially *Escherichia coli* (*E. coli*).

Many steps in the production of raw chicken meat and meat products from slaughtering till reaching the consumers can be responsible for microbial contamination by foodborne bacteria. The current review covers the so far reported data on the incidence of *E.coli* in chicken meat and meat products in Egypt

INTRODUCTION

Escherichia coli (*E. coli*) is a common gastro-intestinal bacteria in both animal and human and it can also found in water, soil and vegetation. Its presence in chicken meat products reflects fecal contamination of meat **(Clarence et al. 2009).**

E. coli has highly incidence rate in food manufacturing sector with noticeable severity on public health **(Abu Elnaga et al. 2014)**. In addition, *E. coli* is mostly nonpathogenic, but some of them considered pathogenic and has the ability to induce a harm on animal and human health.

In humans, the pathogenic strains of *E. coli* have been ensnared in many cases of foodborne infections **(Adzitey et al. 2020)**. Further, *E. coli* considered one of the most isolated bacteria from chicken carcass worldwide **(Davis 2018).** Poultry meat get contaminated at slaughtering during scalding, plucking, evisceration and chilling **(Keener et al. 2004**). Further, contamination during processing and handling by food handlers (**Adeyanju and Ishola 2014**). Therefore, strict hygienic practices should be adopted during primary processing of chicken carcasses to improve the bacteriological aspect of such products.

This review focused only on *Escherichia coli* isolated from chicken meat and chicken meat products, either raw or processed, through the so far available published data in Egypt. It covered the incidence of *E. coli* isola-

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tion, the prevalent serotypes, the antimicrobial susceptibility of the isolates, *E. coli* virulence and antimicrobial resistant genes. This is essential to update the knowledge on prevalence of *E. coli* in chicken meat to develop effective continuous monitoring plans to detect *E. coli* in human food-chain and for planning of effective strategies to control dissemination and spread of antibiotic resistant *Escherichia coli*, especially those with multi-drug resistant patterns, to humans.

E. coli Prevalence in chicken meat and meat products

Ahmed (2004) detected Enteropathagenic *Escherichia coli* (*E. coli*) in 8%, 20% and 12% of luncheon, chicken nuggets and hot wings samples, respectively. While **Ali et al. (2010)** isolated *E. coli* from chicken frozen fillets and chicken frozen legs at 28 and 36%, respectively, *Escherichia coli* O157:H7 were accounted 4 % for each. *Escherichia coli* was isolated from examined chicken thigh and chicken breast samples by 16% and 12%, respectively **(Saad et al. 2011)**. *E. coli* was detected in ready to eat meat (RTE) meat products at 10% total out of 120 examined samples of which represented as 10% (4/40) were from chicken luncheon (**Awadallah et al. 2014**). Further, **Hassanin et al. (2014)** examined chicken thigh and breast samples with the prevalence of *Escherichia coli* of 15% and 10%, respectively. **Gwida et al. (2014)** isolated *Escherichia coli* from chicken carcass from 16% of the tested samples. While in 2015 they recovered *Escherichia coli* from raw chicken meat at 35% (**Gwida and El -Gohary 2015**).Moreover, **Saad et al. (2015)** recorded that *Escherichia coli* was isolated from chicken hot wings, shawerma and nuggets by 5 %, 10 % and 25%, respectively from several hypermarkets at El-Gharbia and El-Dakahlyia, governorates.

Escherichia coli isolated from examined chicken thigh samples in 13.33 % (**Ibrahim et al. 2015)**. **Khalafalla et al. (2015)** reported that *E. coli* was isolated from all examined.

Also, *Escherichia coli* O157:H7 was isolated from 0.8% of chicken carcass, while 2% of chicken meat was positive for non-O157:H7 STEC (**Ahmed et al. 2017**). Out of 90 investigated chicken samples; fresh and frozen, *E. coli* were isolated from 11.7 % from examined samples (**Moawad et al. 2017**).

Abd Elzaher et al. (2018) isolated *E. coli* from 80.35% the internal surfaces and 78.57 % of external surfaces of the examined chicken. The incidence of *E. coli* isolation was higher from the carcass internal surfaces than the carcass external surface. While **Ibrahim et al. (2018)** reported incidence of *E. coli* were 13.3%, 16.6% and 23.3%, in samples of luncheon, nuggets, and shawerma, correspondingly. In addition, **Elbayoumi et al. (2018)** reported that *E. coli* was isolated from breast and thigh samples by 14.3 and 20%, correspondingly. On the other hand, **Atia (2018)** *Escherichia coli* was isolated from 8 and 16 % of the examined breast and thigh samples, and **Elshora (2019)** reported that *E. coli* was isolated from breast samples by 8.57%.

Elsisy (2019) said that *E. coli* was isolated by 30 and 45 % from examined thigh and breast samples correspondingly. On the other side, *E. coli* could be detected in 2(6.66%) of examined nuggets samples (**Gaafar et al. 2019**).

Moustafa et al. (2019) recorded that the isolation of *Escherichia coli* from breast and thigh muscles of chicken carcasses at 40 and 48%, respectively. Similarly, **Abo Elmagd et al. (2019**) stated that out of 600 isolates obtained from 100 examined chicken meat, only 434 isolates were confirmed biochemically as *Escherichia coli* at a prevalence of 72.3 % (434/600), while when based on colonial morphology they reported an incidence of 100%.

Salem et al. (2019) reported high incidence of *Escherichia coli* in chicken fajitas (40%) followed by shishtawook (32%). It was evident from their results that fajitas were the most contaminated ones, followed by Shishtawook. They attributed this to more handling, spices and vegetables added during preparation which may be the source of the contaminating microorganisms.

Out of 100 examined samples, only 4 % were positive for *Escherichia coli* O157:H7,

while 11% of the isolates were other species rather than *E. coli* O157. *E. coli* was detected in 6% and 9% breast and thigh samples, respectively (**Shaltout et al. 2020**). Also, **Abdelkarim et al. (2020)** surveyed a total of 403 chicken carcass samples. The data showed a 66.3% prevalence of *Escherichia coli*. **Arakeeb (2020)** reported that *E. coli* was isolated from 62.5 and 42.8 % of breast and thigh samples, respectively. While, *Escherichia coli* was isolated from 4% raw chicken samples and 12% of chicken ready for consumption samples (**Abd El-fatah et al. 2020**).

E. coli were isolated from 88 % of chicken's meat by (**Shawish et al. 2021**).

Furthermore, *Escherichia coli* O157:H7 was isolated in 1.1% of collected samples from different location in Egypt. The incidence of *Escherichia coli* O157:H7 was 1.2% from examined beef, raw and cooked chicken samples. Through these samples, *Escherichia coli* O157:H7 isolated mostly from cooked chicken samples by 1.9%. Regarding examined samples sources, Hurghada and Luxor Governorate showed the highest incidence of *E. coli* O157:H7 in 8.3% and 6.3%, respectively; however, the incidence in Cairo and Alexandria Governorates was 3.3 and 5.0 %. Lastly, there was no any *E. coli* O157:H7 isolate reported from either Aswan or Sharm El Sheikh Governorates (**Refaay et al. 2022**).

Coliform percentages in poultry meat product samples (n=25) was 76%, 88%, 100%, 100% in chicken pane, luncheon, burger and popcorn, respectively (**Saad et al. 2022**).

Elsenduony et al. (2022) found that all examined chicken breast samples were free from *E. coli* from all farms, while the incidence of *E. coli* were 16.6 % and 25 % from examined chicken thigh samples collected from two farms, correspondingly. While, all samples from breast were free from *E. coli* from all farms.

The prevalence of *E. coli* in chicken product samples was 50% (n=30); it was isolated from 65% (13) of nuggets, 35% (7) of luncheon and 50% (10) of pane samples (**Morshdy et al. 2023**)

Drawing a conclusion based on the comparison of the obtained results among all the above studies will not be consistent due to the vast differences in the sample size, type, collection and storage conditions. However, these data indicate, either high, moderate or low percentages of *E. coli* isolation from chicken or its products that contamination does exist from production till reaching the consumer.

Prevalent E. coli serotypes among chicken meat and chicken meat products

Serotyping is one of the methods used for identifying strains for epidemiological purposes. Identification of *E. coli* by detection of surface antigens using antibodies considered one of traditional technique; flagellar H-antigens, capsular K-antigens and O- polysaccharide antigens. Currently, there are 53 H-types besides 186 different *Escherichia coli* O-groups (**Fratamico et al. 2016**). Further, O-serogroups are related to the virulence factor profile of each strain.

The isolated strains of *E. coli* by **Ahmed (2004)** were serologically identified as 055: K59 0111: K58 and 0124: K72 in addition to untypable isolates.

E. coli O119: K69 and O55: K59 were isolated from breast and thigh, respectively. Thigh and breast O119:K69 and O125:K70, thigh only O26:K60 and breast only O55:K59 and O124:K72 (**Saad et al. 2011**).

Hassanin *et al.* (2014) serologically identified *Escherichia coli* as O55:K59 (enteropathogenic; EPEC), O124:K72 and O125:K70 (enterotoxigenic; ETEC). Furthermore, the incidence of Enteropathogenic *E. coli* which serologically identified (O119:K69 and O55:K59) was 4%, Enterotoxogenic *E. coli* (O125:K70) was 4%, Enterohaemorrhagic *E. coli* (O26:K60) was 4% from the breast only and Enteroinvasive *E. coli* (O124:K72) was 4% from thigh only.

Further, *E. coli* isolated from chicken

luncheon were identified as O55:K59 (3 isolates) and O111:K58 (1 isolate). They were categorized as enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *Escherichia coli* - Shiga toxigenic *Escherichia coli* , respectively **(Hassanin et al. (2014)**.

Incidence of EPEC and STEC in chicken meat may be related to possible cross contamination during food handling and processing or may be during the evisceration process, mainly due to the rupture of the intestine at preparation (**Awadallah et al. 2014**).

Further, 5% of each of the following serotypes were detected in chicken nuggets: O119:H6, O86, O125:H21, O124, O26. Also, O55:H7 and O26 were detected in 5% of the chicken shawarma samples. In addition, O111:H4 was the only serotype in chicken hot wings at 5% as well (**Saad et al. 2015**). Further, serological identification of *E. coli* isolates by **Gwida and El-Gohary (2015)** revealed the presence of EHEC; O26:H11and O103:H2, EPEC; O128:H2 and O111:H2 and ETEC O78.

Non-O157:H7 *E. coli* comprised O26:H11 (2), O111:H8 (3), O113:H21 (1) and O55:H7 (1) (**Ahmed et al. 2017**). Further, Twenty-one *E. coli* isolates from chicken meat samples were serotyped as O1 (2), O18 (3), O20 (1), O78 (1), O119 (1), and O146 (1) (**Moawad et al. 2017**).

As reported by **Ibrahim et al. (2018)**, 4 out of 30 chicken luncheon samples were positive for *E. coli* (13.3%), one of them (25%) identified as Enterotoxogenic *E. coli* (O128:H2),while two of them (50%) identified as Enteropathogenic *E. coli* (O91:H21 and O78) and other isolates (25%) were Enteroinvasive *E. coli* (O124). Moreover, 5 out of 30 s chicken nuggets samples (16.6%) confirmed to be contaminated by *Escherichia coli*, one of them (20%) identified as Enterohaemorrhagic *E. coli* (O26:H11), four of them (80%) were Enteropathogenic *E. coli* (O1:H7 (2), O44:H18 (1) and O78(1), also, 7 out of 30 half cooked chicken shawerma samples were *E. coli* (23.3%), the isolates identified as Enteropathogenic *E. coli* (O91:H21 (1), O127:H6 (1), O55:H7 (1), O44:H18 (1), O153:H2 (2), and O78 (2).

Salem et al. (2019) reported that they could isolate different serotypes of *E. coli* from shishtawook beside chicken fajitas, as O113:H4 (4%), O111:H2 (4%), O127:H6 (4%) ,O26:H11 (4%) and O103 (4%), while O111:H2 (4%), O119:H6 (8%), O113:H4 (4%), O26:H11 (4%), O127:H6 (4%) ,O91:H21 (4%) and O124 (4%) were isolated from fajitas**.**

Serological identification by **Gaafar et al. (2019)** of isolated *E. coli* revealed detection of O78 and O1:H7 (EPEC) in chicken nuggets samples at 6.66%.

While the incidence of other serotypes were O127:H6 (4%), O114:H21 (2%), O26 (2%) and O126 (3%) **(Shaltout et al., 2020)**. Moreover, **Shawish et al. (2021)** reported the most common *E. coli* serotypes were (O128, O86) by 4.5% ; (O26, O 121) by 9%,(O146) by 11.4% ; (O 111 and O91) by 13.6 %;(O119) by 18.2 % and (O45) by 15.9 % .

15 *E. coli* isolates from popcorn, burger, luncheon and fresh pane and serotyped as O142 (20%)**,** O128:H2 (6.66%), O114 (13.33%) and O124 (13.33%) **(Saad et al. 2022)**.

Elsenduony et al. (2022) recorded that by the examination of chicken thigh samples from farm no.4; 8.33% and 16.7% of samples were positive for *E. coli* which serotyped as O2: H6 (EPEC) and O91: H21 (EHEC), correspondingly. While *E. coli* O78 and *E. coli* O44: H18 were isolated from the examined farm no.5 thigh samples with an incidence rate of 8.33% for each serotype.

Elbayoumi et al. (2018) isolate different *E. coli* serotypes (O78 and O26:H11) from breast samples with incidence of 8.6% and 25.71%, correspondingly .While, from thigh muscle samples , the isolated *E. coli* serotypes were O78 , O91:H21 and O2: H6, with incidence of 5.71% for each, correspondingly. **Edris et al. (2015)** said that *E. coli* O78 was isolated from chicken thigh samples and *E. coli* O26, O78 from breast samples.

Such result may well be a small or huge diverse comparing to others because of the huge scale of Escherichia coli serological typing. Phenotypic serotyping methods for differentiating *E. coli* considered not necessarily accurate. Thus, molecular serotyping and subtyping methods for *E. coli* allow more accuracy rather than other methods in addition to the advancement in investigation of outbreaks of foodborne disease and tracing-back the main sources.

Prevalence of E. coli virulence genes among chicken meat and its products

Analysis of different genes associated with virulence in *E. coli* isolates was performed in some not all of the studies. Further, limited number of genes was analyzed per study and there was inconsistency in the detected genes.

Saad et al. (2011) reported that elt gene was detected by molecular examination of isolated *E. coli* (O55: K59 and O119: K69) from examined thigh and breast chicken samples.

Hassanin, et al. (2014) failed to detect *stx*1 and *stx*2 genes in any of the analyzed isolates.

PCR analysis by **Awadallah et al. (2014)** of *E. coli* serotypes showed the presence of *Stx*1 gene and *Stx*2 gene in O111:K58 isolates. The *stx*1 gene was not detected in all *E. coli* O157:H7 isolates or other serotypes rather than O157:H7, while *stx*2 was detected in 100% of the *E. coli* O157:H7, it was detected in 60% of other serotypes rather than O157:H7. In addition, *eae*A and *hly*A were detected in *E. coli* O157:H7 and non- O157:H7 STEC at 100% and 60%, respectively **(Ahmed et al. 2017)**. **Abd Elzaher et al. (2018)** showed that 33.3% (2/6) of *E. coli* isolates from chicken carcasses were positive for the *eae*A gene.

*fli*C gene of *E. coli* is considered as an indicator for *E. coli* O157:H7 (**Carey et al. 2009).** PCR investigation by **Shaltout et al. (2020)** revealed that *fli*C gene was detected in all *E. coli* O157 isolates.

E. coli isolates ECKFGF5 and ECDMGF6 were negative for *stx*1 and *stx*2 genes. While, *stx*2 were detected in *E. coli* ECGHBF1 and ECMFLF4 strains (29%) **(Abdelkarim et al. 2020)**. Moreover, **Refaay et al. (2022)** reported that after examination **of** *E. coli* O157:H7 isolates, the result showed that they harbored *eae* gene and *stx*l gene was not detected. While, only one isolate harbored *stx*2 gene beside *eae* gene, but was not from chicken meat **.**

Antimicrobial susceptibility among E. coli from chicken meat and chicken meat products

Antibiotics have and are still extensively used in poultry farms as therapy and as growth promotors. This greatly inflated the emergence of antimicrobial resistance in food borne pathogens with its hazards on human health and consequent treatment failure. Especially, antimicrobial resistant *E. coli* is considered one of the major challenges in both humans and animals.

Antibiotic susceptibility was mostly carried out using the disc diffusion method, but automated Vitek2 analysis was used by some researchers. Different studies used different antibiotics, however, most of the antibiotic categories were covered. In addition, not all the studies investigated the antibiotic susceptibility of their isolates.

Ali et al. (2010) isolated *E. coli* from poultry products that showed 100% resistance to Cephalexin, Erythromycin and Penicillin G, while 100% sensitivity to Amikacin, Ciprofloxacin and Gentamicin.

Gwida and El-Gohary (2015) showed that raw chicken meat was 100% resistant to Ampicilin, Cefoxitin and Tetracycline, while variably resistant to other tested antibiotics.

Moawad et al. (2017) reported that *E. coli* isolates showed resistance to Tetracycline, Ampicillin, Streptomycin, Trimethoprim/ sulphamethoxazole and Amoxicillin–clavulanic acid with 80, 80, 60, 66.7 and 66.7%, respectively. Eight and seven isolates were susceptible to Ciprofloxacin, Enrofloxacin and Ceftriaxone correspondingly, and all tested isolates were susceptible to Colistin.

Abd Elzaher et al. (2018) showed that *E. coli* isolates were 100% resistance to Penicil-

lin, Trimethoprim/Sulfamethoxazole. While were sensitive to Cefotaxime (35.92%) followed by Chloramphenicol (37.75%), Rifampicin (44.25%), Norfloxacin (50.27%), Ofloxacin (61.77%), Doxycycline (76.97%), Cephradine (93.4%), Amoxicillin (93.7%) and Tobramycin (100%),

The incidence of multidrug-resistant (MDR) strains in chicken meat cuts-up samples was found to be 81%. 3 out of 262 isolates were sensitive to all antibiotic agents, while 212 out of 262 isolates were multi drug resistance. The resistance rates were lowest for Gentamicin (7.2%) and Chloramphenicol (12.6%). On other hand, it was highest for Vancomycin (99%) and Tetracycline (96%). Other antibiotic showed ranged resistance pattern from 24-73% **(Abdelkarim et al. 2020)**.

Abd El-fatah et al. (2020) showed 100% resistance of *E. coli* isolates to Clindamycin and Rifampin and 78.9% resistance to Ciprofloxacin.

Shawish et al. (2021) revealed a strong resistance of *E. coli* isolates against Tetracycline (33.3%), Ampicillin (26.6%), Kanamycin and Trimethoprim-Sulphamethoxazole (20%), Cefotaxime (12%), Chloramphenicol and Ciprofloxacin (8%). Whereas; the lowest degree of resistance was noticed against Streptomycin (6.6%), Ceftazidime (5.3%) and Nalidixic acid (4%).

Antibiotic resistance pattern using Vitek 2 by **Elsenduony et al. (2022)** revealed that all *E. coli* isolates were Extended Spectrum Beta-Lactamase (ESBL) negative and showed resistance to Trimethoprim/ Sulfamethoxazole, Piperacillin, Ticarcillin, and Ciprofloxacin but were sensitive to other antibiotics used.

Refaay et al. (2022) reported that 100 % of *E. coli* O157:H7 isolates showed resistance to Cefixime, Ciprofloxacin, Ampicillin and Cotrimoxazole. 85.7% showed resistance to Pipracillin-Tazopactam, Piperacillin and 57.1% showed resistance to Gentamicin. However, the lowest resistance rate (14.3%) was observed against Aztreonam . Furthermore, the isolates showed high sensitivity to Meropenem (71.4%) and Amikacin (85.7%). *E. coli* O157:H7 isolates showed multidrug resistance.

Generally, MDR was common among all the *E. coli* isolates in the above-mentioned studies with diverse patterns of antibiotic resistance profiles among the isolates that were derived from different sources and/or regions. Monitoring antibiotic usage in poultry production is essential to control the development and spread of MDR bacteria.

Antimicrobial resistance genes harbored by E. coli from chicken meat and chicken meat products

Very few analyses of the antibiotic resistance genes were performed by the papers reviewed here, by using RT PCR, resistance-associated genes were detected during molecular examination of *E. coli* isolates isolated from chicken meat. Five out of ten isolates harbored bla_{TEM} (1 and 104), while $bla_{\text{CTX-M}}$ (1 and 14) was positive in four isolates. Three isolates carried *qnr*B and *qnr*A while $bla_{\text{OXA-1}}$ was detected in three isolates. Genes *qnr*S ,bla_{CMY} and bla_{SHV} were not detected in any isolates. *mcr*-1 gene and $bla_{\text{OXA-23 gene}}$ were not identified in *E. coli* isolates **(Moawad et al. 2017)**. **Abd Elzaher et al. (2018)** showed that *acc6-Ib -cr* genes is detected in 33.3% (2/6) of *E. coli* isolates from chicken carcasses, where *qnr*A gene was not detected in all examined isolates. Further, all *E. coli* isolates obtained from chicken samples contained bla_{TEM} gene but not bla_{CTX} gene **(Abd El-fatah et al. 2020)**.

CONCLUSION

In Egypt, great economic losses to the poultry industry occurred due to Avian pathogenic *E. coli* (APEC) affecting the affordable protein supply to a wide population, while extra intestinal enteropathogenic *E. coli* (ExPEC) causes serious conditions in human. Despite the existence of defining virulence genes shared between APEC and ExPEC of humans, it is not fully ruled out whether APEC is the main source of the ExEPEC infection in human, especially through the food chain suggesting APEC zoonosis. Thus, understanding the prevalence and the phenotypic as well as the genotypic characteristics of *E. coli* isolated from food would greatly unravel the role of

poultry and poultry products in the spread of ExPEC among humans.

Further, monitoring the antibiotic usage in poultry industry is essential to control the spread of antimicrobial resistant bacteria. Moreover, applying strict hygienic conditions during all the production steps of poultry meat processing is essential to control foodborne infections in human.

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