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Original Article *Food hygiene*

Prevalence of Escherichia coli in chicken carcasses from Mansoura, Egypt

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ABSTRACT

Objective: This study ended to detect the prevalence and public health important of *Escherichia coli* in chicken carcasses in Mansoura City.
Procedures: A total of 100 whole frozen chicken carcasses purchased from supermarkets distributed at Mansoura city were examined to detect *Escherichia coli*.
Results: Out of the 100 examined carcasses, 98 chicken carcasses were contaminated with *Escherichia coli* with an incidence rate 98%.

Conclusion and clinical relevance: This study revealed that chicken meat are highly contaminated with *E. coli* and play an important role in transmission of *E. coli* to human, therefore special precautions and hygienic measures must be applied in abattoir during evisceration and dressing of carcasses, and in-home during handling and cooking of chickens to protect consumers from *E. coli* foodborne infection.

Keywords: *Chicken; carcasses; E. coli; PCR*

1. INTRODUCTION

Poultry is one of the most important sources of good quality protein for human being. They are economically cheaper than red meat, low in calories, source of both saturated and unsaturated fatty acids and its protein is good source of essential amino acids, easily digested and has palatable taste. Poultry and poultry products are among the main food sources most often incriminated in outbreaks of human food-borne infection especially that caused by *Escherichia coli* (Panisello et al. 2000).

Escherichia coli is one of the most causes of food-borne pathogens, with a big effect on public health concern, poultry meat was incriminated in the transmition of Escherichia coli worldwide and infection mainly occurs due to cross-contamination from equipment, utensils and via food handlers handling raw carcasses or undercooked poultry meat products (Adeyanju and Ishola 2014). *Escherichia coli* is naturally inhabited in the intestinal tracts of warm-blooded animals and humans, and used as an indicator for fecal contamination, and possibility of contamination by enteric pathogens especially Shiga Toxin-producing *Escherichia coli* (STEC) (Miranda et al., 2008).

Escherichia coli become one of the most microbial agents that are implicated in human extraintestinal infections including urinary tract infections (UTIs), sepsis, and neonatal meningitis which difficult to be treated due to its resistance to the commonly used antibiotics (Johnson et al. 2012 and Manges and Johnson 2012). Due to the potential public health problem of these bacteria, this study is planned to throw the light on prevalence, and incidence of *E. coli* isolates from chicken carcasses distributed in Mansoura city, Egypt.

2. MATERIALS AND METHODS

2.1. COLLECTION OF SAMPLES:

Between December 2016 and May 2017, a total of 100 whole frozen chicken carcasses were purchased from supermarkets distributed at Mansoura city, Egypt. Wherein they are sold retail, in a deep-frozen state. every sample was separately packaged into a polyethylene bag and transferred in icebox to the laboratory for bacteriological examinations, in Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Mansoura University.

2.2. ISOLATION AND IDENTIFICATION OF ESCHERICHIA COLI:

2.2.1. Enrichment:

Whole poultry carcass was separately shacked with sterile tryptone soya broth, 225 ml for each carcass (Oxoid CM0989). Then incubated the resultant suspension at 37°C for 18–24 hours.

2.2.2. Selective plating:

Enriched cultures were streaked onto sterile MacConkey agar (Oxoid CM1169) supplemented with cefixime (final concentration 0.05 mg/L), then the plates were incubated at 37 °C for 24 hours. Every plate was examined for typical E. coli colonies.

2.3. BIOCHEMICAL IDENTIFICATION OF PRESUMPTIVE E. COLI:

A total of 600 colonies exhibiting pink color and able to ferment lactose were culture on nutrient agar (Oxoid CM0003) and then subjected to different biochemical tests purposed for conventional identification of E. coli, (Indole test, Methyl red test, Voges-Proskauer test, and citrate utilization test and Urease test) isolates confirmed biochemically as E. coli if give theses result with (Indole +, Methyl +, Voges -, citrate -, Urease -)

2.4. MOLECULAR IDENTIFICATION OF E. COLI:

One hundred biochemically identified E. coli isolates from chicken samples (each chicken represented by a single isolate) were subjected to molecular identification

2.4.1. DNA extraction:

Firstly, every isolate was cultured in nutrient broth (Oxoid CM0001) and incubated overnight at 37 ^{o} C, 5 ml from each bacterial culture was centrifugation at 3000 rpm for 15 minutes for isolation of chromosomal DNA, discarded the suspensions then suspended the collected cells in 1.5 ml TE (Trise-EDTA) buffer, then transferred into 2-ml centrifuge tube and centrifuged for 1 minute at 8000 rpm for collecting the washed cell pellet. then discarded the supernatant and the bacterial pellet was resuspended with 500 µl of TE and incubated at 37 ^{o} C for 2 hours after the addition of lysozyme (10 mg/ml) and RNase A (50 µg/ml). Then isolated the chromosomal DNA with using of a Maxwell 16 cell DNA purification kit (Promega Corporation, Madison, WI, USA) according the protocol of genomic DNA purification, which supplied by the manufacturer. A volume of the resultant chromosomal DNA was then taken to be diluted 20 times in TE for using as template for PCR amplification of the target gene.

2.4.2. E. coli confirmation

Isolates with typical Escherichia coli biochemical results (+ + - - -) were examined by the use of the specific PCR to determine the presence of uidA gene specific for E. coli. We constructed one primer set for the amplification of uidA gene have the following equence 5´- ATGCCAGTCCAGCGTTTTTGC-3´ and 5´- AAAGTGTGGGTCAATAATCAGGAAGTG -3´ which can amplify an amplicon of 1487 bp, PCR amplification was conducted in a 20-µl reaction mixture using GoTaq Green Master Mix, ready to use solution containing Taq DNA polymerase (Promega Corporation) supplied in 2X Green GoTaq reaction buffer (pH 8.5). PCR reaction mixture consisted of 1 µl (10 pmol) from each of antisense and sense primers, 2 µl DNA template, 10 µl GoTaq Master Mix 2X (1 U Taq DNA polymerase, 400 µM dATP, 400 µM dCTP, 400 µM dGTP, 400 µM dTTP and 3 mM $MgCl_2$) and autoclaved water 6 µl. The mixture was subjected to amplification by 35 cycles of in Gene Amp PCR system 2700 (Applied Biosystems, Foster City, CA, USA). The first cycle was preceded by denaturation 4 minutes at 95 °C for. Each cycle performed for aerolysin (uidA) gene consisted of denaturation at 95 °C for 40 seconds, annealing at 63 °C for 30 seconds, and extension for 1.5 minutes at 72 °C followed by a final extension for 4 minutes at 72 °C followed the last cycle.

2.4.3. Electrophoresis of PCR products

Separated the PCR-amplified products of each reaction mixture by subjecting 4 µl aliquots to agarose (1.5%) gel electrophoresis for 30 minutes at 100 V followed by a 25-minute staining in 1% solution of ethidium bromide solution. Finally, visualization and photographing the separated PCR products under UV illumination. With using E. coli (ATCC 25922) as a positive control in the present study.

3. RESULTS

From the 600 isolates which collected from 100 chicken carcasses only 434 isolates were identified as E. coli by biochemical tests representing a prevalence of (72.3 %; 434/600) with incidence of (100%) represented at figure (1). From 434 biochemically identified E. coli isolates, one hundred E. coli isolates from chicken samples (each chicken represented by a single isolate) subjected to molecular identification, and found that 98 isolates confirmed as E. coli by molecular method with incidence 98% in chicken represented at figure (2).

Figure (1): *Escherichia coli* presumptive isolates confirmed based on conventional identification

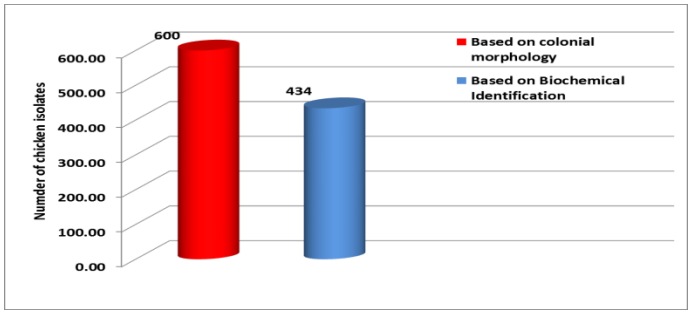


Figure 2: *Escherichia coli* presumptive isolates confirmed based on and molecular identification

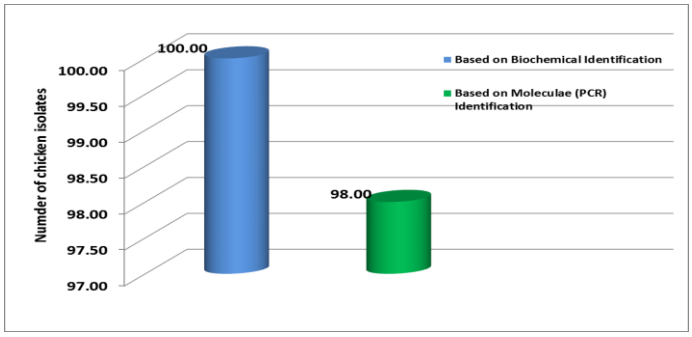
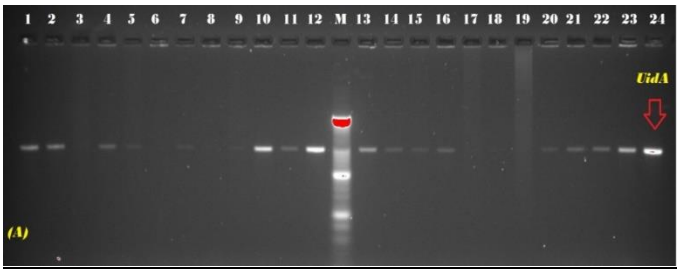


Figure (A),(B)



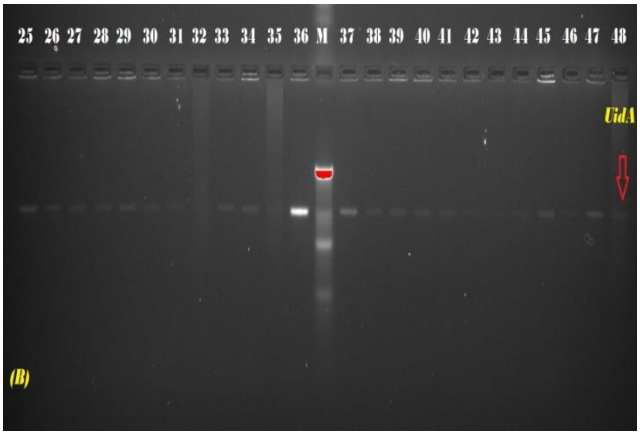


Figure (A), (B) Agarose gel electrophoresis for PCR analysis of (*uidA*) gene in *E. coli* isolated from chicken carcasses. Chromosomal DNA from (n=100) Conventional identified *E. coli* used as a template for PCR amplification using specific oligonucleotide primer for *uidA* gene.

4. DISCUSSION

E. coli has been incriminatory in a variety of diseases in poultry for example colisepticaemia, peritonitis, coligranuloma, pericarditis, air sacculitis, omphalitis and oophoritis, causing about 5-50 % mortality in poultry flocks (Roy et al., 2006). Also, poultry and poultry products are among the main food sources most often incriminated in outbreaks of human food-borne infection especially colibacillosis (Panisello et al., 2000). And due to the potential public health problem of these bacteria, this study is planned to throw the light not only on the prevalence, but also incidence of *E. coli* isolates from chicken carcasses distributed in Mansoura city, Egypt. With using conventional and molecular methods which revealed that chicken carcasses highly contaminated with *E. coli* with incidence 98%.

Also, high incidence of *E. coli* 93.7% was reported by (Abd El-Tawab et al. 2016) when examined 1284 chicken samples from Giza and Qalubia governorates, Egypt, found that 1204 samples positive for *E. coli*. There are several studies in different countries which showed high incidence of *E. coli*, like result of study in Yugoslavia by (Prukner E. 1986) reported high incidence 81.46%, also high incidence of 88.2% was mentioned by El-sukhon et al. (2002) in Jordan. In Egypt Abd El-Aziz et al. (2007) isolated *E. coli* in a percentage of 90%. Barros et al. (2013) could detect *E. coli* in chicken meat by a percentage of 82.5%. Liu et al. (2018) found that *E. coli* incidence was nearly 80 % of the 2,452 meat samples (93% of meat source poultry products).

Intermediate percentages of incidences of *E. coli* were reported by Adeyanju and Ishola (2014) could isolate *E. coli* from chicken meat collected retail markets with an incidence of 43.4% (43/99), (Barbour et al. 1985) isolated *E. coli* with incidence of 45.4%. Abd El Tawab et al. (2015) found that *E. coli* with incidence of 44%, 75% among imported chicks and from local broiler chickens, respectively. Another intermediate result was reported by Saha et al. (2003) who isolated *E. coli* with incidence of 58.18%, Hashem et al. (2012) who isolated *E. coli* in a percentage of 54.55% and by Kegode et al. (2008) who recovered *E. coli* for chicken with an incidence of 68% in Fargo metropolitan area, USA.

Lower incidence value was reported by Abd El Tawab et al. (2015a) who detected *E. coli* in 15.7% and 15.8% of examined apparently healthy broiler chickens collected in winter seasons from December to February and summer seasons (from June to August), respectively from Menofya government, Egypt. Also, reported by Adesiji et al. (2011) who found that *E. coli* incidence was 26% from chicken, beef, goat and pork samples, with 16% in chicken only and by Ukut et al. (2010) who could isolate *E. coli* from poultry by incidence rate of 11.1 %.

The differences in rates of isolation may be attributed to many reasons such as immune status of the bird, vaccination programs which used, use of medication and hygienic status of the poultry abattoir.

The outcome of this study provides that chicken carcasses highly contaminated with *E. coli* isolates, and it is very important to increase the hygienic control to decrease contamination of the chicken carcasses with *E. coli* and to decrease possibility of transmission of *E. coli* to human.

Conflict of interest

Authors declare that they have no conflict of interest

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