OPEN ACCESS
Freely Available Online

JOURNAL OF VETERINARY HEALTHCARE
ISSN NO: 2575-1212

Research

DOI: 10.14302/issn.2575-1212.jvhc-20-3477

Aerobic Plate Count of Contaminants and Molecular Characterization of *Eschereichia Coli* in Raw Chicken Meat in Ismailia, Egypt

El-Sayed A. Afify[1], Fahim A Shaltout[1,*], Zakaria, I. M[1]

[1]Department of Food Control, Faculty of Veterinary Medicine, Benha University. Animal Health Research Institute, Dokki, Giza.

Abstract

A total number of 100 samples from ten random broiler chicken carcasses (breast and thigh) were collected from an automatic poultry slaughtering plant in Ismailia city, Egypt. The mean values of Enterobacteriacae count were 5.9x104±9.7x103 cfu/g and 7.1x 104 ± 1.1x104 cfu/g for chicken breast and thigh samples respectively. The prevalence of *E.coli* were 12% and 9% breast and thigh samples examined, respectively. They are serologically identified as 33.35 and 22.2% O_{157}:H_7 (EHEC) , 16.6% and 11.1% O114:H21 (EPEC), 16.6% and 33.3 %O127:H6 (ETEC) , 0% and 0% O126 (ETEC) and 33.3% and 0% O26 (EHEC) for breast and thigh samples, respectively. The incidence of *E.coli* O_{157}:H_7 was 100% in both serological and PCR methods from biochemical positive *E.coli* samples. Culture is specific and cheap whereas PCR is sensitive and expensive, hence, we recommend both culture and molecular methods, which improve sensitivity and specificity, to enhance detection of foodborne pathogens including *E.coli.*

Corresponding author: Fahim A Shaltout, Department of Food Control, Faculty of Veterinary Medicine, Benha University, Egypt, Email: fahim.shaltout@fvtm.bu.edu.eg

Keywords: Chicken meat, Identification, characterization, E.coli, PCR

Received: Jul 08, 2020 **Accepted:** Aug 31, 2020 **Published:** Sep 20, 2020

Editor: María Del Refugio Castañeda-Chávez, Tecnológico Nacional de México/Instituto Tecnológico de Boca del Río, 94290 Veracruz, Mexico.

Introduction

Chicken meat is one of the most popular foods among developed and developing countries .It contains essential amino acids, minerals including sodium, potassium calcium, iron, phosphorous besides, and traces of vitamins such as vitamin B12 and niacin required for growth and carry on life[1].

Chicken meat is a common source of pathogenic bacteria such as *Escherichia coli* [2].

Poultry meat is an ideal medium for bacterial growth and is known to harbor a large number of bacteria that are pathogenic to human. Typically, contamination with bacteria occur in low sanitation levels, and only pose a threat to the consumer if the product is not handled.

Escherichia coli is considered as a commensal in the alimentary tract of domestic and wild animals as well as man. *E. coli* is one of the important food borne pathogen of public health interest incriminated in poultry meat worldwide[3]. *E. coli O157:H7* has the ability to tolerate acidic condition of the stomach, The infective dose of *E.coli O157:H7* ranges from 10 to 100 cells /g[4].

The detection of foodborne pathogens using conventional culture methods have been considered as the "gold standard" for the isolation and identification of foodborne bacterial pathogens[5]. Culture steps include nonselective enrichment, selective enrichment, selective/differential plating, morphological, biochemical and serological confirmation. Culture isolation and identification is known to be specific and inexpensive, but method is labor-intensive and time-consuming, because it require at least, three working days to produce a negative result and five to ten working days for confirming positive results. Moreover, due to environmental factors, variations in gene expression of microorganisms can occur and may affect the results of biochemical tests. Viable but non cultivable cells are not detected by the conventional cultural methods[6].

Polymerase chain reaction (PCR) is a method used for the *in vitro* enzymatic synthesis of specific DNA sequences by Taq or other thermo resistant DNA polymerases. PCR uses oligonucleotide primers that are usually 20–30 nucleotides in length and whose sequence is homologous to the ends of the genomic DNA region to be amplified. The method is performed in repeated cycles, so that the products of one cycle serve as the DNA template for the next cycle, doubling the number of target DNA copies in each cycle[7]. PCR represents a rapid procedure with high sensitivity and specificity for the immediate detection and identification of specific pathogenic bacteria from different food materials[8].

Material and Methods

Collection of Samples

A total of 100 samples from ten random broiler chicken carcasses (about 2kg in weight) were collected after complete preparation involving (washing in achiller, slaughtering, scalding, defeathering and evisceration), at an automatic poultry slaughtering plant in Ismailia city, Egypt. The samples were kept separately in plastic bags, and transported immediately to the laboratory in an insulated ice box under aseptic conditions.

Bacteriological Examination

Conventional Recovery Methods

Preparation of Samples

The samples were prepared according to the technique recommended ICMSF[9].

Twenty-five grams of a samples was taken by sterile scissors and forceps after surface sterilization by hot spatula, then transferred to sterile polyethylene bags, to which 225 ml of 0.1% of sterile buffered peptone water (0.1%) was aseptically added. Each sample was then homogenized for 2 minutes at 2500 r.p.m. using a sterile homogenizer to achieve 1/10 dilution. The homogenate was allowed to stand for 15 minutes at room temperature. One ml from such dilution was transferred to a second sterile tube containing 9 ml sterile buffered peptone water and mixed well. Further decimal serial dilutions were prepared accordingly. This samples of all groups were subjected to the following examination.

Determination of aerobic plate count[10]: was conducted: using standard plate count agar media. While, Determination of Enterobacteriaceae count[11] was conducted :using violet red bile glucose agar media (VRBG). Isolation and Identification of E.coli[12]: using MacConkey broth and Eosin Methylene blue plates. The metallic green colonies were picked up and identified

biochemically and serologically.

Polymerase Chain Reaction (PCR)

For confirmation of isolated strains and for detection of shiga toxin1 and shiga toxin2 [13, 14].

DNA Extraction

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) according to manufacturer's recommendations with modifications. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl 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 100 µl of elution buffer.

Oligonucleotide Primer

Primers used were supplied from Metabion (Germany) (Table 1)

PCR Amplification

Primers were utilized in a 25µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products.

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. Generuler 100 bp ladder (Fermentas, Germany) was used to determine fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Statistical Analysis

All the obtained results were evaluated statistically using Analysis of variance ("Anova test) statistic "[16].

Results

The initial (cfu/g) mean values of aerobic plate count of fresh chicken breast and thigh samples were $5.1x10^4$ $\pm3.2x10^4$ cfu/g and $6.1x10^5\pm5.6x10^5$ cfu/g respectively (Table2). The initial (cfu/g) mean values of total Enterobacteriaceae count of fresh chicken breast and thigh samples were $5.9x10^4\pm9.7x10^3$ cfu/g (Table 3).*E.coli* was isolated from 12% and 18% of the examined fresh chicken breast and thigh samples respectively (Table 4). Using serology, *E.coli* serogroups isolated from breast samples were 2 (33.3%) O157:H7 (EHEC), 1 (16.6%) O114:H21 (EPEC), 1(16.6%) O127:H6 (ETEC), and 2 (33.3%) belonged to O26 (EHEC) (Table 5).

Similarly, *E.cloi* isolates from thigh samples were 2(22.2%) O157:H7 (EHEC), 1(11.1) O114:H21 (EPEC), 3 (33.3) O127:H6 (ETEC), and 3(33.3) belonged to O126 (ETEC) .All 100% of the identified *Ecoli* $O_{157,}$ isolates from chicken meat samples were positive by PCR (Table 6, Figure 1).

Discussion

Aerobic Plate Count gives an idea about the hygienic measures applied during processing to helps in the determination of the keeping quality of the poultry carcasses. Similar results were reported [17] for chicken thigh samples for where APC was 2.5×10^5 cfu/g and [18] for breast samples where APC was 243.90×10^4cfu/g and 69.60 $\times10^4$cfu/g. On the other hand, higher counts were reported [19] with values of $3.38x10^6\pm1.02x10^6$cfu/g. Aerobic plate counts were $1.75\times10^5\pm1.6\times10^5$cfu/g in freshly slaughtered breast meat samples[20]. Comparatively, lower counts were reported by[21] where APC was 4.2 $\times10^2$ cfu/g in raw chicken breast samples Similarly, it was found that APC in examined chicken thigh samples were 6.84×10^4 $\pm1.65\times10^4$cfu/g [22].

Total Enterobacteriaceae Count (TEC)

Enterobacteriaeace count is more frequently used to assess enteric contamination. Nearly similar results were reported by which *Enterobacteriaeace* counts were 5.26×10^4 in examined chicken breast muscle samples [23]. In a similar study it was reported that *Enterobacteriaeace* counts were $9.5\times10^4\pm0.9\times10^4$cfu/g in examined chicken breast samples[20]. In addition, higher counts of *Enterobacteriacae* counts were reported (3.9 $\times10^5$ and 3×10^5 cfu/g, respectively)[24] in chicken thighs and breast samples tested microbiologically[25]. Another investigator

Table 1 .Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denatur-ation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
E.coli O157:H7 fliC	GCGCTGTC GAGTTCTA TCGAGC CAAC-GGTGACTT TATCGCCA TTCC	625	94°C 5 min.	94°C 30 sec.	57°C 40 sec.	72°C 45 sec.	72°C 10 min.	15

Table 2. Total aerobic plate count (APC) of examined chicken samples (n =100).

Chicken meat samples	Total aerobic count (n=100)				
	Positive samples		Count C.F.U./g		
	No.	%	Min.	Max.	Mean ± SE
Chicken breasts	50	100	$3.5x10^3$	$7.2x10^6$	$5.1x10^4$ ±$3.2x10^4$
Chicken thighs	50	100	$4.6x10^4$	$8.8x10^6$	$6.1x10^5$ ±$5.6x10^5$

Table 3. Total Enterobacteriace count (APC) of examined chicken samples (n =50).

Chicken meat samples	Total Enterobacteriacae count (n=48)				
	Positive samples		Count C.F.U./g		
	No.	%	Min.	Max.	Mean ± SE
Chicken breasts	21	42	$3.1x10^4$	$8.2x10^4$	$5.9x10^4$±$9.7x10^3$
Chicken thighs	27	54	$5.4x10^4$	$9.6x10^4$	$7.1x10^4$±$1.1x10^4$

Table 4. Prevalence of micro- organisms isolated from examined chicken samples (n =50).

Micro oragnisms	Examined chicken samples (n=50)			
	Chicken breasts		Chicken thighs	
	No.	%*	No.	%*
Staphylococcus aureus	5	10	2	4
Salmonella	7	14	4	8
Escherichia coli	6	12	9	18
Klebsiella sp.	0	0	2	4
Enterobacter sp.	2	4	1	2
Proteus sp	1	2	1	2
Shigella sp.	1	2	0	0
Clostridium perfringens	8	16	5	10
total	30	60	24	48

*percent calculated according to total number of samples

Table 5. serotyping of E. coli spp. isolated from examined chicken samples (n =50).

E coli spp.	Examined chicken samples (n=50)			
	Chicken breasts isolates (n=6)		Chicken thighs isolates (n=9)	
	No.	%*	No.	%*
O_{157}:H_7 (EHEC)	2	33.3	2	22.2
O114:H21(EPEC)	1	16.6	1	11.1
O127:H6 (ETEC)	1	16.6	3	33.3
O126 (ETEC)	0	0	3	33.3
O26 (EHEC)	2	33.3	0	0

*percent calculated according to total number of isolates

Table 6. Using PCR for detection of Ecoli o157.

Examined isolates	Number of positive samples		%
	Traditional methods	PCR	
	No.	No.	
Ecoli o_{157}	4	4	100

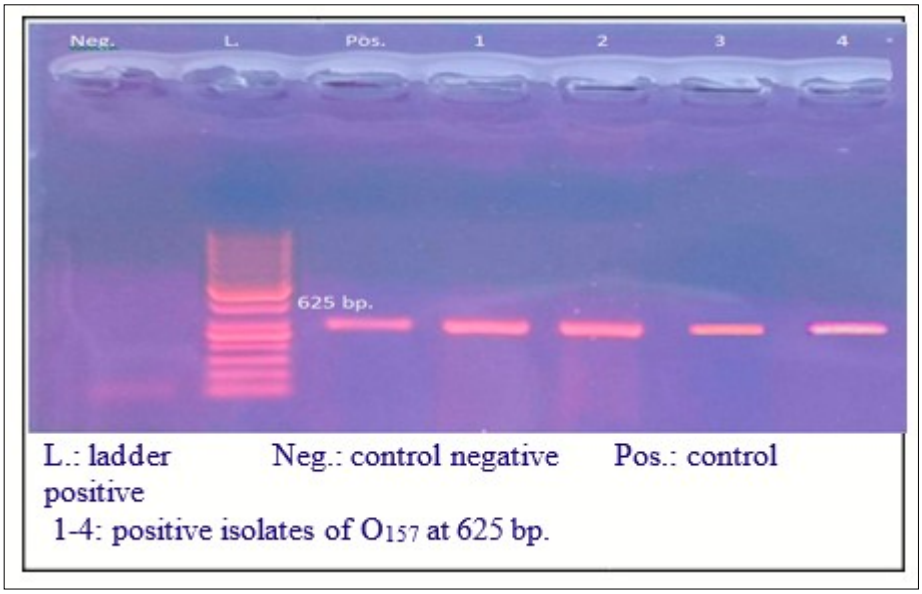

Figure 1. positive gene amplification at 625bp for *Ecoli* $O_{157.}$

reported that *Enterobacteriaeace* counts in examined chicken carcasses samples were 1.57-2.17×10⁶cfu/g[25]. On the other hand, a lower count was reported by [26] in which the mean counts of *Enterobacteriacae* in chicken breast samples was 1.5×10³±2.3×10² cfu/g.

Prevalance and Serotyping of Escherichia coli. Isolated from the Examined Chicken Samples

The presence of *E.coli* in food of animal origin is considered as an indicator of faults during preparation, handling, storage or service[27]. Nearly similar results were reported *E.coli* was isolated from 13.33% thigh samples[28]. Moreover higher percentages of *Escherichia coli* were reported by[29] who founded that the prevalence and load of *E.coli* in chicken meat sold in retail market in Uttar Pradesh was 68% of the examined samples,[30] who reported that 45% of the chicken samples collected from retail outlets were positive for *E.coli.* On the other hand[21] failed to detect *E. coli* O157:H6 targeted samples, whereas only 2% positive samples were reported out of 50 tested[31].

Using serology O_{157}:H_7 (EHEC),1(16.6%) which was belonged to O114:H21(EPEC), O127:H6 (ETEC) and O26 (EHEC) were identified from breast sampls,. while, O_{157}:H_7 (EHEC), O114:H21 (EPEC) ,O127:H6 (ETEC) and O126 (ETEC) were identified from thigh samples.

Enterotoxigenic *E.coli* (ETEC) strains are considered the common cause of traveller`s diarrhea and / or children diarrhea. ETEC may contaminate ready to eat food through a symptomatic carrier, a person who recovers from an ETEC infection and continue to excrete the organism for several months.

On the other hand, Enteroheamorrhagic *E.coli* (EHEC) can cause sever illnesses characterized by sudden onset of severe crampy abdominal pain followed by watery diarrhea, which later on becomes bloody. There may be little or no fever and the duration of illness is 2 to 9 days. Death rate in some reported outbreaks may reach 36%. Since 1982, more than 10650 outbreaks of EHEC were reported in USA [32].

Polymerase Chain Reaction (PCR)

All 100% of *Ecoli* $o_{157,}$ isolates identified serologically from chicken meat samples were positive by PCR. Thus there was complete agreement between the results of serological methods and PCR technique for identification of *Ecoli* $o_{157.}$ Accordingly, the application of one of these trials is sufficient and accurate for identification of such organism.

This agrees with the report[33] who observed

similar findings between multiplex PCR and microbiological/biochemical methods Microbiological method is still the method of choice of isolation and identification of food pathogens owing to its availability and ease of application.

References

1. Food and Agriculture Oraganization "FAO" (2014): The role of poultry in human nutrition available at: www FAO.org/ docrp/ 013/ a1713e/a1713e00.
2. Cason ,J. A. , Hinton, A. and Ingram, K.D.(2000): Coliform, Escherichia coli, and Salmonella concentration in a multiple-tank, counter flow poultry scalder .J. Food Prot.63:1184-1188.
3. Adeyanju, G.T. and Ishola,O. (2014):Salmonella and Escherichia coli contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. Springer Plus,3:13914 7.
4. Doyle, M.P., Robert, L. and Buchanan (1997) : Food borne disease Significance of *E.coli O157 :H7* and other Enterohemorhagic *E.coli*. J.of Food Technology, 51(10).
5. Jasson, V., Jacxsens, L., Luning, P., Rajkovic, A. and Uyttendaele, M. (2011): Alternative microbial methods: An overview and selection criteria. Food Microbiol 27:710-730.
6. Malorny, B., Tassios, P .T., Radstrom ,P., Cook, N. And Wagner, M. (2003): Standardization of diagnostic PCR for the detection of foodborne pathogens. Int J Food Microbiol 83:39-48.
7. Hill,W.E.(1996): The polymerase chain reaction: application for the detection foodborne pathogens. CRC Crit Rev Food Sci Nutrit 36:123–173.
8. McKillip, J.L. and Drake, M. (2004): Real-time nucleic acid-based detection methods for pathogenic bacteria in food. J Food Prot 67: 823-832.
9. International Commission on Microbiological Specification for Foods، ICMSF،(1978): Microorganisms in foods. their significance and methods of enumeration (2nd ed). University of Toronto press. Toronto Canada.
10. USDA (2011) :Quantitative Analysis of Bacteria in Foods as Sanitary Indicators
11. ISO (2001): International Organization for Standardization Microbiology food.
12. American Public Health Association (APHA) (1992): Compendium of methods for microbiological examination of Food. 3rdEd. Brothers, Ann, Arb.
13. Hu,Q., Tu,J., Han X., Zhu Y. and Ding, C (2011): Development of multiplex PCR assay for rapid detection of Riemerella anatipestifer Esherichia coli and salmonella enterica simultaneously from ducks. J. Microbiol.Methods,87:64-69.
14. Dipineto L., Santaniello, A., Fontanella, M., Lagos K., Fioretti, A.,Menna,LF.(2006): Presence of Shiga toxin-producing *E.coli O157: H* in living layer hens. Letters in Applied Microbiol., 43: 293 -295.
15. Fratamico, P.M., Bagi, L.K. and Pepe, T. (2000): A multiplex polymerase chain reaction assay for rapid detection and identification of Escherichia coli O157:H7 in foods and bovine feces. J Food Prot.;63(8):1032-7.
16. Feldman, D (2003): The solution for data analysis and presentation graphics. 2nd Ed., Abacus Lancripts, Inc., Berkeley, USA.
17. El daly ,E.A, Morshedy, A .M .and Sallam ,K .I. (2002):Improving of Sanitary status of broiler carcasses during their processing . 6th Vet. Med . Zagazig. Conference (7-9 sept.2002) Hurghada.
18. Sengupta,R., Da, R., Ganguly, S. and Mukhopadhayay ,S.K. (2011): Survey on microbial quality of chicken meat in Kolkata, India. International J. of Research in pure and Applied Microbiology, 1(3):32-33.
19. Morshdy, A.M.A., Hafez, A.E., Mostafa, A.M. and EL-Sayed, O, A. (2008): Bacterial evaluation of marketed chicken carcasses in Dakahlia Province and improvement with lactic acid. Zag. Vet. J.36 (5): 93-100.
20. EI-Shopary , F. N . (2013) : Effect of antibiotic residues on the sanitary status and storage period of poultry meat. Ph .D .Thesis (Meat Hygiene) .Fac. Vet. Med., Zagazig.Univ.,Egypt.
21. Abd Allah, W.H. and Hassan,A.A.(2000): Sanitary status of ready to eat meat meals in cairo and Giza Governorates. J . Egypt. Vet. Med. 60(7):

95-104.

22. Mousa, M.M., Bkhiet, A.A. and Abd- ElTawab, E. (200): Bacteriological aspect of pre-cooked deboned poultry meat in Damanhour. The second International Scientific Conference. The role of Vet. Med. Mansoura Univ:255-268.
23. Ola, A.e.(2007):Hygienic evaluation of poultry carcasses. Zagazig Univ. Egypt. Fac. Vet. Med.
24. Osman ,E.M.S.(2001): Quality assurance of locally dressed broiler´s cuts and their products.Cairo Univ,Fac of vet Med .
25. Rindhe, S.N., Zanjad, P.N., Doifode, V.K., Siddique, A .and Mendhe M.S. (2008): Assessment of microbial contamination of Chicken products sold in Parbhani city.Veterinary World,1(7):208-212.
26. Tolba, K.(2000):Sanitary status of marketed frozen chicken products exhibited in presentation freezer. J. of Veterinary Medecine, Giza. 217.
27. Tebbut,G.M.(1999): Microbiological contamination of cooked meats International Commission on and environmental site in premise selling both raw and cooked meat products. Int. J. Environm. Health Research 3(4):209-216.
28. Ibrahim, H.M., Amin, R.A., El-Shater, M. A. and Hafez, Salwa. M. (2015) Bacteriological evaluation of freshly slaughtered chicken carcasses. Benha. Vet. Med.J,28 (2):74-82.
29. Joshi ,N. and Joshi ,R.K.(2010): Bacteriological quality of meat sold in retail market in Uttar Pradesh. J. of Veterinarian Public Health, 8 (2):137-139.
30. Ahmed, M.U.D., Sarwar,A., Najeeb, M.I. and Nawaz, M (2013): assessment of microbial load of raw meat at abattoirs and retail outlets.The Journal of Animal &Plant Sciences,23(3):745-748
31. Kiranmayi, C.B. And Krishnaiah,N. (2010): Detection of E.coli O157 H7 prevalance in foods of animal origin by cultural methods and PCR technique. Veterinary World,3(1):13-16.
32. World Health Organization (WHO) (1997): Consultation on prevention and control of EHEC infections. World Health Organization,Geneva, Switzerland.
33. Asensi, G.F , dos Reis, E.M.F. and Del Aguila, E.M. D. (2009): Detection of Escherichia coli and Salmonella in chicken rinse carcasses", British Food Journal, Vol. 111 Issue: 6,pp.517-527.