TRADITIONAL AND RECENT TECHNIQUES FOR DETECTION OF *ESCHERICHIA COLI* IN FRESH CHICKEN CUTS AND GIBLETS

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A B S T R A C T

100 random samples of fresh chicken cuts (breast and thigh) and chicken giblets (liver, gizzard and heart) (25 of each) were collected from different butcher's shops at El-Sharkia Governorates. The mean value of coliforms count varied from ($7.07 \times 10^2 \pm 0.70 \times 10^2$) cfu/g, ($6.71 \times 10^2 \pm 0.65 \times 10^2$) cfu/g , ($6.23 \times 10^2 \pm 0.58 \times 10^2$) cfu/g, ($4.91 \times 10^2 \pm 0.45 \times 10^2$) cfu/g and ($5.88 \times 10^2 \pm 0.53 \times 10^2$) cfu/g for chicken thigh, breast, liver, gizzard and heart samples, respectively. Moreover, the incidence of *E. coli* were 15%, 10%, 25%, 10% and 20% of examined thigh, breast, liver, gizzard and heart samples, respectively. They are serologically identified as O55:k59, O124:k72, O125:k70, O127:k63, O128:k67, O119:k69, O26:k60, O111:K58, O86:k6. The incidence of positively identified *E. coli* were 80% and 65% by both traditional methods (serological examinations) and recent techniques (PCR) from biochemically positive *E. coli* samples. (n=20). Moreover, the serologically identified enteroheamorrhagic strains doesn't have a gene responsible for production of shiga toxin (*stx1* and *stx2* genes).The results cleared that PCR is an ideal method for identification of *E. coli*, as it is effective, less labor, more sensitive, reduces effort and time. The public health significance of isolated microorganisms and the possible sources of contamination of chicken meat cuts and giblets with these organisms as well as suggestive hygienic measures to improve the quality of such items were discussed.

KEY WORDS: *ESCHERICHIA COLI,* chicken giblets

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1. INTRODUCTION

Chicken meat provide an animal protein of high biological value for consumers at all ages, where they contain all the essential amino acids required for growth with high proportion of unsaturated fatty acids and low cholesterol value. (Abou Hussein, 2007). Fecal coliforms can be recorded in great numbers on freshly slaughtered carcasses; their presence in meat generally indicates direct and indirect contamination of fecal origin, improper handling and storage (Charlebois et al., 1991). *E. coli* is a Gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (Singleton, 1999). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recall due to food contamination (Vogt and Dippold, 2005). The term "Pathogenic *E.coli*" means, all the pathogenic strains of *E.coli* which cause bacterial infections, including urinary tract infections, diarrheal disease, and other clinical infections such as neonatal meningitis, pneumonia and bacteremia (Alfredo al., 2010). Certain strains of *E. coli* known as verocytotoxin-producing *E. coli* (VTEC) produce a potent poison, or toxin, which causes illnesses ranging from mild diarrhea to very severe inflammation of the

gut (Ngwa et al., 2012). Food safety is a global health goal and the foodborne diseases take a major crisis on health. Therefore, detection of microbial pathogens in food is the solution to the prevention and recognition of problems related to health and safety (Velusamy et al., 2010). Therefore, the aim of this work is a scope on the contamination of chicken cuts and giblets by coliforms consequently, *E. coli* by traditional methods and recent technique (PCR).

2. Material and Methods

2.1. Collection of samples:

A total of 100 random samples of fresh chicken cuts (breast and thigh) and chicken giblets (heart, liver and gizzard) (20 of each) were collected from different butcher's shops at El-Sharkia Governorates. They were transferred directly to the laboratory in an ice-box under complete aseptic conditions and prepared for detection of *E. Coli.*

2.2. Determination of Coliform Counts:

Coliform counts were determined by using Violet Red Bile Agar media (APHA, 1992).

2.3. Isolation and identification of E. coli:

The technique recommended by APHA (1992) by using Eosin Methyline Blue agar media. Suspected colonies for *E. coli* were morphologically and biochemically identified.

2.3. Serotyping of E. coli :

E. coli isolates were serotyped in Reference Laboratory for Veterinary Quality Control on Poultry Production using commercially available kits (Test Sera Enteroclon, Anti – Coli, SIFIN Berlin, Germany).

2.4. Polymerase chain reaction (PCR)

For confirmation of isolated strains and for detection of shiga toxin1 (stx_1 gene) and shiga toxin2 (stx_2 gene). (Hu et al., 2011 and Dipineto et al., 2006)

3. Results

Total coliforms counts in the examined samples varied from 1.5×10^2 to 1.3×10^3 with an average value of $7.07 \times 10^2 \pm 0.70\times 10^2$ cfu/g for chicken thigh, 1.0×10^2 to 1.3×10^3 with an average value of $6.71\times 10^2 \pm 0.65\times 10^2$ cfu/g for chicken breast, 2.0×10^2 to 1.1×10^3 with an average value of $6.23\times 10^2 \pm 0.58\times 10^2$ cfu/g for chicken liver, 1.0×10^2 to 0.9×10^3 with an average value of $4.91\times 10^2 \pm 0.45\times 10^2$ cfu/g for chicken gizzard and 1.3×10^2 to 1.12×10^3 with an average value of $5.88\times 10^2 \pm 0.53\times 10^2$ cfu/g for chicken heart respectively as shown in table (1).

According to ANOVA analysis, there is no significant difference *($P > 0.05$)* in coliforms count between the examined samples. *E. coli* was isolated from 15%, 10%, 25%, 10% and 20% of chicken thigh, breast, liver, gizzard and heart, respectively depending on the traditional methods as shown in table (2). The results of table (3) indicated that *E. coli* was recovered from 16 (16%) out of total examined 100 samples of fresh chicken cuts and giblets. EPEC constitutes 43.7%, followed by ETEC and EHEC which constitutes 25% of each and finally EIEC which constitutes 6.25% from positive samples of cuts and giblets.

E. Coli strains were serologically identified as (O55:k59, O125:k70, O124:k72, O127:k63, O128:k67, O119:k69, O26:k60, O111:K58, O86:k6). The data recorded in Table (4) and photograph (1&2) revealed that the incidence of positively identified *E. coli* were 80% and 65% by both traditional methods (serological examinations) and recent techniques (PCR) from biochemical positive *E. coli* samples (n=20). Moreover, stx_1 and stx_2 genes failed to be detected in *EHEC* stains as shown in table (5) and photograph (3&4).

Table (1): Total coliform counts (cfu/g) in the examined samples of chicken cuts and giblets (n=20).

Sample/Item	Minimum	Maximum	Mean± SE
A-Chicken cuts			
1- Thigh	1.5×10^2	1.3×10^3	$7.07\times 10^{2\,a} \pm 0.70\times 10^2$
2- Breast	1.0×10^2	1.3×10^3	$6.71\times 10^{2\,ab} \pm 0.65\times 10^2$
B- Giblets			
1-Liver	2.0×10^2	1.1×10^3	$6.23\times 10^{2\,ab} \pm 0.58\times 10^2$
2- Gizzard	1.0×10^2	0.9×10^3	$4.91\times 10^{2\,b} \pm 0.45\times 10^2$
3-Heart	1.3×10^2	1.12×10^3	$5.88\times 10^{2\,ab} \pm 0.53\times 10^2$

Table (2) Incidence of *E. coli* isolated from the examined samples of chicken cuts and giblets (n=20)

Samples		Positive samples	
		NO.	%
Chicken Cuts	Thigh	3	15%
	Breast	2	10%
Chicken Giblets	Liver	5	25%
	Gizzard	2	10%
	Heart	4	20%
Total (100)		16	16%

Table (3) Serology of E. *coli* isolated from the examined samples of chicken cuts and giblets (n=20).

	Thigh		Breast		Liver		Gizzard		Heart		Types	Total	
	No	%	No	%	No	%	No	%	No	%			
O_{86}:k_{61}	-	-	-	-	-	-	-	-	1	5			
O_{119}:k_{69}	-	-	-	-	-	-	-	--	1	5	EPEC	7	43.75
O_{55}:k_{59}	1	5	1	5	2	10	1	5	-	-			%
O_{125}: K_{70}	1	5	-	-	1	5	-	-	-	-			
O_{127}:k_{63}	-	-	-	-	1	5			-	-	ETEC	4	25%
O_{128}:k_{67}	-	-	-	-	-	-	-	-	1	5			
O_{26}:k_{60}	-	-	1	5	-	-	-	-	1	5			25%
O_{111}:k_{58}	-	-	-	-	1	5	1	5	-	-	EHEC	4	
O_{124}:k_{72}	1	5	-	-	-	-	-	-	-	-	EIEC	1	6.25%
Total	3	15	2	10	5	25	2	10	4	20			

Percentages were calculated according to number of positive *samples*. EPEC: Enteropathogenic *E. Coli*, EIEC: Enteroinvasive *E. Coli*, ETEC: Enterotoxigenic *E. Coli*, EHEC: Enterohaemorrhagic *E. Coli*

Table (4): Incidence of *E. coli* by traditional method (serological examination) and recent technique (PCR) for detection of *E. coli* in raw chicken cuts and giblets. (N=20)

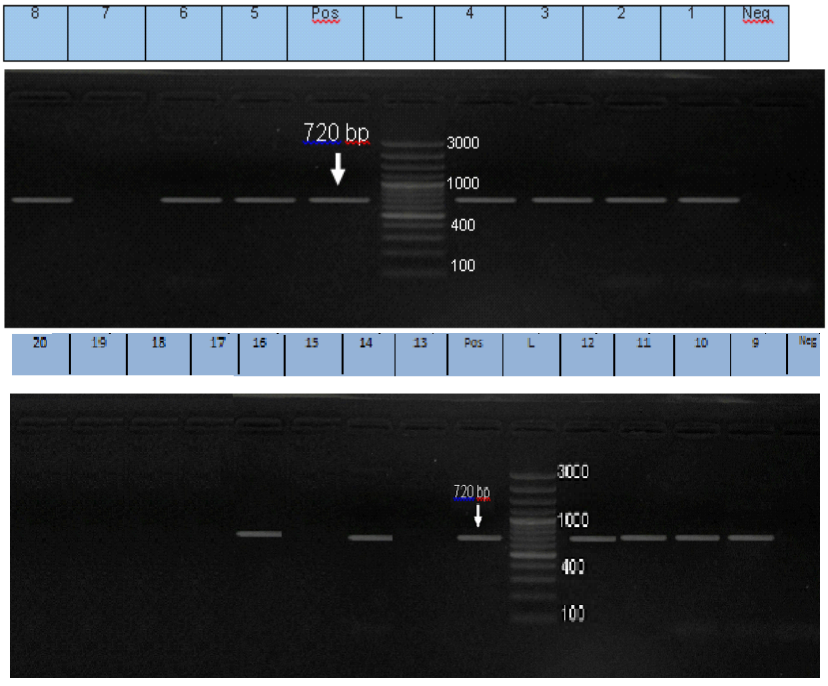
Sample No.	Traditional method (serology)		Recent technique (PCR)	
	No.	%	No.	%
Thigh	3	15 %	3	15 %
Breast	2	10 %	2	10 %
Liver	5	25 %	4	20 %
Gizzard	2	10 %	2	10%
Heart1	4	20 %	2	10 %
Total	16	80 %	13	65 %

Percentages were calculated according to number of biochemical positive *E. coli samples*.

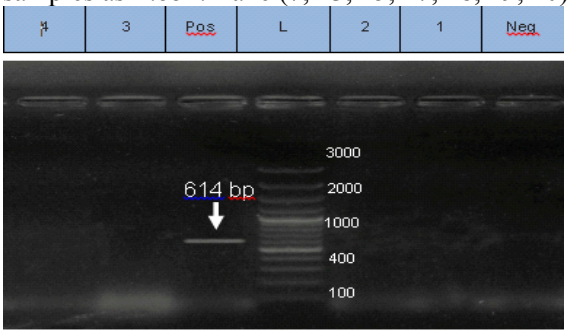
Table (5): Serotyping of isolated EHEC by PCR (n=4).

E.coli serotype	Type of product	Serology		PCR	
		No.	%	No.	%
$O_{26}:k_{60}$	Breast (1) Heart (1)	2	50%	0	0
$O_{111}:k_{58}$	Liver (1) Gizzard(1)	2	50%	0	0
Total	4	4	100%	0	0

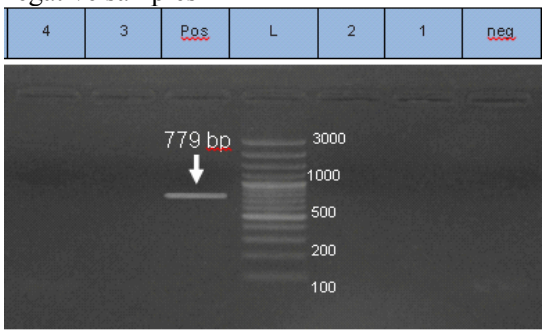
Percentages were calculated according to number of EHEC.



Photograph (1& 2): Agarose gel electrophoresis of PCR amplification products using general primers of *E.coli.* Lane L: 720bp ladder as a molecular DNA ladder. Lane (1,2,3,4,5,6,8, 9,10,11,12,14,16): positive samples as *E.coli.* Lane (7, 13, 15, 17, 18, 19, 20): negative samples



Photograph (3): Agarose gel electrophoresis of PCR amplification products using specific primers of (*stx*$_1$) gene of *E. coli.* Lane L: 614 bp as a molecular DNA marker. Lane (1, 2, 3, 4, 5, 6, 8): negative samples as EHEC producing shiga toxin$_1$



Photograph (4): Agarose gel electrophoresis of PCR amplification products using specific primers of (*stx*$_2$) gene of *E. coli.* Lane L: 779 bp as a molecular DNA marker. Lane (1, 2, 3, 4) negative samples as EHEC producing shiga toxin$_2$

4. DISCUSSION

Coliform act as indicator organisms for unhygienic condition during processing, handling and distribution (ICMSF, 1978). The results in table (1) were nearly similar in chicken cuts with those obtained by Gad (2004); Cohen et al. (2007); Huong et al. (2009), Edris-shimaa(2012). Edris-shimaa (2012) recorded that the average value of coliforms count are $7.84\times 10^2 \pm 0.94\times 10^2$ MPN/g for chicken thigh and $7.36\times 10^2 \pm 0.86\times 10^2$ MPN/g for chicken breast. Higher coliform counts in chicken meat were obtained by Vural et al. (2006) who found that total coliform count was 8.32×10^4 in examined 25 chicken breast meat. Moreover, lower coliform count in chicken meat were obtained by Ruban and Fairoze (2011). It is showed that the total coliforms count of chicken thigh is higher than chicken breast. These results agreed with those obtained by Gad (2004); Nawar (2007); Edris-shimaa (2012). Gad (2004) found that total coliforms counts were $5.12 \times 10^2 \pm 1.94 \times 10^2$cfu/ g for breast and $3.44 \times 10^3 \pm 2.84 \times 10^3$cfu/ g for thigh. High coliforms counts indicate poor hygienic quality of meat. The contamination with coliforms may occur during slaughtering, cutting or dressing of carcasses, soiled hands, shopping blocks or knives used for handling and cutting or contaminated water (Yadav et al., 2006). *E. coli* was previously isolated from chicken meat samples by (Gad (2004); Cohen et al. (2007); Lee et al (2009); Saikia and Joshi (2010); Elsabagh-rasha (2010); Edris-shimaa (2012). Edris-shimaa (2012) isolated 4 *E. coli* isolates from chicken thigh and 3 *E. coli* isolates from chicken breast, while Edris (1992) failed to detect *E.coli* in his examined samples. Results of Table (2) are nearly similar to Edris-shimaa (2012) who recovered *E. coli* with the percentages of (14%) while Higher rates were recorded by Cohen et al. (2007) and Elsabagh-rasha (2010) who recovered *E. coli* with the percentages of 43%and 25% from fresh chicken cuts. In addition, the results obtained in table (2) showed that the examined thigh samples are more contaminated with *E. coli* than other samples and this may attributed to exposure of thigh samples to fecal contamination by worker's hands during

evasiration. The presence of *E. coli* in high numbers indicates the presence of organisms originating from faecal pollution. This is due to improper slaughtering techniques, contaminated surfaces and/or handling of the meat by infected food handlers (Nel et al., 2004). Results in table (3) indicated that EPEC is the most contaminant of the examined samples followed by ETEC and EHEC and finally EIEC. These results differ from lee et al. (2009) who isolated enterotoxigenic *E.coli* (34.6%) followed by enterohaemorrhagic *E. coli* (35.9%) and finally enteropathogenic *E.coli* (20.5%). *E. coli* serotypes O86:K61 (B7), O119:K69 (B19) and O55:k59 (B5) are characterized as enteropathogenic *E.coli* (EPEC), O128:K67 (B12), O125:K70 (B15) and O127:k63 (B8) are characterized as enterotoxigenic *E.coli* (ETEC) while strains causing hemorrhagic colitis O111:K58 (B9) and O26:K60 (B6) are recognized as enterohaemorrhagic *E. coli* (EHEC) (Varnam and Evans, 1991).

EPEC was implicated in cases of gastroenteritis, cystitis, colitis, pyelonephritis, peritonitis and puerperal sepsis as well as food poisoning outbreaks (Doyle, 1990). Enterohaemorrhagic *E. coli* is recognized as the primary cause of haemorrhagic diarrhea and Haemolytic Uremic Syndrome (HUS). The pathogenicity of EHEC appears to be associated with the number of several cytotoxins referred to Shiga- like toxin (SLT) or Vero toxins (VT) (Karmali, 1989). Enterohaemorrhagic *E.coli* has been reported to be probably the most important term of food borne disease (Cliver, 1990). *E. coli* O124 is considered as EIEC, which closely resemble Shigella organisms in causing dysentery like illness. The main difference is that EIEC is much less efficient in their pathogenicity and considered as potential pathogen where 10^9 cells are required to cause illness compared with 10^6 for Shigella (Hoeprich et al., 1994**).** Enterotoxigenic *E. coli* is considered as an important cause of diarrheal disease in adults and infants, particularly, in tropical areas and areas of poor hygiene, as it produces heat labile enterotoxin (LT) and / or heat stable enterotoxin (ST).These strains are common cause of travelers diarrhea in many countries which is a major problem that inhibiting tourism travel to the developing nations (Karmali, 1989). Results in table (4) agreed with those reported by Edris-shimaa (2012*)* who concluded that PCR technique is more accurate than traditional methods for detection of *E. coli.* The traditional methods of *E. coli* identification were able to identify and isolate them, but it was time consuming. On other hand, PCR was more sensitive, more accurate and rapid for bacterial isolation in freshly isolated bacteria as sub culturing of slopes for different times leads to miss of virulence genes on bacterial plasmid lead to false negative result in PCR. The negative results in PCR may be attributed to conventional method show poor sensitivity and sometimes produced false-positive (D'Aoust, 1992). Moreover, PCR based detection mainly depends on the purity and amount of the template DNA used (Estrada et al., 2007). The presence of PCR inhibitors in food samples and incomplete bacterial cell isolation lead to the production of false negative results in PCR based detection and the removal of PCR inhibitors, efficient bacterial cell isolation and efficient DNA extraction is important (Jeníkova et al., 2000).

It concluded that the examined samples of chicken giblets are more contaminated with *E. coli* than chicken cuts and EPEC is the most contaminant of the examined samples followed by ETEC and EHEC and finally EIEC. In addition, PCR is rapid, highly specific, sensitive and accurate in the *E. coli* identification compared to other traditional methods.

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الطرق التقليدية والحديثة لتحديد ميكروب الايشيريشيا كولاى فى لحوم الدجاج الطازج وحوائجها

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الملخص العربي

اجريت هذه الدراسة على عدد مائه (100) عينة عشوائية من قطعيات الدجاج (الصدور والأوراك) وحوائجها (الكبد–القانصة–القلب) من محلات مختلفة من محافظة الشرقية (بمعدل 20 لكل نوع) وقد اسفرت نتائج الاختبارات البكتريولوجية ان متوسط العدد الكلى لميكروبات القولون لعينات الاوراك، الصدور، الكبد، القناصة والقلب على التوالى ($7.07 \times 10^2 \pm 0.70 \times 10^2$) و($6.71 \times 10^2 \pm 0.65 \times 10^2$) و($6.23 \times 10^2 \pm 0.58 \times 10^2$ و $4.91 \times 10^2 \pm 0.45 \times 10^2$) و($5.88 \times 10^2 \pm 0.53 \times 10^2$). كما تم عزل ميكروب الأيشريشيا كولاي من أوراك، صدور الدجاج، الكبدة، القانصة والقلب بنسبة 15%،10%، 25% و10% و20% على التوالى. وكانت العترات المعزولة هي، $_{59}K:_{55}O$ ،$_{60}K:_{26}O$ ،$_{61}K:_{86}O$ ،$_{72}K:_{124}O$ ،$_{58}K:_{111}O$ $_{67}K:_{128}O$ and $_{63}k:_{127}$ ،$_{70}k:_{125}O$ ،$_{69}K:_{119}O$. كما وجد أنه من اجمالى 20 عترة من الايشيريشيا كولاى المعزولة بالاختبارات الكيميائية من قطعيات الدجاج المختلفة وحوائجها 16 عينة كانت ايجابية بالاختبارات التقليدية (السيرولوجية)، بينما اظهرت الاختبارات الحديثة (البى سى ار) 13 عينة ايجابية فقط، من ثم فأن تفاعل البلمرة المتسلسل (بي سي ار) افضل من الطرق التقليدية واكثر حساسية ودقة وموفرا للوقت والجهد فى الكشف عن الميكروبات. وقد تم دراسة ومناقشة الأهمية الصحية للميكروبات المعزولة ومصادر تلوث قطعيات الدواجن وحوائجها بالإضافة إلى اقتراح التوصيات للحد من تلوث هذه القطعيات.

(مجلة بنها للعلوم الطبية البيطرية: عدد 26(2):21- 29, يونيو 2014)