Bacteriological Study on Some Chicken Products Contaminated With *Escherichia Coli* and Its Detection by Using Recent technique

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Abstract

A total of 90 random samples of chicken meat products represented by breast, thigh and drum sticks (30 of each) were collected from different supermarkets in kalyobia governorate for detection of *E. coli* by convential method and PCR assay. The incidence of enteropathogenic *E.coli* in the examined samples, were 6.67%, 10% and 23.33% for breast, thigh and drumstick, respectively. The isolated strains were serotyped as O_{55}: K_{59} (B_5), O78:K80, O_{111}:K_{58} (B_9), O78: $K_{80}O_{124}$: K_{72} (B_{17}), O_{128}: K_{67} (B_{12}). The incidence of isolated strains which produce enterotoxin were 3.33%, 6.67% and 13.33% for breast, thigh and drum stick, respectively. However, enterotoxin producing *E.coli* strains were further studied by immunological method and PCR assay. PCR showed the same results of traditional methods for *E.coli* strain producing heat labile enterotoxin (LT) but PCR were negative for strain producing heat stable enterotoxin (ST).

Introduction

Poultry meat constitutes an excellent source of protein for feeding because of its high meat yield, low cost. Poultry products are considered as a major vehicle of most reported outbreaks of food born diseases and spoilage bacteria and /or food born pathogens (**1, 2**). The processing, handling, distribution, storage as well as marketing of most chicken products constitute a public health hazard either due to the presence of pathogenic bacteria leading to harmful effects as food infection or intoxication among consumers (**3-5**).

Heavy bacterial loads enter the processing operations with the living birds can be disseminated throughout the plant during processing (**6**), the quality assurance of poultry products has been the ultimate goal of all hygienists (**7**), *E.coli* is the most common microorganism implicated in

infants, children diarrheal cases and EHEC bacteria were recognized as zoonotic pathogens and capable of causing heamorrhagic colitis and heamolytic uremic syndrome, EHEC are one of the world wide most important causes of food born infections **(8, 9)**.

Traditional method for detecting and identifying food born pathogenic microorganism require presumptive tests followed by confirmative one which are time consuming , slow which needs many days to confirm its presence of pathogen at low levels in food samples . So, rapid methods as PCR allows several millions fold amplification of target DNA from as little as one copy in 2 to 3 hours.

PCR is highly sensitive, specific and rapid method for detection of food microorganisms , that substitute biochemical and serological characterization of pathogen .In addition, PCR can be applied on fixed tissues (frozen or formalin fixed) reducing the potential dangers involved in transport and handling of specimens with live virulent pathogen, **(10, 11)** .Therefore the present study was throw out light over the bacterial status of some chicken meat products (thigh, breast and drumstick) at Benha city, using polymerase chain reaction (PCR) as a recent technique for the detection of pathogenic bacteria .The health risk of bacteria isolated were also discussed.

Material and Methods

Ninety random samples of chicken meat products represented by breast, thigh and drum sticks, 30 of each) were collected from different super markets in Kalyobia governorate for detection of E. coli by conventional method and PCR assay.

1- Detection of E. coli by traditional immunological method:

Accurately, 25g of each sample were homogenized in 225 ml sterile peptone water and the technique recommended by **APHA (12)** was applied using MacConkey broth as enrichment broth and Eosin methylene blue (EMB) was used as plating medium. The suspected metallic green colonies were identified biochemically and serological according to **Kerig and Holt (13)**. Antisera used for typing of ***E.coli*** were coli test sera poly I, coli test sera poly II and Bacto ***E. coli*** antisera.

2- Testing of E. coli for toxin production:
positive *E.coli* isolates were tested for enterotoxin production by ELISA using the commercially available kits (Oxoid) including:
2.1- VET-RPLA which detects Heat labile enterotoxin (LT) of *E.coli.*
2.2. *E.coli* STEIA which detects heat stable enterotoxin (ST) of *E.coli.*
3- Polymerase chain reaction (PCR) assays **(14).**
Positive LT and ST producing strains of *E.coli* were serogrouped and exposed to PCR technique.
Two synthetic 29-meroliogneuclotide primers were used for the amplification of 195bp fragment from *E.coli* LT gene. The standard reaction mixture was composed of 10 ul test sample, 50 MHCl, 50m MNaCl, 2ml M dithiotreitral, 1m M MgCl, 50m M deoxynuclotide triphasphate, 0.1% triton-X100, 0.2 muM each prima and 2.5 units of Tag polymerase. Moreover, 25 ul of the product was fractionated by electrophoresis on 1.6% agarose gel. The amplified DNA fragment is then visualized by ethidium bromide staining and UV transallumination at 320 nm of the gel and compared with a molecular size marker.

Results

Table (1): Incidence and serotyping of enteropathogenic *E.coli* in the examined samples of chicken meat products (n=30).

Product Serotype	Breast		Thigh		Drumstick		Strain characteristic
	No	%	No	%	No	%	
$O_{55}:k_{59}(B_5)$	-	-	-	-	2	6.67	EPEC (ST producer)
$O_{78}:K_{80}$	1	3.33	-	-	1	3.33	EPEC (LT producer)
$O_{111}:k_{58}(B_9)$	-	-	2	6.67	-	-	EHEC (LT producer)
$O_{124}:k_{72}(B_{17})$	1	3.33	1	3.33	3	10.00	EIEC (non toxin producer)
$O_{128}:k_{67}(B_{12})$	-	-	-	-	1	3.33	ETEC (LT& ST producer)
Total	2	6.67	3	10.00	7	23.33	

EPEC= Enteropathognetic *E.coli*
EIEC= Enteroinvasive *E.coli*
ETEC= Entrotoxigenic *E.coli*
EHEC= Entrohaemoeehagic *E.coli.*

Table (2) Demostration of enterotoxin producing *E.coli* isolated from the examined samples of chicken meat products (n=30).

Chicken meat products	Isolated *E.coli*		Entertoxin producer		Non enterotoxin producer	
	No	%	No	%	No	%
Breast	2	3.67	1	3.33	1	3.33
Thigh	3	10.00	2	6.67	1	3.33
Drum stick	7	23.33	4	13.33	3	10.00
Total (90)	12	13.33	7	7.78	5	5.56

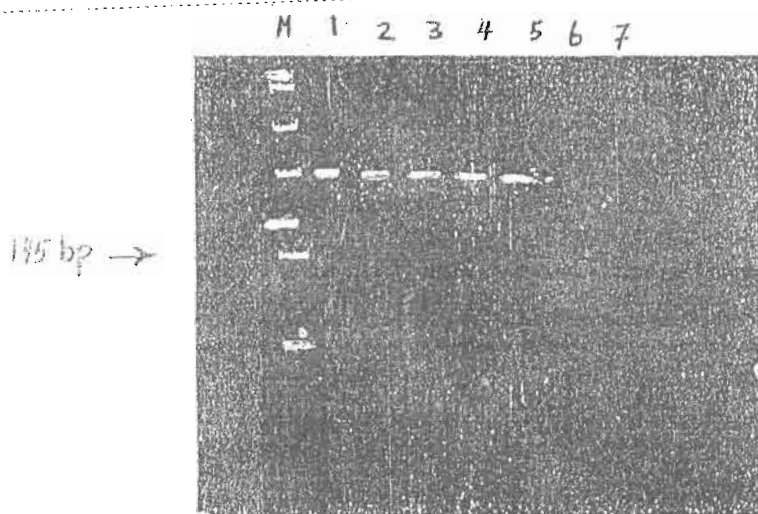

Figure (1): Restriction fragment length polymorphisms of PCR amplification products using meroligonucleotide primers of thermolabile (LT) ETEC.
Lane M.:195bp ladder as a molecules DNA maker.
L1 to 5 : positive samples as TL producers
L6 and 7 : Negative samples
An arrow indicates 195 bp amplified products.

Table (3): Characterization of enterotoxin producing *E.coli* strains by traditional immunological method and PCR assay (n=30).

Chicken meat products	Enterotoxin producing *E.coli* as detected by immunological method				Only LT producing *E.coli* as detected by PCR assay
	LT*	ST**	LT&ST	Total	
Breast	1(O_{78})	-	-	1	1
Thigh	2 (O_{111})	-	-	2	2
Drum stick	1(O_{78})	2(O_{55})	1(O_{128})	4	2
Total	4	2	1	7	5

Discussion

Escherichia coli is an emerging food borne human pathogen which has the ability to tolerate the acidic condition and very few numbers of the organism can cause the disease with infective dose ranged from 10 to 100 cells (**15**).Results achieved in table (1) indicated that enteropathogenic *E.coli* were detected in 6.67%, 10% and 23.33% of the examined samples of breast, thigh, and drum stick, respectively. Accurately, out of 7 strains isolated from drum stick samples, 3strains were serologically identified as O124:K72 (B17), 2as O55: K59 (B5), 1 as O78:K80 and the last strain was O128:K67 (B12). While, O111:K58 (B9) and O124: K72 (B17) strains were recovered from 6.67% and 3.33% of examined thigh samples, respectively. Further, O78:K80 and O124:K72 (B17) strains (3.33% of each) were identified from breast samples.

According to **Byran (16)**, O55 and O78 serotypes are known as Enteropathogenic ***E.coli*** (EPEC), O111 is recorded as Enterohaemorrhagic *E.coli* (EHEC), and O124 is Enteroinvasive ***E.coli*** (EIEC), while O128 strain is recognized as Enterotoxigenic ***E.coli*** (ETEC). In general, Enteropathogenic ***E.coli*** induce sever diarrhea in infants and young children, as well as food poisoning and gastroenteritis among the adults **(17-19)**.

Demonistration of enterotoxine producing ***E.coli*** was recorded in table (2). Therefore, 3.33%, 3.33%, and 10% of examined samples of breast,thigh and drum stick were contaminated with enterotoxin producing

E.coli however , 3.33%, 3.33%and 10% of such samples had non toxin producers. Concerning breast, it had 2 enterotoxin producers represented as O111:K58 (B9) . While, drum stick samples had 4 enterotoxin producer strains represented by 2 (O55:K59 (B5)), 1 (O78:k80 and 1 (O128: K67 (B12)). Enteropathogenic ***E.coli*** was previously isolated by **(20 and 25).**

In general EPEC strains are the major cause of many infantile diarrhea , symptoms appear within 12 to 36 hours and characterized by fever ,nausea , vomiting and watery stools, which occasionally contain mucous, but without gross blood **26.** Positive ***E.coli*** isolates were tested for enterotoxins production by ELISA to detect s Heat labiles (LT),and Heat stable (ST) enterotoxins as well as identification of these enterotoxin producing ***E.coli*** strains by PCR assay. Table (3) and Fig. (1) revealed only LT producing ***E.coli*** detected by PCR assay which represented by 1 (O78) in breast samples ,2 (O111) in thigh samples and 1 (O78),1(O128) of drum stick samples in which O128 produce LT and ST enterotoxin while 2(O55) strain cannot be demonstrated by PCR technique and this could be attributed to the fact that ***E.coli*** (O55: K59 (B5)) doesn't have a gene responsible for production of thermolabile toxin, whereas PCR cannot give positive finding without the presence of this gene. These results were nearly similar to those obtained by **(26 and 29).**

As conclusion, the use of PCR as routine procedure in food microbiology is a very promising tool because of its sensitivity, simplicity and specificity. Whereas, this trail can be useful only for certain ***E.coli*** strain having characteristic genes of LT production but not efficient for other strains of ***E.coli,*** in addition to its relative high cost.

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دراسات بكتريولوجية على بعض منتجات الدواجن الملوثة بميكروب الايشيريشيا كولاى والكشف عنها باستخدام التقنيات الحديثة

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الملخص العربي

ان منتجات الدواجن تعتبر مصدر هام للبروتين الحيواني بما تحتويه من فيتامينات وجلايكوجين وبعض المعادن الهامة ولذلك فهى تعتبر وسط مثالى لنمو كثير من الميكروبات الممرضة ز لذا فقد تم تجميع عدد ٩٠ تسعون عينة عشوائية من الصدور والوراك والدبوس (٣٠ عينة من كل نوع) من محلات مختلفة بمحافظة القليوبية وقد تم فحصها بكتريولوجيا للكشف عن ميكروب الايشيريشيا كولاى وذلك باستخدام الطرق التقليدية للعزل وكذلك استخدام التقنيات الحديثة .

وقد أوضحت النتائج انه تم عزل ميكروب الايشيريشيا كولاى الممرضة التى تم عزلها من منتجات اللحوم كالاتى :

O55 : K59 (B5) , O78: K80 , O111: K58 (B9) , O124: K72 (B17) and O128 :K 67 (B12)

تم تصنيف عترات الايشيريشيا كولاى الممرضة المنتجة للسموم باستخدام الاليزا وكانت نسبة العترات المنتجة للسموم ١٣,٣٣% ، ٦,٦٧% ، ١٣,٣٣% لكل من الصدور والوراك والدبوس على التوالى ، وقد تم اختبار هذه العيناب باستخدام اختبار PCR كوسيلة سريعة ودقيقة ولا تستغرق وقت طويل مثل الطرق التقليدية لعزل الميكروبات الممرضة وذلك للعترات الايشيريشيا كولاي المنتجة للسموم الغير مقاومة للحرارة ولكن لم تعطى نتائج مع عترات الايشيريشيا كولاي المنتجة للسموم المقاومة للحرارة وهذا يعتبر من احد عيوب هذا الاختبار وقد تم دراسة ومناقشة الاهمية الصحية ومصادر التلوث بميكروب الايشيريشيا كولاي .