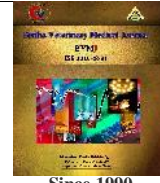




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Molecular studies on some factors of *E. coli* isolated from avian products and infants with diarrhea

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ABSTRACT

Extra-intestinal pathogenic *E. coli* (ExPEC) are facultative pathogens that are part of the normal human intestinal flora which classified into several types, uropathogenic *E. coli* (UPEC), neonatal meningitis *E. coli* (NMEC), sepsis-associated *E. coli* (SEPEC), and avian pathogenic *E. coli* (APEC). The present study was performed on a total number of 150 samples of which 100 samples were collected from poultry products (Gizzard, Liver, Banih, and Nuggets) and 50 samples were collected from infants stool at Gharbia governorate for isolation of *E. coli* and detection of some virulence genes. The results revealed that 26 samples were positive for *Escherichia coli*, 11 from infants stool, 7 from poultry liver, 5 poultry gizzard, 2 Banih and 1 from Nuggets samples. Amikacin 30 and ciproflaxacin 5 (oxid) were the highest efficiency antimicrobial agents used in vitro. Six *E. coli* isolates were serotyped and identified as O₁₅₇, O₁₂₈, O₁₂₅ and O_{86a}. PCR results showed that *traT* and *pic* pathotypic genes (at 307 bp and 572 bp) were detected in all isolates, but *stx2* gene (at 779 bp) was detected only in O₁₅₇ and O₁₂₅. This indicates that *E. coli* strains isolated were extra-intestinal pathogenic strains

1. INTRODUCTION

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped bacterium that belong to family Enterobacteriaceae. It is commonly found as normal inhabitant of GIT of human (Tenaillon et al., 2010). There are many types of *E. coli*, and most of them are harmless, but some of them can cause pathogenicity which called Extra-intestinal Pathogenic *Escherichia coli* (ExPEC). Some strains of *E. coli* O157:H7 may also cause severe anemia or kidney failure, which can lead to death in some times (Pitout et al., 2012). Other strains of *E. coli* can cause urinary tract infections or other strains can cause diarrhea, mastitis, arthritis and meningitis in both humans and animals (Fairbrother et al., 2005 and Nagy and Fekete 2005). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination and lead to GIT infection in human (WHO, 2002). *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards (Widén et al., 2013). The serious problem for researchers who are trying to ascribe Extra-intestinal Pathogenic *E. coli* (ExPEC) transmission to food, people or the environment is to draw the distinction between colonization of ExPEC and infection.

Food safety has an important value for public health both at the production stage, and in the course of its processing and distribution, So the examination of the genetic similarity of ExPEC strains will allow to determine their origin from different sources. (Sarowska et al., 2019).

The aim of our study was to characterize *E. coli* strains isolated from humans (especially infant stool), and avian products (as Liver, Gizzard, Banih, and Nuggets) for the presence of bacterial genes encoding virulence factors in the context of an increasing spread of ExPEC infections.

2. MATERIAL AND METHODS

2.1. Samples:

One hundred and fifty samples Were used in the present study, from which 100 samples were collected from poultry products (25 samples from each of gizzard, liver, banih, and nuggets) from different shops at Gharbia governorate, as well as 50 samples from infants stool suffered from diarrhea collected from different labs at Gharbia Governorate. Each sample was taken alone in sterile packet in ice box and immediately transferred (without delay) for bacteriological examination at animal health research Institute, Tanta branch.

2.2. Bacteriological examination

A small piece of the organ was inoculated into MacConkey broth (oxid) and then incubated at 37°C for 24 hours. Aloopfull from MacConkey broth was inoculated on MacConkey agar then nutrient agar and then incubated at 37°C for 24-48 hours. Cultivation on selective media as EMB and Brilliant green (oxid) for *E. coli*. Suspected colonies were picked up and subcultured on brain heart infusion broth with 50% glycerol and incubated at 37°C for 24 hours, then kept in freezer for further studies (Markey et al., 2013).

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2.3. Differentiation between invasive and non-invasive isolated *E.coli*

Application of Congo red test using Congo red medium (oxoid) which used for differentiation between the invasive and non-invasive *E.Coli* strains, the results showed red colonies for the invasive *E.coli*, and white colonies for the non-invasive *E.Coli* isolation.

2.4. Serological identification of *E. coli* isolates:

E.coli isolates were identified serologically using *E. coli* polyvalent and monovalent O antisera by slide agglutination technique (DENKA SEIKEN CO., LTD.TOKYO, Japan) obtained from Animal Health Research Institute, Dokki, Egypt, were used for sero grouping of *E.coli* suspected isolates using the technique described by (Edward and Ewing,1972)

The tested strains were picked up from 24 hrs old colony on nutrient agar and were emulsified with a loop in the drop of the physiological saline on the slide and mixed thoroughly with a drop of antiserum for one minute according to (Markey et al., 2013)

2.5. In-Vitro anti-microbial sensitivity test

Subculture from the isolated strains were prepared and subjected to the sensitivity test against different antimicrobial discs (Markey et al., 2013)

2.6. Molecular identification of *E.coli* virulence genes by PCR

Oligonucleotide primers used in cPCR; Five pairs of primers were selected (3 infant stool, 2 avian products) and submitted to PCR targeting 5 virulence genes including (*stx1*, *stx2*, *tsh*, *pic*, *tarT*) using primers with specific sequences to each gene supplied from Biobasic (Canada).

2.6.1- Extraction of genomic DNA from *E.coli* isolates

It was performed using QIAamp DNA Mini Kit Catalogue no.51304 according to manufacture instruction.

2.6.2- Amplification & cycling condition for PCR:

It was performed in a thermal cycle according to specific another of each primer and according to Emerald Amp GT PCR mastermix (Takara) Code No. RR310A kit

2.6.3- Detection of PCR products

It was performed according to (Sambrook et al., 1989) and it was detected by electrophoresis on Agarose gel which was photographed by a gel documentation system and the data was analyzed through computer software.

3. RESULTS

The results of isolation as shown in (Table1) indicated that 26/150 (17.3%) were positive for *E.coli* (15 isolates from avian products, and 11 isolates from infant stool), which classified into invasive and non-invasive. Table 2 showed 14/26 (53.8%) were invasive *E.coli* (8 infant stool, and 6 avian products).

The result of serological examination of six invasive isolates were randomly selected (3 from avian products, and 3 of infants stool) showed that 2 *E.coli* strains were O157 (one from Liver, and one from infant stool), one *E.coli* strain of O128 (from Gizzard), one *E.coli* strain of O125 (from infant stool) , *E.coli* strain of O86a (from infant strain), and the last isolate was untyped (Table,3).

Table 1 Incidence of isolated *E.coli* from different samples

Samples	No. of examined samples	No. of positive samples	Percentage of positive samples
Avian product (liver)	25	7	28%
Avian product (gizzard)	25	5	20%
Avian product (banih)	25	2	8%
Avian product (nuggits)	25	1	4%
Infant stool	50	11	22%
Total no. of samples	150	26	17.3%

Table 2 Number and percentage of invasive *E.coli* isolates from examined samples

No. of <i>E.coli</i> isolation/samples	No. of invasive <i>E.coli</i> isolation	No. of non-invasive <i>E.coli</i> isolation	Percentage of invasive isolation/ No. of isolation
7/ liver samples	3	4	42.8 %
5/ gizzard samples	2	3	40 %
2/ banih samples	1	1	50 %
1/ nuggits samples	0	1	0 %
11/ infants stool	8	3	72 %
26/ Total No.	14	12	53.8 %

Table 3 Results of serological examination

No. of samples	Type of samples	polyvalent	Monovalent
1	Avian liver	1	O157
2	Avian banih		Untyped
3	Avian gizzard	1	O128
4	Infants stool	1	O86a
5	Infants stool	1	O157
6	Infants stool	2	O125

The result of In vitro sensitivity tests as showed in table (5) revealed that different sensitivity of strains to antimicrobial agents as the isolated *E.coli* strain (O157) from avian liver showed moderate sensitivity to Amikacin and resistance to Gentamicin (CN) , Erythromycin (E), Ciproflaxacin (CIP) , Ceftriaxone (CRO) , Cefoperazon (CEP) , Rifampin (RA) and Azythromycin (AZM) , While *E.coli* strain O157 that recovered from infant stool showed moderate sensitivity to Amikacin and Ciproflaxacin and were resistant to other antimicrobial agent. Also, the isolated *E.coli* strain (O128) that recovered from avian gizzard showed high sensitivity to Ciproflaxacin, moderate sensitivity to ceftriaxone , cefoperazon , Amikacin , Erythromycin , Azythromycin and resistance to Gentamicin.

The other isolated *E.coli* (O86a) that recovered from infant stool showed high sensitivity to Cip , AZM , E and moderate sensitivity to AK, CRO, CN, RA and has resistant only to CEP , While *E.coli* (O125) that recovered from infant stool showed high sensitivity to AK , CIP , CEP and moderate sensitivity to AZM , CN , RA and resistant to E , CRO (Table 4&5)

PCR results showed that the five examined *E.coli* strains were all positive for *traT* and *pic* genes with amplicon size of 307 bp and 572 bp, respectively (Figure 1,2), While only 2 *E.coli* strains (O157 and O125) obtained from infant stool) were positive for *stx2* gene with 779 bp amplicon size (Figure, 3) , Moreover all *E.coli* strains examined by PCR were negative for *sxt1* and *tsh* genes, respectively (Figure 3 & 4).

All samples showed positive amplification of *traT* gene at 307 bp (fig.1),all samples showed positive amplification of *pic* gene at 572 bp (fig.2),all samples showed negative amplification of *tsh* gene at 620 bp (fig.4),all samples showed negative amplification of *sxt1* gene at 614 bp (Figure 3), and two samples showed positive amplification of *stx2* gene at 779 bp (fig 5).

The avian products represent an important source of nutrition at early aged children as they supplied them by essential ingredients for integrated growth and by increasing consumption of it, we interested in studying the most common bacterial agents as (*E.coli*) that found to affect the raising of chickens and can be transmitted to avian products and then to infants by food borne transmission route which cause serious food poisoning or urinary tract infection by their pathogenic serotypes. There are some studies that reported the avian pathogenic *E.coli* (APEC) and extraintestinal pathogenic *E. coli* (ExPEC) strains, that cause infections in humans, are quite closely genetically related and share some of the same virulence genes (Rodriguez,2005 and Manges, 2016). It is possible that APEC strains may be somewhat influence on the health of humans (Belanger, 2011).

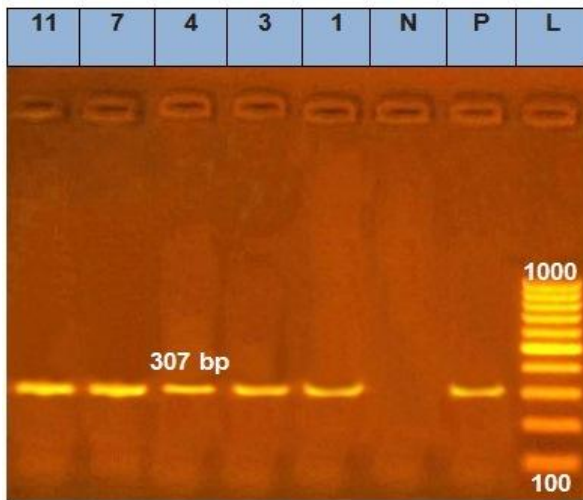


Fig. 1 Agarose gel electrophoresis pattern of PCR for detection of TraT gene of *E.coli* at 307 bp . L: Ladder from 100 bp to 1000 bp. Pos: Positive control: *E. coli* ATCC 25922. Neg: Negative control: Field isolate that were tested and confirmed to be negative by PCR for the related genes in R.L.Q.P. Lane 1, 3,4,7,11: Positive amplification of TraT gene.

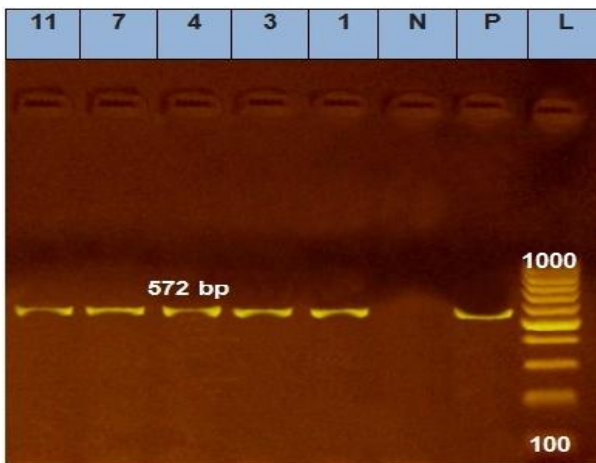


Fig. 2 Agarose gel electrophoresis pattern of PCR for detection of pic gene of *E.coli* at 572 bp. L: Ladder from 100 bp to 1000 bp. Pos: Positive control: *E. coli* ATCC 25922. Neg: Negative control: Field isolate that were tested and confirmed to be negative by PCR for the related genes in R.L.Q.P. Lane 1, 3,4,7,11: Positive amplification of pic gene.

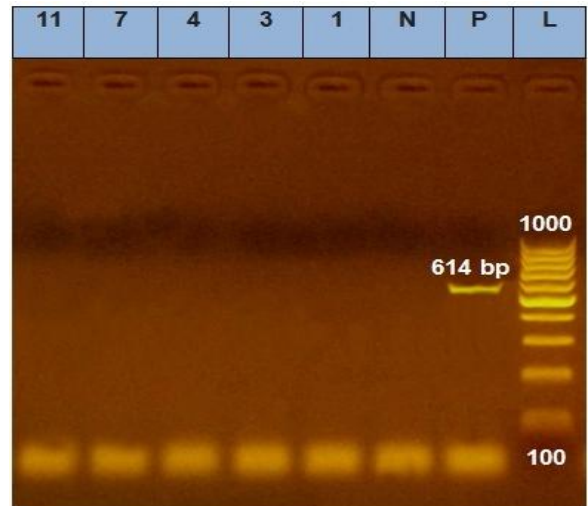


Fig. 3 Agarose gel electrophoresis pattern of PCR for detection of stx1 gene of *E.coli* at 614 bp. L: Ladder from 100 bp to 1000 bp. Pos: Positive control: *E. coli* ATCC 25922. Neg: Negative control: Field isolate that were tested and confirmed to be negative by PCR for the related genes in R.L.Q.P. Lane 1, 3,4,7,11: Negative amplification of stx1 gene.

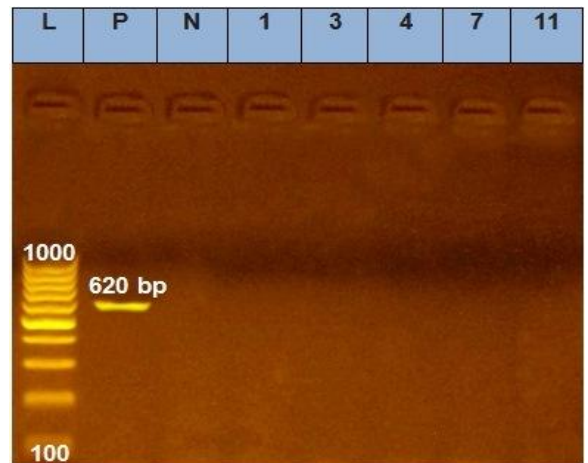


Fig. 4 Agarose gel electrophoresis pattern of PCR for detection of tsh gene of *E.coli* at 620 bp. L: Ladder from 100 bp to 1000 bp. Pos: Positive control: *E. coli* ATCC 25922. Neg: Negative control: Field isolate that were tested and confirmed to be negative by PCR for the related genes in R.L.Q.P. Lane 1, 3,4,7,11: Negative amplification of tsh gene

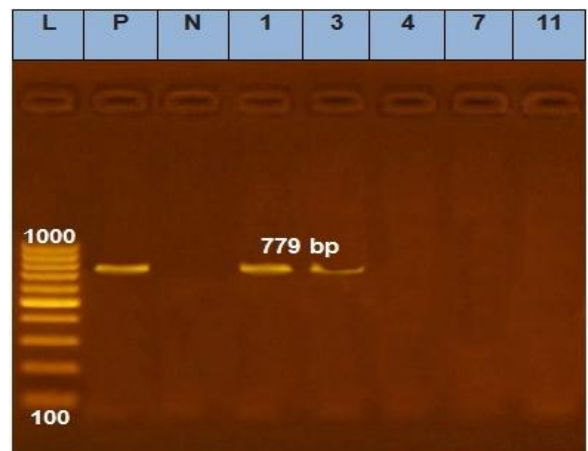


Fig. 5 Agarose gel electrophoresis pattern of PCR for detection of stx2 gene of *E.coli* at 779 bp. L: Ladder from 100 bp to 1000 bp. Pos: Positive control: *E. coli* ATCC 25922. Neg: Negative control: Field isolate that were tested and confirmed to be negative by PCR for the related genes in R.L.Q.P. Lane 1, 3: Positiv amplification of stx2 gene. Lane 4,7,11: Negative amplification of stx2 gene

Table 4 Interpretation of zones inhibition for antimicrobial susceptibility (Modified from CLSI (2017))

Antimicrobial agents	Abbreviation	Disc content in ug	Diameter of inhibition to nearest mm.		
			Resistant	intermediate	Susceptible
Amikacin	AK.	30 ug	14	15–16	17
Gentamicin	CN.	10 ug	12	13–14	15
Erythromycin	E.	15 ug	13	14–17	18
Ciproflaxacin	Cip.	5 ug	15	16-20	>21
Ceftriaxone	Cro.	30 ug	17	18-20	21
Cefoperazon	CEP.	75 ug	14	15-18	19
Rifampin	RA.	5 ug	10	14–17	14
Azythromycin	AZM.	15 ug	14	15–18	19

Table 5 Antimicrobial sensitivity results of six *E.coli* isolates

Strain	CRO	CIP	CEP	AK	RA	AZM	CN	E
1 O157	R	R	R	M	R	R	R	R
2 Untyped	R	M	R	S	R	S	M	R
3 O128	M	S	M	M	R	M	R	M
4 O86a	M	S	R	M	M	S	M	S
5O157	R	M	R	M	R	R	R	R
6 O125	R	S	S	S	M	M	M	R

R = resistant. S =sensitive. M = moderate

Table 6 Results of PCR on examined samples

Sample	<i>tsh</i>	<i>Stx1</i>	<i>Stx2</i>	<i>TraT</i>	<i>pic</i>
1	-	-	+	+	+
3	-	-	+	+	+
4	-	-	-	+	+
7	-	-	-	+	+
11	-	-	-	+	+

Sample 1 = sample no.5 at serological examination table (infant stool) = O157. Sample 3 = sample no.6 at serological examination table (infant stool) = O125. Sample 4 = sample no.4 at serological examination table (infant stool) = O86a. Sample 7 = sample no.3 at serological examination table (avian Gizzard) = O128. Sample 11 = sample no.1 at serological examination table (avian Liver) = O157

4. DISUCSSION

The present results revealed that *E.coli* bacterial agents isolated from different types of avian products (Liver, Gizzard, Nuggits, and Banih) and also *E.coli* bacterial agents isolated from infants stool representing different ages (from 1year-2year) and sexes. The bacterial species isolated were *E.coli* serotyping of (O157) from avian product sample and infants stool, (O128) from avian product, (O86a) and (O125) from infants stool.

PCR examination for detection of gene sequence, the results approved that the presence of mutual genes in all examined serotypes (*traT*, *pic*) which suggest the ability of transmission of extra-intestinal pathogenic *E.coli* (ExPEC) from avian products to infants , So further investigation is needed to confirm the relationship between avian products consumption and infant infection by (ExPEC), Moreover excessive and indiscriminate use of antimicrobials leads to presence of strains of highly resistant to most of them.

Escherichia coli isolates were 15 isolates from avian products as in studies of Makhol et al.,(2010), Abd El Tawab et al.,(2016a) and Halfaoui et al.,(2017).

In spite of Abbott et al.,(2003), Hyma et al.,(2005)and Oaks et al.,(2010) isolated *E.alberti* , and Poulou et al.,(2008), Yamanaka et al.,(2010) isolated *E. hermannii* , and *E. vulneris* was found by Mohanty et al.,(2005) and Kilani et al.,(2008) can't isolate any strains. Also, *E.coli* recovered from infant stool were 11 as reported in (Jackson, et al., 1998)

E.coli distinguished into invasive strains (6 from avian products) and non-invasive strains (9 from avian products) as reported by Berkhoff and Vinal (1986)

Bacteriological examination of *E.coli* isolates, revealed that there are extra-intestinal pathogenic *E. coli* as reported by Köhler and Dobrindt (2011) and Comery et al., (2013).

E.coli isolates are serologically divided into serogroups on basis of their antigenic composition (somatic O antigens) (Mead et al., 1999).

Six *E.coli* isolates were examined serologically and the serogroups were O175, O128, O125, O86a and the last one untyped which not agreed with results of Moawad et al., (2017) who detected O119, O78 and O18 from the examined samples and Younis et al., (2017) who detected O44, but O86a was detected by Hemeg et al., (2018),while Saad et al., (2011) detected O125.

The strain of *E.coli* that serovared as O125 agreed with Abd El Tawab et al.,(2016b) and Ozaki et al.,(2017). Also the serovared O157 agreed with Klien, et al.,(2002)

All results indicated that *E.coli* strains isolated were extra-intestinal pathogenic strains (Morales et al., 2004, Smith, et al., 2007 , Mellata 2013; Delannoy et al., 2017).

Most *E.coli* recovered from all samples (avian products, and infant stool) , It was resistant to almost antimicrobial agents used except amikacin, ciproflaxacin and azithromycin that agreed with Makhol et al.,(2010) and Zhang et al.,(2012).

Antibiotic biogram of the examined *E.coli* showed marked fluctuation to many antimicrobial agents used due to the misuse of these chemotherapeutics in the routine raising of chickens which affect the bird immunity and body gain.

It is a wrong concept of using antimicrobial agents for growth promotion in poultry. The overuse and misuse of these chemotherapeutics to chickens not only effect on them , but also effect on all human beings and animals. So we should limit its use only to sick bird to eliminate chemotherapeutics from human food chain.

Genotypic characterization of tested *E. coli* revealed absence of *stx1* in all isolates, but Fierz *et al.*, (2017) found it, presence of *stx2* gene in two sample as mentioned in Momtaz and Jamshidi (2013), Alonso *et al.*, (2017) and Friesema *et al.*, (2014)

A genetic analysis reported by (Rodriguez-Siek *et al.*, 2005) showed that APEC strains could be a reservoir of virulence genes of ExPEC, pathogenic to humans. This could be the reason for the genetic diversity and genes exchange among pathogenic *E. coli* strains.

5. CONCLUSIONS

The current study concluded that, *E. coli* considered the hidden enemy for poultry as they are commensals and become pathogenic under specific conditions which transmitted to avian products specially those which be unmanufactured as (Liver and Gizzard). Also they become resistant to almost antimicrobial agents that are the only way for treatment.

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