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Original Article

Prevalence and molecular characterization of enteropathogenic *Escherichia coli* isolated from table eggs in Mansoura, Egypt

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ABSTRACT

Objectives: This study was designed to assess the contamination of enterovirulent *Escherichia coli* with table eggs at Mansoura, Egypt.
Materials and methods: A total of 100 commercially available table eggs were randomly collected from various groceries and supermarkets at Mansoura, Egypt. The samples were screened for the presence of *E. coli* through conventional bacteriological and biochemical analyses followed by confirmation by polymerase chain reaction.
Results: Overall, 18% (n=18/100) samples were found to be contaminated with one or more *E. coli* isolates. All possible *E. coli* colonies (n=52) appeared on MacConkey agar plates during the screening process were picked for further analysis. Among the 52 suspected isolates, 24 were confirmed as *E. coli*, which were further serotyped using polyvalent *E. coli* antisera. In this study, 9 different *E. coli* serotypes namely O78, O114, O2, O44, O1, O125, O128, O124 and O26 were identified. Out of these 9 serological strains, 5 (O78, O2, O44, O125, O124 and O26) were positive for *eae* gene, and 3 (O44, O1 and O128) were positive for *stx2* gene. Two serological strains (O44 and O1) were positive for both *stx1* and *eae* genes, while O125 and O114 were positive for *stx2* and *eae* genes. Two strains (O78 and O128) were found to be positive for all three genes (*stx1*, *stx2* and *eae*).
Conclusion: Ensuring hygienic measures can effectively reduce the microbial load from table eggs.

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KEYWORDS

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INTRODUCTION

Eggs are one of the most important foods of animal origin which contain many nutrients that have a role in occupying the first places among foods, this attributed to high nutritive values that meet the human requirement as yolk that act as a stock of omega 3 fatty acids that have important roles in visual and brain function in humans (Maki et al., 2003). Also eggs are important source of vitamins, as vitamins A, D, E, K, and B1, B2, B9, B12. Egg yolk is also important source for minerals as phosphorus and iron. All these nutrients make them a suitable environment for growth of many pathogens.

The eggs can be acquired infection or contamination through shell (horizontally) or vertically (transovarially). The egg shell becomes contaminated during contact with environmental sources as dust and faces (Board and Tranter, 1995). Presence of coliform and enterobacteriaceae give better indication and analysis about the hygienic quality of eggs (Roberts et al., 1995) and suspected public health hazard from eaten raw eggs. There are 30 genera in the bacterial family enterobacteriac*eae* (Holt et al., 2000). Many enteric pathogens have been isolated from eggs especially *Escherichia coli* and *Salmonella* (Hope et al., 2002; Adesiyum et al., 2005). *E. coli* is harmful bacteria that consists of several strains as enteropathogenic (EPEC), enterohaemorrahgic (EHEC), enteroinvasive (EIEC), Shiga toxin-secreting (STEC), enterotoxigenic (ETEC), diarrhea-associated hemolytic (DHEC), enter aggregative (EAAggEC) and cytolethal distending toxin recreating (CDTEC) that have side effects on the health. Infections with Stx producing *E. coli* leads to hemorrhagic colitis in addition to hemolytic uremic syndrome (Butler, 2012) that leads to thrombocytopenia and renal injuries (Noris and Remuzzi, 2005). The initial symptoms of STEC infection are abdominal pain, cramps and non-bloody diarrhea, which observed after incubation period 3-5 days (Griffin, 1995).

Little work has been performed to determine the production of cytotoxin by *E.coli* isolated from table eggs sold from Egyptian markets. Therefore this study was adopted on qualitative analysis of pathogens of family enterobacteriaceae including pathogenic one as *E.coli* and detection some virulent genes of *E. coli* as *eae* and *stx* (*stx1*, *stx2*).

MATERIALS AND METHODS

Samples preparation: One hundred samples of commercial hen eggs (Brown shell, White shell and Baladi eggs) and duck eggs (each 6 eggs represented as one a composite sample) were collected randomly from Mansoura supermarkets and shops. To evaluate microbial contamination of table eggs sold from Egyptian markets and the possibility of presence of enterovirulent *Escherichia coli* strains.

The samples collection occurred under a septic condition and transferred to be examined microbiologically. A total of 180 mL sterile buffered peptone water were poured into the egg samples in plastic bags and good mixed. Egg shells were sterilized by swabbed with 70% ethyl alcohol, flamed and broken with a sterile forceps from the broad ends. Contents from 6 samples were pooled to form one sample and the egg contents were poured into sterile jar and homogenized for 30 sec. A tenfold serial dilution was prepared for egg shells and homogenized contents (Roberts et al., 1995).

Plating out and identification: Enterobacteriaceae counts were done according to Roberts et al. (1995). 1 mL aliquots each diluent were transmitted into sterile separate petridish. Molten violet red bile glucose agar medium (VRBG) was added and mixed well and allowed to set. Further 5mL molten, cooled VRBG was overlaid the solidified media. Plates were incubated at 37°C for 24 h and the typical colonies were observed. Following incubation, pink to red purple colonies with 0.5mm diameter with or without halo were counted as characteristic colonies for enterobacteriaceae. As many as 5 isolates or more from positive samples were selected for purification onto MacConkey for isolation of *E. coli*. Then the pure isolates were preserved on nutrient agar slopes at 4°C for further identification.

Identification and characterization of isolated strains: All isolates were refreshed onto nutrient ager and incubated at 37°C for 24 h. Suspected colonies of *E. coli* were exposed to biochemical testes as Oxidase, Catalase, Indole, Methyl red, Vogus proskouer. Citrate utilization, Hydroben sulphide test and urease test according to Kreig and Holt (1984).

Serological identification of *E. coli*: Positive biochemical isolates were subjected to serological identification**.** The isolates were serologically identified according to Kok et al. (1996) by using ploy valent *E. coli* antisera.

Detection of virulent genes of *E. coli* by PCR:

Extraction of DNA: Extraction of DNA of all samples was done by QIAamp DNA (Mini Kit Catalogue no.51304).

PCR protocol: The serological positive strains were examined for presence of virulent genes that included *eae*, *stx1* and *stx2* genes. The sequences of the three oligonucleotide primers and the suspected size are listed in **Table 5**. Pure colony was refreshed on macConkey agar plates and incubated at 37°C for 24 h. Subsequently, 3-4 colonies from each plate were selected randomly using a sterile toothpick and suspended in 100 µL distilled water and incubated at (15-25°C) for 1 min. Following centrifugation 8000 rpm/min, supernatants were syphoned and acted on them. PCR was performed for *eae* gene in 25 µL volume comprising 6 µL DNA template, 1 µL for each forward and reverse primer (20 pmol), 4.5 µL grade water and 12.5 µL Emerald Amp GT PCR mastermix (2x premix). The cycling conditions for *eae* gene were initial denaturation at 94°C for 5min, 30 cycles (94°C for 30 min, 51°C for 30 sec, 72°C for 30 sec) followed by a final extension at 72°C for 7 min . PCR was performed for *stx1* and *stx2* in 50 µL volume comprising 12 µL DNA template, 1 µL for each forward and reverse primer (20 pmol), 9 µL grade water and 25µL Emerald Amp GT PCR master mix (2x premix). The cycling conditions for *stx1* and

stx2 genes were initial denaturation at 94°C for 5 min, 30 cycles (94°C for 30 min, 58°C for 45sec, 72°C for 1 min) followed by a final extension at 72°C for 10 min. The comb was then removed and the electrophoresis tank was filled with TBE buffer. 20 µL of each uniplex PCR product and 35 µL of each multiplex PCR product in addition to negative control and positive control were loaded to the gel. The power supply was 1-5 volts/cm of the tank length. The run was stopped after about 30 min and the gel was transferred to UV cabinet. The gel was photographed by a gel documentation system and the data was analyzed through computer software.

Statistical analysis: The collected data were exposed to a Microsoft Excel spreadsheet and transferred to SPSS for analysis. Prevalences of *E.coli* were expressed as percentages.

RESULTS AND DISSCUSION

E. coli is Gram-negative, facultative anaerobe, rod-shaped bacterium and the normal habitat in the lower intestine of warm-blooded organisms (Singleton, 1999). Most *E. coli* strains are harmless, but some serotypes are pathogenic and causes serious diseases in human and the main source of contamination is fecal oral route. Most *E. coli* strains are harmless but some pathogenic strains can cause food poisoning as severe abdominal cramps, diarrhea in addition to urinary tract infections, and neonatal meningitis. In rarer cases, virulent strains have a major role in bowel necrosis (Todar, 2007).

Table 1: Prevalence of *E. coli* isolated from examined table eggs.

Type of eggs	No. of samples	Shells/ Contents	*E. coli* contaminated egg (%)
Baladi egg	25	Shell	3 (12)
		Content	1 (4)
Brown Shell egg	25	Shell	2 (8)
		Content	0 (0)
White Shell egg	25	Shell	4 (16)
		Content	1 (4)
Duck egg	25	Shell	5 (20)
		Content	2 (8)

Table 2: Frequency distribution of *E. coli* isolate from examined table eggs samples.

Type of eggs	Enterobacteriac *eae* isolates (No)	Shells/ Contents	*E. coli* isolates (%)
Baladi egg	81	Shell	4 (4.94)
	41	Content	1 (2.44)
Brown Shell egg	75	Shell	4 (5.33)
	68	Content	0 (0)
White Shell egg	38	Shell	4 (10.53)
	88	Content	1 (1.14)
Duck egg	77	Shell	8 (10.39)
	72	Content	2 (2.78)

As shown in **Table 1**, prevalence of *E. coli* in this study was 12, 20, 8, 16% and 4, 8, 0, and 4% of the shell and content of Baladi hen eggs, Duck eggs, Brown shell hen eggs and White shell hen eggs, respectively.

The results of this study not harmony with the prevalence of *E. coli* in Australia eggs as 60.78% of eggs were contaminated with *E. coli* (Gole et al., 2013). Sabrinath et al. (2009) found 13.3%, 45.8% of egg contents collected from large farms and small farms in Grenada were contaminated with *E. coli*, respectively. Cortés et al. (2004) showed that 45% of eggs were contaminated with *E. coli*.

Stępień-Pyśniak (2010) found that 4.3% of egg shells were contaminated with *E. coli* while 19% of egg contents in Shahrekord, Iran were contaminated with *E. coli* (Ghasemian Safaei et al., 2011). On the other hands, these results are inclined with the report of Al-khalaf et al. (2009) who reported that 10% of egg shells were contaminated with *E. coli* while 12% of unwashed Baladi egg shells were contaminated with *E. coli* (Bahobail et al., 2012).

In our study results, most data reveals to the most contamination of *E. coli* occurred in the shells as a several factors were implicated in egg contamination due to the principle way of contamination occurs in short time after laying as the egg shells become within contact environment as soil, dust and dirty nesting material (Smith et al., 2000; Ellen et al., 2000). The growth temperature of this organism is 37°C and mostly isolated from the environment or from the intestinal tract of vertebrate animal. Most of bacterial contamination of eggs contents result from bacterial contamination of egg shells that invade egg contents under improper conditions. Beside the horizontal way egg may contaminated vertically or through ovary and oviduct (transovarially) (Bruce and Drysadale, 1994).

E.coli used as indicators for food qualities and sanitary conditions .Their presence in the eggs indicates poor or improper hygiene (Kornacki and Johnson, 2001; Ricke et al., 2001; Nazir et al., 2005a).

In the present study, many isolates of enterobactericeae had been isolated from table eggs as *Enterobacter, Citrobacter, E. coli, Klebsilla, Protus, Serratia, Shigella and Providenciae* that concided with the findings of Jones and Musgrove (2008), Sabarinath et al. (2009), Bahobail et al. (2012), Maha and AL-Ashmawy (2013) and Rehman et al. (2015), but serological identification and molecular characterization was done for *E. coli* as it is the most harmful one.

E. coli measures quality and sanitary processing condition (Kornacki and Johnson, 2001; Ricke et al., 2001). Also, it is an ideal indicator organism of fecal contamination of human and animal feces (Nazir et al., 2005a, b). *E. coli* can cause egg spoilage (Berrang et al., 1999). Nowadays, the harmful of consumption of contaminated egg not only depend on the number of bacteria on the shell or the content but also depend on their types. *E. coli* especially STEC are considered one of these dangerous types.

As shown in **Table 3** serological typing of isolated E.coli strains revealed to 5 strains belong to serotype O78, O114:H21(3 strains), O2:H6 (1 strain), O44:H18 (4 strains), O1:H7 (2 strains), O125:H21 (4 strains), O128:H2 (2 strains), O124 (2 strains) and O26:H11 (1 strain). The prevalent strains

Table 3: Serotypes of isolated *E. coli* strains from examined eggs.

Pathot-ypes	Suspected *E. coli* isolates	Serotypes	No of strains	No. of *E. coli* contaminated samples							
				Baladi eggs		Red shell hen eggs		White shell hen eggs		Duck eggs	
				Shell	Content	Shell	Content	Shell	Content	Shell	Content
EPEC	52	O78	5	2	-	1	-	1	-	-	1
		O114:H21	3	-	-	-	-	1	-	2	-
		O2:H6	1	-	-	-	-	-	-	-	1
		O44:H18	4	-	-	2	-	-	-	2	-
		O1:H7	2	-	-	1	-	-	-	1	-
ETEC		O125:H21	4	1	-	-	-	1	1	1	-
		O128:H2	2	1	-	-	-	-	-	1	-
EIEC		O124	2	-	-	-	-	1	-	1	-
EHEC		O26:H11	1	-	1	-	-	-	-	-	-
Total			24	4	1	4	0	4	1	8	2

Table 4: Seropathotypes and virulent genes among the isolated strains of *E. coli*.

Pathotypes	Serotypes	No. of strains	Target virulence genes				
			eae	*stx2*	*stx1+eae*	*stx2+eae*	*stx1+stx2+eae*
EPEC	O78	5	4	-	-	-	1
	O114:H21	3	-	-	-	3	-
	O2:H6	1	1	-	-	-	-
	O44:H18	4	2	1	1	-	-
	O1:H7	2	-	1	1	-	-
Total		15	7	2	2	3	1
ETEC	O125:H21	4	3	-	-	1	-
	O128:H2	2	-	1	-	-	1
Total		6	3	1	-	1	1
EIEC	O124	2	2	-	-	-	-
Total		2	2	-	-	-	-
EHEC	O26:H11	1	1	-	-	-	-
Total		1	1	-	-	-	-
Grand Total		24	13	3	2	4	2

Table 5: Oligonucleotide primers sequences for amplification of different target genes of *E. coli*. *Source:* Metabion (Germany).

Target gene	Primers sequences	Amplified segment (bp)	Reference
stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614	Dipineto et al. (2006)
stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779	
*eae*A	ATG CTT AGT GCT GGT TTA GG GCC TTC ATC ATT TCG CTT TC	248	Bisi-Johnson et al. (2011)

were O78 and O125 that coincided with EL-Atrebe (1982) who reported that O78 and O125 as the most predominant serotypes in addition to other serological strains were also isolated (Shalaby and Abd El-Hamid, 1987). The serological difference of isolated *E.coli* may be attributed to locality and environmental condition. Maha and Al-Ashmawy (2013) isolated most similar strains of *E.coli* as O44, O114, O125 and O128 in her study that done in the Mansoura city.

STEC have a major role in disease production associated with gastrointestinal tract, including diarrhea, bloody diarrhea and hemorrhagic colitis (HC), these conditions may be complicated by neurological diseases and renal failure, including hemolytic-uremic syndrome (HUS) (Blanco et al., 2001). EPEC strains are defined as *eae*-haboring diarrheagenic *E. coli* that have the ability to form sever lesions on intestinal cells and that do not possess Shiga toxin genes (Kaper, 1996). *E. coli* O114.H21 strain were classified as enterotoxigenic *E. coli* that produce heat-labile or heat-stable enterotoxins (Beutin et al., 1990; Wolf, 1997) that cause diarrhea in infants and septicemia in calves (Orskov and Orskov, 1966). O78 responsible for colisepticaemia in poultry. Strains of *E. coli* O128 can cause diarrhea in infants (Dziva and Stevens, 2008).

Most serological strains were positive for *eae* gene as O78, O2, O44, O125, O124 and O26 while some strains as O44, O1 and

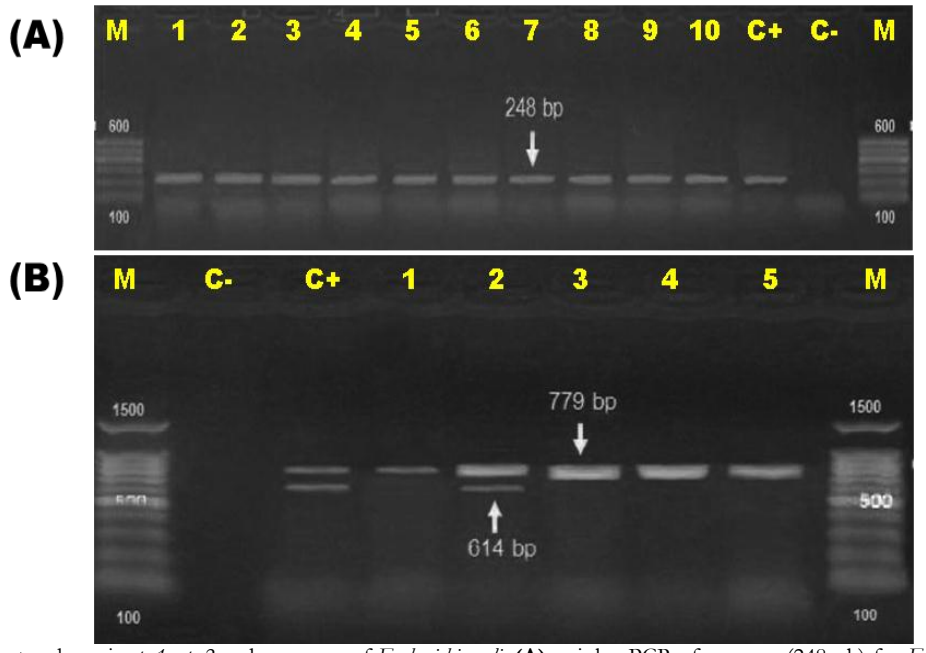

Figure 1. Agarose gel-electrophoresis *stx1*, *stx2* and *eae* genes of *Escherichia coli*. **(A)** uniplex PCR of *eae* gene (248-pb) for *E. coli*. Lane M: Marker (100-bp), Lane C-: negative control, Lane C+: positive control, Lanes 1 to 10: positive strains for *eae* gene. **(B)** duplex PCR of *stx1* (614-bp) and *stx2* (779-bp) genes of *E. coli*. Lane C-: negative control, Lane C+: positive control, Lanes 1 to 5: positive strains for *stx2* gene, Lane 2: positive strain having both *stx1* and *stx2* genes.

O128 were positive for *stx2* and some serological strains were positive for *stx1* and *eae* as O44 and O1 while of O125 and O114 were positive for *stx2* and *eae*. There were strains positive for three genes as O78 and O128, as illustrated in **Table 4**. The data from previous works indicated that only one isolate (8.33%) out of the 12 tested isolates was positive for *stx1*, but *stx2* was detected in 9 (75%) isolates (Zahraei Salehi et al., 2007). The positive strains for *eae, stx1* and *stx2* in the present study agree with the report of Galal et al. (2013) who detected *stx1, stx2* and *eae* genes in 2/19 (10.52%) of the samples of their study that also was done in Egypt. Samanta et al. (2014) detected both *stx1* and *stx2* genes and 11 isolates (n=11/78; 14.1%) possessed *eae* gene.

CONCLUSION

E. coli contamination mostly occurs on egg shells. The contamination may happen due to contact with soil, dust and dirty nesting material. *E coli* measures quality, sanitary processing condition and safety indicator of table eggs production so good hygienic measures should be applied at farm to table to reduce bacterial load on the shells and egg contents in addition to good cooking of foods containing eggs and good refrigeration of foods to avoid public health hazard.

COMPETING INETEST

The authors do not declare any competing interest.

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Nothing to declare.

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