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RESEARCH ARTICLE

Escherichia coli O157:H7 in Raw and Processed Meat with Virulence Genes Detection in Aswan Governorate

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Abstract

Meat and its products are a common source for the most virulent *Escherichia coli* O157:H7 for consumers. Hence, this study aimed to detect the presence of *E. coli* O157:H7 in raw and processed meat and to determine serotypes and some virulence genes of the recovered isolates. A total of 200 samples of raw and processed meat and meat products including minced meat, burger, sausage, kofta, cooked burger, cooked sausage, cooked kofta, cooked luncheon and cooked shawerma (20 of each) were obtained from different shops in Aswan Governorate during 2017. The samples were examined bacteriologically for *E. coli* O157:H7which were characterized serologically and genetically for the confirmation and detection of some virulence genes including *stx1*, *stx2*, *hly*A and *eae*A. Prevalence of *E. coli* O157:H7 versus other *E. coli* serotypes were 14.3% Vs 85.7%. The overall percentage of non-O157:H7 *E. coli* to *E. coli* O157:H7 in raw meat were 27% to 4% while in processed meat were 8% Vs 1%, respectively. *E. coli* O157:H7 and non-O157:H7 *E. coli* were more detected in raw meat than processed ones. Raw meat and meat products still threaten human health via harboring pathogenic and zoonotic *E. coli*, in turn; hygienic and good manufacturing practices should be enforced in meat factories and markets.

Keywords: *E. coli* O157:H7, Raw meat, Meat products, Virulence genes.

Introduction

Escherichia coli (*E. coli*) inhabits the intestine of animals, some may be pathogenic and causing diseases [1-2]. It is facultative anaerobes, harmless to the host but some emerging strains causes diarrhea known as Diarrhogenic *E. coli* [3]. In microbial analysis, the pathogen should be investigated at first as pathogenic *E. coli* before further classification which based mainly on the virulence factors (Enterotoxigenic, Enteropathogenic, Enterohemorrhagic, Enteroinvasive, Enteroaggregative and Diffusely-Adherent). The first four groups are responsible for diseases caused by ingestion of contaminated food [3-4]. *E. coli* O157:H7 is the most important microorganism of Enterohemorrhagic *E. coli* (EHEC) category which characterized by the formation of verotoxins (Shigatoxins) stx1 and stx2 which are responsible for occurrence of several human diseases [5-7]. EHEC group such as *E. coli* O157:H7causes several dangerous diseases for human being such as severe bloody diarrhea, hemorrhagic colitis (HC), thrombotic thrombocytopenic purpura (TTP) and fatal hemolyticuremic syndrome (HUS) which causing renal failure in about 10% of patients' especially young children and elderly [8-12]. Transmission of *E. coli* O157:H7 to humans caused mainly by consumption of raw or undercooked ground beef or hamburger which get contaminated during slaughter, handling and preparation of meat [13-14]. The most significant feature of *E. coli* O157:H7is its very small infective dose (10 CFU/g) [15]. Infection by *E. coli* O157:H7 can occur directly, need no time for propagation and that increase the public health significance of such pathogen [16]. The danger of *E. coli* O157:H7 comes from its ability for production of several virulence proteins such as shigatoxins, hemolysin and adhesion protein (intimin) [6]. Virulence genes such as *stx1*, *stx2*, *rfbE*, *eae*, *hlyA* and *fliCh7* are used for genetic

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identification and confirmation of the *E. coli* O157:H7 [17]. We aimed to study the presence of *E. coli* O157:H7 in raw and processed meat. Serotyping and detection of some virulence genes of the recovered isolates especially those responsible for cytotoxicity and infection were also investigated.

Materials and Methods

Samples

A total of 200 samples of raw and processed meat and meat products including minced meat, burger, sausage, kofta, cooked burger, cooked sausage, cooked kofta, cooked luncheon and cooked shawerma (20 of each) were obtained from different shops in Aswan during 2017. Samples were transferred in ice box to the Microbiology Laboratory, Faculty of Veterinary Medicine, Aswan University for bacteriological, biochemical, serological and genetic assays.

Isolation and Identification of pathogenic E. coli and E. coli O157:H7

Twenty five grams of each meat sample were aseptically transferred to sterile stomacher bag containing 225 ml modified Vancomycin-Trypticase Soy Broth (m-VTSB) (Oxoid, Code:CM0989) as it contained vancomycin (40 mg/L) to suppress Gram-positive bacteria. The bag content was homogenized using a Stomacher® 400 Circulator (Seward Ltd., UK) for 2 min and incubated aerobically in m-VTSB overnight at 37 ºC [18]. A loopful (10 µl) was taken from each m-VTSB enrichment culture after 12 h and streaked on Eosin Methylene Blue Agar (EMB) plates (Oxoid, Code: CM0069) and on Sorbitol McConkey Agar (SMA) (Oxoid, Code: CM0813) plates and incubated at 37°C for 24 h. Olive green colonies with metallic sheen on EMB and colorless colonies on SMA were positive for pathogenic *E. coli* and for *E. coli* O157:H7, respectively. Positive colonies were taken for biochemical and serological investigation. Positive strains were confirmed with Gram's staining, indole production, methyl red, voges-proskauer, simmon`s citrate, urease production, triple sugar irone agar and sugar fermentation especially sorbitol where *E. coli* O157:H7 unable to ferment sorbitol [19]. Positive *E. coli* isolates were investigated for somatic (O) and flagellar (H) antigens by latex agglutination test (Hampshire, UK) [20-21].

Molecular Characterization of E. coli O157:H7

A multiplex PCR assay was performed for detection of four virulence genes of *E. coli* O157:H7 including Shigatoxins (*stx1*, *stx2*), intimin (*eaeA*) and haemolysin (*hlyA*) [22-24]. DNA extraction was carried out by using QIAamp DNA purification kits (Qiagen, Germany) according to the manufacturer`s instructions. Oligonucleotide primers used in multiplex PCR for the detection of virulence genes were illustrated in Table (1). Amplification reaction consists of 12.5 µl master mix (Takara, Code: RR310A), 1 µl of each primer, 3 µl DNA template and nuclease free water till 25 µl volume. Thermacycler (Eppendorf, Germany) was used with initial denaturation step at 95°C for 6 minutes followed by 35 PCR cycles, each consisting of 1 min of denaturation at 95°C; 2 min of annealing at 65°C for the first 10 cycles, decrementing to 60°C by cycle 15; and 1.5 min of elongation at 72°C, incrementing to 2.5 min from cycles 25 to 35. Amplicons were electrophoresed on 2% agarose, stained by ethidium bromide, visualized on UV-transilluminator (Biometra) and analyzed using Biodoc Analyse Biomet [25].

Table (1): Primers used in multiplex PCR for *E. coli* O157:H7 virulence genes

Target gene	Primer	Oligonucleotide sequence (5`- 3`)	Product size (bp)	References
*stx*1	*stx*1 (F)	ACACTGGATGATCTCAGTGG	614	[22]
	*stx*1 (R)	CTGAATCCCCCTCCATTATG		
*stx*2	*stx*2 (F)	CCATGACAACGGACAGCAGTT	779	[22]
	*stx*2 (R)	CCTGTCAACTGAGCAGCACTTTG		
*eae*A	*eae*A (F)	GTGGCGAATACTGGCGAGACT	890	[23]
	*eae*A (R)	CCCCATTCTTTTTCACCGTCG		
*hly*A	*hly*A (F)	ACGATGTGGTTTATTCTGGA	165	[24]
	*hly*A (R)	CTTCACGTGACCATACATAT		

Results

The overall percentage of *E. coli* O157:H7 serotype was 14.3% Vs 85.7% for the other *E. coli* serotypes. For raw meat non-O157:H7 *E. coli* was 27% while O157:H7 was only in 4% of samples. The processed meat were less; 8% Vs 1%, respectively. Percentages of non-O157:H7*E. coli* were (4/20) 20%, (4/20) 20%, (5/20) 25%, (6/20) 30% and (8/20) 40% in raw meat, raw minced meat, raw burger, raw sausage and raw kofta, respectively (Table 2), while they were (1/20) 5%, (2/20) 10%, (2/20) 10%, (1/20) 5% and (2/20) 10% in cooked burger, cooked sausages, cooked kofta, cooked luncheon and cooked shawerma, correspondingly (Table 2). Percentages of *E. coli* O157:H7were (3/20) 15%, (1/20) 5%, (0/20) 0%, (0/20) 0% and (0/20) 0% in raw meat, raw minced meat, raw burger, raw sausage and raw kofta, respectively, even as they were (0/20) 0%, (0/20) 0%, (0/20) 0%, (0/20) 0% and (1/20) 5% in cooked burger, cooked sausages, cooked kofta, cooked luncheon and cooked shawerma, respectively. Latex agglutination test revealed that percentages of O157:H7, O26, O111:H4, O119:H6, O125:H21and O127:H6 were (5/35) 14.3%, (11/35) 31%, (7/35) 20%, (2/35) 5.7%, (5/35) 14.3% and (5/35) 14.3%, respectively (Table 3). Percentages of virulence genes; shigatoxin 1 – forming gene (*stx*1), shigatoxin 2 - forming gene (*stx*2), intimin A - forming gene (*eae* A) and haemolysin A - forming gene (*hly* A) in five *E. coli* O157:H7 isolates were (4/5) 80%, (3/5) 60%, (4/5) 80%, (4/5) 80%, respectively (Table 4) .

Table (2): Percentages of non-O157:H7*E. coli* and *E. coli* O157:H7 in raw and processed meat collected from Aswan market during 2017

Meat sample (No.)	non-O157:H7 *E. coli*		*E. coli* O157:H7	
	Positive No.	Percentage	Positive No.	Percentage
Meat (20)	4	20	3	15
Minced meat (20)	4	20	1	5
Burger (20)	5	25	0	0
Sausage (20)	6	30	0	0
Kofta (20)	8	40	0	0
C[1]-Burger(20)	1	5	0	0
C[1]-Sausage(20)	2	10	0	0
C[1]-kofta(20)	2	10	0	0
C[1]-Luncheon(20)	1	5	0	0
C[1]-Shawerma(20)	2	10	1	5
Total (200)	35	17.5	5	2.5

C[1]: Cooked (Ready-to-eat meat as sold in restaurants)

Table (3): Serotypes of *E. coli* isolated from examined meat samples of Aswan markets during 2017.

Sample	Serotype					
	O157:H7	**O26**	**O111:H4**	**O119:H6**	**O125:H21**	**O127:H6**
Meat	3	1	0	0	0	0
Minced meat	1	1	1	1	0	0
Burger	0	2	1	0	0	2
Sausage	0	2	2	0	1	1
Kofta	0	2	2	1	2	1
C[1]-Burger	0	0	0	0	1	0
C[1]-Sausage	0	1	1	0	0	0
C[1]-kofta	0	1	0	0	0	1
C[1]-Luncheon	0	1	0	0	0	0
C[1]-Shawerma	1	0	0	0	1	0
Total	5	11	7	2	5	5
Percentages	14.3	31.4	20	5.7	14.3	14.3
Total Percentages	14.3			85.7		

C[1]: Cooked (Ready-to-eat meat as sold in restaurants)

Table (4): Distribution of some virulence genes in *E. coli* O157:H7 isolates recovered from raw and processed meat of Aswan markets during 2017

Isolate No. (source)	Virulence genes			
	stx1	***stx2***	***eae* A**	***hly*A**
1 (Meat)	+	+	+	+
2 (Meat)	+	-	+	+
3 (Meat)	+	+	+	-
4 (Minced meat)	+	+	-	+
5 (Shawerma)	-	-	+	+

Discussion

In this study, it is observed that the occurrence of *E. coli* O157:H7is lower than non-O157:H7. Percentages of non-O157:H7*E. coli* were 20%, 20%, 25%, 30% and 40% in raw meat, raw minced meat, raw burger, raw sausage and raw kofta, respectively (Table 2), while they were 5%, 10%, 10%, 5% and 10% in cooked burger, cooked sausages, cooked kofta, cooked luncheon and cooked shawerma, correspondingly (Table 2). Percentages of *E. coli* O157:H7were 15% in raw meat, 5% in raw minced meat and 0% in raw burger, raw sausage and raw kofta, even as they were 0% in cooked burger, cooked sausages, cooked kofta, cooked luncheon and5% in cooked shawerma. Both non-O157:H7*E. coli* and *E. coli* O157:H7 in raw meat were higher than in processed ones, this may be due to processing temperature which may kill most of *E. coli*. Contamination of raw kofta (40%) and raw sausages (30%) by non-O157:H7*E. coli* was higher than other raw meat samples (20%), also, contamination of processed kofta, sausages and shawerma was 10%, for each was higher than other processed meat samples (5%). *E. coli* O157:H7 were found only in raw meat and raw minced meat and not found in raw burger, sausage and kofta while occur only in processed shawerma and not found in processed burger, sausages, kofta and luncheon. These findings may be due to unhygienic manufacturing practices especially fresh kofta and fresh sausages which made in groceries and small meat shops which uses dirty equipments and utensils such as meat mincers, panes, and plastic bags of lower sanitary conditions. Similarly Sallam *et al*. [26] found *E. coli* with percentage of 26.7% in

raw ground beef and lower percentages (13.3%, 14.7% and 8.8%) were reported in beef burgers, beef samples and raw sausages, respectively [27-29]. *E. coli* O157:H7 was detected with percentage of 4% [28], 3.7% and 4%in raw beef meat [30-31], 6.7%in raw meat [32]. Meanwhile, a higher (50%), and a much lower percentage was found (1.1%) were declared in ground beef by Khalifa and Hassan [33] and Chapman *et al*. [17], respectively. Nevertheless, Chinen *et al*. [34] and Willshaw *et al*. [35] do not found *E. coli* O157:H7 in burger or in raw meat and meat products which may be attributed to heat treatments and hygienic conditions during production. Latex agglutination test revealed that percentage of O157:H7 was equal to serotypes O125:H21 and O127:H6 while O26 and O111:H4 were 31.4 % and 20%, respectively and the lowest one is O119:H6 of 5.7%. Out of thirty five *E. coli* isolates, five *E. coli*O157:H7, three in raw meat, one in minced meat and one in cooked shawerma, were identified. El-Gamal *et al*. [36] found 11% of serotypes were O157:H7, 2.6% O26 and O128, 1.3% O111 and O119 and 0% O78. Results showed that the five *E. coli* O157:H7 isolates were positive for the occurrence of virulence genes; the shigatoxin-forming genes (*stx1 and stx2*) (80% and 60%), intimin-forming gene (*eae*A) (80%) and haemolysine-forming gene (*hly*A) (80%), these proteins are responsible for occurrence of pathological effects of the organism. Nearly most *E. coli* O157:H7 isolates were positive for *stx1* (4/5) (80%), *eae*A (4/5) (80%) and *hly*A (4/5) (80%), strain 1 was positive for the four genes while strain 3 was positive for three genes and strain 2, 4, 5 were positive for at least 2 different genes. Similar results reported by El-Gamal *et al.* [36] , Sallam *et al.* [26] who detected *stx*1, *stx*2 and *eae*A from isolates of *E. coli* O157 that recovered from ground beef. *E. coli* O157:H7 able to cause disease by its adhering to the cell membrane (possibly invading host cells) and then producing *stx1* and/or *stx2*. These emphasized that adherence factors, *stx*1 and *stx*2 were critical factors in the pathogenesis of *E. coli* O157:H7 infection [37]. PCR amplification of *eae*A, *stx* and plasmid genes was used frequently for detection of *E. coli* O157:H7 [38].

The occurrence of *E. coli* O157:H7 in beef meat could be attributed to the contamination from feces of infected animals during skinning and evisceration processes at slaughterhouse and this contamination remains on the carcass during subsequent processing [39-40]. Higher incidence of *E. coli* O157:H7 may be attributed to the contamination of meat during slaughtering, evisceration and transportation. Minced meat was more susceptible for contamination during grinding process, partly due to the large surface area exposed to infection and partly due to mixing of different portions of beef from different animals and possible cross contamination [41]. *E. coli* O157:H7 is one of the most important and virulent food borne pathogen worldwide which causes hemorrhagic colitis, and hemolytic uremic syndrome [42]. Hemolytic uremic syndrome was common in children and characterized by three features; acute renal failure, hemolytic anemia and thrombocytopenia [19]. Strict measures should be taken to confirm freedom and safety of meat from the contamination with *E. coli* O157:H7 and other pathogenic *E. coli* to prevent its arrival to the consumers.

Conclusion

Raw meat and meat products especially minced and ground beef are frequently contaminated by *E. coli* O157:H7 and other *E coli* serotypes, so that can be a source for transmission of very dangerous diseases to the consumers. Hence, perfect sanitary measures and adoption of food safety systems in meat factories such as Hazard Analysis and Critical Control Points (HACCP) and Good Manufacturing Practices (GMPs) and good monitoring of meat products must be taken to avoid hazardousfood borne pathogens.

Conflict of interest

The author declares that there are no competing interests.

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الملخص العربى

الكشف عن الأيشيرشيا كولاى O157:H7 فى اللحوم النيئة و المجهزة مع تحديد بعض جينات الضراوة بها فى محافظة أسوان

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تعد اللحوم ومنتجاتها مصدرًا شائعًا لأكثر أنواع الأيشيرشيا كولاىO157:H7 شديدة الضراوة للمستهلكين. وبالتالي ، تهدف هذه الدراسة إلى الكشف عن وجود الأيشيرشيا كولاىO157:H7 في كل من اللحوم النيئة و المطهية ومنتجاتها وتحديد النوع السيرولوجيا و بعض جينات الضراوة للعزلات.أجريت هذه الدراسة على عدد ٢٠٠ عينة من اللحوم النيئة و المطهية ومنتجاتها تشمل كل من اللحوم الحمراء و اللحوم المفرومة و البرجر و السجق و الكفتة و البرجر المطهى و السجق المطهى و الكفتة المطهية و اللانشون المطهى و الشاورمة المطهية (بواقع ٢٠ عينة من كل منهما). تم تجمعيها من محال بيع اللحوم فى محافظة أسوان خلال عام ٢٠١٧. تم فحص هذه العينات بكتريولوجيا للكشف عن الأيشيرشيا كولاىO157:H7 كما تم فحصها سيرولوجيا و جينيا للتأكد من نوعها و للكشف على بعض جينات الضراوة هم *stx1* و *stx2* و *eae*A و *hly*A. أوضحت النتائج أن نسبة الأصابة للأيشيرشيا كولاىO157:H7 الى الأيشيرشيا كولاى الممرضة الأخرى هى ١٤.٣% الى ٨٥.٧% على التوالى و كانت نسبة الأيشيرشيا كولاى الممرضة الى الأيشيرشيا كولاىO157:H7 فى اللحوم النيئة هى ٢٧% الى ٤% على التوالى بينما كانت هذه النسبة فى اللحوم المطهية هى ٨% الى ١% على التوالى. لوحظ أن النسبة المئوية للأيشيرشيا كولاىO157:H7 والأيشيرشيا كولاى الأخرى كانت أعلى فى اللحوم النيئة عنها فى اللحوم المطهية. تهدد اللحوم النيئة ومنتجاتها صحة الأنسان من خلال أحتوائها على الأيشيرشيا كولاى الممرضة لذلك تنصح الدراسة بضرورة ملاحظة الأشتراطات الصحية وممارسات التصنيع الجيد داخل مصانع اللحوم ومحال بيع اللحوم.