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Occurrence, Antimicrobial Susceptibility and Phylogroups of *Escherichia coli* O157:H7 Isolated from Food Outlets in Some Touristic Cities in Egypt

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ABSTRACT

Foodborne illnesses are frequently caused by *Escherichia coli* (*E. coli*). *E. coli* O157 is regarded as a potentially harmful cause of gastrointestinal disorders associated with consumption of foods with animal origin. Therefore, this study was conducted to determine the presence of *E. coli* O157:H7 in food outlets in some touristic cities in Egypt. For this purpose, 648 samples including raw chicken meat, cooked chicken meat, raw beef meat, cooked beef meat, food handlers and equipment swabs were collected from 54 food outlets in some touristic cities in Egypt. *E. coli* O157 was 1.1% (7/648) and 1.2% (5/432) in all examined samples and food samples respectively. Cooked chicken samples were the most contaminated with *E. coli* O157:H7 with an overall prevalence of 1.9% (2/108). The highest prevalence of *E. coli* O157:H7 (8.3%) isolates was recovered from raw chicken and cooked beef meat in Hurghada Governorate followed by Luxor Governorate (6.3%). There is no *E. coli* O157:H7 isolates were identified in Sharm El Sheikh and Aswan governorates. All *E. coli* O157:H7 isolates (100%) showed resistance to ampicillin (AMP), cefixime, ciprofloxacin and cotrimoxazole. Multidrug resistance (MDR) was observed among all *E. coli* O157:H7 isolates. All *E. coli* O157:H7 isolates harbor the *eae* gene with complete absence of *stx1* gene. The most prevalent phylogroup among the *E. coli* O157:H7 strains was B2 identified in raw and cooked beef and cooked chicken, collected from Luxor, Hurghada, and Alexandria governorates, respectively. Whereas, D phylogenetic group *E. coli* O157:H7 was only found in raw chicken sample collected from Hurghada Governorate. In conclusion, the detection of pathogenic MDR *E. coli* O157:H7 in food samples, food handlers and food equipment in some touristic cities in Egypt poses a serious risk to public health. Therefore, it is recommended to focus on identifying practices which increase the risk of food contamination, and on implementing measures to improve the sanitary conditions in the food outlets in touristic cities.

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TR collected, prepared the samples and applied bacteriological analysis and PCR assay. EAE and HAM helped in laboratory work, reviewing and editing. MAS supervised the study, data organization and wrote the manuscript.

Key words
***E. coli* O157, Antibiotic resistance, Phylogroup, Food outlets, Touristic cities, Egypt**

INTRODUCTION

Escherichia coli O157:H7 is one of the most serious foodborne pathogen strains which causes severe infections and significant fatality in humans (Blanco *et al*., 2003; Jo *et al*., 2004). More than 75,000 cases of foodborne illness attributed to *E. coli* O157:H7 occur annually (Perna *et al*., 2001).

Escherichia coli O157:H7 is an entero-hemorrhagic *E. coli* (EHEC) strain that is considered as a subset of Shiga toxigenic *E. coli* (STEC) which may cause severe clinical symptoms, such as hemolytic uremic syndrome (HUS) and hemorrhagic colitis (HC) (Karch *et al*., 2005). However, *E. coli* O157 are not always EHEC but may belong to other pathotypes such as enteropathogenic *E. coli* (EPEC) (Blank *et al*., 2003). Entero-hemorrhagic *E. coli* strains share with EPEC, a leading cause of infant diarrhea in developing countries, the ability to induce the attaching and effacing effect on host cells. This property is specified by a pathogenicity island that includes the *eae* gene encoding the outer membrane adhesin intimin. At the molecular level, EPEC are characterized by the presence of the *eae* gene and the absence of the genes for Shiga toxins (*stx1* and *stx2*) (Kaper, 1996)

Epidemiologically, cattle are considered the primary reservoir of *E. coli* 0157:H7 (Pal and Mahendra, 2016), so

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that, zoonotic transmission of *E. coli* 0157:H7 occurs after consumption of raw or under-cooked meat, inadequately pasteurized dairy products, or contact with contaminated fomites containing the Shiga toxin EHEC (Ameer *et al*., 2018). The contamination of beef may occur during slaughter, and the process of grinding beef may transfer pathogens from the surface of the meat to the interior. Additionally, the organism also could spread from one food item to another by hands, cooking utensils, cutting boards and unclean food preparation surfaces (Pal and Mahendra, 2016). Consequently, failure to implement appropriate food safety management system and applying sanitary conditions during the production process, handling and marketing of food products facilitates the transfer of *E. coli O157* to the different food products (Reilly, 1998).

It is known that early antimicrobial treatment can avoid Shiga toxin producing *E. coli* O157:H7 infection progression to the HUS (Schroeder *et al*., 2002; Amézquita-López *et al*., 2016; Mühlen and Dersch, 2020). However, studies have shown a significant increase in antimicrobial resistance in *E. coli* O157:H7 (Mühlen and Dersch, 2020). This in part may be related to the overuse and misuse of antibiotics by the people and food producing animals (Radostits *et al*., 2000). Moreover, the development of antibiotic resistance in *E.coli* O157: H7 is considered main challenge as it can spread the resistance determinants within the other commensals and pathogens (Ahmad *et al*., 2021).

Clermont *et al*. (2000) earlier created a categorization method based on phylogenetic characterization of *E. coli* strains for monitoring the microbiological source, determining phylogenetic groups, and determining possible pathogenicity among *E. coli* strains. They revealed that *E. coli* isolates are divided into four main groups: A, B1, B2, and D, with seven subgroups: A0, A1, A2, B22, B23, D1 and D2. Clermont *et al*. (2013) then proposed a new phylogenetic grouping technique that comprised four new phylogroups: C, E, F, and Escherichia cryptic clade I.

There is a strong link between the virulence and phylogeny in *E. coli* infections (Pakbin *et al*., 2021), as phylogroup B2 *E. coli* strains, and to a lesser extent phylogroup D, are the most common causes of extra-intestinal infections in humans (Bailey *et al*., 2010). Also, the strains belonging to the phylogroup A are typically commensal (Picard *et al*., 1999).

In Egypt, for many years, tourism has been the main source of economy, but it is now threatened due to food borne illness (Abdelhakim *et al*., 2020). Therefore, Egypt's main challenge is to ensure that it has the capacity to provide safe food for its own people. Several studies have been carried out in Egypt to investigate the incidence of *E. coli* O157:H7 within different food products, but, there are no sufficient reports about the food contamination with *E. coli* O157:H7 in touristic cities in Egypt. Therefore, within some of touristic cities in Egypt, the present study was conducted to investigate the occurrence, antimicrobial susceptibility, virulence genes and the phylogroup of *E. coli* O157:H7 isolated from food outlets.

MATERIALS AND METHODS

Sampling

A grand total of 648 samples were collected from 54 food outlets in Egypt, including raw chicken meat, cooked chicken meat, raw beef meat, cooked beef meat, food handlers' hand swabs and equipment swabs (108 of each). All food samples were received in sterile bags, whereas hand and equipment swabs were placed in 5 ml liquid maximum recovery diluent (MRD) in a sterile screw-capped container (TS/5-31-UK). All samples were carried in ice box to be transferred with a minimum delay to the laboratory for bacteriological examination.

Isolation and identification of E. coli *O157:H7*

The isolation of *E. coli* were done according to procedure using enrichment methods, and then confirmed by PCR. Briefly**,** 25 g of the meat samples (beef and chicken) were transferred to a septic blender jar and 225 ml of 0.1% sterile peptone water was added aseptically (ISO, 2017). After that, each sample was homogenized in the stomacher for 1-2 min at 2000 rpm to produce a homogenate. Phenotype characterization of O157 strains was done using Sorbitol MacConkey Agar (Oxoid, England), 0.1 ml of the prepared samples, as well as hand and equipment swabs were incubated for 24 h at 35-37°C. The suspected colonies were sub-cultured and identified as *E. coli* through Gram's stain films and biochemical tests. Then, *E. coli* isolates were serotyped in the Serology Unit Animal Health Research Institute, Dokki, Giza Egypt, using commercial antisera anti-*E. coli* O157 (SIFIN) according to the manufacturer's instructions.

Antimicrobial susceptibility testing of E. coli *O157:H7*

Antimicrobial susceptibility of *E. coli O157* isolates was determined by the disc diffusion method, according to the guidelines for the Clinical and Laboratory Standards Institute (CLSI, 2012) on trypticase soy agar (TSA) using commercially available discs. Zones of growth inhibition surrounding each antibiotic disc are measured to the closest millimeter after plates are incubated at 37°C for 16–24 h. The isolate's susceptibility and the speed at which the drug diffuses through the agar medium are both correlated with the zone's diameter. The zone diameters of each drug are interpreted using the criteria published by CLSI (2012).

The panel of antibiotics included were ampicillin (AMP) 25µg, ampicillin-sulbactam (SAM) 20µg, piperacillin (PRL) 30µg, pipracillin-tazopactam (TZP) 110 µg, amoxycillin/clavulanic acid (AMC) 30 µg, Aztreonam (ATM) 30 µg, meropenem (MEM) 10 ug, cefixime (CFM) 5µg, ciprofloxacin (CPR) 5 µg, cotrimoxazole (SXT, trimethoprim/sulfamethoxazole) 25µg, gentamicin (GN) 10 µg and amikacin (AK) 30 µg. According to Magiorakos *et al*. (2012) multidrug resistance (MDR) was defined as acquired non susceptibility to at least one agent in three or more antimicrobial categories.

Molecular detection of E. coli *O157:H7*

The EHEC O157 virulence genes *stx1, stx2* and *eae* were assessed by PCR. Descriptions of the targeted genes and primer sequences are listed in Table I. QIAamp DNA mini Kit (catalogue no.51304) was used for extraction of DNA from the recovered strains of *E. coli* O157:H7, the PCR master mix was prepared using Emerald Amp GTPCR master mix (Takara, code No. RR310A).

Phylogenetic group of E. coli *strains determination*

According to Clermont *et al*. (2000) EPEC strains were divided into four main phylogenetic groups (A, B1, B2, and D) based on PCR detection of the *chuA* and *yjaA* genes and DNA fragment TSPE4.C2. Briefly, the primer pairs for *chuA*, *yjaA* and TspE4C2.1 (Table I), were added to the standard PCR mixture, PCR was performed under the following conditions: denaturation for 4 min at 94°C, 30 cycles of 5 s at 94°C and 10 s at 59°C, and a final extension step of 5 min at 72°C. Depending on whether a strain reacted positively or negatively with *yjaA* primers, group B2 or D was assigned to the strains that interacted with the *chuA* primers. Similar to this, the *chuA*-negative isolates were divided into groups B1 or A depending on whether the PCR for TspE4.C2 produced a positive or negative response, respectively.

RESULTS

Occurrence of E. coli *O157:H7*

A total of 7 (1.1%) *E. coli* O157:H7 strains were isolated and confirmed from 648 samples collected from food outlets located in some touristic governorates in Egypt (Table II). The occurrence of *E. coli* O157:H7 in food samples was 1.2% (5 out of 432) including cooked and raw chicken and beef meat, while the other 2 *E. coli* O157:H7 isolates were recovered from both of cutting knife and food handler hand swab from food outlet in Cairo Governorate. Among these samples examined, cooked chicken samples were the most contaminated with *E. coli* O157:H7 with an overall prevalence of 1.9% (2/108). With regard to the source of the samples, the highest prevalence of *E. coli* O157:H7 (8.3%) isolates was recovered from raw chicken and cooked beef meat in Hurghada Governorate followed by Luxor Governorate (6.3%); however, the occurrence of *E. coli* O157:H7 was 3.3 and 5.0 in Cairo and Alexandria Governorates, respectively. There is no *E. coli* O157:H7 isolate identified in Sharm El Sheikh and Aswan Governorates (Table II).

Table I. Oligonucleotide primers sequences for detection of *E. coli* O157:H7 virulence genes and phylogenetic determination.

Gene	Primer sequence 5'-3'	Amplified product	Reference
Stx1	ACACTGGATGATCTCAGTGG	614 bp	(Dipineto *et al*., 2006)
	CTGAATCCCCCTCCATTATG		
Stx2	CCATGACAACGGACAGCAGTT	779 bp	
	CCTGTCAACTGAGCAGCACTTTG		
eaeA	ATGCTTAGTGCTGGTTTAGG	248 bp	(Bisi-Johnson *et al*., 2011)
	GCCTTCATCATTTCGCTTTC		
chuA	GAC GAA CCA ACG GTC AGG AT	279 bp	(Jeong *et al*., 2012)
	TGC CGC CAG TAC CAA AGA CA		
yjaA	TGA AGT GTC AGG AGA YGC TG	211 bp	
	ATG RAG AAT GCG TTC CTC AAC		
TspE4.C2	GAG TAA TGT CGG GGC ATT CA	152 bp	
	CGC GYC AAC AAA GTA TTR CG		
	GCCTTCATCATTTCGCTTTC		

Table II. Occurrence of *E. coli* O157:H7 in food samples obtained from some touristic governorates in Egypt (n=108).

Governorates	Examined sample numbers	Food samples				Food equipment (cutting board and cutting knife) No. (%)	Food handler (positive hand swabs) No. (%)	Total
		Chicken		Beef				
		Raw No. (%)	Cooked No. (%)	Raw No. (%)	Cooked No. (%)			
Cairo	30	0	1(3.3%)	0	0	1 (3.3%)*	1 (3.3%)	3 (1.7%)
Alexandria	20	0	1(5.0%)	0	0	0	0	1 (0.8%)
Sharm El-Sheikh	16	0	0	0	0	0	0	0 (0.0%)
Hurghada	12	1(8.3%)	0	0	1(8.3%)	0	0	2 (2.8%)
Luxor	16	0	0	1(6.3%)	0	0	0	1 (1.0%)
Aswan	14	0	0	0	0	0	0	0 (0.0%)
Total	108	1(0.9%)	2(1.9%)	1(0.9%)	1(0.9%)	1(0.9%)	1(0.9%)	7 (1.1%)

Table III. Antibiotic resistance pattern of *E. coli* O157:H7 isolates (n=7):

Antibiotic class/Antimicrobial agent	Sensitive	Interme-diate	Resist-ant
	No. (%)	No. (%)	No. (%)
β-Lactams (β Ls)	0	0	7(100)
Ampicillin (AMP)			
Ampicillin-sulbactam (SAM)	0	7(100)	0
Piperacillin	0	3(42.9)	4(57.1)
Pipracillin-Tazopactam	0	1(14.3)	6(85.7)
Amoxycillin /Clavulanic acid	4(57.1)	3(42.9)	0
Aztreonam	4(57.1)	2(28.6)	1(14.3)
Meropenem	5(71.4)	0	2(28.5)
Cefixime	0	0	7(100)
Fluoroquinolones (QNs)	0	0	7(100)
Ciprofloxacin			
Folate pathway antagonists (FPAs)	0	0	7(100)
Cotrimoxazole			
Aminoglycosides(AGs)	2(28.6)	1(14.3)	4(57.1)
Gentamicin			
Amikacin	6(85.7)	1(14.3)	0

Antimicrobial susceptibility of E. coli *O157:H7 isolates (n=7)*

The antimicrobial susceptibility investigation of 7 *E. coli* O157:H7 isolates against four different antibiotic classes and 12 commercially available antimicrobial discs revealed that all *E. coli* O157:H7 isolates (100%) showed resistance to ampicillin (AMP), cefixime, ciprofloxacin and cotrimoxazole. An overall resistance of 85.7% and 57.1% was recorded to pipracillin-Tazopactam, piperacillin and gentamicin. However, the lowest resistance was observed against aztreonam (14.3%). Furthermore, the isolates showed high susceptibility to Amikacin (85.7%) and Meropenem (71.4%) (Table III). Multidrug resistance (MDR) was observed among all *E. coli* O157:H7 isolates as 3 isolates showed resistance to three antimicrobial classes and 4 isolates evidenced resistance to four antimicrobial classes (Table IV). It can be also shown in Table IV that there were diverse patterns of antibiotic resistance among the isolates from each source.

E. coli *O157:H7 virulence genes*

Molecular identification of virulence genes revealed that all *E. coli* O157:H7 isolates harbor the *eae* gene with complete absence of *stx1* gene. Whereas, *stx2* was harbored by only one isolate obtained from cooked beef in combination with *eae* gene, in Hurghada Governorate (Table V).

Phylogroup of E. coli *O157:H7 isolates*

The *chuA* gene was found in four strains from groups belonging to B2 and D, but not found in three strains belonging to group A, as a result of this, we were able to distinguish groups B2 and D from groups. Similarly, the *yjaA* gene allowed for complete discrimination between group B2 (42.9 % of the strains were positive) and group D (14.2 % of the strains were negative). Finally, clone TSPE4.C2 was found in four strains, three of which are B2 strains and one of which is a group D strain, whereas it was absent from all group A strains (Table V). In Cairo, phylogenetic group A was predominant among the three *E. coli* O157:H7 isolates recovered from different sources including; cooked chicken, cutting knife and hand swab. However, phylogenetic group B2 was the most prevalent phylogroup among the *E. coli* O157:H7 strains isolated from meat samples, including raw and cooked beef and cooked chicken, collected from Luxor, Hurghada, and Alexandria governorates, respectively. Whereas, D phylogenetic group *E. coli* O157:H7 was only found in raw chicken sample collected from Hurghada Governorate (Table V).

Table IV. Multidrug resistance (MDR) class patterns of *E. coli* O157:H7 isolates (n = 7).

No.	Type of sample	Governorates	Multidrug resistance pattern	No. of classes of antibiotics
1	Raw beef	Luxor	Amp, Cefixime, Gen, Ciprofloxacin, Cotrimoxazole	4 (β Ls, QNs, AGs, FPAs)
2	Raw chicken	Hurghada	Amp, Cefixime, Meropenem, Gen, Ciprofloxacin, Cotrimoxazole	
3	Cooked chicken	Alexandria	Amp, Cefixime, Gen, Ciprofloxacin, Cotrimoxazole	
4	Cooked chicken	Cairo	Amp, Cefixime, Meropenem, Gen, Ciprofloxacin, Cotrimoxazole	
			No. of isolates (%)	**4 (57.1%)**
5	Cooked beef	Hurghada	Amp, Cefixime, Ciprofloxacin, Cotrimoxazole	3 (β Ls, QNs, FPAs)
6	Hand swab (food handler)	Cairo	Amp, Cefixime, Ciprofloxacin, Cotrimoxazole	
7	Cutting knife	Cairo	Amp, Cefixime, Ciprofloxacin, Cotrimoxazole	
			No. of isolates (%)	**3 (42.9%)**

Table V. Characterization and phylogenetic determination of the recovered *E. coli* O157:H7 from different sources and locations.

No	Type of sample	Governo-rates	Virulence genes expressed			Phylogroup	Phyloroup genes		
			Stx1	*Stx2*	*eaeA*		*chuA*	*yjaA*	*tspE4c2*
1	Raw beef	Luxor	_	_	+	B2	+	+	+
2	Cooked beef	Hurghada	_	+	+	B2	+	+	+
3	Raw chicken	Hurghada	_	_	+	D	+	-	+
4	Cooked chicken	Alexandria	_	_	+	B2	+	+	+
5	Cooked chicken	Cairo	_	_	+	A	-	+	-
6	Hand swab (food handler)	Cairo	_	_	+	A	-	+	-
7	Cutting knife	Cairo	_	_	+	A	-	+	-

DISCUSSION

Several researches have suggested that animal-derived foods could be a significant source of human-acquired MDR pathogenic *E. coli* (Rashid *et al*., 2013). Although various studies had been carried out in Egypt to investigate the incidence of *E. coli* O157:H7 within different food products (El-Alfy *et al*., 2013; Ahmed and Shimamoto, 2014; Khalil *et al*., 2015), information about the food contamination with *E. coli* O157:H7 in Egyptian touristic cities is scarce. Therefore, the aims of this study were to determine incidence rate, genotypes, phylogroups and antimicrobial susceptibility patterns in *E. coli* O157:H7 strains isolated from food products, as beef, chicken meat, and other sources, including food handlers and food equipment collected from some Egyptian touristic cities.

In the present study, 648 random samples of meat, food equipment and food handlers obtained from 54 food outlets in some touristic cities in Egypt were investigated for the presence of *E. coli* O157:H7. The total prevalence of *E. coli* O157:H7 was 1.1% (7 out of 648 samples), whereas, this prevalence in meat samples was 1.2% (5 out of 432), including cooked and raw chicken and beef samples. This result was in line with previous studies, which reported that the incidence of *E. coli* O157:H7 in UK was 1.1% of 2075 samples (Chapman *et al*., 2000) and 1.1% of 571 meat samples in the Netherlands (Heuvelink *et al*., 1999). In contrast, our finding as higher than that reported in minced beef samples in Antakya region (1.3%), in southern Turkey (Durmaz *et al*., 2007) as well as in Egypt (0.5%) (Hamed *et al*., 2017).

Regarding the geographical area, a higher incidence of *E. coli* O157:H7 was recorded in meat samples collected from food outlets in Hurghada (2/24, 8.3 %) followed by Luxor (1/32, 3.1%). Hurghada considered as one of the most popular resorts on the red sea coast that attracts tourist from all over the world. Therefore, unfortunately, such incidences of food-borne pathogens might negatively influence the tourism and hospitality industry in Egypt (Abdelhakim *et al*., 2020).

From the obtained results, it can be also noticed that in Cairo Governorate three isolates of *E. coli* O157 were

recovered from cooked chicken, cutting knife (3.3%) and also from food handler's hand swabs (3.3%). However, none of the surface swabs from the cutting boards were positive. In a similar kind of study conducted in Ethiopia, *E. coli* O157:H7 was isolated from 3.6% (4/110) of the surface swabs of wooden cutting boards with complete absence in cutting knives and hand swabs (Beyi *et al*., 2017). However, in Pakistan, *E. coli* O157:H7 was not detected in surface swabs of cutting knives and wooden boards taken from 30 individual retail meat outlet markets (Ali *et al*., 2010). In the current study, in Cairo Governorate, *E. coli* O157 positive cooked chicken sample, hand swab and cutting knife swab were collected in the same visit from food outlet, indicating the possible contamination of the chicken meat from cutting knife and/or food handler or vice versa. Additionally, the presence of *E. coli* O157:H7 in asymptomatic food handler's hand swab may pose a significant public health risk as it increases the possibility of the transmission of this pathogen to tourists when the food handlers un-hygienically handle foods (Oundo *et al*., 2008). Therefore, food handlers must be trained effectively on food safety and hygiene.

All *E. coli* O157 isolates show resistance to ampicillin (AMP), cefixime, ciprofloxacin and cotrimoxazole irrespective to their origin. High resistance was also found against pipracillin-tazopactam (85.7%), piperacillin and gentamicin (57.1% for each). Furthermore, the isolates showed high susceptibility to amikacin (85.7%) and meropenem (71.4%). Similar findings were reported by (Bhowmik *et al*., 2022), who showed that 100% (n=20) of their *E. coli* isolates exhibited resistance to ampicillin, and 41% against gentamicin. In contrast, they observed that their *E. coli* isolates highly sensitive to cotrimoxazole (83%) and ciprofloxacin (58%) and highly resistant to amikacin (66%). Additionally, previous studies in Egypt (Sobhy *et al*., 2020; Elmonir *et al*., 2021), Ethiopia (Haile *et al*., 2022) and Nigeria (Ojo *et al*., 2010) revealed high resistance among *E. coli* isolates to ampicillin, a finding similar to ours. Inadequate antimicrobial selection and abuse can lead to resistance in different bacteria and make it more difficult to treat bacterial infections (Kolář *et al*., 2001). Antimicrobial-resistant bacteria are one of the most serious public health issues, and are predicted to cause the death of 10 million people annually by 2050 (De Kraker *et al*., 2016).

Alarmingly, all the tested *E. coli* O157:H7 isolates (100%) expressed resistance to at least three different classes of antibiotics and were considered as MDR strains. Our finding was similar to that reported in China (100%) (Yu *et al*., 2020), and higher than those reported in Egypt (51.42%) (Elmonir *et al*., 2021), Iran (70.8%) (Pakbin *et al*., 2021) and Ethiopia (57.14%) (Haile *et al*., 2022). This result suggested high risk of transmission of MDR *E. coli* O157:H7 to consumers, including tourists, via food served in food outlets in touristic cities in Egypt. Therefore, MDR *E. coli* has been documented as one of the most significant challenges in food safety (Rashid *et al*., 2013). Furthermore, the transmission of MDR bacteria via the consumption of meat have been proposed as a potential source in Africa (Eibach *et al*., 2018).

The *eae* (encoding intimin) and *stx* (encoding Shiga toxin) harbored in foodborne pathogenic *E. coli* O157:H7 strains are central to the pathogenesis of HUS (Paton and Paton, 1998). Additionally, Shiga toxin produced by *E. coli* O157:H7 can enhance the adherence to epithelial cells and colonization in mice intestines (Robinson *et al*., 2006). In this study, molecular identification of virulence genes revealed that 100% of *E. coli* O157:H7 isolates harbor the *eae* gene with complete absence of *stx1* gene. However, *stx2* was harbored by only one isolate obtained from cooked beef in combination with *eae* gene. This finding was in agreement with Dambrosio *et al*. (2007), who stated that none of the meat STEC isolates harbored *stx1* or *stx2* genes and in contrast with Hessain *et al*. (2015), who reported that 45.45% of *E. coli* O157:H7 isolates recovered from meat samples harbored *stx1* and *stx2*; while *stx1* was present in only one isolate. Interestingly, it is believed that *stx*-negative *E. coli* O157:H7 strains that do not produce Shiga toxin may cause symptoms, such as diarrhea, they are not generally associated with HUS, even though they still carry virulence factors, such as *eae* and *bfpA* genes (Black *et al*., 2010; Ochoa and Contreras, 2011; Ferdous *et al*., 2015) and categorized as EPEC (Bentancor *et al*., 2010). It's interesting to note that the animal aEPEC serogroups O26, O103, O119, O128, O142, and O157 have been linked to human diarrhoea. Additionally, aEPEC has been linked to human infections through consumables such raw meats, pasteurized milk, meat samples, vegetables, and water (Kolenda *et al*., 2015). Therefore, further study is needed to examine whether our isolates carry more virulence genes rather than *stx1*, *stx2* and *eae.*

To fully understand *E. coli* populations, the linkages between strains, their hosts, and disease, and the proven correlation between phylogenetic group and virulence, phylogenetic studies are crucial. Therefore, phylogroup PCR was conducted targeting different marker genes of 7 tested *E. coli* isolates. It was found that phylogroup A and B2 were the dominating groups (42.9% for each) followed by phylogroup D (14.2%). Interestingly, phylogenetic group B2 was the most prevalent phylogroup among the *E. coli* O157:H7 strains isolated from meat samples, including raw and cooked beef and cooked chicken, collected from Luxor, Hurghada, and Alexandria, respectively. However, D phylogenetic group *E. coli* O157:H7 was only

found in raw chicken sample collected from Hurghada. Similarly, a study conducted in India showed that the majority of *E. coli* strains obtained from different food samples belonged to phylogroup B2 (44%) followed by phylogroup B1 (29%), A (16%), and D (3%) (Godambe *et al*., 2017). In Iran, another study also conducted by Pakbin *et al*. (2021) revealed that the phylogenetic group A was the most prevalent (46%) among the *E. coli* isolates and phylogroup D was the least common. It is worth to mention that Phylogroups B2 and D include only virulent *E. coli* strains (Carlos *et al*., 2010). However, phylogroup A characterizes commensal *E. coli* strains (Picard *et al*., 1999), while *E. coli* isolates belonging to the phylogroups B2 most often contribute to extra-intestinal diseases, some strains included in other phylogroups (A and B1) have been identified as causes of diarrheal diseases in humans (Bailey *et al*., 2010). Additionally, in Cairo Governorate the 3 *E. coli* O157:H7 isolates recovered from different sources (cooked chicken, cutting knife and hand swab) belonged to the same phylogenetic group, which is the commensal phylogroup A. This finding might prove also the scenario mentioned above about the possibility of the cross contamination as well as these commensal strains may have gained virulence genes and turned pathogenic. However, further investigation is required to confirm our observation.

CONCLUSION

In conclusion, the detection of pathogenic MDR *E. coli* O157:H7 in food samples, food handler and food equipment in some touristic cities in Egypt poses a great public health problem. Therefore, it is recommended to focus on identifying practices which increase the risk of food contamination, and on implementing measures to improve the sanitary conditions in the food outlets in touristic cities. Further studies are required in comparative genomic analysis including genome sequencing, to recognize the epidemiological sources of *E. coli* O157:H7 and its points of contamination and to define appropriate risk mitigation strategies.

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IRB approval

All food handlers provided oral consent after being told about the usage of hand swab samples. Ethical clearance to use respondents was obtained from the authorized health facility (National Research Centre, Giza, Egypt). The study was conducted in accordance with the ARRIVE recommendations.

Ethical statement

Samples collection protocol was carried out in compliance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, Cairo University, Egypt (VetCU-01102020212).

Statement conflict of interest

The authors have declared no conflict of interest.

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