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Isolation and Characterization of non-O157 Shiga Toxin-Producing *Escherichia coli* in Aswan Governorate with a Zoonotic Approach

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Abstract

Shiga toxin-producing *Escherichia coli* (STEC) infection has a significant negative influence on human wellness and the global economy. The purpose of this investigation was to identify *E. coli* and detect its virulence factors in dairy and meat products as well as, human diarrheal samples. A gross of 200 samples of raw milk, karish cheese, fresh meat, and minced meat were obtained randomly from different localities in Aswan Governorate, Egypt. In addition, 50 diarrhea samples were gathered from outpatients who admitted to medical labs and hospitals in Aswan Governorate. The samples were examined for the presence of non-O157 STEC using different biochemical tests and serotyping. The presence of different virulence genes (*hly*, *eae*, *stx1*, *stx2*) in *E. coli* isolates was investigated using PCR. The results illustrated that 28.8% of the examined samples were tainted with *E. coli* with the acquisition of fresh meat (40%), followed by minced meat and raw milk (20% for each), and finally karish cheese (16%) although it possesses the highest odd ratio (4.846, 1.897-12.379). Depending on serology, twenty different serotypes were detected in overall samples, from the public health point of view, O26, O103, O126, O145, O86, O114, O121, O113, O104, and O118 were serotyped from both food and human samples. The prevalence of *E. coli* in humans was 48%, with insignificant correlation with age, sex, and residence. But the area under the receiver operating characteristic curve (ROC) referring to residence as the riskiest factor to human infection (0.583, 0.424-0.743). Moreover, PCR results demonstrated that the most prevalent gene recognized in *E. coli* strains was *eaeA* (90%) followed by *stx2* (30%), *hlyA* (30%), and *stx1* (10%). In conclusion, our results highlight the risk for non-O157 STEC infections related to consumption of raw milk, karish cheese, fresh meat, and minced meat.

KEYWORDS
E. coli, Fresh meat, Minced meat, Raw milk, kariesh cheese, Human, Virulence genes

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) are zoonotic bacteria that have been identified as a significant cause of foodborne disease and, as a result, have become a major public health concern around the world. In 2016, the EU reported 6,378 assured cases of STEC infections (EFSA, 2017). *Escherichia coli* is a member of the Enterobacteria*ceae* family. This bacterium is a short, non-spore forming Gram-negative bacillus that can grow well on simple culture or synthetic media containing not much more than glycerol or glucose as its primary nutrient. Also, it is as well facultative anaerobe that can be motile through peritrichous flagella or non-motile. Different biochemical features include indole production, a lack of citrate fermentation, a positive methyl red test and a negative urease test, as well as Voges-Proskauer reactions (Steiner *et al*., 2006).

Three essential antigens, O (lipopolysaccharide), K (capsular), and H (flagellar), which may all be broken down into partial antigens, make up the serotyping system of *E. coli*. Despite the fact that there are 50,000 to 100,000 or possibly more *E. coli* serotypes, only a small percentage of these are dangerous and cause gastrointestinal infections. Each of the common categories of diarrheagenic *E. coli* can be mainly categorized based on O:H serotypes, which has been significant in identifying the pathogenesis and epidemiology of enteric *E. coli* infections (Steiner *et al*., 2006). STEC isolates have been detected in animals, as cattle serving as the most substantial reservoir of STEC strains furthermore a source of contamination in food and water (Caprioli *et al*., 2014). There are numerous serogroups were detected in cattle and other animals, but only a few STEC serogroups, such as O26, O45, O103, O111, O121, O145, and O157, are recognized as enterohemorrhagic *E. coli* [EHEC]; because of their potential to cause disease in humans, impose a significant economic burden on food producers due to massive safety problems due to the serious threat to human health (Rivas *et al*., 2016).

Human infection with these bacteria can cause signs ranging from mild diarrhea to life-threatening hemolytic uremic syndrome (HUS) (Mughini-Gras *et al*., 2018). Cattle are responsible for the majority of zoonotic human STEC cases globally. These animals serve as the primary reservoir for O157 STEC as well as some significant non-O157 STEC, including O26, O111, O113, and O103 (WHO, 2019). STEC infection in humans is strongly associated with the ingestion of raw or undercooked meat, raw milk, and their products (Robertson *et al*., 2016). The bacterium can infect food and food products at any stage in the production process, including slaughtering, milking, storage, and packing

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(FAO and WHO, 2022).

The most common STEC serotype related to human illness and the main cause of HUS is *E. coli* O157:H7. But even so, O26, O111, and O103 are also associated in serious human diseases that occur all over the world (Martens *et al*., 2020). In recent years, non-O157 STEC strains like O26, O45, O103, O111, O121, and O145 (termed the "top six" non-O157 STEC) have been exponentially identified as causing food poisoning, bloody diarrhea, HUS, and other gastrointestinal diseases (Valilis *et al*., 2018). Human infections are mostly caused by ingesting contaminated food or water, being exposed to the environment, or coming into immediate contact with animals (Schlager *et al*., 2018). Pathways of transmission include fecal-oral, food-borne, environmental, and person-to-person (Caprioli *et al*., 2005).

There are several of virulence factors that are connected with the ability of STEC strains to cause a disease. Shiga toxins *stx1* and *stx2* (encoded by *stx1* and *stx2* genes) and their variants, are the main virulence factors of STEC which prevent protein synthesis and result in intestinal cell death (Johannes and Romer, 2010). In hig*hly* pathogenic isolates, the protein intimin (encoded by gene *eae*) is discovered which has a role in the tight contact between bacteria and intestinal cells, as well as the effacing lesions on intestinal mucosal cells (McWilliams and Torres, 2014). According to this locus activating host cell signal transduction pathways, *E. coli* attachment to epithelial cells by intimin causes attaching-and-effacing intestinal lesions characterized by cytoskeletal changes like the aggregation of polymerized F-actin (Cepeda-Molero *et al*. 2017). Another virulence-associated factor of these strains is enterohaemolysin, which is a pore-forming cytolysin that aids bacterial invasion into intestinal epithelial cells and is encoded by the *hly* gene (Melton-Celsa, 2014). As a result, the current study attempted to evaluate the incidence of non-O157 Shiga toxin producing *Escherichia coli* in different food sources (raw milk, karish cheese, fresh meat and minced meat) and human in Aswan Governorate, Egypt, using both conventional and molecular methods, in addition serotyping of the obtained isolates.

MATERIALS AND METHODS

Ethical approval and Informed consent

This study was approved by the ethical committee of South Valley University, Qena, Egypt (No. VM/SVU/23(2)-08). Also, Oral consent was obtained from each participant.

Study design and sampling

This research was conducted in Aswan Governorate, which is located in the south of Egypt (Upper Egypt) at a latitude of 24° 5′ 20.18″ N, 32° 53′ 59.39″ E toward 680 km south of Cairo from February 2021 to November 2022. Two hundred food samples of raw milk, karish cheese, fresh meat, and minced meat (50 samples from each) were collected randomly from various markets, butcher shops, dairy shops, and street distributors in Aswan Governorate, Egypt then transmitted instantly to the laboratory in Faculty of Veterinary Medicine, Aswan University for next handling in sterile and closed plastic containers. Twenty-five grams of each sample were appended to 225mL of a selective pre-enrichment medium (buffered peptone water) (Himedia, Code: M028) and thorough*hly* blended by a stomacher before being incubated at 37 °C for 18-24 hours.

Furthermore, the human survey was conducted on 50 diarrheal samples that were gathered in clean cups from confirmed cases to Aswan medical labs and hospitals with gastrointestinal problems and diarrhea then rapidly transmitted to the laboratory in an ice box for more examination. The age, sex, and residence of each patient were documented. The samples were placed in sterile test tubes containing buffered peptone water (BPW) (Himedia) and incubated at 37 °C for 18-24 hours.

Isolation and identification of E. coli species

E. coli species were isolated by extracting a loopful from cultured buffered peptone water tubes and was streaked onto Eosin Methylene Blue Agar (EMB) plates (HIMEDIA, Code: M022) then the cultured plates were incubated aerobically at 37°C for 24 hours. After that typical colonies which were olive green with metallic sheen on EMB were taken and sub-cultured onto nutrient agar plates for purification and incubated at 37 °C for 24 hours, subsequently inspected under a microscope to evaluate the morphology and motility of the isolates under phase contrast microscope and detect Gram-negative bacilli and confirmed biochemically by catalase production, Indole production, Methyl Red, Voges-Proskauer, Simmon`s citrate (Cruickshank, 1968; John *et al*. 1970; Quinn *et al*. 2002).

Serological identification of E. coli

Positive *E. coli* isolates were examined for the presence of somatic (O) antigen performed by slide latex agglutination test by using diagnostic O polyvalent sera (sifin, Germany).

Molecular Identification

This part was conducted in Faculty of Veterinary Medicine, South Valley University.

The ABT bacterial DNA Mini kit was used to extract genomic DNA from *Escherichia coli* cultures (applied biotechnology, ABT001, Korean). The extracted DNA was stored at -20°C until

Table 1. Primer sequences of *E. coli* virulence genes.

Target gene	Primer sequence	Amplification (35 cycles)					(bp)	Reference
		Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension		
stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	94˚C 5 min.	94˚C 30 sec	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min	614	Dipineto *et al*. (2006)
stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	94˚C 5 min.	94˚C 30 sec	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min	779	
eaeA	ATG CTT AGT GCT GGT TTA GG GCC TTC ATC ATT TCG CTT TC	94˚C 5 min.	94˚C 30 sec	51˚C 30 sec.	72˚C 30 sec.	72˚C 7 min	248	Bisi-Johnson *et al*. (2011)
hly	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	94˚C 5 min.	94˚C 30 sec	60˚C 50 sec.	72˚C 1 min.	72˚C 10 min	1177	Piva *et al*. (2003)

needed.

PCR amplification

The primers used in this study, which were obtained from Metabion (Germany) were listed in Table 1. Primers were used in a 25-µl reaction containing 12.5 µl DreamTaq Green PCR Master Mix (2X) (Thermo Scientific, Cat No. K1081). 1 µl of each primer of 20 pmol concentration, 5.5 µl of grade water, and 5 µl of DNA template. Agarose gel 1% was prepared in 100 ml TBE buffer. The negative control, positive control, and 20 µl of each PCR product were laden onto the gel. The power supply ranged from 1 to 5 volts/cm Gel Pilot 100 bp plus ladder (cat. no. 239045) provided from QIAGEN (USA). After nearly 30 minutes, the run was stopped, and the gel was relocated to a UV cabinet. Finally, the gel was photographed using a gel documentation system, and computer software was used to analyze the data.

Statistical analysis

SPSS version 22 was used to analyze data statistically, and all significant levels were regarded at $P < 0.05$. The association between *E. coli* infection and the independent risk factors was predicted by using Chi-square test when the expected value is 5 < 20%, but if it is more than 20% Monte Carlo test was applied at a level of 95% followed by Eta Squared test to estimate the effect size of risk factors which η2= 0.01 indicates a small effect, η2= 0.06 indicates a medium effect, and η2= 0.14 indicates a large effect. The Odd ratio was calculated to estimate the magnitude of the dichotomous independent risk factors with infection and binary logistic regression was applied to assess multichotomous independent risk factors. Nagelkerke R Square used to explain the variability on dependent variable (*E. coli* infection) dependent on the independent variables (risk factors).

RESULTS

Incidence of E. coli in different food and human samples

Represented data demonstrated in Table 2 explained that the overall occurrence of *E. coli* in the investigated samples was 28.8% (72/250) by the conventional method. The current study revealed a significant relationship ($\chi2=19.81$, P= 0.001); Eta Squared test = 0.002 and Nagelkerke R Square test = 0.108 between the existence of *E. coli* in different food sources and infection in humans particularly fresh meat where the highest incidence of *E. coli* was detected (40%) followed by minced meat and raw milk as 20% for each, while the lowest incidence (16%) was detected in karish cheese.

In addition, the presented results proved that the infection percentage of non-O157 *E. coli* 97.22% (70/72) was higher than O157 2.78% (2/72) with a statistically significant difference ($\chi2 =22.680$, P=0.000b) by Monte Carlo test. From all obtained isolates, only two were identified as O157 one from fresh meat samples, and the other from karish cheese samples (Table 3).

Incidence of E. coli in human samples

Data recorded in Table 2 clarified that out of 50 examined diarrhea samples, 48% were positive for *E. coli* which were recognized as non-O157 *E. coli* (Table 3). Insignificantly, the incidence of *E. coli* differed by 50% among males in contrast to 46.4% among females ($\chi2=0.063$, P= 0.802, Odd ratio 0.377- 3.529). Results of the statistical analysis disclosed that *E. coli* was associated with age as a risk factor ($\chi2=1.388$, P= 0.769); with an acquisition in the group of 36-50 years (60%) followed by the age group 21-35 (54.55%), while, among the 5-20 and 51-60 years group, it was 41.67% and 40%, respectively. In addition, incidence of *E. coli* was more frequent in individuals residing in rural (55.17%) areas than

Table 2. Occurrence of *E. coli* in the examined samples by using conventional method.

Sources of the samples		Within source of samples (sub-sample product and human)							
		No. of examined samples	Positive *E. coli*		Chi-square test				
			No.	%	X^2	P	Eta Squared test	Nagelkerke R Square test	Odd ratio
Dairy samples	Raw milk	50	10	20	19.81	0.001	0.002	0.108	3.692 (1.520-8.97)
	Karish cheese	50	8	16					4.846 (1.897-12.379)
Meat samples	Fresh meat	50	20	40					1.385 (0.627-3.058)
	Minced meat	50	10	20					3.692 (1.520-8.97)
Human		50	24	48					References
Total		250	72	28.8					

Significant level at P < 0.05

Table 3. Incidence of O157 and non-O157 *E. coli* isolated from the examined samples.

Sources of the samples		Positive *E. coli*		O157 isolates		Non-O157 isolates		Monte Carlo Sig.	
		No.	%	No.	%	No.	%	X^2	P
Dairy samples	Raw milk	10	20	-	-	10	100	22.680	0.000b
	Karish cheese	8	16	1	12.5	7	87.5		
Meat samples	Fresh meat	20	40	1	5	19	95		
	Minced meat	10	20	-	-	10	100		
Human		24	48	-	-	24	100		
Total		72	28.8	2	2.78	70	97.22		

Significant level at P < 0.05

in urban (38.09%) areas and the variation was not statistically significant (χ2=1.423, P= 0.233, Odd ratio 0.636- 6.286) (Table 4).

The ROC curve values (Table 5 and Fig. 1) elucidated that the most considerable risk factor of *E. coli* infection in individuals was residence with AUC value of 0.583 (0.424-0.743).

Serotyping of E. coli isolates

Serotyping of the isolates using antisera against the O-antigen shown that 20 different serotypes were detected. Milk isolates belonged to O26, O103, O126, O145, O44 and O104, those of karish cheese were serotyped as O86, O121, O157, O119, O142 and O128, while the *E. coli* isolates of fresh meat were O26, O126, O86, O114, O44, O157, O113, O165 and O20. The *E. coli* isolates recovered from minced meat were typed as O26, O103, O126, O145, O86, O114, O125 and O118. The twenty-four human *E. coli* isolates obtained in this study revealed twelve different serotypes with O45 being predominant as 5 (20.8%), followed by O121 as 4 (16.6%). Whereas O26, O103, O145, O111, and O118 represented 2 (8.33%) for each, O126, O86, O114, O113, and O104 serotypes could be found as 1 (4.17%) for each as clarified in Table 6.

Detection of some virulence genes among E. coli isolates

Table 4. Occurrence of *E. coli* in human diarrheal samples according to the age, sex and residence.

Variable		No. of examined cases	No. of positive cases (%)	Chi square		Odd ratio
				X^2	P	
Sex	Male	22	11 (50)	0.063	0.802	1.154 (0.377- 3.529)
	Female	28	13 (46.43)			
Age years	5-20	24	10 (41.67)	1.388	0.769b	0.933 (0.131-6.657)
	21-35	11	6 (54.55)			0.556(0.065-4.755)
	36-50	10	6 (60)			0.444 (0.050-3.976)
	51-60	5	2 (40)			References
Residence	Rural	29	16 (55.17)	1.423	0.233	2 (0.636- 6.286)
	Urban	21	8 (38.09)			

b =Monte Carlo test, ^ = Chi square test, significant level at P < 0.05

Table 5. Area Under the Curve values showing the risk factors effecting on *E. coli* infection in human:

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Sex	.518	.082	.831	.356	.679
Age	.449	.082	.534	.288	.610
Residence	.583	.081	.313	.424	.743

Table 6. The distribution (number and percentage) of O-serogroups of examined samples.

E. coli Serotypes	+ ve Milk samples (10)		+ ve Karish cheese (8)		+ ve Fresh meat (20)		+ ve Minced meat (10)		+ve Human samples (24)		Total -72	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
O26	2	20	-	0	3	15	1	10	2	8.33	8	11.11
O103	2	20	-	0	-	0	1	10	2	8.33	5	6.94
O126	1	10	-	0	3	15	1	10	1	4.1	6	8.33
O145	1	10	-	0	-	0	1	10	2	8.3	4	5.56
O86	-	0	3	37.5	2	10	2	20	1	4.1	8	11.11
O114	-	0	-	0	5	25	1	10	1	4.1	7	9.72
O111	-	0	-	0	-	0	-	0	2	8.3	2	2.78
O45	-	0	-	-	-	0	-	0	5	20.8	5	6.94
O121	-	0	1	12.5	-	0	-	0	4	16.6	5	6.94
O44	1	10	-	0	1	5	-	0	-	0	2	2.78
O125	-	0	-	0	-	0	1	10	-	0	1	1.39
O157	-	0	1	12.5	1	5	-	0	-	0	2	2.78
O119	-	0	1	12.5	-	0	-	0	-	0	1	1.39
O142	-	0	1	12.5	-	0	-	0	-	0	1	1.39
O128	-	0	1	12.5	-	0	-	0	-	0	1	1.39
O113	-	0	-	0	2	10	-	0	1	4.1	3	4.17
O104	3	30	-	0	-	0	-	0	1	4.1	4	5.56
O118	-	0	-	0	-	0	2	20	2	8.3	4	5.56
O165	-	0	-	0	2	10	-	0	-	0	2	2.78
O20	-	0	-	0	1	5	-	0	-	0	1	1.39
Total	10	20	8	16	20	40	10	20	24	48	72	28.8

The existence of variant virulence genes (*hly*, *eaeA*, *stx1*, and *stx2*) was evaluated in the randomly selected 30 *E. coli* isolates showing an overall occurrence of *stx1* and *stx2* as 10 and 30%, respectively with an insignificant association between them ($\chi 2=7.503$, P= 0.822642) (Table 7 and Figs. 2, 3). Only one *E. coli* isolate (O126) was positive for *stx1*gene and one *E. coli* isolate (O103) was positive for *stx2* in raw milk samples (16.67% for each). Two *E. coli* isolates (O119 and O128) in karish cheese samples were positive for *stx1* and *stx2* genes (40% for each), all fresh meat, minced meat, and diarrheal human isolates were negative for the existence of *stx1* gene. The occurrence of *stx2* in minced meat was 20% which detected in one *E. coli* isolate (O118), however, *stx2* was undetectable in fresh meat. On the other hand, the highest occurrence of *stx2* was strongly found in five *E. coli* human isolates (O26, O86, O111, O114, and O145) with a percentage of (62.5%), these findings confirmed that the samples that harbored *stx2* were more than those that harbored *stx1*(Table 7&8). The highest occurrence of virulence gene in represented data was *eaeA* gene (90%) (Table 7, Fig. 4) which was found within all serotypes, and the occurrence of *hly* gene was 30% (Table 7, Fig. 5) which could be detected in O103, O126, and O104 in milk isolates, O119, O86 and O128 in karish isolates, O118, O114 and O126 in minced meat serotypes (Table 8). A strong point in the present result was discovering that the detection of virulence genes differs according to the source of the sample despite belonging to the same serotype.

DISCUSSION

Microbiology is an essential issue in the dairy and meat industries, as current outbreaks of foodborne disease were recorded because of ingestion of these products infected with pathogenic organisms or their toxins in particular *Escherichia coli* which is considered an essential cause of diarrhea in humans. Based on our research, the total occurrence of *E. coli* species was 28.8% of the examined samples by using conventional methods, on other hand the infection of all samples enrolled in current study were contributed about 10.8% as a source of infection due to the Nagelkerke R square was (0.108) so indicated that; the other sources of infection were 89.2% as polluted water or infected birds. A higher incidence was recorded by Bhoomika *et al*. (2016) as 57.87%. While Hamed *et al*. (2017); Karmi and Ismail (2019) recovered it in lower incidences as 24.51% and 16%, respectively. These variations may be returned to the sources from which the organism was isolated.

Although milk is generally a nutritional food for humans, raw milk and raw milk cheeses have frequently been correlated to food-borne disease, especially in developing countries where it is still widely used by agricultural families and workers as Bedasa *et al*. (2018) reported. Orwa *et al*. (2017) documented that contamination of raw market milk may be due to inadequate milking pro-

Table 7. Occurrence of some virulence genes in a few randomly selected *E. coli* samples:

Sources of isolates	No. of *E. coli* isolates	Virulence genes								Monte Carlo Sig	
		stx1		*stx2*		*eaeA*		*hly*		X^2	P
		No.	%	No.	%	No.	%	No.	%		
Raw milk	6	1	16.67	1	16.67	5	83.33	3	50	7.503	0.822642
Karish cheese	5	2	40	2	40	5	100	3	60		
Fresh meat	6	-	0	-	0	6	100	0	0		
minced meat	5	-	0	1	20	5	100	3	60		
Human	8	-	0	5	62.5	6	75	0	0		
Total	30	3	10	9	30	27	90	9	30		

Significant level at P < 0.05

Table 8. Serotyping and genetic characterization of *E. coli* in a few randomly selected samples

Sources of the samples	Serotypes	*stx1*	*stx2*	*eaeA*	*hly*	Sources of the samples	Serotypes	*stx1*	*stx2*	*eaeA*	*hly*
Milk	O26	-	-	+	-	Minced meat	O114	-	-	+	+
Fresh meat		-	-	+	-	Human		-	+	+	-
Human		-	+	+	-						
Milk	O103	-	+	+	+	Milk	O145	-	-	-	-
Human		-	-	+	-	Minced meat		-	-	+	-
						Human		-	+	+	-
Milk	O126	+	-	+	+	Karish cheese	O86	-	-	+	+
Minced meat		-	-	+	+	Fresh meat		-	-	+	-
Human		-	-	-	-	Human		-	+	-	-
Human	O111	-	+	+	-	Human	O45	-	-	+	-
Karish Cheese	O121	-	-	+	-	Milk	O44	-	-	+	-
						Fresh meat		-	-	+	
Minced meat	O125	-	-	+	-	Fresh meat	O165	-	-	+	-
Karish cheese	O119	+	+	+	+	Fresh meat	O20	-	-	+	-
Karish cheese	O142	-	-	+	-	Karish cheese	O128	+	+	+	+
Fresh meat	O113	-	-	+	-	Minced meat	O118	-	+	+	+
Milk	O104	-	-	+	+						

cedures such as milking in a polluted environment with contaminated hands, containers, and contaminated water used for udder washing. The isolation rate of *E. coli* in raw milk samples that were assembled from different markets and farmer houses in Aswan Governorate was 20%. This achieved result almost corresponded with Biruke and Shimeles (2015) who detected *E. coli* in raw milk with a percentage of (18.6%). Lower incidences were detected by Yu *et al*. (2020) (11.1%) and Elbehiry *et al*. (2021) (12.99%). While Hossain *et al*. (2017) and Megersa *et al*. (2019) recorded higher incidences in raw milk at 31.66, and 42%, respectively.

Egypt is one of the most significant and innovative producers of dairy products in particular Karish cheese which is one of the most common local soft cheese. FAO (1990) stated that Karish cheese, an acid-coagulated fresh unripened soft cheese, is produced from raw milk without the addition of starters, but the natural microflora contained in raw milk and the surrounding environment induce fermentation, resulting in the formation of the cheese curd.

Karish cheese samples in our investigation had the lowest isolation rate (16%) of *E. coli* although it had the highest odd ratio with significant issues among samples, it was 4.846 (1.897-12.379) which means the odds of infection occurred in karish cheese was 4.8 times higher than any other kind of samples, this probably due to during karish cheese handling there was hugely direct contact with contaminated hands of workers and distributers more than with other food product samples. This outcome was intimately correlated with that obtained by Al-bajaly and Jabbar (2021) (16.67%). While higher percentages of raw milk cheese were detected by Ombarak *et al*. (2016) (74.5%) and Taha *et al*. (2019) (63.33%). This kind of cheese is prepared from fresh unpasteurized milk and probably under poor sanitation, then distributed in local marketplaces exposed to the elements, potentially contaminating it with a variety of infections. The lower incidence of *E. coli* in karish cheese than in raw milk may be due to the cheese-making process and the characteristics of final products.

While meat contains essential amino acids that the human body needs, it also contains a high level of water which is suitable for the growth of microorganisms. in our study, it was noticed that the isolation of *E. coli* was the highest in fresh meat (40%) when compared to other food samples. Diyantoro and Wardhana (2019) mentioned that certain slaughter processes, such as dehiding and evisceration have the potential to introduce microorganisms to the surface of carcasses and equipment that found in the gut and hide especially if appropriate handling and hygiene standards are not followed. A nearly analogous result was obtained by Park *et al*. (2015) (42.3%), while lower isolation rates from fresh meat were detected by Rani *et al*. (2017) (35%) and Karmi and Ismail (2019) (22%). On the other hand, higher isolation rate was noticed by Babolhavaeji *et al*. (2021) (70%).

Predicated on the obtained findings, *E. coli* species were isolated from minced meat at a rate of 20% which was quite similar to Karmi and Ismail (2019) (18%). However, higher isolation rate was detected by Panahee and Pourtaghi (2017) (23.5%). On the other hand, lower incidences were investigated by Hamed *et al*. (2017) (8%) and Toro *et al*. (2018) (10%).

E. coli O157:H7 is currently the STEC serotype most extremely associated with illness in the United States, Canada, the United Kingdom, and Japan, but other STEC serotypes have been linked with disease and outbreaks in other countries. Our study mentioned that the isolation rate of O157 serotype in overall samples was 2.78% (2 out of 72), one sample was isolated from karish cheese with a percentage of 12.5 and the other one was isolated from a fresh meat sample with a percentage of 5. While non-O157 serogroups were isolated in this paper in 97.22% (70 out of 72) of the examined samples of different sources and showed a significantly higher incidence than O157 ($X2$ = 22.680, P = 0.000b). These outcomes discovered that the isolation rate of non-O157 serogroups were higher than O157 serotype so that this outcome was in coincidence with Kholdi *et al*. (2021), this finding proved that the outbreaks and sporadic cases reported more resulting from non-O157 than O157 serotype. Nevertheless, it disagreed with Joseph and Kalyanikutty (2021) who detected O157 in a higher rate than non-O157.

According to the serotyping of raw milk isolates, six serotypes were identified; O26, O104, O126, O44, O145, and O103 with the acquisition of O104 (30%) while O126, O44, and O145 were recorded in the lowest percentage (10% for each). Ranjbar *et al*. (2018) could type O26 from milk isolates at a percentage of 33.33%, while Mohammed *et al*. (2015) couldn't detect either O26 or O103 from the investigated samples. It is clear to us that O157 couldn't be serotyped in our examined raw milk samples unlike Ranjbar *et al*. (2018) who were able to isolate it. The difference between the serotypes that have been identified in various studies may be due to the difficulties in detecting non-O157 serogroups, such as the lack of routine dependable user-friendly detection procedures, which have occasionally resulted in a lack of expertise about these organisms as Kholdi *et al*. (2021) said, or due to difference between incidence rates of these serogroups in dairy cattle which consider the source of contamination of raw milk.

Six different serotypes were identified in karish cheese; three isolates were serotyped as O86 and five isolates were detected as O121, O157, O119, O142, and O128 (one for each). As is evident from the results, O86 was the most prevalent serotype (37.5%) incompatibly, Elhadidy and Mohamed (2013) detected it as 11.11%. On the other hand, Ranjbar *et al*. (2018) could detect O157 as 47.05%. Whereas the occurrence of O121 in karish cheese in our results was 12.5%, which was higher than that found by Ranjbar *et al*. (2018) (5.88%). Consulting the previously obtained results O26 could not be isolated from karish cheese, while other authors could detect it in the following percentages: 22.22%, and 6.67% by Elhadidy and Mohamed (2013), and Taha *et al*. (2019), respectively.

In regard to fresh meat isolates, nine different serotypes were recorded including O26, O126, O86, O114, O20, O113, O44, O165, and O157. Of these, O114 was the most predominant (25%), followed by O26 and O126 (15% for each), then O86, O113, and O165 (10% for each), while the lowest occurrence rates were reported for O20, O44, and O157 (5% for each). Previously published data recovered the same serotypes from the examined meat samples including Momtaz *et al*. (2013) who detected O26, O113, and O157 as 16.42, 4.48, and 31.34%, respectively. Karmi and Ismail (2019) obtained O26 in 9% of the examined samples and Kholdi *et al*. (2021) detected O26 and O157 at 44.2% and 3.8%.

Regarding minced meat, eight different serotypes were detected from which O86 and O118 were the serotypes most frequently identified (20%), followed by O26, O126, O114, O125, O103, and O145 (10%). The same serotypes were detected by Karmi and Ismail (2019). The STEC population on the surface of the meat is evenly distributed during the mincing process, and if the product is not cooked thorough*ly*, the bacteria positioned in the center may not be subjected to lethal temperatures as Marquezini *et al*. (2022) reported.

Umpiérrez *et al*. (2016) stated Globally, *E. coli* is one of the main causes of infectious diarrhea. The interaction between *E. coli* and the host is defined as commensalism. Yet, in some circumstances, high*ly* adapted strains can produce illnesses such as bloodstream infections, diarrhea, and urinary tract infections (UTI). Indeed, this agrees with the results mentioned in this investigation, where *E. coli* recorded the highest percentage in diarrheic patients (48%) when compared to other food sources. A nearly similar result was obtained by Sudershan *et al*. (2014) (45.6%) while a higher isolation of *E. coli* could be detected by Heydari *et al*. (2020) with percentage of 78%.

Other authors stated lower incidences of *E. coli* as Thakur *et al*. (2018) (21%). Concerning serotyping, 12 different serotypes were obtained as the following O26, O103, O126, O145, O86, O114, O111, O45, O121, O113, O104 and O118. O26, O111, O113, O145 serotypes were also detected in human isolates by Perelle *et al*. (2004), O114 by Farhan *et al*. (2014); Karmi and Ismail

(2019). O103, O121, O118, O126 were detected by Hermos *et al.* (2011).

Among diarrheic serotypes, O45 was detected at the highest rate (20.83%) followed by O121 (16.67%), O26, O103, O145, O111, and O118 (8.33% for each), the other serotypes were detected at 4.17% of the obtained isolates for each. Different occurrence rates of *E. coli* serotypes were recognized by various authors as Farhan *et al*. (2014); Karmi and Ismail (2019) and Elmonir *et al*. (2021) which may be returned to variation in the geographical distribution of STEC strains, sampling technique, or methodology.

From the previous results, we can conclude that different serogroups have been identified in food sources besides its detection in diarrheic patients, most notably O26, O103, O121, O126, O113, O104, O118, O86, O145, and O114 indicating that one of the probable causes of *E. coli* diarrhea could be food samples that harbored these serotypes. Scheutz and Strockbine (2005) mentioned that O4, O5, O16, O26, O46, O48, O55, O91, O98, O111ab, O113, O117, O118, O119, O125, O126, O128, O145, O157 and O172 are the most predominant EHEC serogroups. As a result, non-O157 strains were regarded to have a role in serious infections to consumer, and in some areas, it was more common than O157 in causing diarrhea and HUS, and this opinion was in agreement with Pradel *et al*. (2000).

Among the risk factors connected to *E. coli* infection that were studied in this research were gender, age, and residence. Of 50 investigated patients 22 and 28 were male and female participants, respectively. Half of the investigated males were infected with *E. coli* (50%) which was higher than that in females (46.42%) without a statistically significant difference. This observation concurred with Miri *et al*. (2017) who discovered 32% incidence rate of *E. coli* in males versus 22% in females. Furthermore, Abbasi *et al*. (2017) determined that *E. coli* occurrence rate in males and females was 58% and 43%, respectively. On the other hand, our findings were contradictory with Adesoji and Liadi (2020) findings who recorded *E. coli* in females and males as 53.9% and 46.7%, respectively. There is no role of sex factors as biological confirmation at this deduction, the reason for this finding may be behavioral factors; men travel more often than women and are therefore more susceptible to consuming street food and under-cooked food raw food, and also because of the nature of their work as butchers, Slaughterers, and milk distributors. Mohammed *et al*. (2015) stated that one of the main factors raising the hazard of foodborne pathogens worldwide is travel.

Regarding to age, the highest level of *E. coli* infection was detected in the following age groups; 36-50 and 21-35 years at 60% and 54.55%, respectively, and can be explained as follows; these two age groups travel more frequently and so there is more chance of dining out and consumption of undercooked and contaminated food in high level that contribute to their high susceptibility to the infection. This result concurrent with that obtained by Zhou *et al*. (2021), while Adesoji and Liadi (2020) and Abdul-husin and Abdul-razzaq (2021) detected incompatible results. The infection rate among 5-20 and 51-60 age groups were close, as 41.67% and 40%, respectively. This agreed with Adesoji and Liadi (2020) and Zhou *et al*. (2021) while the opposite result was obtained by Thakur *et al*. (2018). These could be noticed due to a well-developed immune system at a younger age in addition to decrease consumption of street food, undercooked meat, and low level of exposure to travel risks at an older age.

With reference to the residence, the statistical analysis performed in this study revealed that *E. coli* was insignificantly higher ($\chi 2$=1.423, P= 0.233, Odd ratio 0.636- 6.286) in rural regions (55.17%) than urban (38.09%), on other hand the area under the receiver operating characteristic curve was higher in residence risk factor 0.583(0.424-0.743) that explained that; 58.3% of human live in rural area more risky to acquire the infection than urban area, this finding was concordant with Byrne *et al*. (2015) who declared that exposure to the environmental factors and animals is still a significant risk for disease transmission either by direct contact or indirect through instruments contaminated with the fecal matter of farm animals which consider reservoir for *E. coli*. In addition, decreased health education and safety guidelines to control the infection associated with the spreading of disease. Although Zhou *et al*. (2021) recorded a higher level of infection in urban than rural areas at 46.58% and 21.11%, respectively.

Milk and meat were recognized as the most imperative sources of STEC entry into the food chain, Martin and Beutin, (2011) mentioned that. *stx1* and *stx2* are the most important toxins that are typically carried by prophages incorporated into the *E. coli* genome. Khalifa *et al*. (2017) detected that protein intimine (*EaeA*) is essential for bacterial invasion and attachment to intestinal epithelial cells, so it plays a crucial function in the pathogenicity of the disease. In addition, *hlyA* is widely used as an indicator for detecting potentially pathogenic *E. coli* strains as Schwidder *et al*. (2019) proved.

the overall prevalence of 10% *stx1*-positive isolates and 30% *stx2*-positive isolates in our study confirmed the findings demonstrated by Freedman *et al*. (2016) who also stated that *stx2* is more virulent and caused more severe symptoms than *stx1*. In detail, raw milk has the same percentage of *stx1* and *stx2* (16.67%). Lower result of *stx1* in raw milk were documented by Virpari *et al*. (2013), while higher results were recorded by Vanitha *et al*. (2018); Elafify *et al*. (2020) and Elbehiry *et al*. (2021). Furthermore, Virpari *et al*. (2013) identified *stx2* in nearly similar percentage (16%), however, El-Zamkan and Abdel Hameed (2018) could detect it in all obtained isolates, Elafify *et al*. (2020) and Elbehiry *et al*. (2021), additionally Ombarak *et al*. (2016) detected it in variant percentages as 72.22, 30.55, and 0% respectively. On the other hand, *eae* gene in raw milk isolates was detected in a higher percentage (83.33%) than *hly* gene (50%), various percentages of the two genes were recorded by Vanitha *et al*. (2018); Elafify *et al*. (2020) and Elbehiry *et al*. (2021). The higher percentage of *eae* gene in our study emphasized the result obtained by Tavakoli and Pourtaghi (2017) about the direct correlation between the presence of that gene and *E. coli* capacity to inflict serious diseases on humans.

40% *stx1* and *stx2* positive *E. coli* isolates in karish cheese were detected in this study. Contrarily, Ranjbar *et al*. (2018) and Elafify *et al*. (2020); Taha *et al*. (2019) detected them at different rates. All obtained isolates from karish cheese identified as positive *eae* gene. In contrast, Taha *et al*. (2019) couldn't identify any *eae* gene in the recovered isolates. Regarding *hly* gene which was detected as 60%, it was higher than the percentages recorded by Ombarak *et al*. (2016) (2.25%) and Ranjbar *et al*. (2018) (32%), whilst also it was a little lower than that detected by Taha *et al*. (2019) (66.67%).

Isolates obtained from the examined fresh meat and minced meat samples were all positive for *eae* gene. Other genes weren't present in fresh meat isolates Incompatible results in fresh meat were obtained by Cho *et al*. (2020) who observed *stx1*, *stx2*, and *hly* as 12.5%, 12.5%, and 20.83%, respectively, and Babolhavaeji *et al*. (2021) who reported *stx1* and *hly* in percentages of 62.5% and 37.5%, respectively. Moreover, in minced meat *stx2* and *hly* genes were recorded at the following percentages: 20% and 60%, respectively, but *stx1* could not be detected. Variable results were obtained by Toro *et al*. (2018) who detected *stx2* (61%), *hly* (37.5%) and *eae* gene could not be detected in all ground meat samples.

Ferreira *et al*. (2018) stated that the overall differences occurred in food and animals' origin samples can be related to a diversity of herd-related factors such as stress, geographic region, density, and season.

In our study, diarrheal isolates missed the presence of *stx1* and *hly* genes which was compatible with Farhan *et al*. (2014), while Heydari *et al*. (2020) reported *stx1* and *hly* in percentages of 33.3% and 66.7%, respectively. *stx2* was detected in 62.5% of diarrheal samples which was higher than results achieved by Tseng *et al*. (2016) (26.3%) and Falup Pecurariu *et al*. (2019). Samples carrying *eae* genes was detected in the rate of 75% which was higher than that obtained by Farhan *et al*. (2014) (7.1%) while Heydari *et al*. (2020) couldn't detect it. The difference between

virulence genes in the present study was not statistically significant ($\chi 2=7.503$, P= 0.822642).

CONCLUSION

According to this study's findings, Shiga toxin-producing *Escherichia coli* (STEC) could potentially be transmitted to humans through the dairy and meat products in particular O26, O103, O126, O145, O86, O114, O121, O113, O104 and O118 which could be typed among food and human isolates. The obtained serotypes harbored various virulence genes which associated with the ability to cause a disease. So hygienic and control measures either in the farm or during carcass processing, milk collection, and product handling should be performed in the interest of decreasing the incidence of STEC contamination and infection.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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