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Molecular Characterization of Shiga Toxin-producing *Escherichia coli* Isolated from Some Food Products as well as Human Stool in Alexandria, Egypt

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Abstract

The goal of the current investigation was to test various samples of ready-to-eat food and human stool for EPEC. A total of 450 food product samples, including chicken paneeh, chicken burgers, chicken luncheons, beef burgers, minced meat, and kariesh cheese (75 each), and 100 human stool samples (60 from diarrheal people and 40 from healthy people) were randomly gathered from various supermarkets in the Alexandria province. To isolate and identify enteropathogenic *E. coli*, samples were examined bacteriologically. In addition, the recovered isolates underwent a molecular approach employing PCR assay for the simultaneous detection of four virulence indicators, and the antibiogram pattern of the isolates was established. It was found that the highest rate of isolation of *E. coli* was recorded in the examined samples of chicken paneeh (8%) followed by chicken luncheon and minced meat (5.3% of each), Kariesh cheese (4%) and lastly beef burger (2.7%). Concerning stool samples, the rate of isolation was 11.7% and 5% in diarrheic and apparently healthy individuals, respectively. Serotyping of the recovered *E. coli* isolates (n.=21) from food samples revealed the detection of serotype O157:H7 (EHEC) (47.6%), serotype O111:H8 (EHEC) (23.8 %), serotype O26:H11 (EHEC) (19.0%), serotype O125:H21 (ETEC) (4.8%) and serotype O128:H2 (EAEC) (4.8%) while identified serotypes from stool samples (n=9) were O127:H40 serotype (EPEC) (33.3 %), O115:H83 serotype (EPEC) (55.56 %) and O157:H7 serotype (EHEC) (11.1%). Antimicrobial susceptibility of *E. coli* strains to 11 antimicrobial agents was performed. The recorded results clarified that STEC isolated was found to be highly sensitive to Nalidixic acid (76.19%, 77.7%) and Doxycycline (90.5%, 88.89%), while it was moderately sensitive to Ampicillin (52.3%, 44.4%) and Erythromycin (47.6 %, 44.4%). Moreover, it was high resistance to Vancomycin (76.19%, 77.7 %) and cephalexin (81.0%, 77.7%) from food and stool respectively. The recovered *E. coli* isolates from the tested materials, either chicken products or stool, were effectively molecularly characterized using Real time PCR, which included the *Stx1*, *Stx2*, *eae*A, and *hyl*A genes. Despite the relatively low rate of isolation of enteropathogenic *E. coli*, it was determined from the data that retail food products in Alexandria pose a risk to human health.

KEYWORDS
Shiga toxin-producing *Escherichia coli*, Food Products, Human.

INTRODUCTION

The consumption of ready-to-eat food products in many locations poses a concern to public health due to the microbial origin of many food borne diseases. The main cause of contaminated food is conventional processing techniques employed in preparation, inappropriate storage, and/or conservation. *E. coli* is one of the typical pathogenic bacteria that cause foodborne illnesses. Some strains, such Shiga toxin-producing *E. coli* (STEC), create toxins that can cause serious health issues. The most prevalent strain of STEC is *E. coli* O157:H7, although there are numerous other strains as well.

Millions of people live in Alexandria, one of Egypt's most important cities, and they rely on ready-to-eat meat as a quick and delectable supper. Sausage, beef burgers, minced beef and fried chicken are examples of ready-to-eat food items that can be found at markets around Alexandria. These foods may contain a variety of bacteria that are capable of infecting humans and causing a variety of disasters.

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped bacterium that belong to family Enterobacteriaceae. In most mammalian species, humans and birds, *E. coli* is one of the primary gastrointestinal occupants. Most *E. coli* are commensal, however some of them may be dangerous and cause diseases all over the world (Frye and Jackson, 2013).

The majority of *E. coli* strains are non-lethal, however certain serotypes have the potential to infect humans with GIT infections and cause significant food poisoning in their hosts, leading to product recalls. According to Pitout *et al*. (2012), some *E. coli* O157:H7 strains can result in severe anemia or renal failure, both of which can occasionally result in mortality. Moreover, some *E. coli* strains can infect the urinary tract or cause diarrhea, mastitis, arthritis, and meningitis in both people and animals (Nagy and Fekete, 2005).

Based on the production of several virulence factors and the clinical signs they induce, the pathogenic *E. coli* are divided into

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classes; a category of potential pathogenic *E. coli* strains known as enterohaemorrhagic *E. coli* (EHEC) or verotoxins-producing *E. coli* (VTEC) that produce shiga toxins (Detzner *et al*., 2020). According to Karmali *et al*. (2010), they are one of the most prevalent foodborne zoonotic bacteria that can cause a variety of clinical symptoms, including bloody or watery diarrhea, and potentially fatal syndromes like hemorrhagic colitis, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, and acute renal failure.

E. coli is introduced into the environment via fecal waste. For three days, the bacteria develop rapidly in fresh faeces under aerobic circumstances, but after that, it gradually contracts in numbers (Widén *et al*., 2013).

Food safety is crucial for public health at every step of manufacturing, processing, and distribution, therefore analyzing the genetic similarities of ExPEC strains can help identify their distinct origins of origin (Sarowska *et al*., 2019).

The pathogenicity of STEC strains is attributed to the production of different virulence factors including two potent phage-encoded cytotoxins as *Stx1*, and *Stx2*. These toxins are like to those produced by Shigella dysenteriae which inhibit protein synthesis in host cell leading to cell death (Elsyaed and Mounir, 2020). Strains of STEC also carry a pathogenicity island as locus of enterocyte effacement (LEE) that encodes protein of attaching and effacing called *eae* gene which is an outer membrane protein needed for intimate attaching to host intestinal mucosa (Nataro and Kaper, 1998). Alongside the *eae* gene, enterohaemolysin encoded by *hyl*A gene is existed and liberates hemoglobin from RBCs (Castro *et al*., 2017).

Antibiotic resistance (AR) is a global issue, particularly in poor nations. According to Okeke *et al*. (2005), it may affect the prognosis of several illnesses that were previously treated and are still frequent infections. Public health is also at risk from AR in both wealthy and developing nations. Antibiotic resistance is currently the most serious global threat to the effective treatment of bacterial infections. Antibiotic resistance has been established to adversely affect both clinical and therapeutic outcomes, with consequences ranging from treatment failures and the need for expensive and safer alternative drugs to the cost of higher rates of morbidity and mortality, longer hospitalization, and high-healthcare costs (Chinemerem Nwobodo *et al*., 2022).

So, the current work's goal was to explore the function of a variety of food products in the transmission of *E. coli* to consumers beside determining antibiotic sensitivity and molecularly detecting several virulence genes in the recovered isolates of *E. coli*.

MATERIALS AND METHODS

Samples

A total of 450 food samples, comprising chicken paneeh, chicken burger, chicken luncheon, minced meat, beef burger, and kariesh cheese (75 each), were randomly gathered from various shops in the Alexandria province, along with 100 human stool samples (60 from diarrheal individuals and 40 from healthy ones).

Preparation of chicken products samples (APHA, 2001)

Burger and minced meat samples were first thawed by keeping in the refrigerator at 3 to 4°C for an hour. Luncheon and kariesh cheese samples were cauterized by using a hot spatula (surface sterilization) before the cauterized areas were removed. 25 g of each sample were aseptically placed into a sterile blender flask with 225 ml of sterile peptone water 0.1% under strictly aseptic circumstances. The homogenate was then allowed to stand for about 6 minutes at room temperature before being homogenized at 1400 rpm for 2–5 minutes.

Bacteriological examination

In MacConkey broth (Oxoid), 1 ml of homogenate was accurately injected, and the mixture was then incubated for 24 hours at 37°C. A loopful of MacConkey broth was used to inoculate the nutritional agar and MacConkey agar, which were then both incubated at 37°C for 24-48 hours before being used to cultivate the *E. coli* on the selective medium sorbitol MacConkey Agar SMAC (Oxoid). Suspected colonies were isolated, subcultured on brain heart infusion broth containing 50% glycerol, and then preserved in the freezer for further research (Markey *et al*., 2013).

Biochemical identification of E. coli

Biochemical identification of *E. coli* was performed using the tests listed in Table A.

Table A. Biochemical identification of *E. coli*.

Test	Result
Gram Staining	Negative
Motility	Motile
Catalase	Positive
Oxidase	Negative
Methyl Red	Positive
Voges Proskauer	Negative
Indole	Positive
Citrate	Negative
Urease	Negative
H_2S	Negative

Serotyping of E. coli

A total of 21 isolated samples were serologically recognized for the characterization of the enteropathogenic genotypes using quick diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) (Kok *et al*., 1996).

In-Vitro anti-microbial sensitivity test

The Clinical and Laboratory Standards Institute (CLSI) states that on Mueller-Hinton agar, the disc diffusion pattern was used to assess the susceptibility of 21 *E. coli* confirmed strains to 11 antimicrobial medicines. In order to conduct the test, the Muller Hinton agar medium surface was inoculated with bacteria and then streaked with swab sticks. Discs were used to inoculate agar plates, which were subsequently incubated for 24 hours at 37°C. The zone diameters were measured in millimetres using a ruler and the isolators. Isolates were categorized as sensitive or resistant according on the criteria established by CLSI (2017).

Molecular identification of E. coli virulence genes by PCR

Extraction of genomic DNA from *E. coli* isolates was performed using sure fast STEC 4 plex Art No. F5165.

Oligonucleotide primers used in PCR

Two pairs of oligonucleotide primers were used for screening the selected 21 isolates by using PCR. The primer sequences and

Table B. Oligonucleotide primers used in PCR.

Gene	Sequence	Amplified product	Reference
Stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614 bp	Dipineto *et al*. (2006)
Stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779 bp	
eaeA	ATG CTT AGT GCT GGT TTA GG GCC TTC ATCATT TCG CTT TC	251 bp	Panton and Panton (1998)
hylA	GCATCATCAAGCGTACGTTCC AATGAGCCAAGCTGGTTAAGCT	530 bp	

Table C. PCR conditions used in the present study.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
Stx1, 2	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
eaeA	94˚C 5 min.	94˚C 30 sec.	51˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
hlyA	94˚C 5 min.	94˚C 30 sec.	51˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.

amplicon sizes were summarized in Table B.

Amplification and cycling condition for PCR

It was performed in a thermal cycle according to specific another of each primer and according to Emerald Amp GT PCR master mix (Takara) Code No. RR310A kit. The PCR conditions were presented in Table C.

RESULTS AND DISCUSSION

The isolation of pathogenic *E. coli* from food suggested contamination with faeces from both humans and animals. Food items that have fecal contamination are typically thought to pose a greater danger to human health because they are more likely to have enteric pathogens that are unique to humans. Some *E. coli* strains have the potential to cause foodborne illness, ranging from a minor case of enteritis to life-threatening conditions (WHO, 2015).

It was evident from Table 1 that 21 isolates were recovered from the examined samples. the incidence of *E. coli* in the examined food samples of chicken paneeh, burger, luncheon, minced meat, beef burger and Kariesh cheese was 6, 2, 4, 4, 2 and 3 respectively, It was observed that the highest incidence was recorded in paneeh followed by luncheon and minced meat and lastly burger. While the incidence of *E. coli* in the examined stool samples were 7 and 2 from diarrheic and apparently healthy individuals respectively. This result agreed with Nofal (2021) found that the rate of isolation of *E. coli* was 3.2, 1.6 and 5.6 % in the examined samples of minced meat, burger and kariesh cheese, respectively with a total of 21 isolates were recovered from the examined samples. Nearly similar results were obtained by Hemeda (2017) who recorded that the incidence of *E. coli* in the examined samples of luncheon was 16% and El- Ramy (2017) who found that the incidence of *E. coli* in strips and luncheon (processed chicken products) was 12 and 16%, respectively.

On contrary, it was lower than that recorded by Rady *et al*. (2011) who recorded that the incidence of *E. coli* in chicken luncheon was 24 % and Sharaf and Sabra, (2012) who recorded that the incidence of *E. coli* in chicken luncheon was 25%. On the other hand, these results were higher than that recorded by Samaha *et al*. (2012) who could isolate *E. coli* with an incidence of 8 % in chicken luncheon.

Table 1. Recovery rate of coliform bacteria from food products on SMAC.

Food samples	No. of samples	Positive	%
Chicken paneeh	75	18	24
Chicken burger	75	8	10.67
Chicken luncheon	75	10	13.33
Minced meat	75	12	16
Beef burger	75	8	10.67
Kariesh cheese	75	9	12
Total	450	65	14.44

Table 2. Identification of coliform bacteria recovered from food products on SMAC by biochemical tests.

Stool samples	No. of samples	*E. coli*	
		Positive	%
Chicken paneeh	75	6	8
Chicken burger	75	3	4
Chicken luncheon	75	4	5.3
Minced meat	75	4	5.3
Beef burger	75	2	2.7
Kariesh cheese	75	4	5.3
Total	450	23	5.1

Table 3. Identification of coliform bacteria recovered from food products on SMAC by VITEK 2 system.

Food products	No. of samples	*E. coli*	
		Positive	%
Chicken paneeh	75	6	8
Chicken burger	75	2	2.7
Chicken luncheon	75	4	5.3
Minced meat	75	4	5.3
Beef burger	75	2	2.7
Kariesh cheese	75	3	4
Total	450	21	4.7

The rudimentary buildings of the food stalls, where running water, restrooms, and washing facilities are rarely accessible, may be to blame for the prevalence of *E. coli* in the food products under investigation. Hands, utensils, and dishes are frequently washed in bowls or water-filled pots. Additionally, disinfection is seldom performed, and if sewage is not properly disposed of, pests may be drawn to vending locations. Furthermore, because they are improperly hygienic and improperly refrigerated, the goods cooked at these locations offer health hazards. Additionally, food can get contaminated after being heated up by workers, either through direct touch or through the respiratory system from coughing and sneezing.

Serotyping of the obtained isolates of Enteropathogenic *E. coli* was tabulated in Table 4. It revealed the detection 10 of O157:H7 serotype (EHEC) in the examined food samples of with an incidence of 47.6 %, 5 of O111:H8 serotype (EHEC) in the examined samples of with an incidence of 23.8 %, 4 of O26:H11 serotype (EHEC) in the samples with an incidence of 19.0%, 1 of O125:H21 serotype (ETEC) in the samples with an incidence of 4 .8% and 1 of O128:H2 serotype (EAEC) in the samples with an incidence of 4.8 %. While serotypes isolated from stool sample were 3 of O127:H40 serotype (EPEC) with an incidence of 33.3 %, 5 of O115:H83 serotype (EPEC) with an incidence of 55.56 %, one of O157:H7 serotype (EHEC) with an incidence of 11.1%.

Table 4. Serological identification and strain characterization of *E. coli* isolates (n=21) recovered from food products.

Serogroups	Strain characterization	No.	%
O157:H7	EHEC	10	47.6
O111:H8	EHEC	5	23.8
O26:H11	EHEC	4	19
O125:H21	ETEC	1	4.8
O128:H2	EAEC	1	4.8
Total		21	100

The recorded result was in agreement with those of Brusa *et al.* (2013) who isolated *E. coli* O157 from ground beef, Kalender (2013) who isolated STEC O157:H7 from ground beef (2%), Mohammed *et al.* (2014) who studied the prevalence of non-O157 STEC in ground beef and found that 16.7% were positive to non-O157 STEC, Selim *et al*., (2014) who determined the prevalence of STEC in minced meat (1.1%), Eskander (2015) who found that the incidence of *E. coli* was 66% in chicken breasts, Dinçoğlu and Gönülalan (2016) who isolated *E. coli* O157 from chicken meat samples, Bessa (2017) who found that the incidence of *E. coli* was 76% in chicken fillet, Ibrahim *et al.* (2019) who found that the incidence of *E. coli* were 50, 40, 25, 20, 10 and 15% of chicken thigh, pane, nuggets, pane, luncheon and Shawerma, respectively. Also, Ali *et al.* (2020) found that 69% chicken meat parts were positive with *E. coli* and El Sherif and Ali (2020) who detected *E. coli* O157:H7 in Talaga cheese (8%). These results showed that control measures should be developed to prevent contamination with this pathogen in food in this region.

The recorded results in Table 5, clarified the molecular characterization of 21 *E. coli* isolates recovered from food products. *Stx1* and *Stx2* genes were detected in 5 isolates (23.8%), *eae*A was detected in 19 isolates (90.5%) and *hly*A gene was detected in 15 isolates (71.4%). The ability of Shiga toxin producing *E. coli* (STEC) to cause severe human illness had been associated with the production of different Shiga toxins as *Stx1*, *Stx2*, or both. The toxins caused irreversible cytotoxic effects on vero cells and they were structurally and antigenically similar to toxin produced by Shigella dysenteriae (Lee etal., 2016). In addition, Yang *et al.* (2020) reported the identification of a novel *Stx2* subtype, designated *Stx2k*, in *E. coli* strains widely detected from diarrheal patients, animals, and raw meats in China over time. They found that *Stx2k* exhibited varied cytotoxicity in vitro among individual strains.

Table 5. Molecular characterization of *E. coli* isolates (n=21) recovered from food products.

Genes	Positive	%
Stx1	5	23.8
Stx2	5	23.8
*eae*A	19	90.5
hlyA	15	71.4

The antimicrobial susceptibility of *E. coli* strains obtained from the examined food products was tabulated in Table 6. It was found that Doxycycline was the most effective antibiotic as 90.5% of tested isolates were found to be sensitive followed by Nalidixic acid (76.19%) while the least effective antimicrobial agent was Vancomycin as 76.19% of tested isolates were found to be resistant followed by Penicillin G (52.38%).

Table 6. Antimicrobial susceptibility of *E. coli* strains (n=21) isolated from food products.

Antimicrobial agents	Sensitive		Resistant	
	No.	%	No.	%
Streptomycin (S)	11	52.38	10	47.62
Erythromycin (E)	10	47.62	11	52.38
Nalidixic acid (NA)	16	76.19	5	23.81
Penicillin G (P)	10	47.62	11	52.38
Cephalexin (CN)	4	19	17	81
Cefotaxime (CF)	11	52.38	10	47.62
Tetracycline (T)	12	57.14	9	42.86
Ampicillin (AM)	11	52.38	10	47.62
Vancomycin	5	23.81	16	76.19
Ciprofloxacin (CP)	10	47.62	11	52.38
Doxycycline (DO)	19	90.5	2	9.5

As shown in Table 7, the overall recovery rate of *E. coli* from human stool samples on SMAC was 37% (36.7% from diarrheic individuals and 37.5% from apparently healthy individuals). The recorded result in Table 8, clarified the identification of *E. coli* by biochemical tests where only 16 out of 37 isolates are confirmed to be *E. coli* (12 isolates recovered from diarrheic individuals and 4 isolates from apparently healthy individuals). The recorded result in Table 9 shows the identification of *E. coli* by VITEK 2 system where only 9 out of 37 isolates are confirmed to be *E. coli* (7 isolates recovered from diarrheic individuals and 2 isolates from apparently healthy individuals).

Table 7. Recovery rate of *E. coli* from stool samples on SMAC.

Stool samples	No. of samples	Positive	%
Diarrheic individuals	60	22	36.7
Apparently healthy individuals	40	15	37.5
Total	100	37	37

Based on the result of identification of *E. coli* by VITEK 2 system, it was found that the rate of isolation of *E. coli* from human samples was 9%. This result was supported by studies conducted by Byomi *et al.* (2017) (14.2%) and Shaaban *et al.* (2018) (17%).

On contrary, it was lower than that that recorded by Awadallah *et al*. (2014) (64%). Differences in the quantity and health state of human cases, locales, and sanitary practices may be to blame for variations in the prevalence rates of *E. coli* from one research to the next. On the other hand, in stool samples from human clinical laboratories in 10 European nations, the frequency of isolation or detection of *E. coli* by various techniques ranged from 0.4% to 22% (Spina *et al*., 2015).

Table 8. Identification of *E. coli* recovered from stool samples on SMAC by biochemical tests.

Stool samples	No. of samples	*E. coli*	
		Positive	%
Diarrheic individuals	60	12	20
Apparently healthy	40	4	10
Total	100	16	16

The effect of health status of human on the rate of isolation of *E. coli* was presented also in Table 9. It was recorded that the highest rate was recorded in the group of diarrheic patients (11.7%) compared to the group of apparently healthy individuals (5%). This result was consistent with that of Byomi *et al*. (2017), who discovered that the prevalence of *E. coli* was 5.3% in seemingly healthy individuals (non-diarrheic humans) and 11.4% in diarrheic humans, respectively, with a statistically significant difference between the two. The isolation of *E. coli* was found in 30 healthy animal farm employees in another location, but at a substantially greater frequency (73.0%) (Boonyasiri *et al*., 2014).

Table 9. Identification of *E. coli* recovered from stool samples on SMAC by VITEK 2 system.

Stool samples	No. of samples	*E. coli*	
		Positive	%
Diarrheic individuals	60	7	11.7
Apparently healthy	40	2	5
Total	100	9	9

The recorded result in Table 10, illustrated the serological identification and strain characterization of *E. coli* isolates (n=9) recovered from stool samples. It was found that 3 isolates were belonged to O127:H40 (33.33%), 5 isolates were belonged to O115:H38 (55.56%) and 1 isolate was belonged to O157:H7 (11.11%). Different previous researchers from Egypt as Awadallah *et al*. (2014); Merwad *et al*. (2014); Ramadan *et al*. (2016); Ahmed *et al*. (2017) and Hamed *et al*. (2017), identified similar serotypes as O128 (2%) and O55 (8%) from diarrheic persons; O26 (18.1%), O119 (27.2%), O111 (18.1%), O128 (4.5%) and O55 (13.6%) from human; O119 (6.8%), O26 (3.4%), O111 (6.8%) and O113 (3.4%) and untypable (62%) serotypes from diarrheic persons and O26 (14.3%) and O111 (14.3%) and untypable (71.4%) from healthy persons; O55 (4%), O111 (2%) and O157 (2%); and O55 (25%), O111 (25%) and O157 (25%) from diarrheic children). Also, Sharaf and Shabana (2017) identified similar serotypes from diarrheic human including O26 (77.8%), O128 (11%) and O111 (11%) strains.

Table 10. Serological identification and strain characterization of *E. coli* isolates (n=9) recovered from stool samples.

Serogroups	Strain characterization	No.	%
O127:H40	EPEC	3	33.33
O115:H38	EPEC	5	55.56
O157:H7	EHEC	1	11.11
Total		9	100

STEC strains' pathogenicity is attributable to the development of a variety of virulence factors, including the two powerful cytotoxins *Stx1* and *Stx2*, which are encoded by phages. These toxins resemble those produced by Shigella dysenteriae, which cause cell death by inhibiting protein synthesis in the host cell (Elsyaed and Mounir, 2020). Additionally, STEC strains have a pathogenicity island known as a locus of enterocyte effacement (LEE) that encodes the *eae* gene, an outer membrane protein required for close attachment to host intestinal mucosa (Nataro and Kaper, 1998). Alongside the *eae* gene, enterohaemolysin encoded by *hyl*A gene is existed and liberates hemoglobin from RBCs (Castro *et al*., 2017).

Molecular characterization of 9 *E. coli* isolates recovered from stool samples was shown in Table 11. *Stx1* and *Stx2* genes were detected in 1 isolate (11.11%), *eae*A was detected in 9 isolates (100%) and *hly*A gene was detected in 7 isolates (77.78%).

In different studies from Egypt, Mohammed *et al*. (2014) found that 3/5 (60%) of the tested non-O157 *E. coli* were positive to *eae*A gene. While Ahmed *et al*. (2017) reported that *eae*A gene was determined in 2/5 (40%) of the tested *E. coli* O157 strains and in 3/7 (42.9%) from non-O157 *E. coli* and Hamed *et al*. (2017) found that 1/4 (25%) of tested minced meat isolates was carried to *eae*A gene however in comparison to other countries, Cagney *et al*. (2004) found that 41/43 (95.3%) of the tested minced meat were expressed *eae*A gene; Beutin *et al*. (2007) detected *eae*A gene in 5% of the tested minced meat isolates; Kalender, (2013) found that 2% of the examined minced meat were carried to *eae*A gene. High prevalence rates (70.1% and 26.3%) were recorded by Käppeli *et al*. (2011) and Llorente *et al*. (2014), respectively. However, Ibrahim *et al*. (2019) found that PCR results of biochemically positive *E. coli* samples clarified the absence of *Stx1* from all isolated *E. coli* strains, while *Stx2* was present in O44:H18, O114:H21, O119:H4 and O127:H6 isolates and absent from O26:H11, O111:H2, O124 and O125:H18 isolates.

Antimicrobial drugs are crucial in the treatment of infectious illnesses, but their overuse promotes the emergence and spread of antibiotic resistance strains that are linked to serious sickness in human populations. According to Momtaz *et al*. (2013), there are no restrictions on the use of antibiotics in Egypt, whether they are used to treat ill humans, treat animal diseases, or maybe enhance growth in animals used for food. Inappropriate antibiotic usage has the potential to lead to the formation of antimicrobial-resistant zoonotic bacteria in animal-derived commodities, particularly milk and meat, which are often linked to outbreaks around the globe (Abd-Elghany *et al*., 2015).

The presented data in Table 12, showed the antibiogram pattern of selected strains of STEC against 11 antibiotics. The recorded results clarified that STEC was found to be highly sensitive

Table 11. Molecular characterization of *E. coli* isolates (n=9) recovered from stool samples.

Type of samples	No. of tested *E. coli* isolates	*Stx1*		*Stx2*		*eaeA*		*hlyA*	
		No.	%	No.	%	No.	%	No.	%
Stool	9	1	11.11	1	11.11	9	100	7	77.78

to Nalidixic acid (76.19%) and Doxycycline (90.5%), while it was moderately sensitive to Ampicillin (52.3%) and Erythromycin (47.6 %) finally it was less sensitive to Vancomycin (76.19%) and cephalexin (81.0%).

Table 12. Antimicrobial susceptibility of *E. coli* strains (n=9) isolated from stool samples.

Antimicrobial agents	Sensitive		Resistant	
	No.	%	No.	%
Streptomycin (S)	4	44.44	5	55.56
Erythromycin (E)	4	44.44	5	55.56
Nalidixic acid (NA)	7	77.78	2	22.22
Penicillin G (P)	5	55.56	4	44.44
Cephalexin (CN)	2	22.22	7	77.78
Cefotaxime (CF)	5	55.56	4	44.44
Tetracycline (T)	5	55.56	4	44.44
Ampicillin (AM)	4	44.44	5	55.56
Vancomycin	2	22.22	7	77.78
Ciprofloxacin (CP)	4	44.44	5	55.56
Doxycycline (DO)	8	88.89	1	11.11

These results agreed with these results were in agreement with Llorente *et al*. (2014) who tested 57 STEC strains isolated from ground beef against several antimicrobial agents, and they found that all the isolates were susceptible to ciprofloxacin. On contrary, it disagreed with Kalender (2013) who found that all the tested isolates (n.=24) which recovered from ground beef were sensitive to gentamicin and 33.3% of isolates were resistant to Sulphamethoxazol. The obtained results indicated that *E. coli* showed high resistance vancomycin, and these might be attributed to excessive and massive usage of these antibiotic agents in treatment of different clinical cases.

Up to 95% of cases with severe illness are treated without any bacteriological investigations, so regular monitoring of antimicrobial susceptibility is highly advised. Excessive and widespread use of antibiotic agents in livestock lead to the development of antimicrobial resistance in *E. coli*, which is considered to be a growing concern in both developed and developing countries (Dromigny *et al*., 2005).

CONCLUSION

The recorded results revealed that chicken paneeh, chicken luncheon, minced meat, Kariesh cheese, and beef burger has the highest rates of *E. coli* isolation among the food items tested. However, compared to samples from people who seemed to be in good health, the prevalence of *E. coli* isolation from stool samples is greater in samples from those who had diarrhea. Despite the relatively low incidence of isolation of enteropathogenic *E. coli*, it was determined from the data that retail foods in Alexandria pose a risk to human health.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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