Egyptian Journal of Medical Microbiology Volume 32 / No.3 / July 2023 59-69 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Prevalence of E. coli Pathotypes: A Comparative Study between Clinical and Environmental Isolates

Omnia T. Bahgat, Dina E. Rizk*, Hany I. Kenawy, Rasha Barwa
Microbiology and Immunology Department, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

Key words:
Escherichia coli, Pathotypes, DEC, serotyping

Corresponding Author:
Dina Eid Rizk, Microbiology & Immunology Dept., Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt, Tel: +201002605440. Email: dena@mans.edu.eg

ABSTRACT

Background*: The pervasive species of Escherichia coli range from avirulent to extremely pathogenic strains. Pathogenic strains are a serious public health concern globally, causing gastrointestinal infections or disseminate throughout the body, causing urinary tract infections, and sepsis/meningitis. Among bacterial etiologic agents of gastrointestinal infections, diarrheagenic Escherichia coli (DEC) is the predominant cause of severe diarrhea.* ***Objective****: This study aims at determining the prevalence of Escherichia coli pathotypes and serotypes among clinical and environmental isolates.* ***Methodology****: A total of 105 presumptive isolates of E. coli were obtained from different clinical (118) and environmental (217) specimens. Confirmed E. coli isolates were subjected to serological identification, as well as determination of pathotypes. Statistical data analysis was performed applying Fisher's exact test.* ***Results****: Of the 335 presumptive specimens, 31.3% (105/335) were confirmed as E. coli. Seropathotyping of the confirmed isolates showed their distribution as 49.5% EHEC, 26.7% EPEC, 18.1% ETEC, and 5.7% EIEC. Alarmingly, high rate of EHEC and ETEC were observed among dairy and meat products (50% and 20%, respectively), while a low rate belonged to EIEC pathotype. Concerning E. coli clinical isolates, EHEC followed by EPEC were the most prevalent pathotypes. Regarding serotypes distribution, the most prevalent serotype among environmental isolates was O26: H11, whereas the most common serotype among clinical isolates was O128: H2. Serotypes O26: H11 and O125: H21 were significantly more prevalent among environmental isolates than clinical isolates, while serotypes O126: H21, O55: H7, O119: H6, and O128: H2 were significantly more prevalent among clinical isolates.* ***Conclusion****: This research emphasizes the issue of pathogenic pathotypes becoming progressively prevalent in Egypt. We concluded that pathogenic E. coli has been detected not solely in hospitals, but also in food and dairy products rendering them to be possible reservoirs and vehicles for this pathogen.*

INTRODUCTION

Escherichia coli is an important member of the intestinal flora; however, there are also pathogenic strains, including different diarrheagenic *E. coli* (DEC) pathotypes and extraintestinal pathogenic *E. coli* (ExPEC), that infect humans outside the gastrointestinal tract (GI) tract. DEC strains are categorized by serotyping into: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC); enterotoxigenic *E. coli* (ETEC); shiga toxin-producing *E. coli* (STEC); enteroaggregative *E. coli* (EAEC) that causes persistent diarrhea in humans, and diffusely adherent *E. coli* (DAEC), that causes diarrhea in children; enteroinvasive *E. coli* (EIEC); cell-detaching *E. coli* (CDEC); adherent-invasive *E. coli* (AIEC) and ExPEC including: uropathogenic *E. coli* (UPEC), and neonatal meningitis *E. coli* (NMEC) as reviewed in [1].

Pathogenic *E. coli* strains disrupt the intestinal epithelial barrier in a different ways, including adhesion, toxins or effector proteins delivered to host cells, or cytokines generated through infection[2]. Binding of EPEC to the intestinal mucosa leads to the formation of attaching and effacing (A/E) lesions, which in turn cause diarrhea, EHEC are also extremely infectious pathogens that colonize the distal ileum and large intestine in humans and secrete shiga toxin, that is EHEC's key virulence component. Adults and children with EHEC have severe gastroenteritis outbreaks including bloody diarrhea, and later complications can lead to the potentially lethal HUS[3].

Both STEC and ETEC infect the host primarily by releasing toxins. STEC produces shiga toxin (stx). There are two types of stx (stx1 and stx2), with 10 subtypes [4]. ETEC produces thermostable and thermolabile enterotoxins. EAEC causes diarrhea by attaching the intestinal epithelium of the host and forming aggregative adherence known as stacked-brick pattern. EIEC are able to invade the intestinal mucosa of the host and this invasion process triggers an inflammatory response characterized as bacillary dysentery. EPEC and EAEC have been involved in cases of prolonged

diarrhea as well and ETEC is the most common cause of travelers' diarrhea and can result in fatal outcomes for children below the age of 5 [3].

The objective of this research is to assess the prevalence of pathotypes and serotypes of diarrheagenic *E. coli* among environmental and clinical isolates, in Dakahlia Government.

METHODOLOGY

Collection of specimens:

A total of 118 clinical (urine and stool) specimens, 217 environmental specimens were collected over the period between November 2018 to April 2020. The clinical samples were collected from Mansoura University Hospitals in Dakahlia government while environmental isolates were obtained from public supermarkets, different butchers' shops, sewage water, local greengrocers, fish, and chicken markets. Samples were collected in sterile containers and transported immediately to the Microbiology Research Laboratory, Faculty of Pharmacy, Mansoura University for further identification.

Isolation of *E. coli* isolates:

The obtained specimens were cultured in non-differential broth such as nutrient broth tubes and incubated for 24 h at 37ºC [5]. Afterward, a proper inoculum was purified on MacConkey's agar selective medium that involves streaking of inoculum over the surface of medium to obtain pure colonies which will be examined after incubation for 24 h at 37ºC for pink, round medium-sized colonies [6]. The suspected colonies were picked for further confirmation on eosin methylene blue medium [7].

The isolation of environmental food and liquid *E. coli* isolates was performed by homogenization of specimen at 1:10 dilution in nutrient broth. Inoculated broth media were incubated at 37°C for 24 h. After incubation, a proper inoculum was transferred to selective MacConkey's agar medium and incubated at 37ºC for 24 h for further confirmation as previously stated [7].

Identification of *E. coli* isolates

Biochemical confirmation of *E. coli* isolates was carried out using the biochemical laboratory protocols [5]. In this respect, the isolates were tested for their negative Gram stain [8], catalase, indole production, Methyl red/Voges Proskauer (MR/ VP) , and citrate utilization. Pure isolates were cultured in double strength broth and preserved in 50% v/v glycerol [9].

Indole:

In Indole test, confirmed isolates were examined for their ability to produce indole from tryptophan using the enzyme tryptophanase. Peptone water medium containing tryptophan was seeded with the isolate to be examined. The mixture was incubated overnight at 37°C. A few drops of Kovac's reagent were then added to the mixture, and the development of a red ring at the top confirms a positive result [10].

Methyl red/ Voges-Proskauer

The tested bacterium was inoculated into glucose (MR/ VP) peptone medium and incubated for 48 h at 37°C. Three to five drops of MR reagent were added. A positive test result is the appearance of red color. The purpose of the Voges-Proskauer test is to identify the presence of acetoin in a bacterial culture medium. In this test, the bacterial specimen was introduced into a glucose phosphate (MR/ VP) medium peptone medium and cultured for 48 h at 37°C, then, five drops of 40% potassium hydroxide and seven drops of α-naphthol were added to the test medium and vigorously shaken. After 20 mins a positive test result is the appearance of bright red color [11].

Citrate test:

Pure cultures were used to inoculate slants of Simmons citrate agar medium, which were then incubated at 37°C for 48 h. Citrate utilization was identified by the development of blue color[11].

Catalase test:

A pure single colony from an overnight culture was picked, streaked on a sterile glass slide, and a drop of 20% H_2O_2 was added. A positive test is the appearance of air bubbles within a few mins [10].

Serotyping:

To determine the serotypes of all isolates, rapid diagnostic *E. coli* antisera sets from Denka Seiken Co., Japan were employed, following the previously established protocol by Kok *et al.* [12]: briefly, a small colony from the suspect culture was emulsified with physiological saline to produce a smooth suspension. One drop of saline was added to one suspension and mixed as control. One drop of undiluted antiserum was added to the other suspension, which was rotated for 1 min. On a dark background agglutination was observed. A part of a colony that agglutinated extensively with one of the polyvalent serum pools was cultured to be tested with monovalent sera. A dense inoculum of bacteria from each culture was diluted with saline and subjected to slide agglutination tests using diagnostic sera to identify the O-antigen.

Statistical analysis

The fisher exact test was used to detect the hypothesis that the distribution of each serotype was not homogeneous among the different clinical and environmental sources. The analysis was performed with SPSS software (version 20.0; SPSS, Chicago, IL, USA). Statistical significance was determined by considering a p-value of less than 0.05.

Ethical consideration

This study was confirmed and permitted by the Research Ethics Committee of Faculty of Pharmacy with code (2019 – 104).

RESULTS

Incidence of *E. coli*:

The bacteriological examination of 217 environmental specimens revealed that the prevalence of *E. coli* was 18.4% as 40 *E. coli* isolates were identified. They were distributed as follows: 27 isolates from dairy products collected from different supermarkets, 9 isolates from meat, two isolates from sewage water and only one isolate from chicken and fish each. Vegetables, luncheon, and sausage samples were negative for *E. coli.*

Furthermore, examination of 118 clinical isolates revealed that the overall prevalence of *E. coli* was 55.1%, whereas 65 clinical isolates, including 19 isolates from urine (CU) and 46 isolates from stool (CS), were identified as *E. coli*.

Pathotypes prevalence among isolates:

The serological identification of all identified environmental and clinical *E. coli* isolates revealed different pathotypes (figure 1). EHEC was the most common pathotype among both environmental and clinical isolates followed by EPEC, ETEC and EIEC.

EHEC and ETEC pathotypes were detected at high rate among environmental (52% and 20%, respectively) and clinical isolates (47.7% and 17%, respectively). EPEC and EIEC were detected at a higher rate among clinical isolates (29% and 6%, respectively) than in environmental isolates.

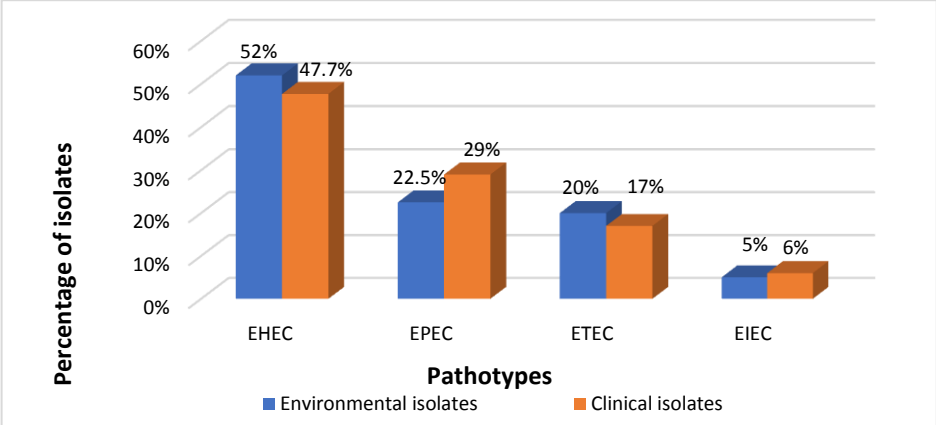

Fig. 1: Distribution of different pathotypes among environmental and clinical *E. coli* isolates.

Correlation between pathotype and different environmental sources

As illustrated in figure 2, the incidence of EHEC isolates was significantly higher in meat products (5/9, 55.55%) than isolates from dairy products (14/27, 51.85%). Both isolates from sewage water were EHEC. EPEC were isolated from dairy products (7/27, 25.9%) at a higher rate than isolated from meat (2/9, 22.22%). Nearby percentages of ETEC were obtained from dairy and meat isolates (18.5% and 22.2%, respectively). Isolates of EIEC were found in dairy products at 3.7% (1/27) but were not found in meat products. Chicken and fish isolates belonged to EIEC and ETEC pathotypes, respectively (Figure 2).

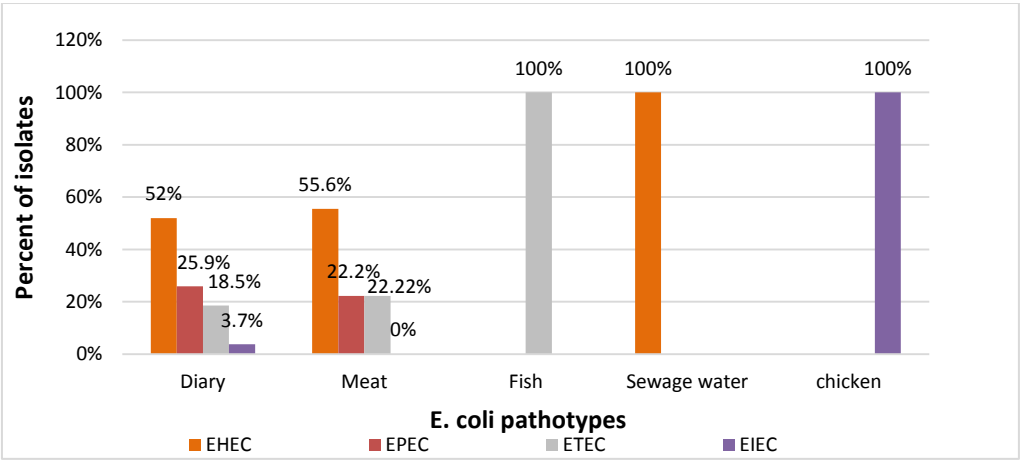

Fig. 2: Distribution of pathotypes among different environmental sources. ETEC: enterotoxigenic *E. coli*, EPEC: enteropathogenic *E. coli*, EIEC: enteroinvasive *E. coli* and EHEC: enterohaemorrhagic *E. coli*.

Correlation between pathotype and different clinical sources

The distribution of different pathotypes among clinical isolates according to specimen sources is illustrated in figure 3**.** EHEC pathotype was significantly higher among urine isolates (57.9%, 11/19) than stool isolates (43.48%, 20/46), while EPEC was isolated at higher rate from stool (30.4%, 14/46) than from urine (26.31%, 5/19) along with ETEC at 19.5% (9/46) from stool and 10.5% (2/19) from urine. Nearby detection percentage of EIEC from both sources was found around 5.9%.

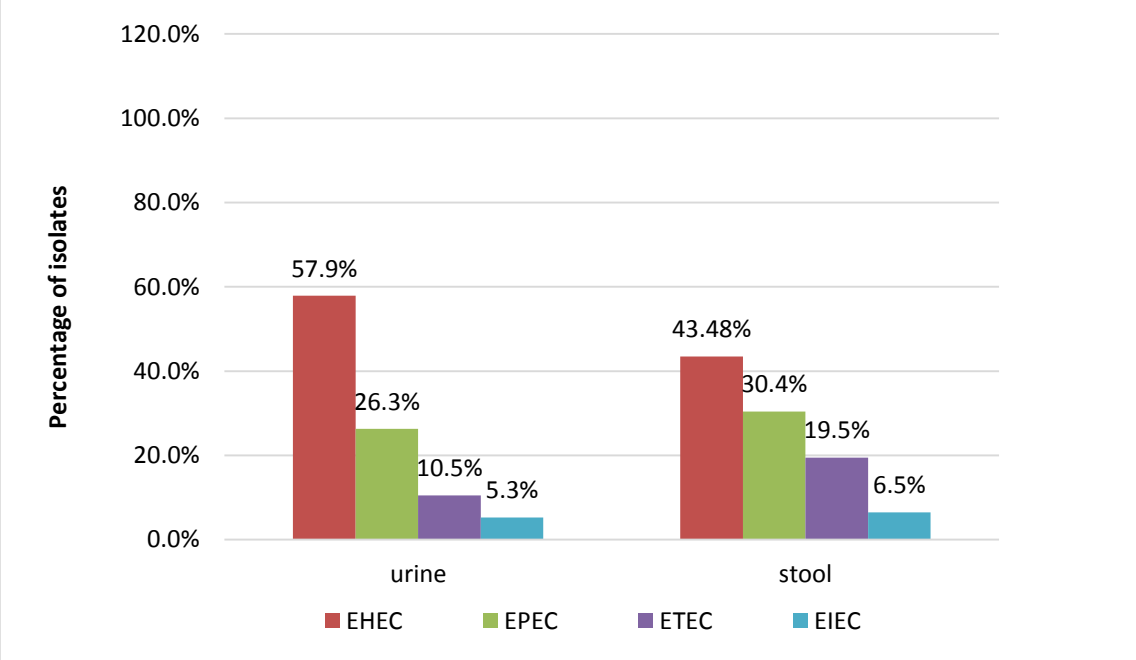

Figure 3: Distribution of pathotypes among different clinical sources. ETEC: enterotoxigenic *E. coli*, EPEC: enteropathogenic *E. coli*, EIEC: enteroinvasive *E. coli* and EHEC: enterohaemorrhagic *E. coli*.

Correlation between serotypes, pathotypes, clinical and environmental *E. coli* isolates:

Regarding serotypes distribution among environmental and clinical isolates, 26 serotypes were observed. Examination of these serotypes revealed that 8 serotypes were shared between environmental and clinical isolates. While 10 serotypes were found in clinical isolates only, the other 8 serotypes were unique in environmental isolates. The most predominant serotype among environmental isolates was O26: H11 (20%), while the most common serotype among clinical isolates was O128: H2 (13.8%). There was a significant difference between environmental and clinical isolates in six serotypes, O26: H11 (p= 0.0230) and O125: H21 (p= 0.0286) were significantly more prevalent among environmental isolates than clinical isolates, and serotypes O126: H21 (p= 0.0079), O55: H7 (p= 0.0022), O119: H6 (p= 0.0006), and O128: H2 (p= 0.0089) were significantly more prevalent among clinical isolates (table 1).

Table 1: Distribution of clinical and environmental isolates within each pathotype

Pathotype (no. of isolates)	Serotype	Number of isolates (%)	Number of clinical isolates (n= 65)	Number of environmental Isolates (n=40)	P value
EHEC (52)	O103: H4	1 (1.9%)	0	1	>0.9999
	O111: H2	11 (21%)	5	6	>0.9999
	O113: H4	1 (1.9%)	1	0	>0.9999
	O117: H4	5 (10%)	4	1	0.2063
	O121: H7	1 (1.9%)	0	1	>0.9999
	O126: H21	5 (9.6%)	5	0	0.0079**
	O26: H11	10 (19.2%)	2	8	0.0230*
	O55: H7	6 (11.5%)	6	0	0.0022**
	O91: H21	12 (23%)	8	4	0.2203
EPEC (28)	O114: H4	3 (10.7%)	0	3	0.1000
	O119: H6	7 (25%)	7	0	0.0006***
	O142	1 (3.6%)	1	0	>0.9999
	O146: H21	1 (3.6%)	1	0	>0.9999
	O153: H2	1 (3.6%)	0	1	>0.9999
	O158	3 (10.7%)	3	0	0.1000
	O173: H2	2 (7.1%)	2	0	0.3333
	O20: H7	3 (10.7%)	3	0	0.1000
	O44: H18	1 (3.6%)	0	1	>0.9999
	O86	4 (14.3%)	2	2	>0.9999
	O78	2 (7.1%)	0	2	0.3333
ETEC (19)	O125: H21	4 (21.1%)	0	4	0.0286*
	O127: H6	2 (10.5%)	0	2	0.3333
	O128: H2	11 (57.9%)	9	2	0.0089**
	O6: H4	2 (10.5%)	2	0	0.3333
EIEC (6)	O124	4 (66.7%)	3	1	0.4857
	O159	2 (33.3%)	1	1	>0.9999

EPEC: enteropathogenic *E. coli*, **EHEC:** enterohaemorrhagic *E. coli*, **EIEC:** enteroinvasive *E. coli* and **ETEC:** enterotoxigenic *E. coli.* ***:** significant difference ($p \leq 0.05$), ****:** moderately significant ($p \leq 0.01$), and *****:** Highly significant difference ($p \leq 0.001$).

Among EHEC pathotype, 9 serotypes were obtained (supplementary table 1). O91:H21, O111: H2 and O26: H11 were the highest detected serotypes (23%, 21% and 19.2%, respectively). For EPEC, 11 serotypes were detected, where O119: H6, O86 were the highest detected at 25% and 14.3%, respectively. Regarding ETEC, 4 serotypes were found. The highest detected serotypes were O128: H2 and O125: H21 (57.9%, 21.1%, respectively). For EIEC only two serotypes were detected with O124 being the most prevalent at 66.7% (table 1).

Supplementary table 1: Seropathotypes and serotypes of all studied clinical and environmental *E. coli* isolates.

Isolate code	Serodiagnosis	Strain characterization
CU1	O117: H4	EHEC
CU2	O91: H21	EHEC
CU3	O26: H11	EHEC
CU4	O55: H7	EHEC
CU5	O111: H2	EHEC
CU6	O126: H21	EHEC
CU7	O117: H4	EHEC
CU8	O91: H21	EHEC
CU9	O91: H21	EHEC
CU10	O111: H2	EHEC
CU11	O55: H7	EHEC
CU12	O173: H2	EPEC
CU13	O20: H7	EPEC
CU14	O119: H6	EPEC
CU15	O128: H2	ETEC
CU16	O20: H7	EPEC
CU17	O158	EPEC
CU18	O159	EIEC
CU19	O128: H2	ETEC
CS1	O55: H7	EHEC
CS2	O55: H7	EHEC
CS3	O113: H4	EHEC
CS4	O91: H21	EHEC
CS5	O117: H4	EHEC
CS6	O91: H21	EHEC
CS7	O126: H21	EHEC
CS8	O117: H4	EHEC
CS9	O91: H21	EHEC
CS10	O126: H21	EHEC
CS11	O26: H11	EHEC
CS12	O111: H2	EHEC
CS13	O126: H21	EHEC
CS14	O55: H7	EHEC
CS15	O126: H21	EHEC
CS16	O91: H21	EHEC
CS17	O55: H7	EHEC
CS18	O111: H2	EHEC
CS19	O111: H2	EHEC
CS20	O91:H21	EHEC
CS21	O158	EPEC
CS22	O6: H4	ETEC
CS23	O119: H6	EPEC
CS24	O124	EIEC
CS25	O128: H2	ETEC
CS26	O119: H6	EPEC
CS27	O119: H6	EPEC
CS28	O128: H2	ETEC
CS29	O142	EPEC
CS30	O124	EIEC
CS31	O128: H2	ETEC
CS32	O128: H2	ETEC
CS33	O6: H4	ETEC
CS34	O158	EPEC

CS35	O20: H7	EPEC
CS36	O124	EIEC
CS37	O173: H2	EPEC
CS38	O128: H2	ETEC
CS39	O119: H6	EPEC
CS40	O86	EPEC
CS41	O119: H6	EPEC
CS42	O128: H2	ETEC
CS43	O86	EPEC
CS44	O128: H2	ETEC
CS45	O119: H6	EPEC
CS46	O146: H21	EPEC
EC1	O26: H11	EHEC
EC2	O91: H21	EHEC
EC3	O26:H11	EHEC
EC4	O111: H2	EHEC
EC5	O26:H11	EHEC
EC6	O26:H11	EHEC
EC7	O111:H2	EHEC
EC8	O26:H11	EHEC
EC9	O91:H21	EHEC
EC10	O103:H4	EHEC
EC11	O26:H11	EHEC
EC12	O111:H2	EHEC
EC13	O128: H2	ETEC
EC14	O124	EIEC
EC15	O125:H21	ETEC
EC16	O125:H21	ETEC
EC17	O125:H21	ETEC
EC18	O114:H4	EPEC
EC19	O78	EPEC
EC20	O114:H4	EPEC
EC21	O78	EPEC
EC22	O125:H21	ETEC
EC23	O114:H4	EPEC
EY1	O26:H11	EHEC
EY2	O91:H21	EHEC
EM1	O26:H11	EHEC
EM2	O111: H2	EHEC
EM3	O111: H2	EHEC
EM4	O111: H2	EHEC
EB	O121: H7	EHEC
Ba	O153:H2	EPEC
EM5	O86	EPEC
EM6	O127:H6	ETEC
EM7	O127:H6	ETEC
M1	O86	EPEC
M2	O44:H18	EPEC
EW1	O91: H21	EHEC
EW2	O117: H4	EHEC
CH1	O159	EIEC
F1	O128:H2	ETEC

CU: urine, **CS:** stool, **EC:** cheese, **EM**: meat, **EW:** sewage water, **EY:** yogurt, **EB:** burger, **CH:** chicken, **F:** fish, **Ba:** pastrami, **M:** milk, **ETEC:** enterotoxigenic *E. coli*, **EPEC:** enteropathogenic *E. coli*, **EIEC:** enteroinvasive *E. coli* and **EHEC:** enterohaemorrhagic *E. coli*.

DISCUSSION

Escherichia coli is a complex facultative anaerobic bacterium, some of which are commensals in humans and animals. Even though, some strains of *E. coli* have become pathogenic by acquiring virulence factors via plasmids, transposons, bacteriophages, and/or pathogenicity islands [13]. In addition to the role of *E. coli* as a causative agent of intestinal diarrheal diseases, most pathogenic isolates cause extra-intestinal diseases such as UTIs [14]. *E. coli* is also regarded an environmental inhabitant due to its ejection out via wastewater or improper disposal of human waste, which contributes to prolonged survival in soil and water [15].

The availability of undercooked fast-food products such as ground beef and burger, meat products, cheese made from unpasteurized milk, and the prominence of raw foods as luncheon. Ground beef contamination may occur following animal slaughter, and cheese contamination may occur during processing[16], while vegetable contamination is commonly caused by irrigation with untreated or sewage water[17]. Furthermore, due to this pathogen tendency for water pipe biofilm formation; it can be released into our drinking water and is considered a water-borne pathogen. This is considered a problem of great concern due to the importance of clean water supply to our society. Thus, the presence of *E. coli* in environmental products that can cause infections is a food safety problem [16].

In our study, 40 environmental *E. coli* isolates were detected among the collected 217 environmental isolates (9 isolates from meat products, 27 isolates from dairy products, 2 isolates from sewage water and only one isolate from both fish and chicken) from different supermarkets and butcher shops. Sixty five out of 118 clinical isolates were identified as *E. coli* (46 isolates from stool and 19 isolates from urine) collected from MDICU and GEC hospital, Egypt.

All *E. coli* isolates were assigned to different pathotypes by serological identification, reflecting their different pathogenesis characteristics. All pathotypes were detected with the exception of EAEC, DAEC, CDEC, and AIEC as previously reported [18].

Alarmingly, it was observed that EHEC was the most prevalent among both environmental and clinical isolates at a percent around 50% (figure 1). According to Clark *et al.* 2003, EHEC has become a crucial pathotype as the frequency of dysentery and HUS outbreaks in both developed and developing countries has risen, and this expansion may be due to excessive consumption of improperly prepared food[19]. Furthermore, high rate (30%) of EHEC was previously reported [17]. This is consistent with various previous reports of STEC in ground beef [20]. EIEC was the least identified pathotype among both categories of isolates. This suggests that this pathotype has a minor influence in diarrheal outbreaks in developing countries [21]. This low rate was consistent with a previous study by Hoseinzadeh *et al.*,2016 [22]. In contrast, a higher incidence of EIEC was found in a previous research [23]. It was observed that EPEC was the second most prevalent among the detected pathotypes (26%). Although ETEC was previously identified as a waterborne than a foodborne pathogen, their presence in environmental isolates (20%) was terrifying, especially within meat isolates (18%). Numerous prior investigations detected ETEC pathotype in food isolates [24]. Among clinical isolates, a high ETEC percentage was detected among stool isolates (11%). This finding is explained by the fact that ETEC is the primary cause of traveler's diarrhea and can be transmitted by the feco-oral route [25].

CONCLUSION

Our results revealed that EHEC was prevalent among environmental isolates, especially dairy products (52%) (figure 2). Lower rates of EHEC isolates have been previously reported among cheese isolates (9.5%) [26], and only 6% in Nigeria [27]. It has been demonstrated that consumption of raw milk or its products is a high risk of EHEC infection and can lead to serious disease and even death [28]. The presence of EHEC in meat products (45.5%) confirms that cattle are the primary source of STEC as reported by Bibbal et al.[29].

Regarding clinical isolates, EHEC was detected at a high rate (57.9%) among urine isolates and 43.48% of stool isolates (figure 3). This could indicate that STEC can survive in the human digestive tract [30]. The prevalence of EHEC in urine isolates was previously detected by Masoumeh et al. 2012 at 2.4% [31]. Furthermore, a previous study carried out in the United States, detected EHEC in 4.2% of stool isolates [32].

All *E. coli* isolates were serotyped. Among the identified 26 serotypes within all environmental and clinical isolates, O26: H11 was the most predominant among environmental isolates and all belonged to EHEC pathotype, while O128: H2 serotype was the most prevalent in clinical isolates and all belonged to ETEC pathotype as stated in (table 1). The serotype O119: H6 (25%) was the most common among EPEC, while O91:H21 (23%) followed by O111: H2 (21%) and O26:H11 (19%) were the most common among EHEC. Previous studies reported that O26, O111 and O103, detected in our study, were more implicated in EHEC infections other than O157:H7 including HUS [33]. Serotype O128:H2 is unique to ETEC represented by 58% and all EIEC identified among our isolates lacked H flagellar antigen and 66% belonged to the O124 serotype. These findings are in accordance with Stenutz et al., 2006**,** who reported that O128:H2 was one of the

most detected serogroups among ETEC isolates with detection of O124 serotype among EIEC isolates [34].

Four categories of DEC were identified among our clinical isolates, with EHEC being the most prevalent (49%), followed by EPEC (29%), ETEC (17%), and EIEC (6%). While EPEC was previously identified at 47.5% and ETEC at 17.5% [35], the bacterial isolation rates of ETEC and EPEC were 9.1% and 6.8%, respectively [36]. On the other hand, Duta *et al*. 2013 showed that EAEC was the most common (5.7%) followed by ETEC (4.2%) and EPEC (1.8%) [37].

High DEC contamination level of food samples was observed in this study (18%), this is noticeably higher than DEC detection (1%) by Canizalez-Roman *et al.*, 2013 [18], 6% reported by Wang et al.,2017 [38], however, higher detection rate (43%) was reported by Kagambega *et al.*,2012 [39]. Supporting our findings, Lee, *et al.* reported that EHEC pathotype was found at 23% while EAEC was absent among food isolates [40].

Until 2013, there had been no food type regarded as a vehicle for EIEC and DAEC infections [3]. It is alarming that our study detected EIEC among chicken and cheese isolates indicating that the public health is under severe warning, while no DAEC nor EAEC pathotypes were found. This is in accordance with previous studies which reported that these pathotypes were absent in all food samples screened [38].

Our study is highlighting that pathogenic *E. coli* strains are not only obtained from hospitals, but also from food and dairy products collected from different locations in Egypt, indicating high levels of fecal contamination in animal source foods and implying that dairy and meat products may be involved in the transmission of foodborne infections, which can be linked to poor hygienic conditions during food processing and storage at stores. Alarmingly, four DEC strains were found among our isolates, particularly EPEC, EHEC, ETEC, and EIEC with EHEC being the most predominant pathotype. Serological studies demonstrated a high incidence of EHEC, EPEC, and ETEC pathovars among our isolates, therefore this finding allowed a better understanding of the pathogens responsible for diarrheal epidemics. The findings in this study might have a substantial influence on the development of preventive strategies for *E. coli* infections caused by DECs through the identification of potential sources that could serve as a vehicle for the transmission of these pathogenic bacteria.

Declarations:

Consent for publication

Not applicable

Availability of data and material

Data are available upon request

Competing interests

The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article none.

Funding

Authors did not receive any grants from funding agencies

REFERENCES

1. Leimbach A, Hacker J, Dobrindt U. *E. coli* as an all-rounder: the thin line between commensalism and pathogenicity. J Curr Top Microbiol Immunol. 2013;358:3-32.
2. Navarro-Garcia F, Serapio-Palacios A, Ugalde-Silva P, Tapia-Pastrana G, Chavez-Duenas L. Actin cytoskeleton manipulation by effector proteins secreted by diarrheagenic *Escherichia coli* pathotypes. Biomed Res Int. 2013;2013:374395.
3. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. Recent advances in understanding enteric pathogenic *Escherichia coli*. J Clin Microbiol Rev. 2013;26(4):822-80.
4. Hernandes RT, Elias WP, Vieira MA, Gomes TA. An overview of atypical enteropathogenic Escherichia coli. FEMS Microbiol Lett. 2009;297(2):137-49.
5. Leboffe M, Pierce B. Photographic Atlas for the microbiology laboratory. 4th editio. J USA: Douglas N Morton. 2011.
6. Jung B, Hoilat GJ. MacConkey Medium. StatPearls StatPearls Publishing; 2021.
7. Adams MR, Moss MO, Moss MO. Food Microbiology, 2nd Edition, Royal Society of Chemistry, Cambridge: Royal society of chemistry; 2000.
8. Smith AC, Hussey MA. Gram stain protocols. J American Society for Microbiology. 2005;1:14.
9. Collee JG, Miles R, Watt B. Tests for identification of bacteria. J Mackie McCartney practical medical microbiology. 1996;14:131-49.
10. Ewing WHJE, Enterobacteriaceae. EsIo. Edwards and Ewing's identification of Enterobacteriaceae. 1986(Edition 4).
11. J Leboffe M. A Photographic: Atlas for the Microbiology Laboratory 4th EDITION. Morton Publishing Company; 2011.
12. Kok T, Worswich D, Gowans E. Some serological techniques for microbial and viral infections. J Practical Medical Microbiology , 14th ed, Edinburgh, Churchill Livingstone, UK. 1996:179-204.

13. Blount ZD. The unexhausted potential of *E. coli*. J Elife. 2015;4.
14. Biran D, Ron EZ. Extraintestinal pathogenic *Escherichia coli*. J *Escherichia coli* O157: H7. 2018:149-61.
15. Berthe T, Ratajczak M, Clermont O, Denamur E, Petit F. Evidence for coexistence of distinct *Escherichia coli* populations in various aquatic environments and their survival in estuary water. J Applied environmental microbiology. 2013;79(15):4684-93.
16. Gould LH, Seys S, Everstine K, Norton D, Ripley D, Reimann D, et al. Recordkeeping practices of beef grinding activities at retail establishments. J Food Prot. 2011;74(6):1022-4.
17. Castro-Rosas J, Cerna-Cortes JF, Mendez-Reyes E, Lopez-Hernandez D, Gomez-Aldapa CA, Estrada-Garcia T. Presence of faecal coliforms, *Escherichia coli* and diarrheagenic *E. coli* pathotypes in ready-to-eat salads, from an area where crops are irrigated with untreated sewage water. J Food Microbiol. 2012;156(2):176-80.
18. Canizalez-Roman A, Gonzalez-Nunez E, Vidal JE, Flores-Villasenor H, Leon-Sicairos N. Prevalence and antibiotic resistance profiles of diarrheagenic *Escherichia coli* strains isolated from food items in northwestern Mexico. J Food Microbiol. 2013;164(1):36-45.
19. Clarke SC, Haigh RD, Freestone PP, Williams PH. Virulence of enteropathogenic Escherichia coli, a global pathogen. Clin Microbiol Rev. 2003;16(3):365-78.
20. Robbins A, Anand M, Nicholas DC, Egan JS, Musser KA, Giguere S, et al. Ground beef recall associated with non-O157 Shiga toxin-producing Escherichia coli, United States. J Emerg Infect Dis. 2014;20(1):165-7.
21. Pourakbari B, Heydari H, Mahmoudi S, Sabouni F, Teymuri M, Ferdosian F, et al. Diarrhoeagenic *E. coli* pathotypes in children with and without diarrhoea in an Iranian referral paediatrics centre. J East Mediterr Health J. 2013;19(7):617-21.
22. Hoseinzadeh T, Ghanbarpour R, Rokhbakhsh-Zamin F. Phylogenetic background of enterotoxigenic and enteroinvasive *Escherichia coli* from patients with diarrhea in Sirjan, Iran. J Microbiol. 2016;8(3):187-92.
23. Vieira N, Bates SJ, Solberg OD, Ponce K, Howsmon R, Cevallos W, et al. High prevalence of enteroinvasive *Escherichia coli* isolated in a remote region of northern coastal Ecuador. J Trop Med Hyg. 2007;76(3):528-33.
24. Gomez-Aldapa CA, Rangel-Vargas E, Bautista-De Leon H, Castro-Rosas J. Presence of non-O157 Shiga toxin-producing Escherichia coli, enterotoxigenic E. coli, enteropathogenic *E. coli* and Salmonella in fresh beetroot (Beta vulgaris L.) juice from public markets in Mexico. J Sci Food Agric. 2014;94(13):2705-11.
25. Lothigius A, Janzon A, Begum Y, Sjoling A, Qadri F, Svennerholm AM, et al. Enterotoxigenic *Escherichia coli* is detectable in water samples from an endemic area by real-time PCR. J Appl Microbiol. 2008;104(4):1128-36.
26. Garbaj AM, Awad EM, Azwai SM, Abolghait SK, Naas HT, Moawad AA, et al. Enterohemorrhagic *Escherichia coli* O157 in milk and dairy products from Libya: Isolation and molecular identification by partial sequencing of 16S rDNA. J Vet World. 2016;9(11):1184-9.
27. Ivbade A, Ojo OE, Dipeolu MA. Shiga toxin-producing *Escherichia coli* O157:H7 in milk and milk products in Ogun State, Nigeria. J Vet Ital. 2014;50(3):185-91.
28. Hlavsa MC, Roberts VA, Kahler AM, Hilborn ED, Mecher TR, Beach MJ, et al. Outbreaks of Illness Associated with Recreational Water--United States, 2011-2012. J MMWR Morb Mortal Wkly Rep. 2015;64(24):668-72.
29. Bibbal D, Loukiadis E, Kerouredan M, Ferre F, Dilasser F, Peytavin de Garam C, et al. Prevalence of carriage of Shiga toxin-producing *Escherichia coli* serotypes O157:H7, O26:H11, O103:H2, O111:H8, and O145:H28 among slaughtered adult cattle in France. J Appl Environ Microbiol. 2015;81(4):1397-405.
30. Singh P, Sha Q, Lacher DW, Del Valle J, Mosci RE, Moore JA, et al. Characterization of enteropathogenic and Shiga toxin-producing *Escherichia coli* in cattle and deer in a shared agroecosystem. J Front Cell Infect Microbiol. 2015;5:29.
31. Navidinia M, Karimi A, Rahbar M, Fallah F, Ahsani RR, Malekan MA, et al. Study Prevalence of Verotoxigenic *E. coli* Isolated from Urinary Tract Infections (UTIs) in an Iranian Children Hospital. Open Microbiol J. 2012;6:1-4.
32. Fey PD, Wickert RS, Rupp ME, Safranek TJ, Hinrichs SH. Prevalence of non-O157:H7 shiga toxin-producing *Escherichia coli* in diarrheal stool samples from Nebraska. Emerg Infect Dis. 2000;6(5):530-3.
33. Gould LH, Mody RK, Ong KL, Clogher P, Cronquist AB, Garman KN, et al. Increased recognition of non-O157 Shiga toxin–producing *Escherichia coli* infections in the United States during 2000–2010: epidemiologic features and comparison with *E. coli* O157 infections. J Foodborne pathogens disease. 2013;10(5):453-60.

34. Stenutz R, Weintraub A, Widmalm G. The structures of *Escherichia coli* O-polysaccharide antigens. FEMS Microbiol Rev. 2006;30(3):382-403.
35. Alikhani MY, Hashemi SH, Aslani MM, Farajnia S. Prevalence and antibiotic resistance patterns of diarrheagenic *Escherichia coli* isolated from adolescents and adults in Hamedan, Western Iran. Iran J Microbiol. 2013;5(1):42-7.
36. Karambu S, Matiru V, Kiptoo M, Oundo J. Characterization and factors associated with diarrhoeal diseases caused by enteric bacterial pathogens among children aged five years and below attending Igembe District Hospital, Kenya. Pan Afr Med J. 2013;16:37.
37. Dutta S, Guin S, Ghosh S, Pazhani GP, Rajendran K, Bhattacharya MK, et al. Trends in the prevalence of diarrheagenic *Escherichia coli* among hospitalized diarrheal patients in Kolkata, India. PLoS One. 2013;8(2):e56068.
38. Wang L, Nakamura H, Kage-Nakadai E, Hara-Kudo Y, Nishikawa Y. Prevalence, antimicrobial resistance and multiple-locus variable-number tandem-repeat analysis profiles of diarrheagenic *Escherichia coli* isolated from different retail foods. Int J Food Microbiol. 2017;249:44-52.
39. Kagambega A, Martikainen O, Lienemann T, Siitonen A, Traore AS, Barro N, et al. Diarrheagenic *Escherichia coli* detected by 16-plex PCR in raw meat and beef intestines sold at local markets in Ouagadougou, Burkina Faso. Int J Food Microbiol. 2012;153(1-2):154-8.
40. Lee GY, Jang HI, Hwang IG, Rhee MS. Prevalence and classification of pathogenic *Escherichia coli* isolated from fresh beef, poultry, and pork in Korea. Int J Food Microbiol. 2009;134(3):196-200.