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ORIGINAL ARTICLE

Detection of *Escherichia coli* O157: H7 from patients with Gastroenteritis

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E. coli *O157:H7 serotype, infectious diseases, hemolytic uremic syndrome (HUS*)

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ABSTRACT

Background: *Acute gastroenteritis is an important public health problem all over the world representing a great economic burden especially in the developing countries. Escherichia coli (*E. coli*) represents about 30% of bacterial causes especially serotype O157:H7. Infected patients may undergo severe complications in the form of dehydration and hemolytic uremic syndrome (HUS). WHO Global Priority Pathogens (GPP) List reported E. coli O157:H7 as a critical pathogen requiring regular monitoring and surveillance.* ***Objectives:*** *The purpose of this cross sectional study is to highlight the occurrence and determinants factors of this serotype isolated from diarrheal cases in Beni-Suef Governate. Frequent monitoring of foodborne bacteria is essential as they may act as a reservoir carrying and transferring resistance genes to humans. Multidrug resistance (MDR) transmission in this manner would make human bacterial infection is very serious and difficult to be treated.* ***Methodology:*** *The study was carried out on a total 457 cases randomly attended to different hospitals of Beni-Suef Governate (Internal Medicine Outpatient Clinic and Emergency Department) over a period of 2 months. Clinically diagnosed patients with gastroenteritis were included. E. coli O157:H7 was identified by API 20E test kit (bioMérieux, France) and serotyped using latex agglutination test (Oxoid, UK). Sociodemographic factors for cases were also assessed.* ***Results:*** *E. coli O157:H7 was isolated with a prevalence rate 11% (41 out of 457) ; aged from 4- 33 years old; (68.3%) males, (51.2%) urban residence and most patients had a habit of outdoor food consumption (70.7%) and a direct contact with animals (82.9%). About 25 cases (61%) needed admission, 10 cases (40%) had moderate dehydration, (13 cases, 52%) had HUS ended with a surviving fate and 2 cases (8%) had HUS ended with death representing the fatality rate. Self-limited gastroenteritis was associated with watery diarrhea with a statistically significant difference; p. value= 0.014. Age among cases who died was lower than survived cases with a significant difference; p. value= 0.006 and all dead patients were children with a statistically significant difference p. value= 0.036. There was no a statistically significant difference regarding sex and residence compared to the fate of admission (p-value> 0.05).* ***Conclusion*:** *E. coli O157:H7 serotype was isolated from Beni-Suef Governate with a relatively high prevalence during the period of the study. Shortage of laboratory facilities to diagnose such pathogen interferes with its definitive diagnosis with subsequent complicated fate for infected cases. Proper handling practices as well as public awareness about the epidemiology of the pathogen should be settled. Vehicles of transmission such as food products and water should be decontaminated to prevent the infection.*

INTRODUCTION

E. coli O157:H7 serotype has been known as the major cause of food and water transmitted diarrheal infections. It is complicated with severe sequels like HUS, pyelonephritis and thrombotic thrombocytopenic purpura (TTP); with a secondary outcome causing renal failure.[1]

Contaminated water or undercooked contaminated food products especially meat products has a great role in its spread. Animal hosts for this pathogen include cattle, sheep, chicken and goats[2].

Therefore, it is important to apply proper hygienic measures at the farms and at the abattoirs on handling or the marketing of these food products to limit human infections[3]. The current study aimed to evaluate the prevalence rate of this pathogen in the governate during the study period as well as assessment of the determinant factors.

METHODOLOGY

The study was carried out on October and November 2023. Patients attended to different Beni-Suef hospitals, complaining from acute diarrhea were included. Acute gastroenteritis case was defined as a patient with ≥ 3 episodes of diarrhea with or without vomiting within the previous 24 h. Patients received antibiotics at the previous week were excluded. Full personal and medical history, anthropometric measurements of children (weight and height) and the body mass index (BMI) (to calculate the degree of dehydration if present) had been assessed. Admitted patients were followed up to record the amount of fluid received per day and the fate of illness.

Stool samples were collected into aseptic cup, transported using an ice-box within 24 hours to the Microbiology Laboratory, Faculty of Medicine Beni-Suef University for examination by ordinary light microscope detecting pus cells and RBCs. A portion of stool sample was inoculated into tubes of selenite broth (Oxoid). After overnight incubation, subcultures onto sorbitol-MacConkey medim (SMAC) (Oxoid) were done and incubated for 24 hours at 35-37°C. Opaque and colorless colonies were identified as sorbitol negative one [4] and were selected for:

- Biotyping as *E. coli* using API 20E test kit (indole production, Simmon's urease, citrate and hydrogen sulphide) (bioMérieux, France) as several species cross-react with O157 [5].
- Serotyping using latex reagents (antibody-coated latex and control latex) (Oxoid) according to the procedures recommended by the manufacturer. If agglutination was reported with the *E. coli* O157 Test Latex and not with Control Latex, this isolate was identified as *E. coli* O157 positive. (Fig 1).
- *E. coli* O157 isolates were incubated on blood agar plates (Oxoid) to enhance flagella protein production.
- Colonies from blood agar plates were examined for *E. coli* H7 antigen using *E. coli* H7 latex reagents. If agglutination was reported with the *E. coli* H7 Test Latex and not with Control Latex, this isolate was considered as *E. coli* O157:H7 positive[4].

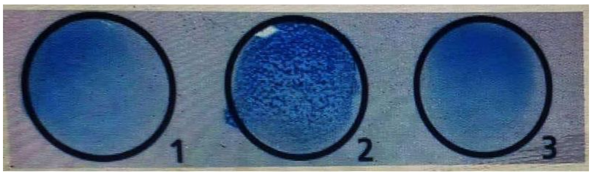

Fig. 1: *E. coli* O157:H7 serotype latex reagents (No 1: Test reagent, No 2: Positive agglutination and No 3: Negative agglutination).

Statistical analysis

Data and variables were statistically analyzed using SPSS (statistical program for social science) to calculate mean, SD, range and percentage. Comparison between variables in parametric data where p value > 0.05 is insignificant, p < 0.05 is significant and p < 0.01 is highly significant.

RESULTS

As shown in table 1, out of a total 457 collected stool samples, forty-one isolates were identified as *E. coli* O157:H7 serotype using API and latex agglutination test. Collected samples were obtained from different hospitals at Beni-Suef Governate.

Table 1: Areas and number of collected samples during study period:

Hospitals	Number of collected samples	*E. coli O157:H7* isolates
Beni Suef University Hospital	161	15
Insurance Hospital	130	11
Alzahraa Hospital	96	10
Beni Suef Hospitals at different centers	70	5
Total	457	41

In the present work, the sociodemographic and some clinical data of the forty one infected *E. coli* O157:H7 serotype cases; with age range from 4-33 years, 68.3% were males, 31.7% were females, 48.8% reside in rural areas, 70.7% with a history of outdoor food consumption and 82.9% with a history of animal contact. In addition, it showed that 61% were complaining from bloody diarrhea while 39 % were with watery diarrhea. The results is shown in table 2.

Table 2: Sociodemographic and some clinical data of infected *E. coli* O157:H7 serotype cases:

Variables	Category	N (%)
Age in years	(Mean ±SD)	**17.95±7.79**
	Minimum-maximum	**4-33 years**
	Children (<18 years)	**22 (53.7)**
	Adults (≥18 years)	**19 (46.3)**
Sex	Male	**28 (68.3)**
	Female	**13 (31.7)**
Residence	Rural	**20 (48.8)**
	Urban	**21 (51.2)**
Outdoor food consumption	Yes	**29 (70.7)**
	No	**12 (29.3)**
Handling and animal contact	Yes	**34 (82.9)**
	No	**7 (17.1)**
Type of diarrhea	Watery diarrhea	**16 (39)**
	Bloody diarrhea	**25 (61)**

Table 3 showed that twenty-five out of forty-one infected *E. coli* O157:H7 patients were hospitalized*;* ten cases with moderate degree of dehydration only, thirteen cases had severe degree of dehydration complicated with HUS and survived while two cases showed severe dehydration and complicated with HUS and died representing the case fatality rate of about 8%.

Table 3: Causes of hospitalization for infected *E. coli* O157:H7 cases:

Variables	Category	N (%)
Need to admission (N =41)	Yes	25 (61)
	No	16 (39)
Fate after admission (N =25)	Moderate dehydration	10 (40)
	Dehydration with HUS and survival	13 (52)
	Dehydration with HUS and survival	2 (8)

Table 4 showed that infected hospitalized cases complained with a bloody diarrhea (p-value < 0.05) but there were insignificant difference between those cases regarding the other data (p-value> 0.05).

Table 4: Comparison between infected cases regarding some clinical data and fate of infection (N=41):

Variables		Need of admission (N= 25) N (%)	Self-limited (N= 16) N (%)	p-value*
Outdoor food consumption	**Yes**	19 (65.5)	10 (34.5)	0.354
	No	6 (50)	6 (50)	
Handling and animal contact	**Yes**	20 (58.8)	14 (41.2)	0.534
	No	5 (71.4)	2 (28.6)	
Type of diarrhea	**Watery diarrhea**	6 (37.5)	10 (62.5)	0.014*
	Bloody diarrhea	19 (76)	6 (24)	

* statistically significant

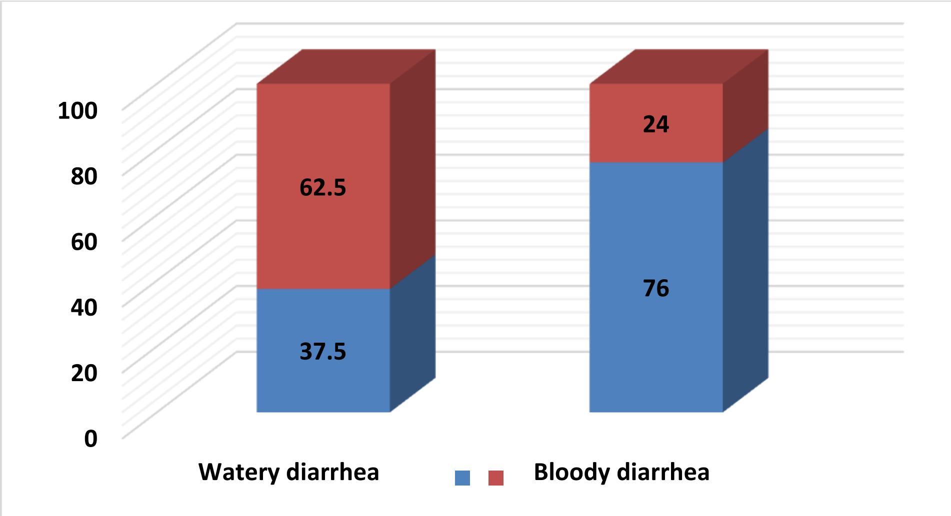

Fig. 2: Type of diarrhea compared to the outcome of infected cases

A significant difference between the age of infected cases and development of complications either dehydration or HUS (p-value < 0.05); as shown in table 5.

Table 5: Comparison between infected hospitalized cases regarding some clinical data: (N=25)

Variables		Moderate dehydration N= 10 N (%)	HUS with surviving N= 13 N (%)	HUS with death N= 2 N (%)	p-value*
Age	(Mean ±SD)	23.15±6.75	13.84±8.78	4.75±1.06	0.006*
	Children (<18 years)	3 (20)	10 (66.7)	2 (13.3)	0.036*
	Adults (≥18 years)	7 (70)	3 (30)	0	
Gender	Male	5 (29.4)	10 (58.5)	2 (11.2)	0.234
	Female	5 (62.5)	3 (37.5)	0	
Residence	Rural	4 (25)	10 (62.5)	2 (12.5)	0.105
	Urban	6 (66.7)	3 (33.3)	0	

*statistically significant

DISCUSSION

Categorization of *E. coli* producing Shiga toxins as an important food-borne pathogen made it a major health threat[6]. The current study was planned to be focused on this serotype, which resides in the intestinal tract of healthy animals, so it is easily present in the environment with subsequent transmission to humans via consumption of contaminated food. Therefore, it is important to relay sensitive, diagnostic, prognostic and therapeutic approaches for it. Moreover, multiple researches showed that antimicrobial resistant *E. coli* isolates in humans were primarily of an animal origin.[7]

Johar et al.[8] emphases that integrons are involved in developing and disseminating antibiotic resistance genes between enteric bacteria, spreading this resistance elements to humans consuming infected animal products.

Therefore, detection of the occurrence rates of such pathogens and improvement the quality of food processing events for further application of safe food, is mandatory.[9]

Annual surveys relay that *E. coli* O157:H7 infections have been reported all over the world[10]**.** Metz et al.[11] proved the increased rates of this infection in any community refer to both poor water quality employed in the handling and processing of food products either meat or vegetables and to the improper hygienic measures of food handlers.

It was noted that HUS is a serious sequel of this serotype and up to 50% of HUS cases may develop permanent renal dysfunction or disorders in blood pressure. [12]

Concerning prevalence of *E. coli* O157:H7 existence during study period at Beni-Suef governate, it was noted as 11% (42 out of 457).

Different prevalence rates were reported in different researches; it was noted by Shimaa et al.[13] in Egypt, that this serotype was isolated from human stool samples of smallholders' animals contacts with a rate of 14% (7/50) while from farm workers with a rate of 16.7% (5/30). Prevalence rate at Bahir-Dar town, Ethiopia was about 50% (124 out of 250)[14]. While the prevalence is much lower in a study done by Havelaar et al.[15] reporting only 2 isolates out of 322 samples were *E. coli* O157:H7. Furthermore, Adwan et al.[16] found a prevalence rate of over 7% and also in Zarya with a prevalence rate of 2.2% [17]. In Poland, Katherine et al., [18] in a study done on children complaining from gastroenteritis reported that *E. coli* O157:H7 serotype was not isolated at all. Similarly, Muloi et al.[19] could not isolate *E. coli* O157:H7 from any of 606 diarrhea cases in Italy.

Khalid et al.[20] study in Egypt from the period of November 2021 to March 2022 reported that meat marketed in Beheira Governorate, Egypt, was contaminated with multi and extensively drug resistant Shiga toxigenic *E. coli* O157:H7 with a prevalence rate 9.1%; 10 out of 110 testes samples.

Regarding Abong'o and Momba[21] discrepancy in their prevalence rates was explained by sample size difference, ages of study population. Moreover, this study reported that *E. coli* O157:H7 prevalence is also affected by bacterial genome, the ability of the community to prevent the foodborne epidemics and the medical conditions of infected patients e.g hydration status which was aggravated by fluid loss.

Shimaa and Gamal [22] relay that this serotype may be associated with either fluid or semiliquid diarrheal motions containing RBCs or WBCs.

In the current study, O157:H7 serotype was detected in the warm seasons of fall. This finding agrees with Courtney et al.[23]. While other studies stated that the incidence was peaked in the warmer months of the year[24].

Also, Ongeng et al.[25] mentioned that ability of serotype to survive efficiently in the environment, animals and meat at different seasonal conditions.

Different seasonal spread was proved according to nature and some biological characteristics of the organism as low infective dose, ability to have different virulence factors and the long survival time at different environmental conditions (temperature and Ph).[26]

This seasonal pattern was previously reported demonstrating that infections had occurred during two seasons; in spring (March to June) and in the fall (September to December)[27]. Also, at United States, most of *E. coli* O157:H7 infections in the had been estimated in the fall (September to November) followed by the spring seasons[28].

E. coli O157:H7 was more prevalent in males especially who are in contact with animals.[29] Moreover, CDC, 2016 stated that the main route of transmission of this pathogen relay on consumption of contaminated undercooked meat or vegetables and raw milk.[30]

Heba et al.[31] found that *E. coli* O157 isolates mainly in patients lived in rural cities especially raw milk consumers. Other researchers reported that the main age in positive cases was 2.5 years and cases were more frequent in boys[32].

The ability of such pathogen to withstand the different degrees of acidic environment favors it to settle in the intestine with development of negative selection and multidrug resistant strains. Nonetheless, acquisition of multiple virulence genes by horizontal gene transfer aggravates the condition[33].

In our work following up of cases showed that hospitalized cases with moderate degree of dehydration received IV fluids of about 500ml /person/day and survived. While cases complicated with severe degree of dehydration took IV fluids of about 2 liters/person/day. This agrees with a research done by Chandler et al. [34]

HUS is a thrombotic microangiopathy; triad of thrombocytopenia, hemolytic anemia and ischemic organ damage. Gastrointestinal infection by *E. coli* O157: H7 is called typical HUS differed from atypical HUS which complicates alternative complement pathway malfunction and secondary HUS accompanying various pathological conditions.[35]

The danger of typical HUS lies in the unique characters of its components; associated anemia is sudden, severe and may need urgent blood transfusion, thrombocytopenia is great with sever risk of platelet transfusion and other biochemical changes are displayed e.g elevated lactate dehydrogenase (LDH), elevated indirect bilirubin levels and haptoglobin[36]. All these features if not proper assessed eventually it will require dialysis or even renal transplantation thus urgent diagnosis and management of this pathogen is mandatory[37].

In our work available laboratory findings for hospitalized cases complicated with HUS and associated with *E. coli* O157:H7 gastroenteritis revealed that leucocyte count was strongly raised. Chemical analysis noted evidence of hemolytic anemia with markedly elevated serum urea and creatinine, hematuria and proteinuria. None of the patients required dialysis. The two cases who died aged 2 years old. The cause of death was severe metabolic acidosis affecting renal and brain function with mortality rate 8%[23].

It was observed that HUS complicates up to 10% of *E. coli* 0157:H7 serotype cases, with higher risk was noted in younger children, especially those under 5 years. Once HUS is developed, the risk of mortality approaches 5%[3].*E. coli* O157:H7 serotype is a public health problem with a mortality rate reach up to 15%–33% in adult and pediatric populations[32].

Nowadays, the increase in the rate of antimicrobial drugs resistance requires further efforts to classify specific causes and practices that aggravate the problem. Whether such factors are professional, infrastructural, social or personal is not yet fully known.[7]

Antibiotics are shown to induce HUS worsening of disease due to either significant release of shiga toxin following bacterial cell lysis, or changes occurred in commensal intestinal flora allowing the toxin easily attached to the l epithilium[12]. A contrasting hypothesis stated, earlier elimination of *E. coli* leads to reduced production of shiga toxin and subsequent decreased severity of STEC-HUS.[1]

On the contrary, Dos Santos et al.[38] analyzed that early use of trimethoprim showed no significant progression to STEC-HUS, improvement or even any change in shiga toxin production. While bactericidal antibiotics like ß-lactams given in the first 3 days of illness was associated with development of HUS in a case-control study of 195 patients.

Sydney et al.[39] found after doing interviews with *E.coli* O157:H7 cases it was established that about (70 cases out of 84 cases) (83%) had a history of fast food eating the week preceding the diarrheal illness onset. Undercooked animal meat is a widely recognized as an important risk factor for acquiring *E. coli* O157:H7. Bacterial contamination may occur during any time in the farm-to-table passage either from environmental, animal, or human sources and cause foodborne illness.

According to Ranya et al.[40] acute infectious diarrhea is a very common infection in developing countries and it is the second most common cause of death in children.

CONCLUSIONS

Laboratory investigations are critical for better understanding the epidemiology of *E. coli* 0157:H7 serotype which greatly affect the fate of illness. In addition, *E. coli* have a significant burden on patients and the healthcare system, so prompt recognition, and appropriate treatment are necessary. Public health officials, regulatory agencies, and health educators for good food supplies must reevaluate current prevention strategies.

N.B: On behalf of all authors, there is no conflict of interest in the article. In addition, a written consent was obtained from the patients included in our study. Manuscript has not been published elsewhere. All authors approved the content of the manuscript and have contributed significantly in the work.

The study was approved by Research Ethical Committee at Faculty of Medicine, Beni-Suef University, Egypt; under Approval number: FMBSUREC/03102023/ Abdel-Aziz.

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