

Identification of *E.coli* O157:H7 in Intestinal and Urinary Tract Infection in Samawah City .

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Abstract

This study was conducted to isolate *E.coli* O157: H7 as an important zoonotic pathogen from 150 samples (75 bloody stools and 75 urine samples) of patients at many age groups range from one to 50 years old and for both sexes were collected from patients suffering from diarrhea and urinary tract infection who attend the Samawah Teaching Hospital for pediatrics and Gynecology of AL-Muthanna Governorate.

The results revealed that 120 out of 150 were positive to *E.coli* O157:H7 at a percentage (80%). The number of *E. coli* isolates in bloody stool were 67(89.3%) and urine samples were 53(70.6%) gave positive results to *E.coli* O157:H7 .

Key words: Human, *E.coli* O157:H7, Bloody stool samples, Api 20E.

الخلاصة

اجريت هذه الدراسة لعزل بكتريا القولون *E.coli* O157: H7 والتي تعتبر احد العوامل الممرضة المهمة حيث تم عزل 150 عينة (75 براز دموي و75 عينة بول) من مرضى باعمار تتراوح ما بين 1-50 سنة لكل من الجنسين جمعت من المرضى الذين يعانون من الإسهال والتهاب المسالك البولية الذين حضروا مستشفى السماوة للاطفال والولادة في محافظة المثنى.

اشيرت النتائج ان 120 عينة من اصل 150 كانت موجبة للبكتريا بنسبة (82,7%). اما اعدادها كانت 67 (89,3%) لعينة البراز الدموي و53 (70,6%) لعينة البول موجبة لبكتريا *E.coli* O157: H7 .
الكلمات المفتاحية: بشري، بكتريا القولون *E.coli* O157: H7، عينات براز دموي.

Introduction

Escherichia coli O157:H7 were considered very important zoonotic disease that induced a clinical signs ranged from limited watery to severe bloody diarrhea and hemolytic colitis in the patients, also it can be caused extra-intestinal infection generally hemolytic ureamic syndrome (HUS) and kidney failure especially in children besides neurological disturbances and other complications of the infection (Masoumeh *et al*, 2012) .

E. coli O157:H7 infections were emergency disease (Manning *et al.*, 2008) and showed highly virulence factors ,therefore low dose (100 organisms) may take place infection and express clinical signs in humans and furthermore the high virulence factors of *E.coli* O157:H7 which associated with severe illness of humans was shiga toxins (Restaino *et al*, 1999).

The present study aimed to determine the isolation of *Escherichia coli* O157:H7 from bloody stool and urine samples. Biochemical confirmation of *Escherichia coli* O157 suspect colonies by Api 20E and chromagar *E.coli* O157:H7 .

Materials and methods

Collection of samples

This research enrolls 150 patients who had attended Samawah Teaching Hospital for pediatrics and Gynecology in AL-Muthanna governorates from February 2014 to January 2015 and labeled before brought to laboratory for processing according to the standard methods. All patients were (1-50) years for both genders and clinically diagnosed as suspected bloody diarrhea and urinary tract infection. A

total of 75 stool samples and 75 urine samples are collected .

All the samples were collected in sterile containers. The stool samples were collected in sterile screw capped test tubes and the urine samples were collected from patients and instructed on hand to collect the midstream urine into sterile bottles. The samples were then transported to the laboratory with ice packs in sterile container.

Processing of the samples

Primary growth

Primary growth of all kinds of bacteria is performed in nutrient broth , MacConkey agar , Eosin methylene blue agar and Chromagar *E.coli* O157:H7. Biochemical confirmation of *Escherichia coli* O157 suspect colonies by Api 20E (Merchant and Packer, 1967 and Ewing, 1986).

Results and Discussion

Phenotypic characteristics of *E.coli* O157:H7:-

Colonies Characteristics

The samples were collected and promptly inoculated onto McConkey agar. After overnight incubation, bright pink or red colonies were chosen to be *E. coli*.

The presumptive colony on McConkey agar was subcultured successively onto Eosin Methylene Blue (EMB) agar for presumptive identification of *E. coli*. The greenish-black colonies with metallic sheen on EMB agar were presumptively selected *E. coli*. This result was similar to bacterial colonies of *E.coli* recorded by Adam *et al.*, (2008) and Son *et al.*,(2000).

Culturing on Chrom agar™ *E .coli* O157: H7:

The culturing of isolated *E.coli* on CHROM agar showed different color colonies like *Ecoli* O157:H7 showed mauve color, while other species of *E.coli* showed blue color colony .

Our results showed the importance of Chrom agar media in diagnosis of *E.coli* O157:H7. Similar results are reported by other researchers. Bettelheim, (1998) found that *E. coli* O157:H7 utilizes one of chromogenic substrates which produce mauve colored colonies. The growth of mauve colored colonies is considered presumptive identification for *E. coli* O157:H7 on Chrom agar™ O157. Non-*E. coli* O157:H7 bacteria may utilize other chromogenic substrates resulting in blue to blue green colored colonies or, if none of the chromogenic substrates are utilized, colonies may appear as a natural color. Tarr *et al* .,2005 ; Philips *et al* .,(2005) showed that The improved diagnostic performance and efficiency of Chrom agar would allow more appropriate management of *E. coli* O157 cases and outbreaks. A similar study of Tavakoli *et al.*, (2008) showed that chromogenic media have more advantages and can be an appropriate alternative for conventional and routine procedure. Researches conducted all over the world have shown that these techniques have higher specifically, exclusivity and performance as compared with other techniques and indicated that Chrom agar is an effective supplemental medium for the isolation of probable STEC strains (Ngwa *et al* ., 2013).

Biochemical characteristics (Api 20 E System).

Based on this system, a total of 120/150 (82.7%) of the total bloody stool and urine samples were diagnosed as *E. coli* while the proportion of other species of bacteria which was diagnosed in the Api 20 E system showed that coliform 16(11%) and *Proteus* 9(6.3%) as shown in the table (2,5) (Quinn *et al* 2004).

Table (5): Biochemical characteristics of *E.coli* isolated from bloody stool and urine samples (Api 20 E system).

Bacteria	O N P G	A D H	L D C	O D C	C I T	H 2 S	U R E	T D A	I N D	V P	G E L	G L U	M A N	I N O	S O R	R H A	S A C	M E L	A M Y	A R A	Culture no.
<i>Escherichia coli</i>	+	-	+	+	-	-	-	-	+	-	-	+	+	-	+	+	+	+	-	+	8101

(+): positive result

(-): negative result

In this research, a total of 150 specimens were collected and examined from patients who suffered from bloody diarrhea and urinary tract infection. The results revealed that the percent of *E.coli* O157:H7 were (89.3% , 70.6%) from bloody stool and urine samples respectively (Table 1) .

E. coli O157:H7 infection has been reported in 2–7% of sporadic cases and 20% of outbreaks in the world (Paton and Paton, 1998).

Table (1): Number and percent of isolates of *E.coli* O157:H7 from different clinical samples (n=150) .

Clinical samples	No. of tested specimens	No. of +ve samples	%	No. of –ve samples	%
Bloody stool	75	67	89.3	8	10.6
Urine	75	53	70.6	22	29.3
Total	150				

The percent of isolation revealed that the most prevalent bacterial isolates from bloody stool and urine samples are *E.coli* O157:H7 120/150 (80%) , coliform 21/150 (14%) and *Proteus* 9/150 (6%) (table 2). In Turkey, there is insufficient information about the prevalence of *E. coli* O157 in humans. However, a prevalence of 0–4% has been reported in recent studies (Erdog˘an *et al.*,2008; Eksxi *et al.*, 2007; Gu˘ney *et al.*, 2001; Hascelik *et al.*, 1991).

Table (2): Distribution of isolated bacterial species in bloody stool and urine specimens (n=150) .

Bacterial species	No. of isolates (+ve)	% of isolation
<i>E.coli</i> O157:H7	120	80
coliform	21	14
<i>Proteus</i>	9	6
Total	150	100

The total clinical samples with bloody diarrhea according to the age groups were (8.95%, 13.43%, 14.92%, 10.44%, 11.9%, 7.46%, 4.47%, 5.97%, 11.9%, 10.44%) respectively. The majority of cases occur between the ages (10-14) years . While, the total urinary tract infection according to the age groups were (3.77%, 13.2%, 9.43%, 5.66%, 22.6%, 11.3%, 15%, 9.43%, 7.5%,1.88%) respectively. The majority of cases occur between the ages (20-24) years (Table 3).

This evidence was in agreement with AlWgaa,(2014) who recorded that 8(3.50%) out of 228 urine samples of humans were *E.coli* O157:H7 positive isolates, also the present study found that the percentage of *E.coli* O157:H7 isolates from stool samples of young patients was higher than those recorded in adult patients, this result may be due to development of acquired immunity in adults or due to difference in concentration of Globotriaosylceramide (Gb3) in digestive system according to age, this result was in agreement with Paton and Paton,(1998) who showed the ratio of *E.coli* O157:H7 infection in children was higher as compared with adults.

Also Faten, (2013) collected 230 stool samples from diarrhetic children and she showed that 14(6%) out of 230 were positive *E.coli* O157:H7. Slutsker *et al*; (1997) found that the highest age-specific isolation proportions from fecal specimens for *E. coli* O157:H7 were in patients at 5 to 9 years of age (0.90%) and 50 to 59 years of age (0.89%).

Table (3): Age distribution of patients enrolled in the research of bloody diarrhea and urinary tract infection caused *E.coli* O157:H7.

Age (years)	Bloody diarrhea				Urinary tract infection				Total	
	+ve samples		-ve samples		+ve samples		-ve samples		No.	%
	No.	%	No.	%	No.	%	No.	%		
1-4	6	8.95	0	0	2	3.77	1	4.5	9	6.1
5-9	9	13.43	1	12.5	7	13.2	3	13.63	20	13.3
10-14	10	14.92	1	12.5	5	9.43	6	27.27	22	14.6
15-19	7	10.44	2	25	3	5.66	4	18.18	16	10.6
20-24	8	11.9	0	0	12	22.6	1	4.5	21	14
25-29	5	7.46	1	12.5	6	11.3	5	22.7	17	11.3
30-34	3	4.47	0	0	8	15	0	0	11	7.3
35-39	4	5.97	2	25	5	9.43	1	4.5	12	8.1
40-44	8	11.9	0	0	4	7.5	0	0	12	8.1
45-50	7	10.44	1	12.5	1	1.88	1	4.5	10	6.6
Total	67	100	8	100	53	100	22	100	150	100

Out of 75 bloody diarrhea samples were 67 positive for *E. coli* O157:H7. The males' samples were 25 (37.4%) . While , the females' samples were 42 (62.6%) . The higher proportion of the occurrence was found among the females' samples (Table 4).

The percent of isolation from urine samples were 53/75(70.6%) according to the gender groups. The males' samples were 37 (69.9%) . While , the females' samples were 16 (30.18%) . The higher proportion of the occurrence was found among the males sampled (Table 4).

It was reported in the present study that the ratio of bacterial isolates from male patients was more than those isolated from the female ,this result was in agreement with results of the CDC Atlanta surveys 2009 that found *E.coli* O157:H7 in males were more than in females .

We Suggested that the males were more exposed to sources of infection by this pathogens ,this evidence was coincident with the results of bacterial distribution among rural and urban patients, in the present study , that investigated a high number of *E.coli* O157:H7 isolates in the former as compared with later one .this result may be due to the rural individuals were more in contact with sources of infections such as carrier animals and their dairy or meat products.

This evidence was in agreement with Giacometti *et al.*,(2012) who recorded that 10% of systemic infection was associated with consumption of raw milk, also Ijaz,(2013) recorded that unpasteurized milk was considered essential route of the shigatoxigenic group of *Escherichia coli* (STEC) O157 infection.

Table (4): Rate of occurrence of *E. coli* O157 among bloody diarrhea patients and urinary tract infection based on gender.

Patients	Bloody diarrhea	%	Urinary tract infection	%	Total
Male	25	37.4	37	69.9	62
Female	42	62.6	16	30.18	58
Total	67	100	53	100	120

Conclusions

The direct examination alone is not efficient in the diagnosis of bloody diarrhea and urinary tract infection and this emphasizes the need for laboratory culture of specimens on the differential media such as Chromagar *E.coli* O157:H7 and Api 20E.

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