Dicle Tıp Dergisi /
Dicle Medical Journal

2016; 43 (2): 205-211
doi: 10.5798/diclemedj.0921.2016.02.0668

ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

The Investigation of Pathogenic *E. coli* serogroups in Patients with Diarrhea

Diyareli Hastalarda Patojenik E. coli Serogruplarının Araştırılması

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ABSTRACT

Objective: Diarrheal diseases express a major health problem especially in developing countries. The real reasons of diarrheal disease are largely due to low hygiene or sanitation as well as low budgets of primary and secondary health care. In this study, it was aimed to determine the presence of bacterial enteropathogens especially diarrheagenic *Escherichia coli* serogroups in stool samples taken from patients with diarrhea in our geographic region.

Methods: 343 stool samples were collected from the patients who were diarrhea. Stool samples were subjected to macroscopic and microscopic examinations, and then were cultured into EMB Agar, MacConkey Agar and Selenite F to isolate and distinguish *E.coli* from other intestinal pathogens. Finally, all isolated *E.coli* species were identified by using specific antisera.

Results: 343 (156 female, 187 male) stool samples were bacteriologically and parasitologically examined. Only *E. coli* presence was detected in 262 (76.4%) samples. 77 (29.4%) of total isolated 262 *E.coli* strains were identified with latex agglutination test. Most common EHEC, EPEC, ETEC and EIEC strains were detected as following; O26, O55, O128 and O152 respectively. *E.coli* O157:H7 serovar was not detected.

Conclusion: As a consequent, just usage of O antisera (except H7) is not adequate to detect all the pathogenic bacteria. However, determination of bacterial serogroups which often seen in a region may lead to draw the attention of the clinicians on these bacteria and provide an opportunity for more accurate diagnosis and treatment. The main way to prevent diarrheal *E. coli* infections is to obey the hygiene rules.

Key words: Diarrhea, *E. coli*, O and H antigens

ÖZET

Amaç: Diyare ile ilişkili hastalıklar başta gelişmekte olan ülkeler olmak üzere birçok ülkede ciddi bir sağlık problemidir. Diyare ile ilişkili hastalıkların gerçek nedeni, birincil ve ikincil sağlık hizmetlerinin düşük bütçelerinin yanı sıra sanitasyon ve kötü hijyen olarak tespit edilmiştir. Bu çalışmada, bölgemizdeki ishalli hastalardan alınan dışkı örneklerinde, başta ishal oluşturan *Escherichia coli* suşları olmak üzere bakteriyel enteropatojenlerin varlığının tespit edilmesi amaçlanmıştır.

Yöntemler: İshal şikayeti olan 343 hastadan dışkı örnekleri toplandı. Toplanan dışkı örnekleri ilk olarak makroskobik ve mikroskobik incelemeye tabi tutuldu. Ardından, örneklerin EMB Agar, MacConkey Agar and Selenite F besiyerlerine ekim işlemleri gerçekleştirildi. Son olarak, E.coli bakterileri spesifik antiserumlar kullanılarak tanımlandı.

Bulgular: Parazitolojik ve bakteriyolojik incelemeye tabi tutulan 343 dışkı örneğinin 156'sı kadın, 187'si erkek hastalara aitti. 262 dışkı örneğinde (%76,4) yalnızca E.coli bakterisi tespit edildi. E.coli tespit edilen 262 hastanın 77'sinde (%29,4) lateks aglütinasyon yöntemi ile sınıflandırma yapıldı. En çok tespit edilen EHEC, EPEC, ETEC ve EIEC suşları sırasıyla O26, O55, O128 ve O152 alt serotipleri olarak tanımlandı. Bu çalışmada, E.coli O157:H7 suşu tespit edilmedi.

Sonuç: Sonuç olarak, sadece O antiserumunun kullanılması tüm patojenik bakterilerin tanımlanması için yeterli olmamaktadır. Ancak, bölgemizdeki bakteriyel serogrupların tanımlanması, konu hakkında klinisyenlerin dikkatini çekecek ve doğru tanı ve tedavi konusunda daha doğru sonuçlara ulaşılmasına olanak sağlayacaktır. İshal oluşturan *E.coli* suşlarının sebep olduğu infeksiyonların önüne geçebilmenin en temel yolu, hijyen kurallarına özen göstermektir.

Anahtar kelimeler: Diyare, *E.coli*, O ve H antijenleri

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Geliş Tarihi / Received: 23.03.2016, Kabul Tarihi / Accepted: 11.04.2016

INTRODUCTION

Diarrheal diseases express a major health problem especially in developing countries and are also a risk for tourists who visit these countries [1]. It has been predicted that more than one billion incidents of diarrhea occur annually and it causes nearly two million deaths per year [2]. The real reasons of diarrheal disease in developing countries are largely due to low hygiene or sanitation as well as low budgets of primary and secondary health care [3]. Additionally, some bacterial agents of infectious diarrhea may cause critical long-term problems including Guillain-Barre syndrome, Hemolytic Uremic Syndrome and malnutrition. The wide varieties of bacterial agents that may lead diarrheal complications confirm surveillance and diagnosis. Moreover, describing etiology of acute diarrhea is important to therapy and prevention of this disease [4].

Diarrhea is caused by enteric pathogens including bacteria, viruses, parasites and fungi. The clinical appearance can be described with vomiting, watery or semi-formed stool or bloody stool that can be accompanied by systemic symptoms like fever, fatigue, nausea and malaise. The pathogenesis of bacterial diarrhea depends on adherence, enterotoxins and colonization factors [5]. The important causes of bacterial diarrhea are diarrheagenic *Escherichia coli* (DEC), Campylobacter spp., Salmonella spp., Shigella spp., Vibrio spp., *Yersinia enterocolitica* and *Clostridium difficile* [6]. DEC strains are divided into six pathotypes: enteropathogenic *E.coli* (EPEC), Shiga toxin-producing (or enterohemorrhagic) *E.coli* (STEC or EHEC), enterotoxigenic *E.coli* (ETEC), enteroaggregative *E.coli* (EAEC), enteroinvasive *E.coli* (EIEC) and diffusely adherent *E.coli* (DAEC) [7].

Acute gastroenteritis occurred by bacteria and parasites are one of the most seen diseases in our country and our region [8]. Routine laboratory diagnosis of most enteritis factors can be performed; however, there are some problems about the microbiological diagnosis of viral agents and some bacterial agents especially *E.coli*. In this study, it was aimed to determine the presence of bacterial enteropathogens especially diarrheagenic *Escherichia coli* serogroups in stool samples from patients with diarrhea in our geographic region.

METHODOLOGY

Study design

The patients were selected from persons with diarrhea complaints admitted to different clinics of Ataturk University, Faculty of Medicine, Erzurum, Turkey. A total of 343 stool samples were collected from the patients who were diarrhea. The main part of this study was to identify the pathogenic *E.coli* strains which were detected after bacteriological and parasitological examinations. In this study, *E.coli* ATCC-12798 was used as a control strain.

Mediums

Selenite F Broth, Sheep Blood Agar (SBA), Eosin Methylene Blue Agar (EMB) and MacConkey Agar were used to isolate *E.coli* or other bacterial pathogens from the patient's stool samples and Sorbitol-MacConkey Agar (SMAC), Triple Sugar Iron (TSI) Agar, Mannitol Agar, Simmons Citrate Agar, Tryptophan Broth, Urea Indole Broth and Clarks-Lubs Broth were used to determine the biochemical properties of these isolated bacteria in this study.

Commercial Kits

Monovalent antisera (SEIKEN, Denka Seiken Company, Tokyo, Japan) which were obtained from rabbits and contained 0.08% sodium azide in 1 milliliter as a preserver were used to detect *E.coli* strains. The names of antisera were *E.coli* O8, O25, O26, O55, O78, O111, O115, O124, O125, O126, O127a, O128, O136, O142, O152, O157 and *E.coli* H7 respectively.

Macroscopic and Microscopic Examinations

Stool samples were macroscopically examined in terms of consistency, color, bloody or mucoid forms and detecting adult helminthes forms. On the other hand, parasitological examination was performed to detect cystic and trophozoite forms of protozoa and helminth eggs under the microscope by 10X and 40X magnification. During microscopic examination, leukocytes, erythrocytes and yeast cells were detected as well as some parasites. These findings were recorded on the evaluation form.

Culture

Stool samples were cultured to EMB Agar, MacConkey Agar and Selenite F Broth to isolate and distinguish *E.coli* from other intestinal pathogens.

After 6-8 hours, new passages were performed from Selenite F Broth to MacConkey Agar. Then, plates were left to aerobic incubation at 37°C for 24 hours. Then, morphological examination and some specific tests (TSI Agar, Mannitol Agar, Simmons Citrate Agar, Tryptophan Broth, Urea Indole Broth and Clarks-Lubs Broth) were performed to identify the bacteria. All findings were recorded to evaluation forms.

Predominant *E.coli* colonies which were cultured on EMB Agar and MacConkey Agar were subcultured to SMAC Agar for preliminary determination of *E.coli* O157:H7 and incubated at 37°C for 24 hours. Then, *E.coli* O157:H7-suspected transparent colonies presence was investigated.

E.coli Serogroups Determination

E.coli serogroups were identified by using specific antisera (SEIKEN, Tokyo, Japan). At first, for detection O antigens, 8-10 bacterial colonies suspended in 3 ml physiological saline and heated 100°C for 1 hour. Then; heated suspension centrifuged at 900 g for 20 minutes, supernatant discarded, and precipitate suspended with 0.5 ml physiological saline (antigenic suspension) respectively. A drop each of monovalent serums placed onto a cleaned glass slide. An antigenic suspension (5-10 µl) placed onto the serum on the glass slide. Finally, the reagents mixed by tilting the glass slide back and front for 1 minute and agglutination pattern were observed. For detection H7 antigen, 3 drops of H7 antiserum were put into separate test tubes using the syringe attached to the containers and then 0.5 ml of the cell suspension were added to each. After mixing thoroughly, the tubes were kept in a water bath (50°C) for 1 hour and agglutination was observed.

Statistical Analysis

The statistical analysis (Chi-square (χ^2) test) was performed by using SPSS for Windows Version 17.0 (Statistical Package for Social Sciences version 17.0).

RESULTS

Culture Results

A total of 343 (156 female, 187 male) patient's stool samples were bacteriologically and parasitologically examined. 26 (7.6%) samples were detected as negative in terms of any bacterial or parasitic agents. Only *E.coli* presence was detected in 262 (76.4%) stool samples while only other bacterial/parasitic agent's presence was detected in 55 (16%) stool samples.

Serogroups Identification Results

77 (29.4%) of total isolated 262 *E.coli* strains were identified with latex agglutination test. *E.coli* O157:H7 serovar was not detected in this study. The distribution of *E.coli* strains is seen in Table 1.

Table 1. The distribution of *E.coli* strains in diarrheal patients

Category	Number	%
Enteropathogenic *E.coli* (EPEC)	32	41.5
Enterotoxigenic (ETEC)	23	29.9
Enterohemorrhagic *E.coli* (EHEC (O26))	13	16.9
Enteroinvasive *E.coli* (EIEC)	9	11.7
Total	77	100

The most common EHEC strain was O26 (16.9%); EPEC strain was O55 (15.6%); ETEC strain was O128 (7.8%) and EIEC strain was O152 (5.2%). There was no significantly difference between *E.coli* strains and gender. The distribution of *E.coli* serogroups according to the gender is seen in Table 2.

Table 2. The relationship between *E.coli* serogroups and gender

Serogroups	Male	Female	Total	%
O26	7	6	13	16.9
O55	5	7	12	15.6
O111	5	3	8	10.4
O142	3	2	5	6.5
O8	3	1	4	5.2
O25	1	4	5	6.5
O78	1	1	2	2.6
O115	2	1	3	3.9
O125	1	2	3	6.5
O126	2	3	5	2.6
O127a	1	1	2	7.8
O128	3	3	6	3.9
O124	1	2	3	2.6
O136	1	1	2	5.2
O152	2	2	4	0
O157	0	0	0	100
Total	38	39	77	

53 (68.8%) of total *E.coli* strains identified patients was under 16 years-old while 24 (31.2%) of them was above 16. When statistical analyses were performed, it was seen that there was a significant difference between *E.coli* strains and age (p<0,005). *E.coli* strains were significantly higher in children group (under 16 years) (χ^2: 21,844; p<0,005) (Table 3).

Table 3. The relationship between *E.coli* strains and age groups

Strains	Serovar	0-2 years		3-5 years		6-14 years		15+ years	
		n	%	n	%	n	%	n	%
EPEC	O55	5	6.5	3	3.9	0	0	4	5.2
	O111	6	7.8	1	1.3	0	0	1	1.3
	O126	1	1.3	1	1.3	1	1.3	2	2.6
	O127a	2	2.6	0	0	0	0	0	0
	O142	2	2.6	1	1.3	0	0	2	2.6
Total		16	20.8	6	7.8	1	1.3	9	11.7
ETEC	O8	3	3.9	0	0	0	0	1	1.3
	O25	1	1.3	1	1.3	2	2.6	1	1.3
	O78	0	0	0	0	0	0	2	2.6
	O115	1	1.3	1	1.3	1	1.3	0	0
	O125	0	0	0	0	2	2.6	1	1.3
	O128	2	2.6	1	1.3	2	2.6	1	1.3
Total		7	9.1	3	3.9	7	9.1	6	7.8
EIEC	O124	1	1.3	0	0	0	0	2	2.6
	O136	0	0	0	0	0	0	2	2.6
	O152	0	0	1	1.3	0	0	3	3.9
Total		1	1.3	1	1.3	0	0	7	9.1
EHEC	O26	9	11.7	2	2.6	0	0	2	2.6
	O157:H7	0	0	0	0	0	0	0	0
Total		9	11.7	2	2.6	0	0	2	2.6
Grand Total		33	42.8	12	15.6	8	10.4	24	31.2

Microscopic Results

Leukocytes and erythrocyte positivity were detected during microscopic examination of stool samples. A majority of leukocytes positivity was seen in samples which EPEC strains were isolated. Erythrocyte positivity was not seen only in samples which ETEC strains were isolated (Table 4).

On the other hand, there were some other microbial agents except *E.coli* strains. The most common protozoon was *Giardia lamblia* (8.2%) for this study. *Entamoeba histolytica* is known as one of the most common agent which may lead enteritis, was detected at low rate (0.6%). *Trichomonas vaginalis*, is one of the infectious agent, was only detected in 1 patient (0.3%). Some bacterial agents such as *Shigella* spp. was detected at high rate while *Salmonella* spp. was detected at low rate. The most common (0.9%) helminthes was *Ascaris lumbricoides* (Table 5).

Table 4. Leukocytes and erythrocytes positivity after the microscopic examinations

Category	Leukocytes positivity		Erythrocytes positivity	
	Number	%	Number	%
EPEC (n=32)	29	90.6	4	12.5
ETEC (n=23)	9	32.1	0	0
EHEC (n=13)	7	53.8	1	7.7
EIEC (n=9)	9	100	4	44.4
Total	54	70.1	9	11.7

Table 5. The microbial agents that isolated from stool samples and their rates

Isolated Microorganisms	Positivity	
	n	%
Giardia lamblia	28	8.2
Shigella sonnei	8	2.3
Shigella dysenteriae	3	0.9
Shigella flexneri	3	0.9
Candida albicans	4	1.2
Ascaris lumbricoides	3	0.9
Entamoeba histolytica	2	0.6
Salmonella typhi	1	0.3
Hymenolepis nana	1	0.3
Taenia saginata	1	0.3
Trichomonas vaginalis	1	0.3
Total	55	16.2

DISCUSSION

Diarrheal diseases are really associated with high mortality and morbidity in both endemic and epidemic settings especially in infants and children all over the world. Additionally, it has been estimated that nearly 12.000 children die a day in Asia, Africa and Latin America continents. Mostly viruses, bacteria and parasites may lead diarrhea; however, ETEC and rotaviruses are the most common microbial agents in developing countries while *Norwalk virus*, *Campylobacter jejuni* ve cytotoxigenic *Clostridium difficile* are most common in developed countries. *Shigella, Salmonella, Cryptosporidium, Giardia* species are the most isolated other diarrhea agents [9-11].

Acute gastroenteritis, occurred by bacteria and parasites, are one of the most seen diseases in our country, our region. Our aim was to determine the presence of bacterial enteropathogens especially diarrheagenic *E. coli* serogroups in stool samples from patients with diarrhea, aiming to establish the prevalence of them in our geographic region. Total 343 samples were collected and 262 *E. coli* strains, 55 other microbial agents were isolated. 77 *E. coli* strains (32 EPEC, 23 ETEC, 13 EHEC and 9 EIEC) were identified by using latex agglutination test. When the current data were checked, our findings were parallel with Robins et al. findings [12]. Although, the obtained data were nearly similar with many studies in the literature, the distribution rate of microbial agents which may lead gastroenteritis may change from country to country, region to region. Bacterial agents are mostly responsible for the etiology of the disease in developed countries while viral agents are mostly responsible in developing countries [10,13].

There was no *E.coli* O157:H7 and EAEC strains in our study; however, in one study, 9 verotoxigenic *E.coli* O157:H7 strains (VTEC) were detected as the diarrhea agent [14]. Another study performed in Netherland reported that Shiga-toxigenic *E.coli* O157:H7 strains (STEC) were detected from 1250 diarrhea incidents every year for 10 years [15]. On the other hand, EAEC O126:H7 strain was responsible from diarrhea in hospitalized children in a study performed in Israel [16].

Some studies in the current literature about *E.coli* serogroups identification were nearly containing same findings with our study [7,17-20]. These studies reported that ETEC, EHEC, EPEC and EIEC were identified from *E.coli* positive stool samples. On the other hand, in this study, EHEC O26 serovar, EPEC O55 serovar, ETEC O128 serovar and EIEC O152 serovar were the most identified serovar. Parallel with these findings, 2 studies reported from our country that EPEC O55 is one of the most isolated *E.coli* serovar [21,22].

According to some literature findings, diarrhea agent's incidence has been changed. In our study (Table 5), most common other diarrhea agent was *Giardia, Shigella species, Candida, Ascaris, Entamoeba* and *Salmonella* respectively. However, *Vibrio cholera, Shigella dysenteriae* and *rotavirus* were mostly common isolated other diarrhea agents in Indian children while *Campylobacter, Salmonella, Shigella*, *Vibrio* and *Plesiomonas* were most common isolated other diarrhea agents in Thai children [23, 24]. This means that diarrhea agent incidence may be variable from region to region. *E.coli* infections are also effect children who are under 2 years as well as in all acute diarrheal diseases. Our findings showed that there were significantly differences between *E.coli* strains and age groups (Table 3). *E.coli* strains were isolated higher in children than teenagers. Same findings from literature are available that younger ages are more under at risk for diarrheal death [25].

There are some differences in terms of isolated diarrheal agents in our country. Isolated microorganisms and isolation rates may vary from region to region. Parallel with this, these factors may be different from country to country. The reasons of this variety are the lack of studies to determine the viral enteritis agents and not to routinely investigate all bacterial pathogens. Some disruptions such as not to transport the samples to laboratory in time and under appropriate conditions, lack of technical knowledge and equipment may affect the test results. In this study, our aim was to investigate the prevalence of *E.coli* strains from diarrheal patients. However, we also tried to identify other gastrointestinal system pathogens. As a summary, most common *E.coli* serogroups were EPEC, ETEC, EHEC, EIEC and most common serovars were O26, O55, O111 and O128 respectively in our region. Additionally, we determined that these pathogens were most isolated from 0-2 year's age group.

As a consequent, just usage of O antisera (except H7) is not adequate to detect all the pathogenic bacteria. H antigen serotyping and other virulence factors detection methods should be used for healthy data. However, determination of bacterial serogroups which often seen in a region may lead to draw the attention of the clinicians on these bacteria and provide an opportunity for more accurate diagnosis and treatment. The main way to prevent diarrheal *E. coli* infections is to obey the hygiene rules. The importance of true hand washing, toilet and body cleaning after defecation should be described efficiently not only for patients but also whole community. Otherwise, scientifically; it should be performed more efficient study to find the possible mechanisms of these infections and possible treatment alternatives.

Acknowledgments

We acknowledge the invaluable support of staff of the laboratory at the Ataturk University, Faculty of Medicine and Department of Microbiology in Erzurum.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

Financial Disclosure: No financial support was received.

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