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The Frequency of Diarrheagenic *Escherichia coli* Isolates in Children with Acute Diarrhea

Akut İshalli Çocuklarda Diyarejenik *Escherichia coli* İzolatlarının Sıklığı

Ayşegül Koç(İD), Salih Maçin(İD), Hatice Türk Dağı(İD)

Department of Medical Microbiology, Selçuk University Faculty of Medicine, Konya, Türkiye

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Abstract

Objective: This study aimed to detect the frequency of diarrheagenic *Escherichia coli* pathotypes [enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) Shiga toxin-producing *E. coli (STEC)*, enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC) and diffuse adherence *E. coli* (DAEC)] in children with acute diarrhea under five years in Konya, Türkiye.

Material and Methods: Three thousand and sixty-four stool samples taken from children with acute diarrhea under the age of five years sent to the Medical Microbiology Laboratory of the Medical Faculty Hospital, Selçuk University between 1 January and 31 December 2019 were scanned. *E. coli* isolates were included in the study that predominantly growing in the feces of 312 children. *E. coli* was defined by conventional methods and VITEK® 2, compact automated system. DNA isolation was performed with a commercial kit. The primers of *eae*, *pEAF*, *daaC*, *aatA*, *elt*, *est*, *ipaH*, *stx1* and *stx2* genes were studied by real-time multiplex polymerase chain reaction (PCR) method. Detection of *eae* plus *pEAF* genes was accepted typical EPEC (tEPEC), only *eae* atypical EPEC (aEPEC), *stx*1 and/or *stx*2 genes STEC, *eae* plus *stx1* and/or *stx2* genes EHEC, *elt* and/or *est* genes ETEC, *ipaH* gene EIEC, *aatA* gene EAEC and *daaC* gene DAEC. Results were confirmed by monoplex PCR and gel electrophoresis.

Results: Of the 312 *E. coli* isolates, 56 had single pathotype, 254 had mixed pathotypes and two isolates could not be typed. Two hundred and eighty-one DAEC, 187 EPEC, 159 EAEC, 43 EIEC, 31 EHEC, four ETEC and two STEC were detected in 312 samples. The most frequently detected single pathotypes were DAEC (47) and EAEC (7), while mixed pathotypes were DAEC+EPEC+EAEC (108), DAEC+EPEC (58) and DAEC+EIEC (24) respectively.

Öz

Giriş: Bu çalışmanın amacı Konya, Türkiye'de, beş yaş altı çocuklarda 2019 yılında, akut ishale yol açan diyarejenik *Escherichia coli* patotiplerinin [enteropatojenik *E. coli* (EPEC), enterotoksijenik *E. coli* (ETEC), enteroinvaziv *E. coli* (EIEC), Shiga toksin üreten *E. coli* (STEC), enterohemorajik *E. coli* (EHEC), enteroagregatif *E. coli* (EAEC) ve diffüz aderan *E. coli* (DAEC)] sıklığının araştırılmasıdır.

Gereç ve Yöntemler: Selçuk Üniversitesi Tıp Fakültesi Hastanesi, Tıbbi Mikrobiyoloji Laboratuvarına 1 Ocak-31 Aralık 2019 tarihleri arasında gönderilen akut ishalli beş yaş altı çocuklardan alınan 3064 dışkı örneği tarandı. Üç yüz on iki çocuğun dışkısında baskın üreyen *E. coli* izolatları çalışmaya alındı. *E. coli* konvansiyonel yöntemler ve VITEK® 2 compact otomatize sistemi ile tanımlandı. DNA izolasyonu, ticari bir kitle yapıldı. *eae*, *pEAF*, *daaC*, *aatA*, *elt*, *est*, *ipaH*, *stx*1 ve *stx*2 primerleri gerçek zamanlı multipleks polimer zincir reaksiyonu (PZR) yöntemi ile çalışıldı. Bir izolatta *eae* genin saptanması aEPEC, *eae* ve *pEAF* genlerinin saptanması tEPEC, *stx*1 ve/veya *stx*2 genlerinin saptanması STEC, *eae*, *stx*1 ve/veya *stx*2 genlerinin saptanması EHEC, *elt* ve/veya *est* genlerinin saptanması ETEC, *ipaH* genin saptanması EIEC, *aatA* genin saptanması EAEC ve *daaC* geninin saptanması DAEC olarak tanımlandı. Gerçek zamanlı multipleks PZR, monopleks PZR ve jel elektroforezi ile doğrulandı.

Bulgular: Toplam 312 *E. coli* izolatının 56'sında tek, 254'ünde miks patotip saptanırken iki izolat tiplendirilemedi. Üç yüz on örnekte 281 DAEC, 187 EPEC, 159 EAEC, 43 EIEC, 31 EHEC, dört ETEC ve iki STEC saptandı. En sık tespit edilen tekli patotipler DAEC (47) ve EAEC (7) iken miks patotipler DAEC+EPEC+EAEC (108), DAEC+EPEC (58) ve DAEC+EIEC (24) olarak belirlendi.

Correspondence Address/Yazışma Adresi

Salih Maçin

Department of Medical Microbiology,
Selçuk University Faculty of Medicine,
Konya, Türkiye

E-mail: salihmacin@hotmail.com

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Conclusion: DAEC was single and also DAEC plus tEPEC/aEPEC plus EAEC were mixed predominant pathotypes in the diarrheagenic *E. coli* isolates in children with acute diarrhea under five years in Konya, Türkiye. This is the first study to investigate all diarrheagenic *E. coli* pathotypes in Türkiye. The most important limitation of our study is that single-centered. Multicentre studies are needed to determine which pathotypes are common in Türkiye.

Keywords: Acute diarrhea, diarrheagenic *Escherichia coli*, multiplex polymerase chain reaction

Sonuç: Konya, Türkiye'de beş yaş altı akut ishalli çocuklarda DAEC'in baskın patotip olduğu ve miksenfeksiyonlar da ise en sık DAEC+EPEC+EAEC birlikteliği saptanmıştır. Bu çalışma Türkiye'de tüm diyarejenik *E. coli* patotiplerinin araştırıldığı ilk çalışmadır. Çalışmamızın en önemli kısıtlılığı tek merkezli olmasıdır. Türkiye'de hangi patotiplerin yaygın olduğunun belirlenmesi için çok merkezli çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Akut ishal, diyarejenik *Escherichia coli*, multipleks polimeraz zincir reaksiyon

Introduction

Acute diarrhea is the most common cause of childhood morbidity and mortality after lower respiratory tract infections and is an important public health problem for the whole world (1). Each year, 1.700.000 diarrhea cases are reported worldwide, with 525.000 deaths in children aged 0-5 years (2). Various bacterial agents, including *Escherichia coli, Salmonella*, *Shigella, Clostridioides difficile* and *Campylobacter* spp.; and viral agents, including rotavirus, adenovirus and norwalk virus; protozoal parasites, *Giardia lamblia*, *Cryptosporidium parvum/hominis*, *Blastocystis hominis*, might lead to diarrhea (3). Among the bacterial agents, diarrheagenic *E. coli* (DEC) is one of the main causes of diarrhea in developing countries, especially in children under the age of five (4).

DEC is an important cause of diarrhea in children (5). DEC isolates have various types of pathogens that cause different clinical pictures and carry a number of virulence factors. DEC strains are generally classified into six pathotypes: Enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC) including Shiga toxin producing *E. coli* (STEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffuse adherent *E. coli* (DAEC). EPEC strains are divided into typical EPEC (tEPEC) and atypical EPEC (aEPEC) strains based on the presence or absence of the EAF plasmid (6).

The role of some types of *E. coli* in the etiology of acute diarrhea is better understood every day. Detection of virulence factors that distinguish pathogen *E. coli* pathotypes from normal intestinal microbiota elements in children under five years of age with acute diarrhea is important for the diagnosis and treatment of the disease.

Data on the prevalence and importance of the pathogen *E. coli* that cause diarrhea in our country is limited, and the identification of these strains will contribute significantly to epidemiological studies, diagnosis and treatment of the disease. The prevalence and epidemiological characteristics of these pathogens differ in different regions even within the same country. The aim of this study is to investigate the frequency of pathotypes of DEC (EPEC, ETEC, EIEC, STEC, EHEC, EAEC and DAEC) in children under five years of age with acute diarrhea in Konya, Türkiye.

Materials and Methods

Watery, bloody or mucoid stool samples of 3064 patients sent to Selçuk University Medical Faculty Hospital Medical Microbiology Laboratory between 1 January-31 December, 2019 were scanned in children under the age of five. Stool samples of 310 children with acute diarrhea without *Salmonella*, *Shigella*, *Campylobacter*, rotavirus, adenovirus and parasites were included in the study. *E. coli* isolates were identified with conventional methods (motility, indole, simmons citrate, TSI, urease and oxidase tests) and VITEK® 2 automated system (Biomerieux, France).

DNA isolation was performed according to Genomic DNA isolation kit (RTALABS, Türkiye) protocol. The extracts were transferred to sterile eppendorf tubes and stored at -40°C until molecular analysis was performed. Negative and extraction controls were used in each run.

Real-time multiplex polymerase chain reaction (PCR) was performed on Light-Cycler 96 (Roche, Germany) thermal cycler. Each 40 µl reaction mix contains 20 µl Fast essential DNA green master mix (Roche, Switzerland) and 2 µl DNA. Using Win 7+Win XP operating systems, amplification curves with absolute quantification module and melting curves with TM calling end point genotyping HRM, Qual Detection were analyzed.

Primers of intimin (*eae*), a bundle-forming pilus geni (*pEAF*), diffusely adherent gene (*daaC)*, aggregative gene (*aatA)*, enterolable toxin *(elt)*, enterostable toxin (*est)*, invasive plasmid antigen H (*ipaH)*, Shiga toxin 1 (*stx*1) and Shiga toxin 2 (*stx*2) were studied by real time multiplex PCR method. Melting analysis showed nine different peaks for all target genes. Detection of *eae*+*pEAF* genes tEPEC, only *eae* gene aEPEC, *stx*1 and/or *stx*2 genes STEC, *eae*+*stx*1 and/or *stx*2 genes EHEC, *elt* and/or *est* genes ETEC, the *ipaH* gene EIEC, *aatA* gene EAEC and *daaC* gene were accepted as DAEC. Real-time multiplex PCR was confirmed by monoplex PCR and gel electrophoresis.

Our study was approved with the decision of the Ethics Committee of Selçuk University Medical Faculty dated 26.09.2018 and numbered 2018/326.

Statistical Analysis

Chi-square test and Fisher's exact test were used when necessary, using SPSS Statistics 21 (Windows 32 bit) software to statistically analyze the data. $p< 0.05$ was considered statistically significant.

Results

Stool samples of 3064 children were scanned and 310 *E. coli* isolates were pathotyped. DAEC 281 (90.6%), EPEC 187 (60.4%), EAEC 159 (51.3%), EIEC 43 (13.9%), EHEC/STEC 33 (10.6%) and ETEC were detected in four (1.3%) patients.

When the distribution of patients according to age groups was examined, the findings were as such: 100 zero year olds (32.4%), 73 one year olds (23.5%), 50 two year olds (16.1%), 59 three year olds (19.0), 14 four year olds (4.5%), and 14 five year olds (4.5%). The difference between ages was statistically significant (p= 0.000). The number of boys was found to be higher than that of girls [174 (56.1%) boys, 136 (43.9%) girls] the difference between sexes was statistically significant (p= 0.031).

DEC species were detected higher in summer (40.0%) and spring (26.4%) compared to other seasons [autumn (19.4%) and winter (14.2%)]. The highest rates were observed in June (12.3%), July (15.8%), August (11.9%), and the lowest rates were in December (4.5%), January (6.5%) and February (3.2%). The difference between the months and the number of cases was found to be statistically significant (p= 0.000).

The distribution of DEC pathotypes by months is shown in Table 1. The difference between the months and EPEC, EAEC, EIEC, and EHEC was found to be statistically significant (p= 0.000) .

Distribution of DEC pathotypes by age is shown in Table 2. There was no statistically significant difference between the

Table 1. Distribution of diarrheagenic *E.coli* pathotypes by months

Pathotypes / Months	DAEC n (%)	aEPEC n (%)	tEPEC n (%)	EAEC n (%)	EIEC n (%)	EHEC n (%)	STEC n (%)	ETEC n (%)	Total n (%)
January	18 (6.4)	6 (4.0)	0 (0.0)	4 (2.5)	1 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	20 (6.5)
February	9 (3.2)	1 (0.7)	0 (0.0)	2 (1.3)	5 (11.6)	0 (0.0)	0 (0.0)	0 (0.0)	10 (3.2)
March	10 (3.6)	1 (0.7)	0 (0.0)	7 (4.4)	3 (7.0)	0 (0.0)	1 (50.0)	0 (0.0)	13 (4.2)
April	28 (10.0)	2 (1.3)	1 (2.6)	7 (4.4)	21 (48.8)	2 (6.5)	0 (0.0)	0 (0.0)	33 (10.6)
May	31 (11.0)	10 (6.7)	1 (2.6)	14 (8.8)	10 (23.3)	9 (29.0)	1 (50.0)	1 (0.25)	36 (11.6)
June	33 (11.7)	7 (4.7)	24 (63.2)	12 (7.5)	1 (2.3)	7 (22.6)	0 (0.0)	0 (0.0)	38 (12.3)
July	45 (16.0)	30 (20.1)	12 (31.6)	28 (17.6)	1 (2.3)	5 (16.1)	0 (0.0)	2 (0.50)	49 (15.8)
August	35 (12.5)	33 (22.1)	0 (0.0)	29 (18.2)	0 (0.0)	4 (12.9)	0 (0.0)	0 (0.0)	37 (11.9)
September	23 (8.2)	18 (12.1)	0 (0.0)	21 (13.2)	1 (2.3)	4 (12.9)	0 (0.0)	0 (0.0)	23 (7.4)
October	20 (7.1)	20 (13.4)	0 (0.0)	16 (10.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	20 (6.5)
November	17 (6.0)	16 (10.7)	0 (0.0)	12 (7.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.25)	17 (5.5)
December	12 (4.3)	5 (3.4)	0 (0.0)	7 (4.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	14 (4.5)
Total	281 (90.6)	149 (48.1)	38 (12.3)	159 (51.3)	43 (13.9)	31 (10)	2 (0.6)	4 (1.3)	310 (100.0)
p	0.288	0.000	0.000	0.000	0.000	0.011	0.216	0.607	0.000

DAEC: Diffuse adherence *E. coli*, aEPEC: Atypical EPEC, tEPEC: Typical EPEC, EAEC: Enteroaggregative *E. coli*, EIEC: Enteroinvasive *E. coli*, EHEC: Enterohemorrhagic *E. coli*, STEC: Shiga toxin-producing *E. coli*, ETEC: Enterotoxigenic *E. coli*.

Table 2. Distribution of diarrheagenic *E. coli* pathotypes by age

Pathotype	0 n (%)	1 n (%)	2 n (%)	3 n (%)	4 n (%)	5 n (%)	Total n (%)	p
aEPEC	47 (31.5)	37 (24.8)	21 (14.1)	28 (18.8)	9 (6.0)	7 (4.7)	149 (100.0)	0.778
tEPEC	12 (31.6)	10 (26.3)	6 (15.8)	7 (2.3)	0 (0.0)	3 (7.9)	38 (100.0)	0.668
ETEC	1 (25.0)	0 (0.0)	0 (0.0)	3 (75.0)	0 (0.0)	0 (0.0)	4 (100.0)	0.121
EHEC	11 (35.5)	8 (25.8)	5 (16.1)	6 (19.4)	1 (3.2)	0 (0.0)	31 (100.0)	0.867
STEC	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0.517
EIEC	12 (27.9)	11 (25.6)	5 (11.6)	11 (2.6)	2 (4.7)	2 (4.7)	43 (100.0)	0.630
EAEC	48 (30.2)	38 (23.9)	23 (14.5)	34 (21.4)	9 (5.7)	7 (4.4)	159 (100.0)	0.713
DAEC	91 (32.4)	65 (23.1)	46 (16.4)	53 (18.9)	13 (4.2)	13 (4.2)	281 (100.0)	0.990
Total	100 (32.4)	73 (23.5)	50 (16.1)	59 (19.0)	14 (4.5)	14 (4.5)	310 (100.0)	0.000

aEPEC: Atypical EPEC, tEPEC: Typical EPEC, ETEC: Enterotoxigenic *E. coli*, EHEC: Enterohemorrhagic *E. coli*, STEC: Shiga toxin-producing *E. coli*, EIEC: Enteroinvasive *E. coli*, EAEC: Enteroaggregative *E. coli*, DAEC: Diffuse adherence *E. coli*.

ages and DEC pathotypes (p> 0.05). There was no statistically significant difference between the gender and DEC pathotypes (p> 0.05) Single pathotype was detected in 56 of 310 *E. coli* isolates and mixed pathotype in 254 of them.

Discussion

Gastrointestinal infections due to pathogenic *E. coli* are an important cause of outbreaks and sporadic cases worldwide, especially in children particularly in developing countries (7,8). Differences in the frequency of DEC infection worldwide; study populations, geographical region and socioeconomic classes, age distributions, result from the methods used in diagnosis (Table 3).

It has been observed that younger patients are more prone to DEC infections. This condition has been attributed to age-related immunity. Muni et al. have examined the distribution of DEC isolates by age in India, and found that DEC isolates were more common in children aged 0-12 and 49-60 months (9). Patzi-Vargas et al. have examined the prevalence of DEC isolates by age in 831 children with diarrhea under the age of five in Mexico, 23% were less than six months old, 30% were 6-12 months old, 27% were 13-24 months old, and 31% were older than 24 months. The EAEC pathotype was more common in children younger than six months between 6-24 months of age (p= 0.008), and children aged 13-24 months were found to have less mixed DEC infection than children of other age groups (p= 0.012). It tended to be more frequently infected with tEPEC strains in children younger than six months (p= 0.052) (10). Canizalez-Roman et al. examined the distribution of DEC isolates with acute diarrhea in the 0-99 age group in Mexico, and found that DEC strains were more common in children ≤2 years of age (7). In India, Mandal et al. found DEC isolates more common in children one year old, and it was found to be less with age (11). In China, Zhou et al. found that DEC isolates were more common in children 0-11 months old (p< 0.001). In addition, EPEC, the predominant pathotype, tended to be more common in children under 24 months (66.7%) (4). In our study, when the age distribution of 310 DEC pathotypes was examined, 32.4% were 0 years old, 23.5% were one year old, 16.1% were two years old, 19.0% were three years old, and 4.5% were four years old and 4.5% were determined to be five years old. DEC strains were found to be more common in children aged ≤1 years, Presumably, it was detected less due to increased immunity as the age got older.

When the distribution of DEC cases by gender was examined, it was found that male patients under the age of five were more prone to infection than female patients, and it was found to be statistically significant in some studies. Mandal et al. examined the distribution of DEC by sex, it was found that 348 (54.9%) were boys and 285 (45.1%) were girls, and a significant relationship was found between DEC cases and sex (11). Singh et al. determined that 73 (60.8%) of 120 children under the age of five with acute diarrhea were boys and 47 (39.2%) were girls (12). In our study, when the distribution of 310 DEC patients by sex was examined, it was found that 174 (56.1%) were boys and 136 (43.9%) were girls, and the difference was statistically significant (p= 0.031).

Table 3. Distribution of DEC pathotypes according to studies in the literature

	Country	Age	Number of Samples	DEC n (%)	EPEC n (%)	EAEC n (%)	ETEC n (%)	DAEC n (%)	EHEC n (%)	STEC n (%)	EIEC n (%)	Mix n (%)
Muni et al. (2014)	India	<5	120	110 (91.6)	54 (49.1)	12 (10.9)	6 (5.5)	38 (34.5)	1 (1.5)			
Tobias et al. (2015)	Sweden	<5	307	59 (19.2)	28 (47.5)	27 (46.0)	3 (5.0)					
Canizalez-Roman et al. (2016)	Mexican	0-99	1037	242 (23.3)	53 (21.9)	126 (52.1)	43 (17.8)	15 (6.2)		3 (1.2)	2 (0.8)	
Mandal et al. (2017)	India	<5	633	191 (30.2)	16 (8.4)	132 (69.1)	20 (10.5)		5 (2.6)		3 (1.6)	15 (7.8)
Konaté et al. (2017)	Burkina Faso	<5	192	31 (7.4)	8 (25.8)	15 (48.5)	1 (3.2)		3 (9.6)		4 (12.9)	
Singh et al. (2017)	India	<5	120	106 (88.3)	87 (72.5)	76 (63.33)	55 (45.83)		11 (9.16)		3 (1.6)	
Zhou et al. (2018)	China	<5	684	54 (7.9)	27 (50.0)	11 (20.9)	8 (14.8)		2 (3.7)		2 (3.7)	4 (7.4)
Tutak and Tuğrul (2015)	Türkiye	0-99	1318	29 (10.3)	2 (75.0)		1 (25.0)					
Our study	Türkiye	<5	3064	310 (100.0)	187 (60.4)	159 (51.3)	4 (1.3)	281 (90.6)	31 (10.0)	43 (13.9)	2 (0.6)	

DEC: Diarrheagenic *E. coli*, EPEC: Enteropathogenic *E. coli*, EAEC: Enteroaggregative *E. coli*, ETEC: Enterotoxigenic *E. coli*, DAEC: Diffuse adherence *E. coli*, EHEC: Enterohemorrhagic *E. coli*, STEC: Shiga toxin-producing *E. coli*, EIEC: Enteroinvasive *E. coli*.

When seasonal variations of DEC cases are examined, it has been found that they are common especially in the spring/summer period and are strongly associated with environmental factors such as temperature and humidity. Patzi-Vargas et al. have detected DEC isolates most frequently in the spring and summer seasons in Mexico (10). In China, Zhou et al. have examined the seasonal distribution of DEC (54) cases and found it in summer (33.4%), spring (14.8%), winter (12.9%) and autumn (38.9%) (4). In our study, it was found that DEC is mostly seen in summer and spring seasons, temperature and humidity affect the development of DEC infection and it was found to be compatible with other studies.

When the frequency of DEC pathotypes is examined, the sixth pathotype is known as DAEC associated with diarrhea. Ochoa et al. have detected 30 (31.6%) DAEC among 95 DEC pathotypes in their study (13). Muni et al. have detected DEC pathotypes in 110 (91.6%) of 120 children under the age of five with diarrhea in the Katihar, Kosi region of Bihar, 34.5% of which was DAEC (9). The most common pathotype detected in our study is DAEC with a rate of 90.6%. These findings show that DAEC is a common pathotype in our region. However, the epidemiology of DAEC remains uncertain as there is no data on the frequency of DAEC infections in our country.

Enteropathogenic *E. coli* continues to cause diarrhea worldwide and pose a serious risk for children under two years of age. In a study conducted by Tobias et al. in Sweden, 59 (19.2%) DEC was found in patients with diarrhea under the age of five, and approximately half of the isolates (47.5%) were EPEC pathotypes (14). In a study conducted by Wang et al. in China, three groups of diarrhea patients (755 children under five years old from Henan, 1422 children under five years old and 1047 adults over 18 years old from Beijing) were included in the study. EPEC was found to be the most common factor in children (4.5% in Beijing, 3.6% in Henan province) and the second most common (2.1%) agent in adults (15). In a study conducted by Bozçal et al., 146 *E. coli* were detected from the stool samples of 102 patients, 62 of whom were children, 40 of whom were adults in İzmir, Türkiye. EPEC and STEC were examined among DEC pathotypes, and it was reported that three were EPEC (2.05%) strains (16). In our study, EPEC was detected in 60.4% of DEC isolates detected in children under five years of age with acute diarrhea. These findings showed that EPEC is the second most common pathotype in our region.

For many years, aEPEC has been thought to be dominant in industrialized countries, although it is rare in developing countries (17). However, recent data have shown that aEPEC infections are more common than tEPEC diarrhea in both developing and developed countries (12). Tobias et al. reported that aproximately half of 59 DEC isolates were aEPEC (46%) (14). In Türkiye, Bozçal et al. found that all of the EPEC (2.05%) strains detected in 102 patients with diarrhea were aEPEC (16). In our study, 60.4% of EPEC was detected in DEC isolates, 79.7% of which was aEPEC and 20.3% was tEPEC. The results of our study showed that the aEPEC pathotype is more common than the tEPEC pathotype, consistent with other studies.

Enteroaggregative *E. coli* strains have been associated with tourist diarrhea in both developing and industrialized countries. EAEC is a pathogen responsible for acute and chronic diarrhea (18-20). In the study conducted in India, DEC was detected in 110 (91.6%) of 120 children under five years of age with diarrhea, and the second most common DEC pathotype (10.9%) was found to be EAEC (9). Tobias et al. have determined that EAEC was the second most common DEC pathotype (46%) in 307 patients with diarrhea under the age of five in Sweden (14). It was found by Mandal et al. in Bihar and Konaté et al. in Burkina Faso that the most common pathotype detected in children with diarrhea under the age of five was EAEC (69.1%-48.5%) (11). In our study, EAEC (51.3%) was one of the most common pathotypes among DEC pathotypes in children under five years of age with acute diarrhea and consistent in other countries.

EIEC causes endemic infections in developing countries and sporadic infections in developed countries. In the study conducted by Wang et al. in China, it was found that EIEC (0.35% in children in Beijing, 0.48% in adults and 0.3% in children in Henan) was the third most common pathotype in three groups of patients with diarrhea (15). In Burkina Faso, Konaté et al. stated that EIEC (12.9%) ranked third in 315 children with acute diarrhea under the age of five years and it was among the most common pathotypes (6). In Türkiye, Tutak and Tugrul identified 254 *E. coli* from 1318 patients with complaints of diarrhea in different age groups. EIEC virulence gene could not be detected by molecular method in 11 EIEC (5.4%) pathotypes diagnosed by slide agglutination test (20). In our study, EIEC (13.9%) is in the fourth place among the factors detected in DEC pathotypes.

The prevalence of EHEC is especially common in developed countries, in children under the age of five and in the summer season. In India, Mandal et al. have reported EHEC (2.6%) among the least common DEC pathotypes in 633 children under five with diarrhea (11). Tobias et al. have identified at least EHEC pathotypes (1.5%) in 307 patients with diarrhea under the age of five in Sweden (14). In Türkiye, EHEC was not detected in 91 children with acute diarrhea under the age of five and 60 healthy persons in Gaziantep (21). In Türkiye, Erdogan et al. did not detect the EHEC serotype in 1815 patients, Tas in two (1.0%) of 200 patients, and Aydoğan et al in three (3%) of 100 patients (22-24). In our study, EHEC was found in 10% among DEC pathotypes in children under five years of age with acute diarrhea. The results of our study were similar to the studies abroad, but higher than the studies conducted in our country. The reason for this is thought to be due to the lack of studies on this subject in our country for a long time.

STEC has the highest worldwide incidence of HUS in children under the age of five and is common in developing countries. In Scotland, about 40% of STEC pathotype outbreaks associated with meat or dairy products or environmental contamination have been demonstrated by foodborne, 54% by environmental contact and 6% by both transmission routes (25). In Iran, STEC was the most frequent pathotype (35.4%). ETEC (14.0%) and EPEC (13.1%) were the second and the third most frequent pathotypes, respectively (26). In a study by Bozcal et al. STEC pathotype was found to be the most common (3.42%) in 102 patients with diarrhea in Türkiye (16). In our study, the least STEC (0.6%) pathotype was found among the DEC pathotypes in children under five years of age with acute diarrhea. It has been determined that the prevalence of the STEC pathotype in diarrhea in our region is low.

The prevalence of ETEC is the most important cause of bacterial diarrhea, especially in children under two years of age in developed and developing countries with high morbidity rates (1). Zhou et al. reported that ETEC ranked third (14.8%) in 684 patients under the age of five with acute diarrhea in China (4). Low rate (1.5%) of ETEC was determined in patients with diarrhea under the age of five in Sweden (14). In Burkina Faso, a low rate of ETEC (3.2%) was detected in 315 children under the age of five with acute diarrhea (6). Children samples showed EAEC, having high phenotypic frequency of (12%) followed by ETEC, (5.3%) and EPEC (3.3%) the least being mixed infections enteroaggregative/enterotoxigenic *E. coli* and enteroaggregative/enteropathogenic *E. coli* with (1.3%) respectively in Kenya (27). In our study, ETEC was found with a rate of 1.3% in children under five years of age with acute diarrhea and a lower rate than other studies. The reason for this has been attributed to ETEC being a more travel-related diarrhea agent.

This is the first study to investigate the entire DEC pathotypes in Türkiye. The most important limitation of our study was single center therefore multicenter study must be performed whether pathotypes are widespread in Türkiye. Hospital and community-acquired DEC infections should be regularly monitored through annual surveillance studies, necessary precautions should be taken and treatment protocols should be arranged according to these results. The routine use of a multiplex PCR system for the detection of DEC in medical microbiology laboratories will be an important diagnostic tool. Although the cost poses a significant constraint for developing countries, it will ensure that diarrhea cases are correctly diagnosed and targeted.

Ethics Committe Approval: This study was approved by the Selçuk University Faculty of Medicine non-Interventional Clinical Research Ethics Committee (Decision no: 2018/18, Date: 29.09.2018).

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