

PATHOGENIC *ESCHERICHIA COLI* AMONG ASYMPTOMATIC CHILDREN AND ASSOCIATED FACTORS

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Although Escherichia coli is a part of the commensal flora of the gastrointestinal tract, pathogenic types of E. coli can cause diarrhea, especially in children. Pathogenic types are found also in healthy individuals, but prevalence of pathogenic E. coli among asymptomatic children varies and has not been studied in Latvia. The aim of the study was to determine prevalence of pathogenic E. coli among asymptomatic children and identify factors associated with presence of bacterium. Children (aged 0.5–8 years) without acute gastrointestinal symptoms were included in a cross-sectional study. Parents were asked to answer a questionnaire (demographic data, parental education, type of delivery, breastfeeding, antibacterial therapy, and allergic diseases) and bring a faecal sample of their child. The prevalence of pathogenic E. coli was detected by polymerase chain reaction and analysed in respect to risk factors. Statistical analyses included Chi-Square test, one-way ANOVA, and logistic regression. The patient sample group contained 245 children, mean age 4.5 SD ± 2.1; 46.5% (114/245) had allergies. In total, 16% (39/245) of isolates were positive for pathogenic E. coli. Prevalence of pathogenic types of E. coli was significantly higher among children without allergy compared to children with allergy: 21% (27/131) vs. 11% (12/114), p = 0.03. Prevalence did not differ significantly in respect to other studied factors. In logistic regression analysis pathogenic E. coli positivity was inversely associated with presence of allergy (OR = 0.45, CI: 0.21–0.94, p = 0.03). Asymptomatic carriage of pathogenic E. coli was identified in our paediatric patient sample and was inversely associated with an allergic disease. Microbiota changes related to pathogenic E. coli, as well as duration of carriage of bacterium, should be studied further.

Key words: Commensal flora, enteropathogenic *E. coli*, children, asymptomatic, allergy.

INTRODUCTION

Escherichia coli is one of the dominant commensal microorganisms that colonises the newborn's gastrointestinal tract already during the first hours of life. At the same time, pathogenic types of *E. coli* can cause diarrhea, especially in children (Nataro and Kaper, 1998).

Up to now, five diarrhoeagenic types of *E. coli* have been characterised: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC) (known also as Shiga toxin producing *E. coli*) and enteroaggregative *E. coli* (EAEC)

(Nataro and Kaper, 1998). Moreover, the latest classification of *E. coli* is based on the presence/absence of virulence genes (for example, *E. coli* adherence factor plasmid), and enteropathogenic *E. coli* are further classified as typical (tEPEC) and atypical enteropathogenic *E. coli* (aEPEC) (Hu and Torres, 2015).

Several diarrhoeal outbreaks caused by different types of pathogenic *E. coli* have been reported both in children and adults. In Brazil, pathogenic *E. coli* was identified in 40% of children and in 39% of adults with diarrhea (327 fecal samples, in total) (Spano *et al.*, 2017). In a similar study in

the Andaman Islands, pathogenic *E. coli* was found in 6.82% of patients with diarrhea, the majority of them being EAEC (70.1%), while EPEC and ETEC was isolated in 19.6% and 10.3% cases, respectively (Ramya Raghavan *et al.*, 2017).

In a prospective study in United States of America, 9.4% of patients with diarrhea were positive for pathogenic *E. coli*. However, the same study demonstrated presence of pathogenic *E. coli* also in 6.8% persons in a control group (Nataro *et al.*, 2006). Moreover, in the Netherlands, in collections of faecal samples in 28 day care centers during a period of three years, regardless of presence of gastrointestinal symptoms, enteropathogenic *E. coli* was detected in 19.9% of children. The authors concluded that asymptomatic infection with enteropathogens in day care attendees is not a rare event (Enserink *et al.*, 2014). This finding was supported also by a study in Germany, showing that prevalence of pathogenic *E. coli* is similar (17.4%) in control and diarrheal patients (Hu and Torres, 2015).

A review of several studies (Hu and Torres, 2015) showed that pathogenic *E. coli*, especially aEPEC, can be found in similar proportions in symptomatic and asymptomatic individuals.

Although meat and vegetables are shown to be the causes of diarrhoeal outbreaks (Crohen *et al.*, 2013), factors associated with asymptomatic carriage of pathogenic *E. coli* are not well established. It has been shown that type of delivery and infant feeding (breast vs. formula feeding) affect the microbiota composition in newborns (Brown *et al.*, 2013). Further, it was shown that breast feeding decreases the risk of *Enterobacteria* infection in infants, because human milk oligosaccharides significantly decrease enteropathogenic *E. coli* attraction to epithelium. Furthermore, mice, who received human milk oligosaccharides, were less colonised with enteropathogenic *E. coli* compared with a control group (Manthey *et al.*, 2014). Some data show that antibacterial therapy during infancy induces changes in intestinal microbiota, thus possibly increasing the risk of colonisation of pathogenic microorganisms (Brown *et al.*, 2013).

Asymptomatic carriage of pathogenic *E. coli* goes should also be considered under the so-called hygiene hypothesis, which suggests that individuals with bacterial infections have less allergic diseases (Brown *et al.*, 2013).

The aim of the study was to determine the prevalence of pathogenic *E. coli* among children without gastrointestinal symptoms and analyse factors associated with the presence of pathogenic *E. coli*, in particular, allergic disease.

MATERIALS AND METHODS

Study design. A cross-sectional study was performed in primary health care centres, kindergartens and allergologist consultation at the Children's Clinical University Hospital, Rīga, Latvia. Children's parents were asked to answer a questionnaire and bring a faecal sample of their child. The

prevalence of pathogenic types of *E. coli* was detected by polymerase chain reaction and presence of pathogenic *E. coli* was analysed in respect to presence of allergy and different environmental factors.

Patients. Children (aged five months to eight years) without acute gastrointestinal symptoms were included in the study. Presence of allergy was considered if allergy was diagnosed by the doctor at the allergologist consultation or doctor diagnosed allergy was reported by parents in a questionnaire.

Infection with a hemorrhagic type of pathogenic *E. coli* was considered if EHEC and/or *E. coli* O157:H7 was isolated from the faecal sample of the child.

Methods. The questionnaire included questions about demographic data, child health history (in particular, presence of allergic diseases), family and parental education, type of delivery, duration of breast feeding, and previous treatment with antibiotics.

The presence of pathogenic types of *E. coli* in clinical material was detected by real time polymerase chain reaction. Clinical material was stored in a freezer in temperature -60°C . In the laboratory DNA was extracted from 100–300 g of clinical material and the presence of different pathogenic types of *E. coli* (EPEC, ETEC, EHEC, EAEC, *E. coli* O157:H7) was detected using GI-Bacteria (II) Assay based on dealer instructions for the diagnostic method (Anonymous, 2020).

Statistical analyses. Statistical analysis (one-way ANOVA, Chi-Square test, descriptive statistics and logistic regression analysis) was conducted using IBM SPSS Statistics 23 and MedCalc.

The prevalence of pathogenic *E. coli* was compared in individuals with/without allergy and other factors by the Chi-Square test. Variables with a p -value ≤ 0.1 were included in a logistic regression analysis model and the 95% confidence interval was calculated. The p value < 0.05 was considered significant.

Ethics. The study was approved by the Research Ethics Commission of the Institute of Cardiology and Regenerative Medicine, University of Latvia. Parents of children signed informed consent prior to inclusion in the study.

RESULTS

The final patient sample included 245 children with mean age of 4.5 years ($\text{SD} \pm 2.1$); 52% (128/245) were girls, and 46% (114/245) were children with allergies.

Pathogenic types of *E. coli* were detected in 16% (39/245) of children. The prevalence did not differ between children below and above 60 months of age: 16% (23/138) and 15% (16/107), respectively ($p = 0.71$). The majority had infection with EPEC (64% (25/39)). The prevalence of other types of pathogenic *E. coli* is shown in Figure 1.

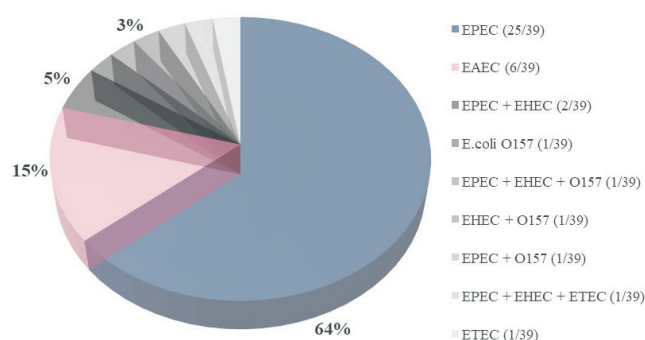


Fig. 1. Prevalence of pathogenic types of *Escherichia coli*.

EPEC, enteropathogenic *E. coli*; EAEC, enteroaggregative *E. coli*; EHEC, enterohemorrhagic *E. coli*; ETEC, enterotoxigenic *E. coli*; O157, enterohaemorrhagic *E. coli* serotype O157:H7

In six samples more than one type of pathogenic *E. coli* was identified simultaneously. A combination of two different types was found in four children (EPEC and EHEC was detected in two samples, EHEC and *E. coli* O157 in one sample, and EPEC and *E. coli* O157 in one sample). A combination of three different types was found in two children: EPEC, EHEC and *E. coli* O157 in one sample and a combination of EPEC, EHEC and ETEC in the other sample. The mean age of children with several types of pathogenic *E. coli* was four years and ten months; both children with combination of three types of pathogenic *E. coli* were six years old.

Prevalence of pathogenic *E. coli* was significantly higher among children without allergy compared to children with allergy: 21% (27/131) vs. 10.5% (12/114) ($p = 0.03$). The mean duration of exclusive breast feeding in the pathogenic *E. coli*-positive group was 3.96 (SD \pm 2.14) months compared to in the pathogenic *E. coli*-negative group ($p = 0.57$). Prevalence of pathogenic *E. coli* with respect to the other studied factors is shown in Table 1. Detailed characteristics of children with more than one type of pathogenic *E. coli* with respect to the presence of risk factors is shown in Table 2.

Analysing the subgroup of individuals infected with a hemorrhagic type of pathogenic *E. coli* (individuals with EHEC and/or *E. coli* O157) ($n = 7$), 14% (1/7) had received antibacterial therapy during the first six months of life; 57%

Table 1. Prevalence of pathogenic *E. coli* in relation to some studied risk factors

Studied factor	Pathogenic <i>E. coli</i> % (n/total)	<i>p</i> value
Sex	male 12% (16/129)	0.11
	female 20% (23/116)	
Allergic disease	yes 11% (12/114)	0.03
	no 21% (27/131)	
Siblings	no 12% (13/107)	0.10
	yes 19% (26/136)	
Parental education	Secondary 19% (17/91)	0.38
	Higher 14% (22/153)	
Type of delivery	vaginal 17% (32/186)	0.42
	C-section 12% (7/55)	
Duration of exclusive breast feeding	≤ 4 months 15% (25/159)	0.97
	> 4 months 15% (13/82)	
Duration of breast feeding in total	≤ 5 months 16% (28/174)	0.97
	> 5 months 16% (10/62)	
Antibacterial therapy during the previous year	yes 15% (19/38127)	0.52
	no 18% (19/105)	
Antibacterial therapy during the first six months of life	yes 13.0% (12/92)	0.25
	no 19% (16/139)	

(4/7) during the previous year and 14% (1/7) during the previous month.

The variables sex, presence of siblings and presence of allergic disease were entered into the logistic regression analysis model. Pathogenic *E. coli* positivity was inversely associated only with presence of allergy (OR 0.45, CI 0.21–0.94, $p = 0.03$).

DISCUSSION

Asymptomatic carriage of pathogenic *E. coli* in children was identified in our paediatric patient sample group and the detected prevalence was comparable to data from other countries. Presence of pathogenic *E. coli* was inversely associated with presence of allergic disease, but was not significantly related to number of siblings, parental education, type of delivery, duration of breast feeding, and previous antibacterial therapy.

Table 2. The characteristics of children with more than one type of pathogenic *E. coli*

Combination of pathogenic types of <i>E. coli</i>	Allergy	Antibacterial therapy			Type of delivery	Number of siblings
		First six months of life	Previous year	Previous month		
EPEC + EHEC	No	No	No	No	Vaginal	0
EPEC + EHEC	Yes	Yes	Yes	Yes	C-section	1
EPEC + EHEC + O157	No	No	Yes	No	Vaginal	1
EHEC + O157	No	No	Yes	No	Vaginal	1
EPEC + O157	Yes	No	Yes	No	Vaginal	2
EPEC + EHEC + ETEC	No	No	No	No	Vaginal	1

EPEC, enteropathogenic *E. coli*; EAEC, enteroaggregative *E. coli*; EHEC, enterohemorrhagic *E. coli*; ETEC, enterotoxigenic *E. coli*; O157, enterohaemorrhagic *E. coli* serotype O157:H7.

Pathogenic *E. coli* may cause gastrointestinal symptoms, but asymptomatic carrying of pathogenic types of *E. coli* was observed among 16% of Latvian children. The detected prevalence of asymptomatic carriage of pathogenic *E. coli* in our patient sample is comparable to data from some other European countries, for example, in Germany (17%) and in Norway (10%) (Hu and Torres, 2015; Ochoa *et al.*, 2008), while lower prevalence data (below 10%) was shown in the United States of America (6.8%) (Nataro *et al.*, 2006), Brazil, Peru, Vietnam, Mozambique and Iran (Ochoa *et al.*, 2008). In contrast, prevalence of pathogenic *E. coli* was rather high (27.1%) in day care centres in Netherlands (Enserink *et al.*, 2014), showing the importance of the close person-to-person contact in the spread of the infection.

Factors associated with asymptomatic carriage of pathogenic *E. coli* are not studied widely, the majority of studies being about symptomatic patients. Next to person-to-person contact, transmission via food products and water is considered as an important route (Croxen *et al.*, 2013).

A study in Brazil Lima found that factors like age, sex, rotavirus vaccination, recent use of antibiotics and previous contact with pets were not associated with the presence of pathogenic *E. coli*, but higher income was inversely related to presence of EPEC (Lima *et al.*, 2019). Another study showed seasonal differences of infection with pathogenic *E. coli*, while other factors associated with pathogenic *E. coli* were not identified (Enserink *et al.*, 2014). However, analysing data about paediatric EHEC infections and haemolytic-uraemic syndrome in England, no relationship was found with socioeconomic status, while association was demonstrated only with early age, type of bacterium and presenting symptoms (Adams *et al.*, 2019).

Similarly, in our study sample we could not demonstrate association between presence of EPEC and age, parental education, size of family, previous antibacterial therapy, and type of delivery. However, in the univariate analysis, pathogenic *E. coli* was non-significantly associated with presence of siblings and lower parental education, which was found also in other studies (Lima *et al.*, 2019). We can speculate that the association could be significant in a larger patient sample.

Nevertheless, we found that presence of allergic disease was inversely associated with pathogenic *E. coli*. The possible association between pathogenic *E. coli* and allergy could be explained by the following ways. On the one hand, presence of allergic disease could be a marker of higher socioeconomic status, which has been shown to be inversely associated with EPEC in some studies (Lima *et al.*, 2019). On the other hand, the observed association conforms to the so-called hygiene hypothesis, which considers that development of allergic disease is partly influenced by gastrointestinal tract microflora (Fujimura *et al.*, 2015). Although there is no consensus about the exact immunological mechanisms that can explain the association between atopic diseases and gastrointestinal tract microbes, it has been suggested that microbiota have the potential to modulate responses at mul-

tipl points during the course of allergic disease (Wesemann and Nagler, 2016). Thus, several studies have shown that pathogenic *E. coli* interacts with the innate immune system and that some of the effects are even more expressed compared to wild-type EPEC (Bashir *et al.*, 2004; Ruchaud *et al.*, 2007; Sabharwal Harshana *et al.*, 2016). Further, it has been shown that EAEC, especially mixed infection, results in significant microbiota alterations (Mathew *et al.*, 2019). Therefore, the presence of pathogenic *E. coli* could also be a marker of more profound changes in gastric microbiota associated with allergic disease. More detailed analysis of intestinal microbiota in relation to pathogenic *E. coli* should be studied further by molecular biological methods.

Finally, the tactic used in cases of asymptomatic carriage of pathogenic *E. coli* should also be discussed. It has been shown that carriage of EPEC is often transient, with shedding of bacterium persisting for up to seven months (Abu Sin and Takla, 2012). However, it would be important to analyse duration of infection with pathogenic *E. coli* in a prospective study.

CONCLUSIONS

Asymptomatic carriage of pathogenic *E. coli* was identified in our paediatric patient sample and was comparable to data from other countries. Presence of pathogenic *E. coli* was inversely associated with allergic disease, but was not related to parental education, number of siblings, duration of breast feeding, previous antibacterial therapy.

Microbiota changes in the case of pathogenic *E. coli*, as well as duration of carriage of bacterium, should be studied further.

ACKNOWLEDGEMENTS

The study was supported by the grant from Latvia State Research Programme, "Biomedicine." We are grateful to all the children and their parents, as well as all the doctors (in particular Sarmīte Kupča, Rita Seske) who participated in our study. We also thank kindergartens, primary health care centers and Children's Clinical University Hospital in Rīga for their cooperation.

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Received 21 January 2020

Accepted in the final form 22 February 2020

ASIMPTOMĀTISKA PATOĢĒNO *ESCHERICHIA COLI* NĒSĀŠANA BĒRNIEM UN AR TO SAISTĪTIE FAKTORI

Lai gan *Escherichia coli* pieder pie normālas gastrointestinālā trakta mikrofloras, tās patogēnie tipi var izraisīt diareju, īpaši bērniem. Patogēno *E. coli* atrod arī veselīem indivīdiem, turklāt tās prevalence asimptomātiskiem nēsātājiem ir atšķirīga dažādās valstīs, bet Latvijā līdz šim nav pētīta. Pētījuma mērķis bija noteikt patogēnās *E. coli* prevalenci asimptomātisku bērnu vidū un ar to saistītos faktorus. Šķēsgriezuma pētījums veikts primārās aprūpes centros, bērnudārzos, alergologa konsultācijā Bērnu klīniskajā universitātes slimnīcā. Pētījumā iekļauti bērni (0,5–8 gadu vecumā) bez akūtas gastrointestinālas simptomātikas. Bērnu vecākus lūdza aizpildīt anketu (demogrāfiskie dati, vecāku izglītība, dzemdības, krūts barošana, antibakteriālā terapija, alerģijas) un atnest bērna fēču paraugu. Patogēno *E. coli* noteica ar PĶR un analizēja dažādus riska faktorus bērniem ar un bez patogēnās *E. coli*. Statistiskā analīze: Chi-Square tests, ANOVA, loģistiskā regresija. Pētījuma pacientu kopa: 245 bērni vecumā (vidēji 4,54 (SD ± 2,1) gadus veci); 46,5% (114/245) ar alerģiju. Kopumā 16% (39/245) paraugu tika atrasta patogēnā *E. coli*. Tās prevalence bija ticami augstāka bērniem bez alerģijas, salīdzinot ar bērniem ar alerģiju: 21% (27/131) vs. 11% (12/114), $p = 0,03$. Savukārt, prevalence ticami neatšķīrās, analizējot citus faktorus. Loģistiskās regresijas analīzē patogēnās *E. coli* pozitivitāte bija negatīvi saistīta ar alerģijas klātbūtni (OR = 0.45, CI:0.21–0.94, $p = 0.03$). Pētāmajā populācijā tika novērota asimptomātiska patogēno *E. coli* nēsāšana, turklāt tā bija retāk sastopama bērniem ar alerģiskām saslimšanām. Turpmāk būtu nepieciešams padziļināti pētīt gan mikrobiotu, gan baktērijas nēsāšanas ilgumu.