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Invasive Properties, Adhesion Patterns and Phylogroup Profiles Among *Escherichia Coli* Strains Isolated from Children with Inflammatory Bowel Disease*

Zdolności adhezyjne i inwazyjne oraz przynależność do grup filogenetycznych szczepów *E. coli* izolowanych od dzieci z nieswoistymi zapaleniami jelit

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. A great deal of evidence indicates a link between *Escherichia coli* (*E. coli*) and Crohn's disease in adult patients, but there is lack of information on the association of these bacilli with inflammatory bowel disease (IBD) among children.
Objectives. The study was carried out to determine the distribution of phylogenetic group, the adherence patterns and invasive properties of *E. coli* isolated from children with IBD and non-IBD chronic bowel diseases.
Material and Methods. A total of 22 *E. coli* isolated from biopsy specimens from children with IBD and 21 *E. coli* strains obtained from children with indeterminate colitis and intestinal polyps were examined for adherence and internalization to the Int407 cell line. Genes involved in epithelial cell invasion and genes specific to *E. coli* phylogroups were determined by polymerase chain reaction (PCR).
Results. The undefined adherence pattern predominated among the isolated *E. coli*, although most of them demonstrated the *afaD* and *aggB* genes encoding invasions of diffusely adhering and enteroaggregative *E. coli*. Regardless of the clinical entity, most *E. coli* were internalized by Int407 epithelial cells and belonged to the B2 and D phylogroups.
Conclusions. The wide distribution of adhesive *E. coli* capable of entering Int407 cells but also having genes encoding adhesins and invasins characteristic to pathogenic *E. coli* strains seems to indicate that these *E. coli* may represent a large group of pathogenic *E. coli* strains contributing to chronic intestinal disorders (**Adv Clin Exp Med 2012, 21, 5, 591–599**).

Key words: inflammatory bowel disease, invasion, *E. coli*, adherence pattern, invasion genes, phylogroups.

Streszczenie

Wprowadzenie. Wiele wyników badań wskazuje na związek *Escherichia coli* z chorobą Leśniowskiego-Crohna u osób dorosłych, jednak nadal brakuje danych na temat roli tych pałeczek w nieswoistych zapaleniach jelit (IBD) u dzieci.

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Cel pracy. Charakterystyka zdolności adhezyjnych i inwazyjnych oraz przynależność do grup filogenetycznych szczepów *E. coli* izolowanych od dzieci z nieswoistymi zapaleniami jelit oraz innymi przewlekłymi chorobami przewodu pokarmowego.
Materiał i metody. Test adhezji i inwazji *in vitro* do komórek nabłonka jelita linii Int407 wykonano dla 43 szczepów *E. coli* izolowanych z bioptatów błony śluzowej jelita 22 dzieci z rozpoznaną chorobą Leśniowskiego-Crohna i wrzodziejącym zapaleniem jelita grubego oraz 21 dzieci z nieokreślonym zapaleniem jelita i polipami jelit. Geny kodujące inwazyny oraz geny klasyfikujące badane szczepy do konkretnej grupy filogenetycznej wykrywano testem PCR.
Wyniki. Wśród badanych izolatów *E. coli* przeważał nieokreślony typ adhezji, chociaż większość szczepów posiadała geny kodujące inwazyny, tj. *afaD* i *aggB* typowe dla szczepów o rozsianym i agregacyjnym typie adhezji. Niezależnie od jednostki chorobowej, z której izolowano badane szczepy, większość z nich była internalizowana przez komórki nabłonka jelita i należała do grup filogenetycznych B2 i D.
Wnioski. Z uwagi na to, że większość badanych szczepów *E. coli* wykazała zdolność adhezji i inwazji do komórek nabłonka jelita oraz prezentowała geny kodujące inwazyny typowe dla patogennych szczepów *E. coli*, prawdopodobnie badane izolaty mogą reprezentować większą grupę szczepów związanych z przewlekłymi chorobami przewodu pokarmowego (**Adv Clin Exp Med 2012, 21, 5, 591–599**).

Słowa kluczowe: nieswoiste zapalenie jelit, inwazja, *E. coli*, typ adhezji, geny inwazji, filogrupy.

There have been several reports on increased numbers of *Escherichia coli* (*E. coli*) colonizing the intestinal mucosa of patients with inflammatory bowel disease (IBD) [1, 2]. Bacterial adherence to the intestinal mucosa is an essential step in the initiation of colonization and infection involving a wide variety of distinct bacterial adhesins. Fimbrial and nonfimbrial adhesins that mediate attachment to host cells are produced by most pathogenic and non-pathogenic *E. coli* strains. Many of these adhesins are associated with specific adherence patterns to epithelial cells, i.e. the diffuse adherence pattern, aggregative adherence pattern or localized aggregative adherence pattern, which are characteristic to particular *E. coli* pathotypes [3]. Recently a new category has been described: adherent-invasive *E. coli* (AIEC), associated with ileal mucosa in Crohn's disease [4–6]. AIEC strains are able to adhere to and invade intestinal epithelial cells, and are also able to survive in macrophages. Moreover, many AIEC have shown a mannose-resistant diffuse adherence pattern to intestinal epithelial cells, characteristic of the DAEC (diffusely adhering *Escherichia coli*) pathotype.

About 25% of all IBD patients will present before the age of 20 years. Childhood IBD, like adult IBD, comprises two phenotypes: Crohn's disease (CD) and ulcerative colitis (UC). Although the pathophysiology of IBD is unknown, several studies have provided evidences that IBD may be a result of a genetic predisposition associated with defects of the innate response of the intestinal mucosa, conferring susceptibility to undefined environmental factors such as luminal bacteria [7, 8]. There is a lot of evidence linking *E. coli* and CD in adult patients, but there is lack of information on the association of these bacilli with inflammatory bowel disease IBD among children.

The present study was carried out to determine the adherence patterns and invasive properties as well as the distribution of phylogenetic groups among of *E. coli* strains isolated from biopsy specimens obtained from children with IBD and non-IBD chronic bowel diseases.

Material and Methods

Biopsy Specimens and *E. coli* Strains

Biopsy specimens from a total of 43 consecutive untreated children diagnosed on the basis of clinical, endoscopic and histological criteria of IBD at Wroclaw Medical University's 2nd Department and Clinic of Pediatrics and Gastroenterology (Wrocław, Poland) were examined. CD was diagnosed in 11 children (mean age 12 years, ranging from 3 to 18 years) and UC was diagnosed in 11 children (mean age 12 years, ranging from 2 to 15 years). Eleven *E. coli* isolates were obtained from 11 children (mean age 11 years, ranging from 3 to 13 years) with indeterminate colitis; ten *E. coli* strains were from 10 otherwise healthy children (mean age 10 years, ranging from 2 to 17 years) with intestinal polyps. In the study these 21 *E. coli* were categorized as non-IBD group. Biopsy samples were taken during endoscopy from sites with visible macroscopic inflamed ileal mucosa. Immediately after being obtained, the biopsy specimens were plated onto MacConkey agar. After overnight incubation, five lactose-positive colonies were isolated from every sample and defined as *E. coli* by standard biochemical testing. All the isolated *E. coli* were tested in an adherence assay to determine their adherence types. A total of 43 *E. coli* strains representing 43 patients were included for further analysis. The following strains containing genes encoding invasins were used as controls in the polymerase chain reaction (PCR) and internalization assays: *ipaH*-positive *Shigella flexneri* strain (ATCC™12022), *invE*-positive *E. coli* O29:NM

(ATCC™43892), and *afaA*, *afaC* and *afaD*-positive, but *ipaH*-, *tia*-, and *aggB*-negative wild *E. coli* strain 48-2 of diffuse adherence pattern (DAEC) isolated from a child with diarrhea, enterotoxigenic *tia*-positive *E. coli* (ATCC™ 35401) and *E. coli* 17-2 reference strain of aggregative adherence pattern was used as a source of the *aggB* gene. The *E. coli* K-12 C_{600} strain was used as a noninvasive control and negative control in all the PCR experiments. All bacterial strains were stored in Luria broth (LB) with glycerol 15% v/v at –80° C and were cultured routinely in LB overnight at 37°C.

Ethical Considerations

The Ethical Committee of Wroclaw Medical University (Wrocław, Poland) approved the study, and the written consents of children's parents were obtained in every case in the study.

Adherence Assay

The *in vitro* adherence assay to embryonic human epithelial cell line Int407 (ATCC CCL6) was performed as described by Cravioto et al. [9]. A chamber slide was seeded with cell suspension at a density ca. 4×10^5 that was grown overnight to a near confluent monolayer at 37°C in an atmosphere with 5% CO_2 in Dulbecco modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (FBS, Lonza; Germany) and antibiotic-antimycotic solution (penicillin 100 U, streptomycin 100 U, amphotericin B 0.25 µg per ml; Invitrogen). Before the test was performed, the cells were washed twice with phosphate-buffered saline (PBS; pH 7.2; Sigma-Aldrich) and the cell culture medium in the chamber slides was replaced with 0.5 ml DMEM without antibiotics but supplemented with 2% FBS and 1% methyl α-D-mannopyranoside (Sigma-Aldrich). The bacterial strains to be tested were grown overnight at 37°C in LB medium. Twenty-five µl of bacterial culture was added to each chamber and incubated for 3 or 6 hours at 37°C in an atmosphere with 5% CO_2. Then the slides were washed three times with PBS, fixed with 70% methanol for 10 minutes and stained with 10% Giemsa (Sigma-Aldrich) for 30 minutes. After staining the slides were washed, mounted on glass slides and examined under the oil immersion objective of an Olympus BX51 light microscope. The assay was repeated twice for each *E. coli* strain, with incubation periods of 3 hours and 6 hours. The sample results are presented in Figure 1.

Bacterial Internalization Assay

The invasive ability of *E. coli* isolates to the Int407 cells were evaluated by the standard gentamycin protection assay with some modifications. This test is based on the principle that the gentamycin has limited capacity to penetrate to eukaryotic cells and internalized bacteria are thus protected. The minimal inhibitory concentration (MIC) of the gentamycin for all strains examined was determined by an agar dilution technique on Mueller-Hinton agar according to the Clinical Laboratory and Starndards Institute (CLSI) recommendations and the antibiotic was used at 100-fold the MIC value. The Int407 cells were grown to a confluence in minimal essential medium (MEM) with 10% FBS and antibiotic-antimycotic solution (penicillin 100U/l, streptomycin 100 µg/ml, amphotericin B 0.25 µg/ml) at 37°C in a humidified atmosphere containing 5% CO_2. The cells were harvested by trypsinization and seeded at a density of 4×10^5 cells/ml into a 24-well tissue culture plate, then incubated overnight to near (80%) confluence. Before infection, the cells were washed three times with pre-warmed PBS (pH 7.4) and fresh culture medium supplemented with FBS 1% v/v, and methyl α-D-mannopyranoside 0.5% v/v without antibiotics was added. The *E. coli* isolates were harvested from overnight cultures in LB and resuspended in PBS to a density of 9×10^8cfu/ml. The Int407 cells were then infected at a multiplicity of infection (MOI) of 100 bacteria per cell. After centrifugation at 1200 rpm for 10 minutes to obtain efficient invasion, the cells were incubated at 37°C in a 5% CO_2 atmosphere. After a 4-hour incubation period the cells were washed three times with PBS, and fresh culture medium containing 100 µg/ml gentamycin was added to kill extracellular bacteria. After a 1-hour incubation period the cells were washed three times and lysed by vigorous pipetting and shaking for 10 minutes at room temperature, after the addition of 1% Triton X-100 in deionized water. The cellular lysates were serially diluted in PBS, placed onto MacConkey agar and incubated overnight at 37°C to determine the number of bacterial colony forming units (CFU). The number of bacteria internalized was expressed as the mean percentage of the initial inoculum that was internalized. Each assay was run in duplicate and repeated three times.

PCR Assay

Genes involved in the invasiveness of *E. coli* were determined by colony polymerase chain reaction (PCR). Oligonucleotides used for amplification of *ipaH, invE* and *tia*-specific sequences

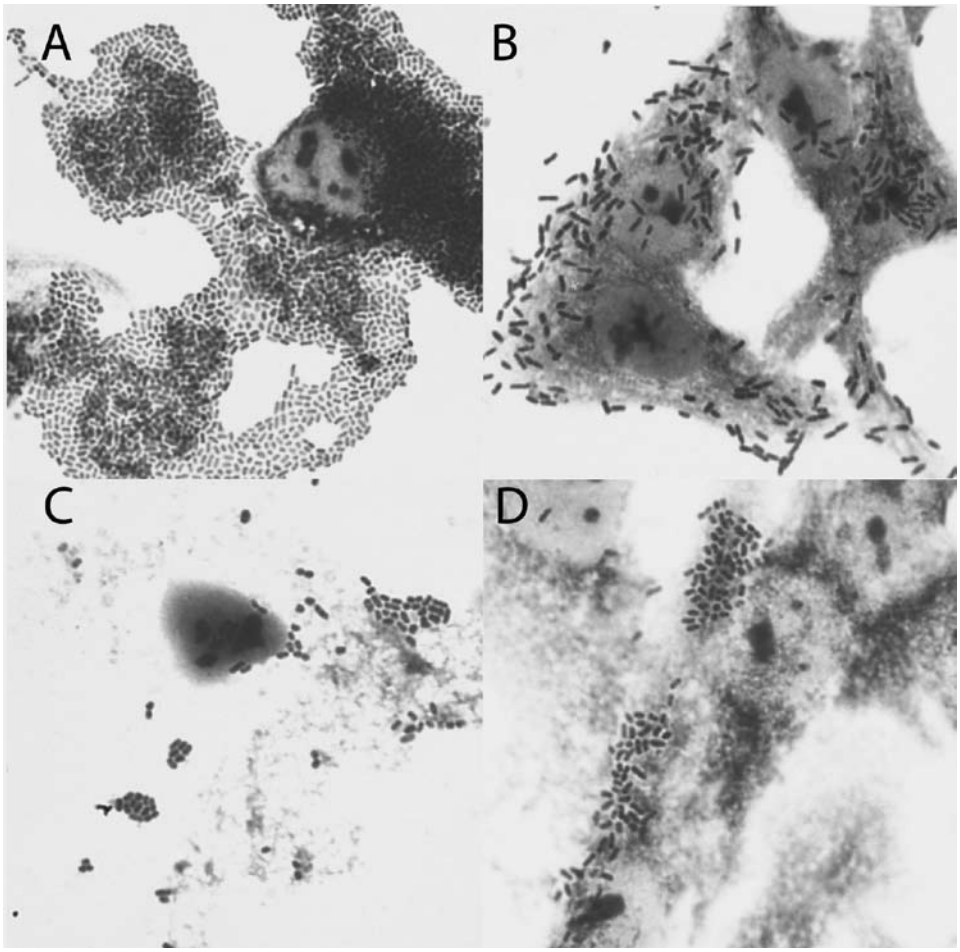

Fig. 1. Adherence patterns of the E. coli strains examined. A) aggregative adherence (AA); B) diffuse adherence (DA); C) cell-detaching pattern (CDT); D) undefined adherence pattern (UD). Giemsa stain. Magnification 100×

Ryc. 1. Typy adhezji badanych szczepów E. coli; A, agregacyjna adhezja (AA); B, rozsiana adhezja (DA); C, szczep odrywający komórki od podłoża (CDT); D, nieokreślony typ adhezji (UD). Barwienie metodą Giemzy. Powiększenie 100×

Table 1. Primer sequences and amplification conditions used in the study

Tabela 1. Sekwencje starterów oraz warunki reakcji zastosowane w badaniach

Gene (Gen)	PCR primers (Startery PCR)	PCR conditions (Warunki PCR)	Product size – bp (Wielkość produktu – bp)	References (Piśmiennictwo)
aggB	(F) GCATATTACCGATGTCCTGCG	95°C 1 min, 42°C 1 min,	421	this study
	(R) CCTCTTGATATTAGACATTCA	72°C 2 min (29 cycles)		
afaD	(F) GTCACCTGCGGGATGTTACT	94°C 1 min, 60°C 45 s,	392	this study
	(R) GCTCCGCCAACCGAAAAC	72°C 45 s (34 cycles)		
ipaH	(F) GCTGGAAAAACTCAGTGCCT	94°C 1 min, 56°C 2 min,	424	[29]
	(R) CCAGTCCGTAAATTCATTCT	72°C 1 min (29 cycles)		
tia	(F) ACCAGCGCTTCCGTCAGG	94°C 1 min, 56°C 1 min,	382	[4]
	(R) GCCAGATTCATTCCAGGAGG	72°C 1 min (30 cycles)		
invE	(F) ATATCTCTATTTCCAATCGCGT	94°C 30 s, 47°C 1 min,	382	[30]
	(R) GATGGCGAGAAATTATATCCCG	72°C 1.5 min (25 cycles)		

were synthesized on the basis of published nucleotide sequences. The *aggB* anad *afaD* genes were amplified using primers designed in the authors' laboratory (*aggB* Forward: positions 3460 to 3480 and Reverse: positions 3857 to 3880 Gen-Bank accession no. U12894; *afaD* Forward: 7479 to 7498 and Reverse: positions 7853 to 7870 Gen-Bank accession no. X76688). The sequences of primers used, PCR conditions and the expected amplicon sizes are presented in Table 1. Triplex PCR with primers for *chuA*, *yjaA* and anonymous cryptic DNA fragment TSPE4.C2 for the phylogenetic groups was performed as described by Clermont et al. [10]. All PCR amplifications were performed in a DNA-Engine PT200 thermal cycler (MJ Research Waltham, MA, USA). The PCR products were visualized after electrophoresis on 2% agarose gel in Tris-acetate-EDTA buffer by staining with SYBR Green I Nucleic Acid Gel Stain. The distribution of genes identified among the *E. coli* isolates is shown in Table 2.

Statistical Analysis

The significance of differences observed in the prevalence of genetic determinants of invasiveness and phylogenetic groups among the *E. coli* examined was assessed using the Pearson chi-square test (Excel 2007) with $p \leq 0.05$ considered statistically significant. The data are reported as mean values with standard deviation (SD).

Table 2. Distribution of the genes identified in the study and the adherence patterns of *E. coli* strains from children with IBD and non-IBD groups

Tabela 2. Występowanie genów oznaczanych w badaniach oraz typy adhezji szczepów *E. coli* od dzieci z grup IBD i nie-IBD

Characteristic/*E. coli* group (Cecha/grupa *E. coli*)	№ of strains (Liczba szczepów) %				
	CD (n = 11)	UC (n = 11)	IC (n = 11)	PO (n = 10)	total (n = 43)
Adherence pattern (Typ adhezji)					
UD	7 (63.6)	5 (45.4)	6 (54.5)	4 (40)	22 (51.2)
AA	2 (18.2)	4 (36.4)	2 (18.2)	0	8 (18.6)
DA	1 (9.1)	1 (9.1)	1 (9.1)	2 (20)	5 (11.6)
CDT	1 (9.1)	1 (9.1)	2 (18.2)	4 (40)	8 (18.6)
Invasion genes (Geny inwazji)					
aggB	8 (72.7)	4 (36.4)	3 (27.3)	2 (20)	17 (39.5)
afaD	5 (45.4)	5 (45.4)	4 (36.4)	4 (40)	18 (41.9)
tia	1 (9.1)	3 (27.3)	0	1 (10)	5 (11.6)
ipaH	1 (9.1)	1 (9.1)	1 (9.1)	0	3 (7.0)
invE	0	0	0	0	0
Phylogroups (Grupy filogenetyczne)					
A	0	0	1 (9.1)	2 (20)	3 (7.0)
B1	0	0	0	0	0
B2	7 (63.6)	8 (72.7)	6 (54.5)	5 (50)	26 (60.5)
D	4 (36.4)	3 (27.3)	4 (36.4)	3 (30)	14 (32.6)

Adherence patterns: UD: undefined; AA: aggregative; DA: diffuse; CDT: cell-detaching;
CD: Crohn's disease; UC: ulcerative colitis; IC: indeterminate colitis; PO: polyps.
Typy adhezji: UD, nieokreślony; AA, agregacyjny; DA, rozsiany; CDT, odrywający komórki; CD, choroba Crohna; UC, wrzodziejące zapalenie jelit; IC, nieokreślone zapalenie jelita grubego; PO, polipy.

Results

Adherence Assay

The distribution of the *E. coli* isolates' adherence patterns is shown in Table 2. Undetermined adherence (UD), in which adhering bacteria do not form any characteristic pattern (Fig. 1), was the most prevalent, associated with 22 (51.2%) of the *E. coli* isolates ($p = 0.0015$). This adherence pattern was also the one most frequently associated with *E. coli* from CD children, although there was no statistical difference in the distribution of the *E. coli* presenting the UD pattern among the strains isolated from CD, UC, IC and PO groups ($p \geq 0.05$). *E. coli* with aggregative adherence (AA) and cell-detaching (CDT) strains were isolated with equal frequency, i.e. 8 (18.6%); whereas diffusely adhering *E. coli* were isolated less frequently: 5 (11.6%) strains. There was no statistical difference in the frequency of isolation of *E. coli* strains presenting AA, CDT and DA adherence patterns among the 43 *E. coli* strains examined ($p \geq 0.05$).

Invasion Assay

All the *E. coli* strains examined in the *in vitro* invasion assay were susceptible to gentamycin (MIC ≤ 2 μg per ml). The invasion assay demonstrated that 42 (97.7%) of the 43 *E. coli* isolates were internalized by epithelial cells Int407 at the level ≥ 0.1%, although there were differences in the invasion efficiency among isolates (Figure 2). *E. coli* isolates from CD and UC patients demonstrated similar invasion efficiency (3.85% and 3.39%, respectively), but lower than the mean invasion levels of *E. coli* strains from patients with IC and PO (4.09% and 5.25%, respectively). Interestingly, *E. coli* isolated from children with PO, who were considered an otherwise healthy control group, showed the highest invasion efficiency (5.25%) as compared with the other *E. coli* strains examined ($p < 0.05$). An invasion efficiency (> 6%) comparable to the *ipaH*-positive *Shigella flexneri* 12022 reference strain was demonstrated by 4 (40%) *E. coli* isolates from the PO group, 3 (27.3%) *E. coli* from the UC and IC groups, and only 1 (9.1%) strain from the CD patients ($p > 0.05$). As many as 5 (45.5%) *E. coli* from the CD group showed mean invasion levels ranging from 2% to 6%, comparable to the mean invasion level of the *invE*-positive *E. coli* 43892 reference strain. In contrast, mean invasion levels ranging from 2% to 6% were demonstrated by 3 (27.3%) *E. coli* isolates from the IC group and only one isolate from the UC group and the PO group ($p > 0.05$). Similarly, *E. coli* from CD patients most frequently showed mean invasion levels ranging from 1% to 2%, comparable

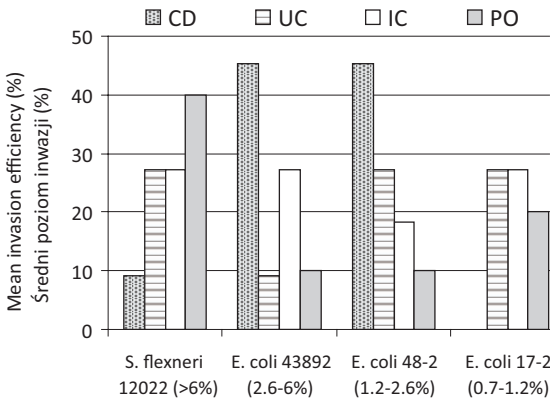

Fig. 2. The mean invasion efficiency of *E. coli* strains isolated from children with CD (Crohn's disease), UC (ulcerative colitis), IC (indeterminate colitis) and PO (polyps) in comparison with reference strains used in the study: *S.flexneri* 12022 *ipaH*-positive, *E. coli* 43892 *invE*-positive, *E. coli* 48-2 *afaD*-positive, *E. coli* 17-2 *aggB*-positive

Ryc. 2. Średni poziom inwazji szczepów *E. coli* izolowanych od dzieci z CD (chorobą Crohna), UC (wrzodziejącym zapaleniem jelita grubego), IC (nieokreślonym zapaleniem jelit) oraz PO (polipami jelit) w porównaniu z referencyjnymi szczepami zastosowanymi w badaniach: *S.flexneri* 12022 *ipaH*-dodatni, *E. coli* 43892 *invE*-dodatni, *E. coli* 48-2 *afaD*-dodatni, *E. coli* 17-2 *aggB*-dodatni

with *afaD*-positive *E. coli* 48-2 strain, although this was not statistically significant ($p > 0.05$) in comparison with *E. coli* from UC, IC and PO patients. Mean invasion levels comparable with *aggB*-positive *E. coli* 17-2 strain, ranging from 0.7% to 1.2%, were shown by 3 (27.3%) *E. coli* from the UC and IC groups, but by none of the *E. coli* strain from CD patients. The lowest internalization levels < 0.06% to Int407 epithelial cells were demonstrated by single *E. coli* strains isolated from the UC and PO groups. Generally, all *E. coli* isolated from CD patients demonstrated mean invasion levels ranging from 1% to > 6%. In contrast, *E. coli* isolated from UC and PO patients showed mean invasion levels ranging from < 0.7% to > 6%, whereas *E. coli* from IC group were internalized by Int407 cells at mean levels from > 0.7% to > 6%. However, these differences were not statistically significant ($p > 0.05$).

Distribution of Genes Encoding for Invasins

The analysis of the invasion gene distribution demonstrated that *afaD* gene encoding the outer membrane protein involved in the internalization

of diffusely adhering *E. coli* (DAEC) strains [11, 12] and *aggB* gene encoding the outer membrane protein AggB considered as an invasion protein of enteroaggregative *E. coli* (EAEC) strains [13] were most frequently detected among *E. coli* isolates associated with 18 (41.9%) and 17 (39.5%) *E. coli* strains, respectively (Table 2). Moreover, the *aggB* gene was associated statistically more frequently with *E. coli* from CD patients than with IC ($p = 0.03$) and PO ($p = 0.01$) *E. coli* strains, although there was no significant difference ($p > 0.05$) in the distribution of the gene among *E. coli* from CD and UC patients. The *ipaH* invasion plasmid antigen associated with the invasiveness of *Shigella* and enteroinvasive *E. coli* (EIEC) strains [14, 15], and the *tia* gene encoding the outer membrane protein engaged in the invasion of ETEC strains [16] were detected in a minority of *E. coli* isolates (Table 2). There was no statistical difference ($p > 0.05$) in the distribution of these genes among the *E. coli* strains examined. None of *E. coli* examined showed the presence of *invE* regulator for cell invasion of enteroinvasive *E. coli* (EIEC). It is also worth noting that in some *E. coli* strains isolated in the study, the authors were unable to detect any of the invasion genes examined, although these isolates demonstrated invasion capability. This may indicate that these isolates may present other invasins not identified in this study.

Phylogroups Distribution

The *E. coli* species has been divided into four main phylogenetic groups based on an anonymous DNA fragment designated the TSPE4.C2, *chuA* gene, required for heme transport in EHEC O157:H7 *E. coli*, and the *yjaA* gene of unknown function present in nonpathogenic *E. coli* K12 strain. The A or B1 phylogroups comprise most commensal *E. coli* strains, whereas isolates associated with extraintestinal infections (ExPEC) derive predominantly from the B2 phylogroup and to a lesser extent the D phylogroup. Pathogenic *E. coli* responsible for acute and severe diarrhea are distributed between the A and B1 phylogroups, but isolates causing mild and chronic diarrhea are distributed across all four phylogroups [10, 17]. In the present study, overall, 26 (60.5%) *E. coli* examined belonged to phylogenetic group B2 and 14 (32.5%) to D phylogroup. Only 3 (7%) of the strains studied belonged to phylogroup A, and none of the examined isolate belonged to phylogenetic group B1 (Table 2). *E. coli* isolates from CD and UC patients derived from the B2 and D phylogroups, whereas *E. coli* from IC and PO patients, comprised isolates from the A, B2 and D groups. There was no statistically significant difference in the phylogroup distribution among *E. coli* strains isolated from CD, UC, IC and PO patients ($p > 0.05$).

Discussion

In biopsy specimens from patients with IBD a high concentration of bacteria invading intestinal mucosa have been demonstrated [18–20]. Bacterial invasion of host cells induces a cascade of events to up-regulate innate immune defences, recruiting inflammatory cells responsible for epithelial barrier disruption and mucosal inflammation through the released cytokines [21]. A new category of *E. coli*, i.e. AIEC, associated with the ileal mucosa of adult patients with Crohn's disease has been thoroughly investigated [4–6]. AIEC strains efficiently invade a wide range of human epithelial cell lines and have a unique capability to survive and replicate within macrophages [5, 22]. Moreover, AIEC strains present diffuse adherence pattern to intestinal epithelial cells, although only some of these strains harbor virulence genes characteristic of the DAEC pathotype, which confers this type of adhesion. AIEC strains have been isolated from 36.4% of adult patients with CD vs. 6% of controls [4, 23].

Most of the *E. coli* strains isolated in the current study, regardless of the clinical diagnosis, showed undefined adherence patterns and moderate levels of invasion into Int407 epithelial cells, most probably associated with invasion genes characteristic to diffusely adhering *E. coli* DAEC and/or EAEC. Only some of the *E. coli* strains in the study presented defined adherence patterns – i.e. aggregative or diffuse patterns – and invasion levels corresponding to *Shigella* bacilli. Generally, when comparing *E. coli* isolates from children with IBD and other chronic bowel diseases (indeterminate colitis or polyps), the results of the study showed no striking differences among these isolates. None of the adherence patterns was significantly associated with any of the disorders. Moreover, all but one of the *E. coli* isolates in the study showed some degree of invasion capability, even the strains from children with polyps. In spite of the fact that the *aggB* and *afaD* genes were more frequently detected among *E. coli* from IBD than among *E. coli* isolated from children with indeterminate colitis or polyps, generally none of the genes examined was significantly associated with any of these clinical entities. Although they demonstrated adhesive and invasive capabilities, *E. coli* isolates from children with IBD and other chronic bowel diseases seem to be different from *E. coli* strains isolated from adults with IBD. First, in the current study *E. coli* isolates from UC as well as

CD patients were internalized by non-polarized, non-differentiated Int407 cells, which is a contrast to the results demonstrated by Darfeuille-Michaud et al. [4] for adult patients with UC. According to their study AIEC were associated with none of colonic specimens from patients with UC and 1.9% of the specimens from healthy controls. However, an increased number of mucosa-associated *E. coli* in adult patients with UC was found in studies by Swidsinski et al. [24] and Kotlowski et al. [25]. Next, AIEC strains present diffuse adherence and, even more importantly, they lack known invasive determinants. In contrast, most of the *E. coli* isolates in the current study demonstrated at least one of the well-characterized invasion genes. Thus, it seems that the only similarity to AIEC shown by the *E. coli* isolated in the study from children IBD is the affiliation to phylogroups: Most of the *E. coli* from children with IBD belonged to phylogropus B2 and D, which is similar to *E. coli* from adults IBD. However, both these phylogroups include *E. coli* associated with extraintestinal infections (ExPEC). Martinez-Medina et al. [26] showed that AIEC strains carry many virulence-associated genes characteristic of ExPEC, suggesting a close relationship between these both *E. coli* subgroups. Interestingly, Abe et al. [27] detected among some ExPEC isolates several virulence genes characteristic of the EAEC pathotype, e.g. the *aatA* gene encoding the dispersin transporter. The dispersin protein is responsible for dispersing EAEC across the intestinal epithelium, whereas the *aatA* gene is required for the proper function of aggregative adherence fimbriae of EAEC [28]. This may indicate that at least some ExPEC strains arose from the EAEC pathotype. In the current study the *aggB* gene, characteristic of the EAEC pathotype, was the second most common invasion gene detected in the *E. coli* isolated.

The results of the study indicate that *E. coli* from IBD children do not differ significantly from *E. coli* isolated from other chronic bowel diseases, but do differ from adult IBS *E. coli*. Taking into consideration that children become infected with many intestinal pathogens more frequently than adults, the authors hypothesize that the differences among the *E. coli* colonizing children's and adults bowels depend on immunological, behavioral and nutritional divergences associated with these age groups. On the other hand, AIEC as well as the *E. coli* isolated in the study seem to be well adapted to colonize human intestinal tissue.

In summary, the study characterized the adherence patterns and invasion capabilities of *E. coli* isolated from children with IBD and other chronic intestinal diseases. However, on the basis of the results obtained, the comparison of these strains with AIEC associated with IBD in adults is too preliminary and needs further investigation. Nevertheless, taking into consideration the fact that invasive *E. coli* of undefined adherence patterns were isolated in the study not only from children with IBD but also from children with other intestinal problems, e.g. polyps, it can be assumed that these strains may represent a larger group of pathogenic *E. coli* strains contributing to chronic intestinal disorders.

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