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Escherichia coli isolates from patients with inflammatory bowel disease: ExPEC virulence- and colicin-determinants are more frequent compared to healthy controls

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ABSTRACT

A set of 178 *Escherichia coli* isolates taken from patients with inflammatory bowel disease (IBD) was analyzed for bacteriocin production and tested for the prevalence of 30 bacteriocin and 22 virulence factor determinants. Additionally, *E. coli* phylogenetic groups were also determined. Pulsed-field gel electrophoresis (PFGE) was used for exclusion of clonal character of isolates. Results were compared to data from a previously published analysis of 1283 fecal commensal *E. coli* isolates.

The frequency of bacteriocinogenic isolates (66.9%) was significantly higher in IBD *E. coli* compared to fecal commensal *E. coli* isolates (54.2%, $p < 0.01$). In the group of IBD *E. coli* isolates, a higher prevalence of determinants for group B colicins (i.e., colicins B, D, Ia, Ib, M, and 5/10) ($p < 0.01$), including a higher prevalence of the colicin B determinant ($p < 0.01$) was found. Virulence factor determinants encoding fimbriae (*fimA*, 91.0%; *pap*, 27.5%), cytotoxic necrotizing factor (*cnf1*, 11.2%), aerobactin synthesis (*aer*, 43.3%), and the locus associated with invasivity (*ial*, 9.0%) were more prevalent in IBD *E. coli* ($p < 0.05$ for all five determinants). *E. coli* isolates from IBD mucosal biopsies were more frequently bacteriocinogenic (84.6%, $p < 0.01$) compared to fecal IBD isolates and fecal commensal *E. coli*. PFGE analysis revealed clusters specific for IBD *E. coli* isolates ($n = 11$), for fecal isolates ($n = 13$), and clusters containing both IBD and fecal isolates ($n = 10$).

ExPEC (Extraintestinal Pathogenic *E. coli*) virulence and colicin determinants appear to be important characteristics of IBD *E. coli* isolates, especially the *E. coli* isolates obtained directly from biopsy samples.

1. Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are the most common inflammatory bowel diseases (IBD) of the human gastrointestinal tract. Over 1 million people in the USA and 2.5 million people in Europe are estimated to have IBD (Kaplan, 2015). The etiology of IBD remains unclear, however, host-genetic, exogenous, and microbial factors are known to be involved (Kotlowski et al., 2007; Vejborg et al., 2011).

Several bacteriological analyses of fecal microbiota in IBD patients have shown that a wide range of bacterial species can be implicated in IBD, including *E. coli,* which has been found to be in greater numbers in the stool of IBD patients (Eaves-Pyles et al., 2008; Giaffer et al., 1992; Gorbach et al., 1968; La Ferla et al., 2004; Pascal et al., 2017).

E. coli isolates from IBD patients have been shown to be different from *E. coli* isolated from healthy individuals and a comparative genomic analysis of IBD *E. coli* showed that these isolates represented a heterogeneous population that was more similar to extraintestinal pathogenic *E. coli* (ExPEC) than to the classic diarrheagenic pathotypes (Vejborg et al., 2011). Most IBD *E. coli* strains belonged to phylogenetic groups B2 and D, which are typical for ExPEC strains (Kotlowski et al., 2007).

Previously published studies (with diverse sets of ExPEC strains including isolates from urogenital tract, isolates from skin and soft

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tissue infections, respiratory infections, intra-abdominal infections, genital smears, and blood-derived isolates) described an association between *E. coli* strains harboring ExPEC virulence factors and the frequency of bacteriocinogeny, as well as the prevalence of specific bacteriocin determinants (Azpiroz et al., 2009; Budič et al., 2011; Micenková et al., 2014; Micenková et al., 2016a; Micenková et al., 2016b; Micenková et al., 2017; Šmajs et al., 2010).

Bacteriocins are antimicrobial peptides or proteins (Cascales et al., 2007; Šmarda and Šmajs, 1998). Some bacteriocins have also been shown inhibit growth of pathogenic bacteria (Bosák et al., 2013; Bosák et al., 2016; Patton et al., 2007; Rendueles et al., 2014; Šmajs and Weinstock, 2001a,b). In addition, the inhibitory activity of some bacteriocins has been used as potential therapeutics for treatment of IBD patients (Brown et al., 2012; Kotlowski, 2016).

Nedialkova et al. (2014) described that inflammatory bowel disorders and pathogen infections result in conditions that increase colicin synthesis and also increase susceptibility to colicins in the competitor bacteria. Moreover, colicin synthesis appears to be an important bacterial fitness factor in the *Enterobacterial* blooms in the inflamed gut (Nedialkova et al., 2014).

In this study, we used 178 fecal or biopsy *E. coli* isolates from inflammatory bowel disease patients, and determined the frequency of bacteriocinogeny and the prevalence of 30 bacteriocin determinants. In addition, the clonal character of the isolates was examined using pulsed-field gel electrophoresis (PFGE). The virulence potential of these *E. coli* isolates was analyzed by testing for the presence of 21 virulence factor determinants. The distribution of *E. coli* phylogenetic groups (A, B1, B2, D, C, E, F, and clade I) was also analyzed. Lastly, results from the IBD *E. coli* isolates were compared to a previously characterized set of 1283 human fecal *E. coli* isolates (Micenková et al., 2016a).

2. Material and methods

2.1. E. coli isolates

Human *E. coli* isolates (n = 178) from patients with IBD, including Crohn's disease (n = 112) and ulcerative colitis (n = 66), were collected between 2006 and 2012 from fecal samples of patients being treated at the University Hospital in Brno, Czech Republic (n = 139), and from of mucosal biopsy samples from patients being treated at the Louis Pasteur University Hospital in Košice, Slovak Republic (n = 39) (Table S1). From each sample, a predominant single *E. coli* was isolated and identified using the biochemical ENTEROtest16 (Erba Lachema, Czech Republic). The set of *E. coli* strains isolated from IBD patients was compared to a set of human fecal commensal *E. coli* (n = 1283), collected in the same geographical region, and which had been characterized in a previously published study (Micenková et al., 2016a).

All human clinical samples were collected after patients gave written informed consent regarding their participation in the study. All patient data used in the study were anonymized and the study was approved by the ethics committee of the Faculty of Medicine, Masaryk University, Czech Republic.

Indicator strains *E. coli* K12-Row, C6 (ϕ), B1, P400, S40, and *Shigella sonnei* 17 were used for identification of bacteriocinogeny on agar plates (Micenková et al., 2014; Šmajs et al., 2010).

For PCR determination of colicin and microcin types, known bacteriocin producers were used as positive controls: *E. coli* BZB2101pColA - CA31, BZB2102 pColB - K260, BZB2103 pColD - CA23, BZB2107 pColE4 - CT9, BZB2108 pColE5 - 099, BZB2150 pColE6 - CT14, BZB2120 pColE7 - K317, BZB2279 pColIa - CA53, BZB2202 ColIb - P9, BZB2116 pColK - K235, PAP1 pColM - BZBNC22, BZB2123 pColN - 284 (original source: A. P. Pugsley), *E. coli* 189BM pColE2 - P9 (B. A. D. Stocker), *E. coli* 385/80 pColE1, pColV (H. Lhotová), *E. coli* 185M4 pColE3 - CA38 (P. Fredericq), *E. coli* W3110 pColE8, *E. coli* W3110 pColE9 (J. R. James), *E. coli* K-12 pColS4 (D. Šmajs), *Shigella boydii* M592 (serovar 8) pColU (V. Horák), *E. coli* K339 pColY (D. Friedman), *Shigella sonnei* (colicinotype 7) pColJs (J. Šmarda), *E. coli* pCol5, *E. coli* pCol10 (H. Pilsl), *E. coli* 449/82 pColX (microcin B17), *E. coli* 313/66 pColG (microcin H47), *E. coli* 363/79 pColV (microcin V, original source: H. Lhotová), *E. coli* TOP10F' pDS601 (microcin C7), *E. coli* D55/1 (microcin J25), and *E. coli* B1239 (microcin L, D. Šmajs) (Micenková et al., 2014; Šmajs et al., 2010).

Positive controls for PCR detection of virulence factors were taken from our laboratory stock and comprised the following strains: *E. coli* B2917 (pCVD432), *E. coli* B3428 (*α-hly*), *E. coli* B3406 (*afaI*), *E. coli* B3427 (*aer*), *E. coli* B3410 (*cnf1*), *E. coli* B3418 (*sfa*), *E. coli* B3406 (*pap*), *E. coli* B3430 (*ial*), *E. coli* B2541 (*st*), *E. coli* B2802 (*lt*), *E. coli* B1804 (*bfpA*), *E. coli* B2905 (*eaeA*), *E. coli* B2987 (*ipaH*), *E. coli* B3411 (*iucC*), *E. coli* B3423 (*fimA*), *E. coli* 1369 (*stx1, stx2, ehly*), *E. coli* B1201 (*fepC*), *E. coli* B1254 (*ireA*), *E. coli* B1201 (*fyuA*), and *E. coli* B2843 (*pks* island).

2.2. Detection of bacteriocin types

IBD *E. coli* isolates (n = 178) were inoculated by agar stab method and cultivated at 37 °C for 48 h. Subsequently, the macrocolonies were killed using chloroform vapors and overlaid with a top TY agar layer (0.7%, w/v, soft agar) containing 10^7 cells from one of 6 indicator strains *E. coli* K12-Row, C6 (ϕ), B1, P400, S40, and *Shigella sonnei* 17 (Micenková et al., 2014; Šmajs et al., 2010). Next, the plates were incubated at 37 °C for 24 h and bacteriocinogenic isolates were identified. Zones of at least 1.5 mm width were considered as bacteriocin inhibition zones.

Bacteriocin producers, which were phenotypically identified by agar stab method, were subsequently investigated by PCR method for identification of bacteriocin determinants encoding 23 colicins (A, B, D, E1-9, Ia, Ib, Js, K, L, M, N, S4, U, Y, and 5/10) and 7 microcins (H47, M, B17, C7, J25, L, and V). The PCR protocol was as follows: 94 °C (2 min for the DNA-PCR method; 5 min for colony PCR); 94 °C (30 s), 60 °C (30 s), 72 °C (1 min), 30 cycles; 72 °C (7 min).

Since microcins H47 and M were shown to be sensitive to chloroform vapors (Patzer et al., 2003) and since chloroform vapors were used during phenotypical identification of bacteriocinogeny, all *E. coli* isolates were also tested (using colony PCR) for the presence of determinants encoding microcins H47 and M.

PCR products of closely related colicin-encoding genes (E2-9, Ia–Ib, and U–Y) were sequenced using dideoxy chain terminator sequencing with amplification primers (Table S2). Bacteriocin sequence analysis was performed using Lasergene software (DNASTAR, Inc., Madison, WI).

Determinants encoding two additional competition systems were also tested among IBD isolates. PCR detection of *cdi* operon (encoding contact-dependent inhibition) was performed with the primers described by Aoki et al. (2005). The prevalence of determinants encoding type VI secretion systems (T6SS1, T6SS2 and T6SS3) was analyzed by a method described in Ma et al. (2013).

2.3. Detection of virulence determinants

The presence of 22 virulence determinants encoding 21 different virulence factors (*α-hly* – α-hemolysin; *afaI* – afimbrial adhesin; *aer* – aerobactin synthesis; *cnf1* – cytotoxic necrotizing factor; *sfa* – S-fimbriae; *pap* – P-fimbriae; pCVD432 plasmid; *ial* – locus associated with invasivity; *lt* – thermolabile enterotoxin; *st* – thermostabile enterotoxin; *bfpA* – bundle forming pilus; *eaeA* – intimin; *ipaH* – locus associated with invasivity, *iucC* – aerobactin synthesis; *fimA* – fimbriae type I; *stx1* – shiga toxin 1; *stx2* – shiga toxin 2; *ehly* – enterohemolysin; *fyuA* – Fe-Ybt/pesticin receptor FyuA; *ireA* – iron-regulated outer membrane virulence protein; *fepC* – ferric enterobactin transport ATP-binding protein, and *pks* island – colibactin) were screened for all IBD *E. coli* isolates. The spectrum of *E. coli* virulence factors detected in this study was based on previously published data and on virulence determinants that were previously detected in other studies analyzing fecal