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Toxins of Extraintestinal *Escherichia coli* Isolated from Blood Culture

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Abstract

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is one of the main etiological agents of bloodstream infections caused by Gram-negative bacilli. ExPEC pathogenicity is due to the presence of genes, located on plasmids or chromosomes that encode virulence factors. *E. coli* virulence factors such as adhesins, toxins, invasins are able to modify the metabolism of host cell, contributing to disease. In this study, 80 strains of *E. coli* were isolated by hemoculture from septicemic patients and examinated by polymerase chain reaction to identify the virulence factors genes encoding toxins and to determine the phylogenetic group. We characterized genes encoding 3 different types of toxins: α-hemolysin (hlyA), cytotoxic necrotizing factor type 1 (cnf1) and five subtypes of cytolethal distending toxins (cdt-I to cdt-V). 23.75% of *E. coli* strains contained cnf1 gene and 22.5% hlyA. Genes cdt-I and cdt-IV were detected in 1 of 80 strains. Phylogenetic classification showed that *E. coli* strains fall into 4 groups (A, B1, B2, D) based on PCR detection (chuA and yjaA genes and DNA fragment TSPE4.C2). Virulent ExPEC belong mostly to groups B2 and D. Our results confirmed this fact: 56% of *E. coli* strains belonged to group B2 and 24% to group D.

Keywords: *E. coli*; Hemoculture; Toxin; Phylogenetic group

Introduction

Pathogenic *Escherichia coli* strains cause intestinal or extraintestinal infections in many host species. Intestinal diseases are caused by *E. coli* strains that enter the host together with food, colonize and remain in the intestinal epithelium causing infection. Strains that cause extraintestinal infections are involved in a diverse spectrum of diseases, including urinary tract infections (UTI), newborn meningitis, abdominal sepsis, and septicemia. Most extraintestinal pathogenic *E. coli* (ExPEC) strains are opportunistic, and the site of infection is not necessarily the site of colonization [1,2]. ExPEC represent one of the main etiological agents of bloodstream infections caused by Gram-negative bacilli worldwide [3].

Pathogenicity of *E. coli* is related to the presence of genes, located on plasmids or chromosomes that encode virulence factors (VFs). ExPEC possess genes encoding putative VFs, which include adhesins, toxins, polysaccharide coatings, siderophores or iron acquisition systems, serum resistance mechanisms, and invasins [4]. Bacterial toxins and proteases are important VFs that define virulence properties among ExPEC. They participate in different biological activities carried by bacterial pathogens that include cell adhesion, iron accumulation, cell invasion, as well as modulation and induction of the cell cycle, inflammatory reactions, and apoptosis [5]. Several toxins of *E. coli*, called cyclomodulins, are attracting growing attention because they interfere with the eukaryotic cell cycle. Four kinds of cyclomodulins are known in *E. coli*: the cytotoxic necrotizing factors (CNFs) 1 to 3, the cycle-inhibiting factor (Cif) and two kinds of genotoxins, the cytolethal distending toxins (CDTs) I to V and the colibactin [6]. CDTs, Cif and colibactin block mitosis and CNFs promote DNA replication without cytokinesis. They are encoded by mobile genetic elements that belong to flexible genetic pool of *E. coli* [7]. CNFs activate Rho GTPases, which leads to cytoskeletal alterations, formation of multi-nucleation of cells and enlargement of eukaryotic cells in cell culture. Human intestinal and extraintestinal pathogenic *E. coli* produce CNF 1 [8,9]. CDTs induce DNA damage probably through DNAse activity, which causes cell cycle arrest and leads to further changes (cell distension and death, apoptosis) depending on the cell type [10]. Five subtypes of CDT (I to V) were reported in *E. coli*. CDT is composed of three subunits (CDTs A, B, C) encoded by three adjacent or slightly overlapping genes (*cdtA*, *cdtB*, *cdtC*) arranged in an operon. Genes encoding CDT-III are located on a large virulence plasmid (pVir), genes encoding CDT-I, -II, -IV and -V on a chromosome [11,12]. Toxins belonging to RTX (repeat in toxin) family disrupt host cell integrity through their poreforming and catalytic activities. The prototype of RTX proteins, α-hemolysin (HlyA), is produced mainly by ExPEC and causes hemolysis by forming pores in the erythrocyte membrane. The *hly*CABD operon that is necessary for the production and secretion of HlyA is located on chromosomal pathogenicity islands [13].

The majority of ExPEC strains including bloodstrains from septicemic patients belong mostly to B2 and less D phylogenetic groups, whereas commensal intestinal strains belong to groups A or B1 [14,15]. Strains of groups B2 and D often carry virulence factors that are lacking in strains of groups A and B1 [16].

In this study, we determined virulence factors genes encoding toxins and phylogenetic origin of 80 *E. coli* strains isolated by hemoculture from septicemic patients. We characterized genes encoding 3 different types of toxins: α-hemolysin (*hly*A), cytotoxic necrotizing factor type 1 (*cnf1*) and five subtypes of cytolethal distending toxins (*cdt-I* to *cdt-V*). Phylogenetic classification was based on PCR detection (*chuA* and *yjaA* genes and DNA fragment TSPE4.C2).

Materials and Methods

Bacterial isolates

We examinated in our study 80 *E. coli* strains isolated from hemoculture of septicemic patients. Automated blood culture system (Bactec 9050) was used in hemocultivation. *E. coli* strains of positive hemoculture were isolated and identified by standard biochemical methods ENTEROtest 16, ENTERO-Rapid 24 (*Lachema*, Czech Republic).

Virulence factor genes

PCR was used to detect genes encoding these toxins: HlyA (gene *hly*A), CNF1 (gene *cnf1*) and CDT-I to -V (genes *cdt-I* to *cdt-V*). Template DNA was extracted by the boiling-water method [17]. All *E. coli* strains were screened for presence of genes encoding toxins by a simplex and multiplex PCR assays using specific primers in appropriate conditions (Table 1). The following *E. coli* strains were used as positive controls in PCR assays: strain EB28 [18] for *hlyA* and *cnf1* genes, strain 6468/62 [19] for multiplex *cdt* and *cdt-I*, strain 6797/96 [20] for *cdt-II*, strain 1404 [21] for *cdt-III*, strain 28c [22] for *cdt-IV*, and strain 493/89 [23] for *cdt-V*. Strain C600^{Rif} was used as negative control in all PCRs.

Target gene	Encoding product	Amplicon size, bp	Reference
hlyA	α-hemolysin	1177	[18]
cnf1	cytotoxic necrotizing factor	498	[18]
Multiplex *cdt*	cytolethal distending toxin	466	[22]
cdt-I	cytolethal distending toxin type I	411	[22]
cdt-II	cytolethal distending toxin type II	556	[22]
cdt-III	cytolethal distending toxin type III	555	[22]
cdt-IV	cytolethal distending toxin type IV	350	[22]
cdt-V	cytolethal distending toxin type V	748	[23]
chuA	heme transport in enterohemorrhagic O157:H7 *E. coli*	279	[14]
yjaA	protein with unknown function	211	[14]
TSPE4.C2	putative DNA fragment in *E. coli*	152	[14]

Table 1: Primers used in PCR assays

Phylogenetic classification

ECOR phylogenetic classification of *E. coli* strains was determined by multiplex PCR assay using primers targeted at three markers, *chuA*, *yjaA*, and TSPE4.C2 (Table 1). The phylogenetic grouping was made on the basis of the presence of specific PCR-amplified fragments as follows: group B2 (*chuA+*, *yjaA+*, TSPE4.C2±), group D (*chuA-*, *yjaA+*, TSPE4.C2±), group B1 (*chuA-*, *yjaA±*, TSPE4.C2+), and group A (*chuA-*, *yjaA±*, TSPE4.C2-). *E. coli* strains HE26 and HE27 were used as positive controls, and strain C600^{Rif} was used as negative control for detection of appropriate markers.

Detection of PCR products

Specific gene products were separated using electrophoresis on 1.5% agarose gels and visualised by staining with EcoSafe (Biotium) under UV light. A 100-bp DNA ladder (Biolabs) was used as a molecular size marker. After gel electrophoresis PCR products were analysed using software system GelQuant and GelCapture.

Results and Discussion

All 80 *E. coli* strains were isolated from septicemic patients older than 20 years (mean age of 61.7 years). Causes of septicemia were different: UTI in 17 (21.25%) patients, oncological diseases in 17 (21.25%) patients, GIT diseases in 8 (10%) patients, endocrine glands diseases in 8 (10%) patients, transplantations and extracorporeal dialysis in 6 (7.5%) patients, circulatory system diseases in 5 (6.25%) patients, musculoskeletal system diseases in 4 (5%) patients, respiratory diseases in 3 (3.75%) patients. The origin was difficult to define among the remaining patients.

Phylogenetic classification based on PCR detection showed that *E. coli* strains fall into 4 groups (A, B1, B2, D). Virulent ExPEC mainly belong to groups B2 and D [14]. Our results confirmed this fact: phylogenetic group B2 was confirmed in 45 of the 80 *E. coli* strains (56.25%), group D in 19 strains (23.75%). 9 strains (11.25%) belonged to group A and 7 strains (8.75%) belonged to group B1. As would be expected, phylogenetic group B2 was the most prevalent in *E. coli* strains isolated from hemoculture. The overall rate of 56.25% is similar to the 63% reported by Moreno et al. [24] and 65% reported by Johnson et al. [1]. In total, 80% of isolates belonged to groups B2 and D, a fact that reinforces the suggestion [25] that these groups represent pathogenic lineages for majority of ExPEC.

In fact, the toxin studies, genes *hlyA* and *cnf1* were present in 22.5% and 23.75% of *E. coli* strains. Gene *hlyA* is frequently detected in ExPEC and we demonstrated this gene in 18 of 80 *E. coli* strains.

Product of this gene, HlyA, is a pore-forming bacterial exotoxin that may contribute to the virulence of bacteria during bloodstream infection and sepsis. It causes lysis of the targeted cell, contributes to penetration of epithelial barriers, evasion of the immune system, induction of inflammation and hence to the manifestation of sepsis [26]. Gene *cnf1* encoding CNF1 is very common in ExPEC, like *hly*A. In our study, this gene was detected in 19 of 80 *E. coli* strains. During urosepsis, CNF1 induces a severe controlled inflammatory response [6,27]. Moreno et al. [24] found that urosepsis isolates exhibited a high prevalence of VFs including *cnf1* (56% in patients with normal status, 38% in patients with compromised status) and hlyA (56% and 50%). They suggest that *E. coli* strains causing bacteremia of urinary tract origin need toxins (*cnf1*) to invade and remain in the bloodstream. Presence of both genes was confirmed in 13 *E. coli* strains (16.25%). Ananias and Yano [28] detected both genes in 12 of 60 isolates from patients with sepsis. This association is probably due to the presence of the pathogenicity island (PAI) IIJ96-like domain, in which the *cnf1* gene is located just downstream of the *hlyA* gene [29].

CDT is genotoxin characterized by the ability to cause DNA damage in the target cells. Five subtypes of CDT were reported in *E. coli* [11]. In our *E. coli* strains, genes *cdt-I* (n=1) and *cdt-IV* (n=1) were confirmed, no *cdt-II*, *cdt-III* or *cdt-V* were found. CDT-producing *E. coli* are isolated in a low percentage from patients with extraintestinal infections, including sepsis [6,30] and our results correspond with these studies. Weak prevalence of *cdt* genes suggests that CDTs are not major virulence factors for urosepsis. In several studies, *cdt-I* to *cdt-IV* genes were detected in enteropathogenic (EPEC) and in shiga toxin producing *E. coli* (STEC) isolated from patients with diarrhea and in ExPEC causing different extraintestinal infections (meningitis, UTI, sepsis), and *cdt-V* gene only in STEC [6,23,31-33].

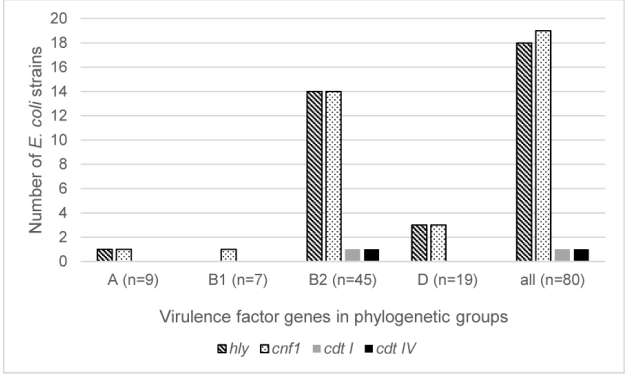

Figure 1: Distribution of genes encoding toxins according to the phylogenetic groups of *E. coli* strains.

Distribution of genes encoding toxins within phylogenetic groups is presented in Figure 1. Phylogenetic group B2 of *E. coli* contains strains responsible for severe infections, and their genetic background is adapted to the acquisition and/or maintenance of numerous virulence factors [16]. In most frequent phylogenetic group B2 (n=45) of 80 *E. coli* strains used in this study we observed the highest incidence of genes encoding toxins. 14 *cnf1*- and 14 *hlyA*-harboring strains were associated with group B2, representing 31.1% and 31.1% of *E. coli* B2 strains. Similarly, Dubois et al. [6] observed 37% *cnf1*-harboring *E. coli* strains in phylogenetic group B2 among *E. coli* isolates recovered from patients with urosepsis. Their urosepsis strains were significantly more likely to harbor hlyA and cnf1 associated with Hly than the fecal strains. Sannes et al. [34] detected 40% (32 of 80) *cnf1*-harboring *E. coli* strains in group B2 among bacteremia isolates. Moreover, 22.2% of our B2 strains (n=10) possessed both genes, *cnf1* and *hlyA*. In our study, genes *cdt-I* and *cdt-IV* were observed also in group B2, without association with genes *cnf1* and *hlyA*. Phylogenetic group D with 19 strains was determined by presence of 3 strains (15.78%) harboring genes *cnf1* and *hlyA*.

In conclusion, 80 *E. coli* strains isolated from septicemic patients were used in present study. As we expected, phylogenetic group B2 with 56.25% of isolates was most prevalent and 80% of isolates belonged to groups B2 and D, pathogenic lineages for majority of ExPEC. Whereas only *E. coli* and no other bacteria were isolated from hemocultures, we suppose that they represent real pathogens responsible for causing sepsis.

Our investigation showed a high prevalence of *cnf1*- and *hlyA*-harboring *E. coli* B2 strains. It suggests therefore a possible role of *HlyA* and *CNF1* producing *E. coli* in pathogenesis of sepsis. As previously reported for ExPEC, pathogenicity of these strains is due to presence of virulence genes, pathogenicity islands, and plasmids associated with the virulence, therefore the study of genetic factors is important for better understanding of these important pathogens. Our further research should be based on the comparison of the phylogenetic distribution and epidemiologic associations of virulence genes encoding toxins between *E. coli* strains isolated from septicemic patients and control *E. coli* strains isolated from healthy individuals.

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