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The Carrier State of Shiga-like Toxin II (SLT II) and Hemolysin-producing Enteroaggregative *Escherichia coli* Strain

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Abstract

Shiga-like toxin-producing (SLTEC) *Escherichia coli* strains are one of the most important food borne emerging pathogens. One hundred and fifty-seven *E. coli* strains isolated from 39 children with diarrhea of unknown origin and one hundred and five *E. coli* strains from 20 healthy children were examined for Shiga-like toxin production in Vero cell line assay. The synthesis of Shiga-like toxin was observed on Vero cell line and confirmed by PCR for one of 262 *E. coli* strains tested. The shiga-like toxin II-positive *E. coli* strain was isolated from 2-years old healthy child with no symptoms of gastrointestinal tract infection.

Key words: *E. coli*, toxin SLT II

Shiga-like toxin-producing *Escherichia coli* (SLTEC) are an important public health threat pathogens, causing hemorrhagic colitis and hemolytic uremic syndrome (Karch *et al.*, 1999).

During the year 2002, 39 stool samples from randomly allocated children with diarrhea of unknown origin hospitalized in Pediatric Gastroenterology Clinic, Wrocław, Poland, and stool samples from 20 healthy children with no symptoms of gastrointestinal tract infection and not treated at least for two weeks before sample obtainment, were collected. The mean age of examined children was 18 months. From each stool sample two to six sorbitol-fermenting and all sorbitol-negative colonies were picked up from sorbitol – MacConkey agar (SMAC) and identified biochemically as *E. coli*. All *E. coli* isolates tested were serogrouped with antisera for somatic O antigens of enteropathogenic *E. coli* strains and serotype of *E. coli* O157. The supernatants of overnight tryptose-soy broth cultures of *E. coli* examined were tested on Vero cell line according to method of Konowalchuk *et al*. (1977).

None of 157 (n = 7 sorbitol-negative and n = 150 sorbitol positive) *E. coli* strains isolated from children with diarrhea showed characteristic cytopathic effect on Vero cell line (in comparison with the reference *E. coli* O157:H7 EDL 933 strain), and none of sorbitol-negative *E. coli* belonged to serogroupe O157.

Among 105 *E. coli* strains (n = 14 sorbitol negative and n = 91 sorbitol positive) received from healthy children, the supernatant obtained from sorbitol-positive *E. coli* isolate after 24 h incubation on Vero cell line showed typical for shiga-like toxins cytopathic effect. The observation of the cytopathic effect was hindered by hemolysin production what was ascertain on blood agar. All hemolysin-producing *E. coli* strains isolated in the study (n = 75 of 262 *E. coli* strains) made detach and rounding Vero cells after 1 to 3 h of incubation, and the effect was unchangeable after 72 h of incubation. In the case of SLT II-producing *E. coli* strain (designated 50/4) after 24 h of incubation on Vero cell line the cytopathic effect (shrink and death the cells) was clearly distinguishable from hemolysin effect. Shiga toxin synthesis by the strain *E. coli* 50/4 was confirmed by PCR and detection of *slt 2* gene (Linquist R 1997; Weaver and Rowe, 1997; Wieler *et al.*, 2000). The results of examination of genes for SLT I, intimin, and enterohemolysin were negative. The *E. coli* 50/4 strain did not belong to enteropathogenic *E. coli* or O157 serogroupe and produced clear zone of hemolysis on blood agar after 3 h of incubation (α-hemolysin). Further examination

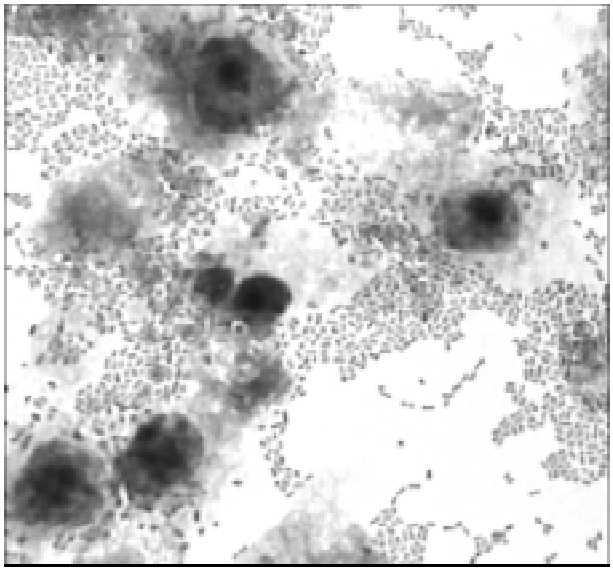

Fig. 1. The aggregative pattern of adherence to HEp-2 of examined *E. coli* 50/4

of the strain showed the presence of P fimbria (characteristic for uropathogenic *E. coli* strains) and mannose-resistant hemagglutination of sheep, rabbit, goat, horse, and cattle erythrocytes, and human group A red blood cells, but when cultured in Luria broth only (there was no hemagglutination with strain cultured on solid agar). Beside of that, the *E. coli* 50/4 strain showed clump formation of the surface of broth cultures that is characteristic for enteroaggregative *E. coli* strains (EAEC) (Albert *et al.*, 1993). In the 3 h adherence assay to HEp-2 cells performed according to Cravioto *et al.* (1996) the strain presented aggregative pattern of adherence (Figure 1).

The *E. coli* 50/4 strain was resistant to ampicilin and piperacilin in disc diffusion test, and sensitive to amoxycilin/clavulanate, tetracycline, trimetoprim/sulphamethoxazole, cefuroxime, ceftriaxone, ciprofloxacin, chloramphenicol and furazolidone.

The isolation of shiga-like toxin producing enteroaggregative *E. coli* strains (of O111:H2 and O86:HNM serotypes) was reported by Morabito *et al*., 1998 and Iyoda *et al*., 2000. In both cases these strains were isolated from patients with hemolytic uremic syndrome.

In the data, surprisingly the SLT II and hemolysin-producing enteroaggregative *E. coli* strain was isolated from healthy 2-years old child. The interview with child's parents (both of them had no history of diarrhea during the time of child's stool sample obtainment) reveal that the child had no contact with wild or domestic animals and was not attend to nursery school. The source of infection was undefined (the most probably food of animal origin). Cattle are the major reservoir of SLTEC and humans food borne infections are the most frequent but healthy persons carrying shiga-like-producing *E. coli* strains can be a source of SLTEC especially for their families (Ritchie *et al.*, 1992). The unusual adhesive properties of enteroaggregative *E. coli* together with shiga-like toxin production make these strains a potent pathogens. To our knowledge this is a first report of asymptomatic carrying of SLT II-producing enteroaggregative *E. coli* strain.

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