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Brief communication

# Cytotoxic factor secreted by *Escherichia coli* associated with sepsis facilitates transcytosis through human umbilical vein endothelial cell monolayers

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ABSTRACT

Culture supernatant of sepsis-associated *Escherichia coli* (SEPEC) isolated from patients with sepsis caused loss of intercellular junctions and elongation of human umbilical vein endothelial cells (HUVEC). The cytotoxic factor was purified from culture supernatant of SEPEC 15 (serogroup O153) by liquid chromatography process. PAGE (polyacrylamide gel electrophoresis) showed that the purified SEPEC cytotoxic factor had a molecular mass of ~150 kDa and consisted of at least two subunits. At the concentration of 1 $CD_{50}$ (40 μg/mL) did facilitate transcytosis through the HUVEC cells monolayer of SEPEC 15 as much as *E. coli* K12 within 30 min without affecting cell viability. These results suggest that this cytotoxic factor, named as SPF (SEPEC's permeabilizing factor), may be an important SEPEC virulence factor that facilitates bacterial access to the bloodstream.



Bacterial sepsis is a condition in which bacteria invade the bloodstream and infect several organs. In these cases, the most frequently isolated microorganism from patient's blood is *Escherichia coli*.[1,2] Sepsis-associated *E. coli* (SEPEC) belongs to the extra-intestinal pathogenic *E. coli* (ExPEC) group[1,3,4] and is phylogenetically and epidemiologically different from both intestinal pathogenic *E. coli* and commensal *E. coli*, which are part of human microbiota.[1,2] A variety of virulence factors related to human SEPEC include secreted toxins such as HlyA (α-hemolysin), Sat (secreted autotransporter toxin) and CNF-1 (cytotoxic necrotizing factor 1).[2,5,6] These toxins can change the host cell shape and/or function, thereby contributing to the biological processes stimulated by the pathogen.[7] Despite its medical importance, SEPEC is a poorly studied pathotype and it is still not known how these bacteria cross the endothelial barrier and gain access to the bloodstream.

In this study, we observed that culture supernatant of all SEPEC strains isolated from patients with sepsis (HC/UNICAMP, Campinas, SP, Brazil),[3] were cytotoxic to human umbilical vein endothelial cells (HUVEC), causing loss

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of intercellular junctions, cellular elongation and death. In this way, we examined whether SEPEC's cytotoxic factor facilitates transcytosis of these strains in HUVEC monolayers, possibly indicating how SEPEC may reach the bloodstream during sepsis.

Sepsis-associated *E. coli* 15, serogroup O153,[3] was selected among other SEPEC strains because this strain induced more intense cytotoxic effects on HUVEC cells (Fig. 1). This strain was grown in tryptic soy broth (TSB) for 5 h at 37 °C. The culture supernatant obtained by centrifugation was concentrated by a rotary evaporator (Marconi, SP, Brazil) and dialyzed (Spectrum, USA) against deionized water. This material was submitted to Mono-Q column chromatography (GE Healthcare), equilibrated with 0.04 M Tris–HCl, pH 8.8, and eluted with a linear gradient of 0–1 M NaCl in the same buffer. The elution profile was then monitored at 280 nm and fractions (1 mL/tube) were collected at a flow rate of 0.5 mL/min.

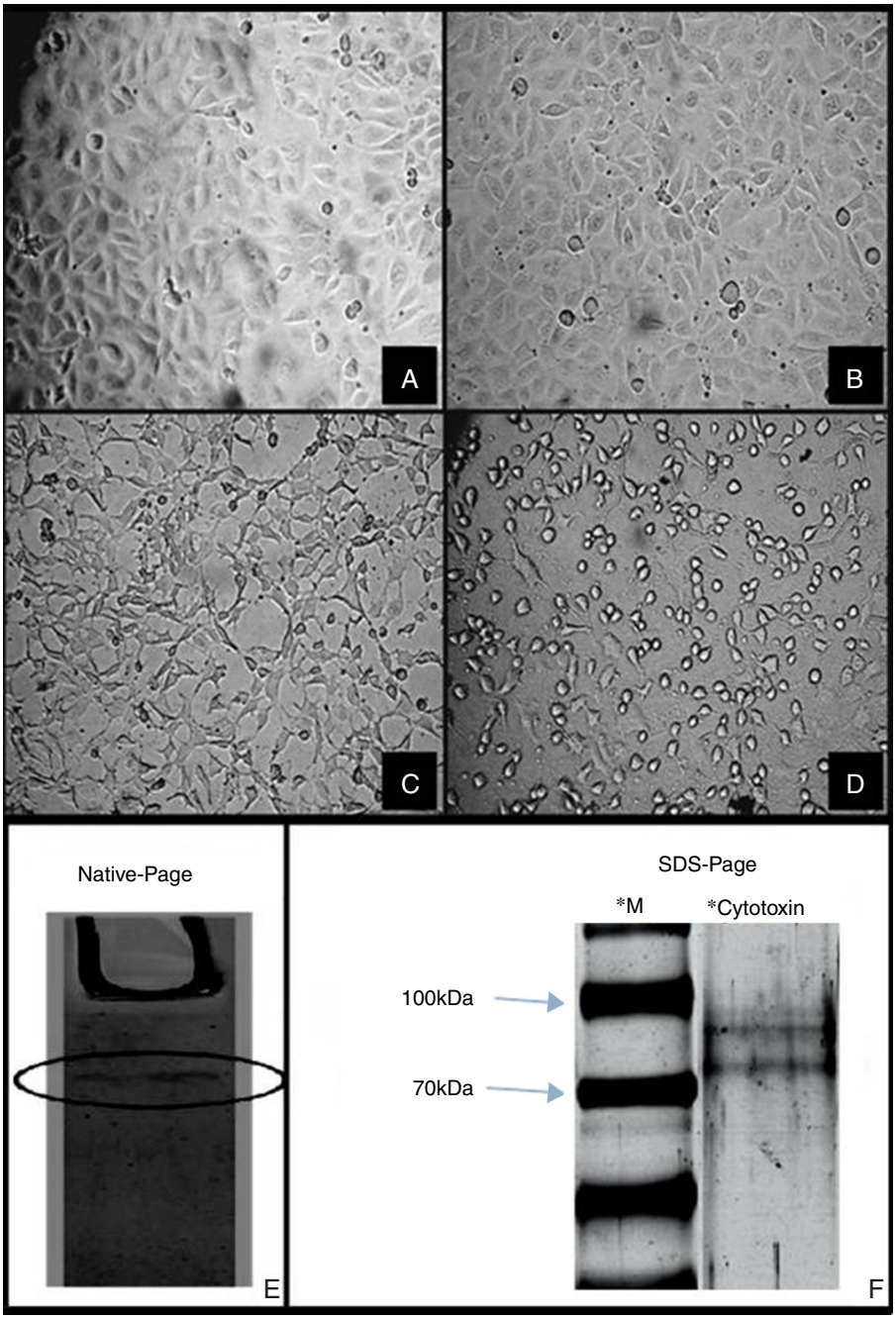


**Fig. 1 – Cytotoxicity of SEPEC cytotoxic factor on HUVEC. (A) HUVEC Cell Control, (B) HUVEC treated with *E. coli* K12 C600 culture supernatant, (C) HUVEC treated with chromatographic fractions (Superose 12 10/300GL), (D) HUVEC treated with SEPEC 15 culture supernatant. After treatment, all cells were incubated for 24 h. Magnification for all images: 200×. Electrophoretic profile of purified cytotoxic factor in (E) native PAGE showing a single protein band and (F) SDS-PAGE showing two protein bands with molecular masses between 70 and 100 kDa. **Active fractions obtained by gel filtration on Superose 12 10/300GL were run in both cases. *M – molecular mass markers.**