Title: One protease, two functions: the Secreted autotransporter toxin (Sat) as a versatile virulence factor enrolled in bloodstream infections caused by Extraintestinal *Escherichia coli*.

Authors: Claudia A. Freire, Rosa M. Silva, Angela S. Barbosa, Waldir P. Elias

Institutions: Laboratório de Bacteriologia, Instituto Butantan, São Paulo, SP, Brazil; Disciplina de Microbiologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil.

Extraintestinal *Escherichia coli* is one of the main pathogens involved in bloodstream infections (BSI) and uses several strategies to evade the mechanisms of innate immunity found in the blood, such as the complement system. Among them, two members of the Serine Proteases Autotransporters of Enterobacteriaceae (SPATEs) family, named Pic and EspP, have been described presenting proteolytic effects against complement proteins. However, high frequencies of *sat*, which encodes the SPATE Sat (Secreted Autotransporter Toxin), have been detected in strains of *E. coli* isolated from bacteremia, suggesting its involvement in the pathogenesis of BSI. Sat has already been characterized for its cytotoxic action on different cell lines, including endothelial cells, but its possible immunomodulatory effects have not yet been investigated. For that reason, our study aimed to evaluate the role of Sat in the pathogenesis of BSI and sepsis caused by *E. coli*. EC071 was selected from a previously characterized *E. coli* collection isolated from patients with bacteremia due to the absence of other SPATEs-encoding genes besides *sat*. Initially, Sat production was confirmed by Western Blot using culture supernatant of EC071. Also, this strain was resistant to the bactericidal activity of normal human serum (NHS). Sat was purified in its native form from EC071 culture supernatant and used in proteolytic assays against proteins of the complement system. Cleavage products were analyzed by Western Blot using specific antibodies against the target substrates. The proteins C2, C3, C3b, C4, C4b, C5, C6, C7, C8 and C9 were cleaved by Sat in a time and dose-dependent manner and these cleavages were inhibited by the serine protease inhibitor phenylmethylsulfonyl fluoride. Still, Sat pre-treated human serum did not kill the non-virulent *E. coli* DH5α after 60 minutes of incubation. An EC071 *sat* mutant was obtained and further complemented with the *sat* gene cloned from EC071. EC071 and its derived strains were tested in a murine sepsis model showing that the *sat* mutation reduced the animal lethality by 50%. Still, the *sat*-complemented mutant partially restored the effect observed in the wild strain (100% lethality). The results presented herein show that Sat is involved in the establishment of BSI and sepsis and in addition to the cytotoxic effects, it can also provide protection against the immune system of the host by the direct cleavage of proteins of the complement system.

Key words: *Escherichia coli*. Bacteremia. Sepsis. Sat. SPATEs.

Financial support: FAPESP, CNPq and CAPES