

Title: Characterization of enteroaggregative *Escherichia coli* (EAEC) strains harboring the SepA autotransporter-encoding gene

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Abstract:

Enteroaggregative *Escherichia coli* (EAEC) are recognized worldwide as an agent of acute and persistent diarrhea in children and adults. These strains can be classified as typical or atypical, based on the presence or absence of *aggR*, respectively. The SPATES (Serine Proteases Autotransporters of Enterobacteriaceae) proteins, also known as autotransporters (AT), are virulence factors that EAEC strains may express. Among the AT, the SepA encoding-gene (*sepA*), described in *Shigella flexneri* 2a and EAEC, has been associated with diarrheal cases caused by EAEC. However, the role of SepA in pathogenicity is unknown. The present study aimed to determine the frequency of *sepA* gene in a large collection of EAEC strains. A total of 193 strains (108 typical and 85 atypical) isolated from two studies on the etiology of acute diarrhea in children were selected. Initially, all strains were searched for the presence of *sepA* by PCR. Following, the *sepA*-positive strains were further characterized in terms of the presence of other AT genes, also by PCR. Among the 193 EAEC, *sepA* was detected in 26 (13.5%) strains (10/9.2% typical and 16/18.8% atypical). The *sepA*-positive strains presented the following combinations of different AT-encoding genes: 5 strains harbored only *sepA*, 10 *sepA-pic*, 2 *sepA-espl*, 1 *sepA-pet*, 2 *sepA-pic-sat*, 2 *sepA-pic-pet*, 3 *sepA-pic-sat-espl*, and 1 *sepA-pet-pic-sat*. Two of the typical EAEC *sepA*-positive strains (BA198 e BA732) were chosen for further characterizations, based on the presence of the smaller number of additional AT genes. These strains presented the *sepA-pic* genetic combination. The plasmid profile and *sepA* location were determined in both of them by gel electrophoresis of plasmid extraction and Southern Blotting, respectively. Strain BA198 harbored three plasmid bands of 2.8, 6 and 88 MDa; while BA732 was devoid of plasmids. Hybridizations with the *sepA* probe demonstrated the presence of that gene in the 88 MDa plasmid of BA198 and in the chromosome of BA732. DNA sequencing analyzes, in comparison with *sepA* of the reference *Shigella flexneri* 5a (M90T), showed high identities between these *sepA* sequences, *i.e.* 96% for BA198 and 97% for BA732. We can conclude that the *sepA* gene occurred in low frequency in our EAEC strains, being more frequent in the atypical group. Also, in these strains *sepA* may be either plasmidial or chromosomal and their DNA sequences showed high similarity with the corresponding gene of *Shigella flexneri* 5a.

Key words: enteroaggregative *Escherichia coli*, autotransporters, SepA, *Shigella flexneri*

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