Enterotoxigenic *Escherichia coli* – An Overview

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Enterotoxigenic Escherichia coli *is an important cause of traveler's diarrhea and diarrheal illnesses in children in the developing world. In this presentation we will focus on the main virulence attributes of this pathogenic category of* E. coli, *and discuss the evolution of studies conducted in our laboratory.*

Key words: enterotoxigenic *Escherichia coli* - virulence factors - enterotoxins - adhesins

Diarrheagenic *Escherichia coli* belongs to different categories that are classified based on their virulence mechanisms, epidemiology and serotypes. Currently, six distinct categories of diarrheagenic *E. coli* are recognized: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Nataro & Kaper 1998).

The importance of ETEC as a major cause of diarrhea was recognized in the late 60's. Since then, a large body of information on the epidemiology and pathogenesis of this group of bacteria has been accumulated.

ETEC strains are a worldwide cause of acute diarrheal disease in both human and animals, responsible for a high rate of infantile mortality in the developing countries. They are also an important agent of diarrhea among travelers from industrialized countries visiting tropical or subtropical areas of the world.

Two virulence attributes that characterize ETEC are the colonization of the small intestine surface and the production of enterotoxins that induce a net secretion of electrolytes and water into the gut lumen.

The enterotoxins produced by these organisms belong to two major classes of heat-labile and heat-stable toxins (LT and ST, respectively), that differed in regard to structure, antigenicity and mechanisms of action, usually related to cyclic nucleotides (Acheson 1992, Spangler 1992, Sears & Kaper 1996).

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LT, which is closely related to cholera toxin (CT), is an oligomeric protein of 85 kDa composed by five identical B subunits, arranged in a ring and in one A subunit. The B subunits bind strongly to the intestinal receptor GM1, while the A subunit is responsible for the enzymatic activity of the toxin, and is proteolitically cleaved to yield A_1 and A_2 peptides joined by a disulfide bond. After binding to the host cell membranes, LT enters into the cell through an endocytic process and the A_1 peptide ADP-ribosylates the alpha subunit of the GTP-binding protein Gs, leading to activation of adenylate cyclase in the enterocyte and accumulation of cyclic AMP. The intracellular accumulation of cAMP elicits secretion by crypt cells and decrease absorption by villus tip cells. In this family of toxins two distinct classes were described LT-I and LT-II, that although very similar present some differences specially in the B subunit.

In contrast to LTs, the STs are small, monomeric toxins of approximately 5 kDa, and two different classes were also distinguished based on biological and chemical properties: ST-I and ST-II. ST-I is methanol soluble and active in the infant mouse model, and differences in the aminoacid sequence of the toxin led to the identification of two genetic variants ST-Ih and ST-Ip, described initially in association with strains isolated from humans and pigs, respectively. However, subsequent studies showed that both variants can be produced by human ETEC strains, although ST-Ih seems to prevailed. ST-II, which is methanol insoluble and causes a positive response on pig and rat intestinal loops, is associated primarily with diarrheal disease in pigs, but some human ETEC strains expressing ST-II have also been reported.

It is well known that in ETEC strains the colonization of intestinal mucosa is related to the expression of several different proteinaceous surface structures, called colonization factors (CFs), which recognize specific receptors on the intestinal epithelial surface. A great variety of CFs has

been described in ETEC strains from human origin, which can present fimbrial, nonfimbrial or fibrillar structures. Most of CFs are fimbrial proteins and consist of a single antigen as CFA/I, while others such as CFA/II and CFA/IV are formed by a complex of different antigens, named coli surface antigens (CS). The terminology of CFs is rather confusing since no uniform system exists to classify these antigens. Terms such as colonization factor antigens (CFAs), coli surface antigens (CS), and putative colonization factors (PCFs) are used to describe these adhesins. Recently, a new designation of ETEC CFs was proposed by MM McConell, considering the order in which the CFs were identified over time, i.e., CFA/I and CS1 to CS22 (Gaastra & Svennerholm 1996, Pichel et al. 2000). CF genes are usually encoded on high-molecular-weight plasmids, which also encode the enterotoxins ST and/or LT. Moreover, surveys on ETEC strains have shown that most CFs are associated with a limited number of serotypes and enterotoxin type (Wolf 1997).

After this brief introduction, the evolution of ETEC studies that have been conducted at the Escola Paulista de Medicina, Universidade Federal de São Paulo will be presented.

In the late 70's, the studies to determine the prevalence of ETEC strains among children with diarrheal disease in São Paulo were based on the identification of LT and ST expression by using Y1 culture cells and the infant mouse assays, respectively. CFs detection was performed by agglutination assays using human and bovine erythrocytes, and confirmation with specific CFA/I and CFA/II polivalent antisera (Reis et al. 1982). The usefulness of polivalent antisera in detecting ETEC serogroups was evaluated, but due to the great diversity of serotypes observed, specially among LT strains, was not effective for ETEC diagnosis (Guth & Trabulsi 1985). In the 80's, the search for genetic sequences related to LT and ST toxins using hybridization assays (Gomes et al. 1991) was established in the laboratory, and the association of biological and hybridization assays led to the detection and characterization of LT-II enterotoxin in *E. coli* strains, isolated from food and humans with diarrhea (Guth et al. 1986a, b). However, subsequent studies showed that this toxin seems not to be frequent among *E. coli* strains isolated from different origins (Sato 1987, Cerqueira et al. 1994). The characterization of *E. coli* strains belonging to serogroups O128 and O29 showed that the subgroup, H antigen and the biochemical behaviour were useful markers for distinguishing among enterotoxigenic and other pathogenic categories of *E. coli* (Guth et al. 1985, 1989).

In the 90's, a larger range of CFs has been identified in São Paulo by the use of monoclonal antibodies in several immunological assays (Giraldi & Guth 1993, Guth et al. 1994, Nunes 2000). The ability of ETEC strains expressing CFs to adhere to different cell lines has been described (Darfeulle-Michaud et al. 1990, Guth et al. 1994, Viboud et al. 1996), and among the several lines assayed Caco-2 seems to be the most suitable. A high frequency of adherence to Caco-2 and/or HeLa cells was detected in a recent study conducted in São Paulo, and adherence to Caco-2 cells was preferentially observed. Although almost 50% of the adherent strains carried a known CF, there are still a great number of strains, especially among the ST and LT ones, where no CF was identified (Nunes 2000). However, studies are been carried out in order to identify the structures involved in the adherence ability of these ETEC strains.

Studies on the electrophoretic profiles of outer membrane proteins (OMP) and lipopolysaccharides (LPS) of ETEC strains suggested that these characteristics can represent important tools in the clonal analysis of these group of bacteria. Moreover, the observed homogeneity of OMP and LPS profiles among ETEC strains of the same serotype but isolated at different periods of time in São Paulo, suggested that only few clones were disseminated in our population (Nishimura et al. 1996). The use of random amplification of polymorphic DNA (RAPD) confirmed these observations, and also revealed intraserotype-specific variations, undetectable by the combination of several phenotypic typing methods (Pacheco et al. 1997). Moreover, studies on the characterization of OMP profiles and Enterobacterial Repetitive Intergenic Consensus (ERIC) sequence polymorphism of O128ac ETEC strains isolated worldwide, showed the occurrence of O128ac characteristic clones, disseminated in different geographic regions (Nishimura et al. 1999). Recently, using the molecular and phenotypic methods, it was possible to demonstrate the relevance of ETEC as an etiologic agent of acute diarrhea in children and adults in Brazilian Amazon, showing the importance of a broad bacteriological investigation of diarrhea in this region (Vicente et al. 2000).

Because the CFs play a key role in the pathogenesis of ETEC, and the prevalence of these CFs varies according to the geographical region, a detailed description of distribution of ETEC carrying the various CFs in different parts of the world is essential for the development of vaccines against diarrheal diseases in children in developing countries. Indeed, numerous approaches have already been explored for the construction of CF-based

ETEC vaccines. Thus, the perspectives on ETEC future researchs will certainly focus on the identification of new virulence markers, such as adhesins and the design and development of effective CF vaccines.

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