Revista Latinoamericana de Microbiología

Volumen *Volume* **47** **Número** *Number* **3-4** **Julio-Diciembre** *July-December* **2005**

Artículo:

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Revista Latinoamericana de MICROBIOLOGÍA

REVIEW ARTICLE

Vol. 47, No. 3-4
July - September. 2005
October - December. 2005
pp. 140 - 159

Enteroaggregative *Escherichia coli* an emergent pathogen with different virulence properties

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ABSTRACT. Enteroaggregative *Escherichia coli* (EAEC) is an emergent bacterial pathogen. The first studies in developing countries with EAEC strains, showed that this bacterium was associated with persistent diarrhea. However, new studies showed that EAEC may be associated also with acute diarrhea, with both nosocomial and community outbreaks worldwide, and as an important pathogen of diarrheal disease in human immunodeficiency virus-infected adults. EAEC strains are recognized by their characteristic aggregative adherence or "stacked-brick" pattern to epithelial cells. Although the pathogenesis of EAEC infection is not well understood, cellular changes observed in animal models and *in vitro* assays, suggested that the alterations in the intestinal mucosa during EAEC infection are associated with adherence factors and toxins production. The damage has been associated with the release of inflammatory mediators, which may contribute also to the intestinal illness. The dissemination of the high pathogenicity island from *Yersinia pestis* evolutionary group to EAEC has been show; different studies suggest that it may contribute to the virulence of EAEC strains. Molecular methods to investigate the presence of plasmid and chromosomal EAEC-associated virulence markers, have been used for the characterization and epidemiological studies of EAEC strains. Although the clinical and epidemiological importance of EAEC have been demonstrated in different studies, *Escherichia coli* strains with adherent agreggative phenotype are commonly isolated from healthy children and environmental sources. This support the necessity to study virulence factors no related with the cells adherence pattern, that show the specific EAEC pathogenic clones associated whit intestinal disease.

Key words: Enteroaggregative *Escherichia coli*, diarrhea, intestinal damage.

RESUMEN. *Escherichia coli* enteroagregativa (EAEC) es un patógeno emergente. Los primeros estudios realizados con cepas EAEC en países en desarrollo, mostraron que esta bacteria está asociada con diarrea de tipo persistente. Nuevos estudios han mostrado que estas bacterias pueden, además, ocasionar diarrea aguda, brotes de diarrea tanto en comunidades como de tipo noS socomial en diferentes partes del mundo, incluidos países industrializados. También han sido asociadas como agentes causantes de diarrea en individuos adultos infectados por el virus de la inmunodeficiencia humana. Las cepas de EAEC son reconocidas por el patrón agregativo o de "ladrillos-apilados" que presentan sobre células epiteliales en cultivo. La patogénesis de la infección por EAEC no está del todo entendida. Las alteraciones celulares en ensayos *in vitro* y modelos animales sugieren que el daño, en la mucosa intestinal, está relacionado con la adherencia mediada por adhesinas y la producción de toxinas. Por otro lado diferentes observaciones señalan que durante la infección por EAEC se presenta la liberación de mediadores de la inflamación, su presencia en heces sugiere que pudieran contribuir por un lado al daño del tejido y de manera consecuente a la enfermedad intestinal. Como parte de los factores de virulencia de las cepas de EAEC, se ha señalado la existencia de una isla de patogenicidad que le fue transferida por el grupo evolutivo de *Yersinia pestis*. Entre los genes que presenta dicha isla, se encuentran algunos relacionados con sistemas de captación y transporte de hierro. El uso de métodos moleculares para la detección de genes de adhesinas y/o toxinas presentes en plásmido o cromosoma, han sido de utilidad para la caracterización y la realización de estudios epidemiológicos de EAEC. Sin embargo, las cepas de *Escherichia coli* con patrón de adherencia agregativo se aislan frecuentemente de niños sanos y muestras ambientales. Por tal motivo es necesario realizar estudios sobre factores de virulencia, que permitan contar con un marcador específico que facilite la identificación de las clonas de EAEC responsables de enfermedad intestinal.

Palabras clave: *Escherichia coli* enteroagregativa, diarrea, daño intestinal.

INTRODUCTION

Escherichia coli (*E. coli*) is the main facultative anaerobe of the large intestine and colonizes the gastrointestinal tract during the first hours of life.[123] It functions at a microbiotic level in the intestine, and has been linked to macrobiotic functions in terms of nutrition, acting as a vitamin producer.[66]

E. coli that belongs to the *Enterobacteriaceae* family is a short Gram-negative bacillus, non-spore forming, fimbri-

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Received June 22, 2005; received in revised form September 5, 2005; accepted October 17, 2005.

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ate with peritrichous flagellum and a capsule or microcapsule often present. It grows readily on simple culture or synthetic media with glycerol or glucose as the sole carbon source and energy. On solid media, colonies are circular and smooth with a complete edge; some strains produce mucoid colonies. On media containing washed erythrocytes, cell-associated β-hemolysin production can be demonstrated. Different antigens are used to type *E. coli* strains, being the most commons the somatic (O), capsular (K) and flagellar (H) antigens, which contribute to the serotyping scheme introduced in 1944 by Kauffmann. Bacteriophage and colicine typing are also employed to characterize *E. coli* strains, but they have not come into general use.[95,112,131]

Although regarded as part of the flora of the human intestinal tract, several highly adapted *E. coli* clones have evolved, which have developed the ability to cause disease in several areas of the human body. Most of these diseases are related to mucosal surfaces. However, mucosal colonization of the intestine and urinary tract often may be asymptomatic.[114,146]

Theodore Escherich, suspected the association of *E. coli* with intestinal infections early in 1885 when the bacteria wasidentified for the first time. However, it was until 1945 when Bray and other researches demonstrated its involvement in intestinal disease. *E. coli* associated with enteric diarrheal disease includes strains of many different serotypes, categorized into five major groups according to their virulence mechanisms: enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohemorragic (EHEC), and enteroaggregative (EAEC). Other strains, such as diffusely adherent *E. coli* (DAEC), are less well established as pathogens.[60,63,93,180]

ENTEROAGGREGATIVE *ESCHERICHIA COLI*

Classical EAEC strains are recognized by their characteristic aggregative adherence (AA) or "stacked-brick" adherence (Fig. 1 A), to HEp-2 culture cells monolayers.[110,116-119] Recently, Gioppo *et al*.,[59] described a variant of the AA pattern called "chain-like adherence" (CLA), this new adherence pattern frequently has been observed in our laboratory also (Fig. 1 B).

EAEC strains are an emergent pathogen causing diarrhea.[32,113,128,132] The first studies with EAEC strains showed that this bacteria was associated with persistent (≥ 14 days) diarrhea in children,[18,36,99,110] however, new studies show that EAEC may be associated with acute diarrhea.[157] EAEC also is associated with a number of both nosocomial and community outbreaks worldwide.[33,81] In addition, the participation of EAEC as the causative agent of diarrheal disease in human immunodeficiency virus (HIV)-infected adults in the developed world has also been described.[42,187,188]

The polysaccharide O-antigen from EAEC is composed of tetrasaccharide repeating units.[178] EAEC strains belong to a large number of different serogroups such as O44, O55, O86, O111, O125, O126, and O128.[27,45,104,160,161,166,179,193] In EAEC have been reported characteristic serotypes, as example O44:H18, which resulted positive when was examined by adherence patterns on HEp-2 cells and DNA hybridization with EAEC probes.[13,35,166] Suzart *et al*.,[173] observed heterogeneity both in serotypes and outer membrane protein (OMP) profiles, and suggested a great genetic diversity of EAEC. Kahali *et al*.,[87] showed that the majority of EAEC isolates from diarrhea patients in Kolkata, India, were not serotypeable (62%), this support that EAEC strains are an heterogeneous group. Recently, Yatsuyanagi *et al*.,[193] and Elias *et al*.,[46] using molecular methods (EAEC probes and PCR assays) have detected the presence of plasmid and chromosomal EAEC-associated virulence markers among classical EPEC O serogroups. This suggest the horizontal transference of virulence genes, between different pathogenic serogroups difficulting the epidemiological studies.

EPIDEMIOLOGY

Epidemiological studies of diarrhea associated with EAEC strains have been described worldwide implicated

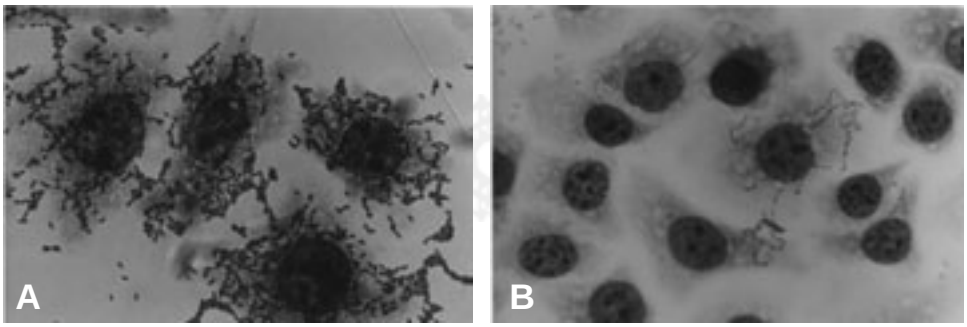

Figure 1. *HEp-2 cells adherence assay.* ***A****, Classical aggregative adherence pattern (AA) or "stacked-brick" of EAEC.* ***B****, Chain-like phenotype variant of the AA. Giemsa stained, 100X light microscopy.*

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as etiologic agents of infant gastroenteritis, in both developed and developing countries. In developing countries with endemic problems of infection, the EAEC strains infection sources may be associated with foods and water.[3,50] In a study of Chilean children, Nataro *et al*.,[110] studied a total of 516 *E. coli* strains isolated from feces of 154 patients and 66 controls, and found that EAEC strains were associated with persistent diarrhea. In India, Bhan *et al*.,[18-20] showed the participation of EAEC in persistent diarrhea of children. Cravioto *et al*.,[36] studying children less than two years of age in a household surveillance study in Morelos, Mexico, found that 51% of isolated EAEC strains were associated with persistent diarrhea. Also, Wanke *et al*.,[186] in a study of a household surveillance of children < 5 years old, in Fortaleza, Brazil, showed that 20% of isolated EAEC strains were associated with persistent diarrhea. Other studies in Bangladesh, India, Brazil, Iran, Ethiopia, Kenya, and Israel showed an association of EAEC and persistent diarrhea in children.[6,11,21,24,25,50,51,55,58,59,94,151]

However, other studies in developing countries, like Bangladesh, Peru, and Thailand showed that EAEC was not identified more frequently in persistent than in acute diarrhea.[8,12,92] A high frequency of severe acute diarrhea caused by EAEC strains has been also seen in India[88,135] and Venezuela.[65] More recently, Okeke *et al*.,[126,127] studied 131 EAEC strains isolated from Nigerian children under five years of age with acute diarrhea and from control subjects. Their results showed that EAEC strains are an important causal agent of acute diarrhea. Scaletsky *et al*.,[158] in a paired case-control study, examined the association between HEp-2 adherent *E. coli* strains and diarrhea. Their results showed a high frequency of EAEC strains in children with diarrhea (20%) when compared with healthy controls (3%) ($p < 0.0001$). Recent studies showed that EAEC is the most prevalent pathotype among diarrhea caused by *E. coli* strains in Congo, Mongolian, Brazilian, and Thai children.[84,142,143,147,152]

In developed countries, a study by Chan *et al*.,[30] in east London, England, showed that EAEC was frequently detected in stool samples from children with persistent and acute diarrhea. In other study in the same country Knutton *et al*.,[91] performed a phenotypic and genetic characterization of EAEC strains isolated from children. In western Europe (Germany), Huppertz *et al*.,[78] recovered EAEC from 2% of children with diarrhea, Presterl *et al*.,[138] in Austria, and Pabst *et al*.,[132] in Switzerland also isolated EAEC from patients with diarrhea.

Annually, many people travel from developed to developing countries. Traveler's diarrhea is a common disorder in these persons, with an important increase of such cases being seen around the world.[2,4,34] Mathewson *et al*.,[96,97] initially described the association of EAEC strains with traveler's diarrhea in adults traveling from USA to Mexico. Gascon *et al*.,[56] studied 165 returned Spanish travelers with diarrhea and EAEC was associated with this cases of diarrhea. Aldachi *et al*.,[2] showed EAEC prevalence in 26% of traveler's diarrhea in three different regions of the world (636 US, Canadian or European travelers with diarrhea): 218 in Guadalajara, Mexico, 125 in Ocho Rios, Jamaica, and 293 in Goa, India. Other studies made in Spain, USA, Jamaica, and Asian countries have showed that traveler's diarrhea is caused by EAEC strains in adult patients traveling from developed to developing countries.[125,141,172,181]

In the last few years, a growing number of reports have shown that EAEC is a potential cause of diarrhea in adults infected with human immunodeficiency virus. Mayer *et al*.,[101] proposed EAEC as a possible cause of diarrhea in HIV-positive patients. Other studies have showed that EAEC could be recovered from diarrhoeal stools from VIH infected patients.[42,57,100,107,108,137,187,188] Wanke *et al*.,[187] studied 68 HIV infected adults with diarrhea and 60 without diarrhea to identify the presence of EAEC strains as determined by HeLa cell adherence. EAEC strains were present in stools of 30 patients with diarrhea and in 18 without diarrhea ($p = 0.05$), these results found a significant association between EAEC and diarrhea in these patients.

Several diarrhea outbreaks caused by EAEC strains have been described. In Mexico, two persistent diarrhea outbreaks were reported, in these five of the infected children died as a consequence of the diarrhea. Both occurred in the malnutrition ward of a pediatric hospital, and were associated with EAEC strains.[48] In France, EAEC O111 strains produced an outbreak of diarrhea and hemolytic-uremic syndrome in children.[23,106] In the United Kingdom, Smith *et al*.,[165] described four outbreaks involving 19, 10, 51 and 53 patients and were associated with EAEC strains. Cobeljic *et al*.,[33] studied an outbreak of diarrhea in 19 neonates and infants less than 6 months in a neonatal nursery ward from Serbia. Pai *et al*.,[133] described an outbreak of diarrhea associated with EAEC in 20 patients from south India. Also, Spencer *et al*.,[167] in England described four outbreaks of diarrhea in adult students and infants with diarrhea. In Tajimi City, Gifu, Japan, Itho *et al*.,[81] described an outbreak in 2,697 schoolchildren with diarrhea in 16 schools. EAEC were isolated of stool specimens from 30 children. Twenty-seven strains of EAEC were isolated from 12 patients all of serotype, O non determited (OND):H10.

CLINICAL MANIFESTATIONS AND TREATMENT

The infection with EAEC strains is associated with watery, mucoid and secretory diarrhea, low-grade or absence of fever and some times vomiting.[19,135] Bloody stools are present in approximately one-third of EAEC infected pa-

tients.[36] Volunteers infected with prototype EAEC strains (O44H:18, O42 strain), showed mucoid diarrhea of low volume and without hidden blood or fecal leukocytes; all patients remained afebrile, during this study was showed that the incubation period was between 8 and 18 hours.[114] Bhan *et al*.,[19] during a study performed in Anapur-Palla, northern India, observed that the lasting diarrhea period was of 17 days. On the other hand, Steiner *et al*.,[168] observed that a high percentage of patients infected with EAEC strains showed fecal lactoferrin and high levels of interleukin-8 (IL-8) in their feces.

The treatment of individuals with diarrhea caused by EAEC is the oral rehydratation, utilizing glucose and electrolytes. Some studies have showed that rifaximin[80,134] and ciprofloxacin[61] decreases the lasting period of diarrhea by EAEC.

HISTOLOGICAL STUDIES

The pathogenesis of EAEC infection is not well understood. A better understanding may be found by histological examination of intestine samples from infected patients or employing animal models, in which several candidate virulence factors have been described.

Tzipori *et al*.,[177] observed in gnotobiotic piglets, orally inoculated with EAEC strains of human origin, that the bacteria caused diarrhea and death in the majority of animals. The post-mortem histological examination revealed moderate hyperemia of the distal small intestine and cecum, swelling of the small intestinal villi, and layers of aggregated bacteria stacked together in a mucus gel-like matrix overlying intact epithelium. Although some of the animals did not experience diarrhea, all of them developed an unusual mucoid gel closely adherent to the small intestinal epithelium. In addition, the intestinal epithelium displayed pitting of goblet cells, suggesting that hyper-secretion of mucus had been stimulated.

Tickoo *et al*.,[175] showed that EAEC strains of human origin colonize the small and large intestine of rabbits, causing diarrhea, moderate villous stunting and karyorrhexis. Vial *et al*.,[180] showed strains using the rabbit and rat ileal loop models resulted in destructive lesions, characterized by shortening of the villi, hemorrhagic necrosis of the villous tips, and a mild inflammatory response with edema and mononuclear infiltration of the submucosa. Additionally, pitting of goblet cells and embedding of aggregating bacteria within a periodic acid-Schiff staining blanket was also observed.

Recently, Sainz *et al*.,[150] utilizing BALB/c mice as infection model inoculated with the prototype EAEC O42, strain[109] showed that 50% of the animals were colonized. The intestine necropsy of some infected animals presented mucus and blood. Histopathologic evidence confirms the stimulation of mucus hypersecretion, increased amount of goblet cells, the presence of bacterial aggregates in the apical surfaces of intestinal epithelial cell and edema in the submucosa. Similar histological alterations have been observed in autopsy specimens of ileum samples, from children who died as consequence of persistent diarrhea associated with EAEC infection.[48]

Hicks *et al*.,[75] examined the interaction between EAEC and the human intestine using the *in vitro* organ culture (IVOC) model. Intestine mucose biopsies obtained from children were cultured with five different EAEC strains isolated from infants with diarrhea, and with two well-described prototype EAEC strains (17-2 and JM221). The prototype strains adhered to jejunal, ileal, and colonic mucosa. However, the wild-type strains showed a variation in adhesion location: two adhered to all intestinal levels, one adhered to the jejunum and ileum, another adhered to the ileum only, and the last one adhered to the ileum and colon. Adherence was in an aggregative or stacked-brick pattern, resembling those seen on HEp-2 cells. Electron microscopy of the infected small intestinal mucosa revealed bacteria in association with a thick mucus layer above an intact enterocyte brush border, which contained extruded cell fragments. The mucus layer was not present in the control samples. EAEC adherence to the colonic mucosa was associated with cytotoxic effects including microvillous vesiculation (but without evidence of an attaching/effacing lesion), enlarged crypt openings, the presence of intercrypt crevices, and increased epithelial cell extrusion.

Nataro *et al*.,[115] observed in polarized monolayers of CaCo-2 and T84 intestinal epithelial cells inoculated with the 042 EAEC strain, that the bacteria adhered strongly to the T84 cells but not to the CaCo-2 cells. On the other hand, T84 cells infected with 042 displayed marked toxic effects, most prominently in areas where bacteria were adhering. The apical membrane of damaged cells exhibited vesiculation and shedding of microvilli, the cells cytoplasm displayed subnuclear vacuolization, and in some cases, nuclei of affected cells became separated from the surrounding cytoplasm. Severely affected cells release their nuclei, and vacuolated remnant cells were also seen.

VIRULENCE FACTORS

Adherence. EAEC posses hemagglutinating activity indicative, of their adhesive properties to the intestinal mucose surface. Qadri *et al*.,[139] studied EAEC strains isolated mostly from children with diarrhea in Bangladesh, India, Thailand, Central America, and South America. Most of these strains showed mannose-resistant hemagglutination (MRHA), which was inhibit by sialic acid-containing com-

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pounds, suggesting that these compounds may be the receptors for EAEC on erythrocytes and possibly on the intestinal mucose.

EAEC strains cultured at 37°C produced an unusual fibrillar MRHA of 2.5 nm estimated diameter, that may be participate in adherence.[130] Knutton *et al*.,[90] examined a collection of 44 EAEC strains isolated from infants with diarrhea in India and the United Kingdom (Forty-three EAEC strains were positive with a DNA probe and MRHA). These strains were studied to see their ability to adhere *in vitro* to human intestinal mucosa. All strains adhered only to human colonic mucosa forming localized aggregates. Examination by electron microscopy of infected colonic mucosa indicated that fimbrial structures mediated the adhesion. The study showed also the existence of four morphologically distinct kinds of fimbriae: 6- to 7-nm-diameter hollow cylindrical rodlike fimbiae, 5- to 6-nm-diameter rodlike fimbriae, 2- to 3-nm-diameter fibrillar fimbriae, and bundles of fine 2- to 3-nm-diameter fibrils.

Nataro *et al*.,[111] showed that the AA of EAEC is associated with the presence of a 60-MDa plasmid (pAA). A region of approximately 12 kb on the pAA cloned in HB101 (pJPN31) was implicated in the AA phenotype. Transmission electron microscopy of HB101 (pJPN31) revealed the presence of bundle-forming fimbriae. A 14-kDa protein was seen upon polyacrylamide gel electrophoresis and immunoblotting of surface shear preparations from fimbriate clones. This is a flexible, bundle-forming fimbrial structure of 2 to 3 nm in diameter, which was designated aggregative adherence fimbriae I (AAF/I). AAF/I mediate HEp-2 adherence and human erythrocyte hemagglutination in strain 17-2.[112,113] Moreira *et al*.,[105] have showed the role of AAF/I in the AA patern and in EAEC biofilm formation. The *agg* genes for AAF/I are organized as two separate gene clusters on the 60-MDa plasmid of strain 17-2, separated by a 9 kb DNA.[112] Region 1 contains a cluster of genes required for fimbrial synthesis and assembly, including the structural subunit of the fimbriae itself (*aggA*).[155] Nucleotide sequence analysis of the region 1 cluster suggests that AAF/I is a member of the Dr family of adhesins, so called because they mediate adherence to the Dr blood group antigen. Region 2 include AggR, a transcriptional activator of aggregative adherence fimbria I (AAF/I) expression.[155] AggR is a mememberr of the AraC/XylS family of transcriptional regulators.[113] Although the AAF/I fimbriae are bundle-forming fimbriae do not show homology with other members of the so-called type 4 classes of fimbriae. Using immunogold electron microscopy and a DNA probe derived from the biogenesis cluster of AAF/I, it has been found that only a minority of EAEC strains expresses AAF/I.[112,113] Czeczulin *et al*.,[37] identified a second plasmid encoding a fimbriae of 5 nm in diameter (AAF/II), which was morphologically, antigenically and genetically distinct from AAF/I. The *aaf* genes encoding AAF/II are also organized as two unlinked regions; however, in this case, the fimbrial subunit is removed by more than 15 kb from the required biogenesis gene cluster. The region 1, comprising *aafA* (the corresponding AAF/II structural fimbrial subunit) and *aafR* as well as the chaperone *aafD*; and region 2, which contains the usher protein *aafC*. The biogenesis of AAF/II also requires the activation of the transcriptional activator *aggR*.[44] The *fis* gene is required for expression of *aggR*,[163] which encoded an activator of AAF/II expression acting at the level of the chaperone-encoding gene *aafD*.[44] The product of the *yafk* gene also is required for transcription of AA/FII-encoding genes.[163] Ruiz-Perez *et al*.,[148] have developed an *in vitro* culture method to characterize the expression of EAEC strain 042 genes under conditions mimicking the colonic environment, and showed that *aggR* expression is increased under low pH and relative low osmolarity in the presence of commensal bacteria. DNA probe analysis showed that AAF/I and AAF/II were present in only a minority of EAEC strains and thus, as it is the case of ETEC strains, intestinal colonization by EAEC appears to be mediated by one or more fimbrial antigens.

Bernier *et al*.,[17] identified a third different aggregative adhesion fimbriae (AAF/III) in a typical EAEC strain (isolate 55,989). The analysis of the *agg*-3 gene cluster encoding AAF/III fimbriae which is plasmid borne, showed this cluster to be closely related to *agg* and *aaf* which encode AAF/I and AAF/II, respectively. The biogenesis of AAF/III also requires the activation of the transcriptional activator *aggR*. A collection of 25 EAEC strains isolated from HIV-infected patients presenting persistent diarrhea showed that only 36% of the strains carried similar sequences to those of the *agg* and *aaf* operons.

Sheickh *et al*.,[164] showed that EAEC strains secrete a 10.2-kDa protein, witch is encoded by the *aap* gene, localized immediately upstream from the AggR transcriptional activator. The *aap* gene was originally designed as *aspU* (EAEC secreted protein U) by Czeczulin *et al*.[38] The *aap* product has a typical signal sequence and is secreted when it remains noncovalently attached to the bacterial surface. This protein is exported by a putative ABC transporter complex, which is encoded in a genetic locus of the plasmid that comprises five genes (aat-PABCD).[124] Infection of colonic biopsies with the wild-type EAEC strain 042 and its *aap* mutant, revealed an intense autoagglutination of the mutant compared with that observed in the wild-type parent. This data suggest that the *aap* gene product participate in the formation of a surface coat that acts to disperse the bacteria. By the specific activity of the *aap* gene product it was named “dispersin”, suggesting that it could be

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representative of a new functional class of colonization factors. Serum samples obtained from volunteers naturally infected with EAEC strains that are dispersin producers, reacted with the 10.2-kDa protein. This protein could be considered as a possible immunogen to prevent EAEC infections.[164]

Wai *et al*.,[184] examined the surface of three EAEC and three EPEC strains by electron microscopy freeze-substitution technique and observed an electron dense surface layer on EAEC but not on EPEC strains. The SDS-PAGE analysis of the outer membrane proteins (OMP) revealed the existence of a 38-kDa protein in EAEC strains. Using immunoelectron microscopy the EAEC surface was seen to react with an anti 38-kDa-protein serum raised in mice. The protein could be easily extracted from the bacterial surface with 5 M LiCl treatment at room temperature. The hydrophobic surface feature of the EAEC strains was lost when the 38 kDa was extracted. These observations suggested that the surface protein layer is participating in the expression of the aggregative phenotype of EAEC strains. Chart *et al*.,[31] showed that in EAEC (serotypes O44:H18 and O126:H27) magnesium ions were essential for the adhesion to HEp-2 cells, and the expression of an outer membrane-associated protein of 18-kDa was necessary for the pellicle formation when growing in L-broth. On the other hand, Grover *et al*.,[68] described an 18-kDa adhesin that binds specifically to a galactose molecule.

Toxins. The cellular alterations observed in animal models, or in *in vitro* assays as human intestinal explants or cultured T84 cells infected with EAEC, are not accompanied by internalization or by an intimate attachment of the bacteria. These and other experimental results suggested that the alterations in the intestinal mucosa observed during EAEC intestinal infection are associated with toxin production.

ENTEROAGGREGATIVE HEAT-STABLE TOXIN

Studies using different models showed that to produce cellular alterations, genes encoded on the aggregative plasmid were required. Savarino *et al*.,[153,154] identified an open reading frame encoding a 4,100-kDa protein on the 65-MDa plasmid, a homologue of the heat-stable enterotoxin from ETEC. This 38-amino acid protein featuring four cysteine residues instead of six, which are characteristic of *E. coli* producing ST, and was, designated EAST1 (Enteroaggregative heat-stable toxin-1). EAST1 caused increases of the short circuit current (Iscc) and of the potential difference (PD) on the rabbit mucosa with the Ussing chamber model. However, its participation in human disease has not been demonstrated. Savarino *et al*.,[155,156] showed that EAST1 is present in approximately 40% of EAEC strains and in a similar proportion of nonpathogenic *E. coli* strains. Other *E. coli* categories, most notably EHEC, elaborate EAST1 with a higher frequency than EAEC.[194]

HEAT-LABILE TOXIN ANTIGENICALLY RELATED TO *E. COLI* HEMOLYSIN

Haque *et al*.,[69] studied the hemolytic activity of EAEC strains. These strains showed K- sensitive contact hemolysin activity with sheep erythrocytes when were cultured in both; Casamino Acids-yeast extract broth supplemented with 1 mM calcium chloride and in nutrient broth medium. Fernandez-Prada *et al*.,[54] showed that hemoysin-positive EAEC strains caused oncosis in human monocyte-derivated macrophages and apoptosis in J774 cells. On the other hand, Baldwin *et al*.,[10] identified a protein of approximately 120-kDa produced by non-hemolytic EAEC strains. Using Western blot assays, was observed that the protein reacted with antibodies raised against the C-terminal region of *E. coli* hemolysin. When cultured HEp-2 cells were treated with the culture supernatants of different EAEC strains, was observed an increase of intracellular calcium and the induction calcium-dependent protein phosphorylation. However, the participation of this protein in intestinal damage has not been determined.

PLASMID-ENCODED TOXIN

From an EAEC strain isolated during two independent outbreaks of diarrhea in a Pediatric Hospital in Mexico City, Eslava *et al*.,[48] identified two proteins of high molecular weight (108-kDa and 116-kDa). These proteins tested in a rat ileal loop model, caused shortening of the villi, hemorrhage, necrotic alterations and ulceration of the upper epithelium. Similar intestinal lesions were described in *post mortem* studies of one child who died during the diarrhea outbreaks. The microscopic study showed that the ileum was the mainly region affected with necrosis of the superficial epithelium, ulcerated areas and severe inflammatory response consisting of lymphocytes, plasma cells and eosinophiles in both, the lamina propria and the submucosa, and hyperplasia of the lymphoid follicles of these former areas. The colon of this child showed focal destruction of the crypts and lymphocytic infiltration. Genetic studies developed by Eslava *et al*.,[49] found that the gene for the 108-kDa toxin was on the 60-MDa adherence-related plasmid of the O42 EAEC strain. Immunological studies using serum samples from the children infected with EAEC during the Mexican outbreaks,[48] and other samples obtained from naturally infected human volunteers,[114] showed that this protein is recognized by the serum (Fig. 2). These results have been confirmed in a study from children in Brazil.[14] The DNA sequence analysis of

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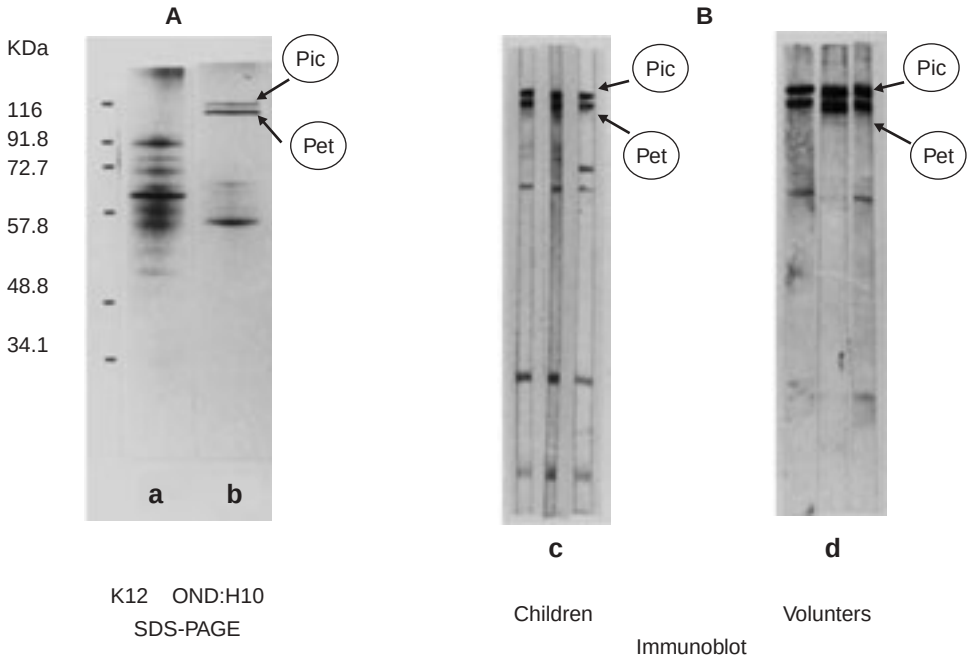

Figure 2. *Pet and Pic autotransported proteins from EAEC strains.* ***A****, SDS-PAGE of ammonium sulfate precipitate supernatant from* ***a,*** E. coli *HB101 and* ***b,*** *EAEC (OND:H10) isolated from a died child as consequence of persistent diarrhea.* ***B****, western blot of ammonium sulfate precipitate supernatant from EAEC OND:H10 developed with:* ***c,*** *serum samples obtained from children naturally infected during two diarrhea outbreaks;* ***d,*** *serum samples from volunteers naturally infected with EAEC strains Pet and Pic producers.*

the *pet* gene, showed that Pet toxin is a member of the autotransporter (type V) protein family, included in the serine protease autotransporters of the *Enterobacteriaceae* (SPATEs) subgroup.[72, 73] The product of the *pet* gene is synthesized as a 140-kDa precursor molecule which is processed at the N and C termini during secretion, allowing the release of a mature protein into the culture supernatant. The molecular weight of this protein deduced from the gene sequences was 104 kDa (GenBank nucleotide sequence accession no. AF056581).

The Ussing chambers model demonstrated that Pet induces an enterotoxic effect on rat jejunal tissue.[122] Studies with cultured HEp-2 and HT29 C1 cells treated with Pet, showed a release of the cellular focal contacts from the glass substratum, followed by complete rounding of the cells and detachment from the glass.[120] However, using stained techniques, it was revealed that > 90% of rounded cells were viable. A fluorescein-labeled phalloidin analysis of the same cells showed contraction of the cytoskeleton and loss of actin stress fibers. The preincubation of Pet with phenylmethylsulfonyl fluoride (PMSF) a serine protease inhibitor, abrogated the cytopathic effects completely. Similar results were observed using a toxin obtained from the mutant Pet S260I (deficient in protease activity) where the serine (the catalytic residue) was changed by an isoleucine. This protein did not induce cytopathic effects, cytoskeletal damage, or enterotoxic effects in Ussing chambers.[116,120]

An *in vitro* assay showed that Pet cleaves pepsin and human coagulation factor V.[40] A study performed by Villaseca *et al.*,[182] showed that Pet produces spectrin and fodrin (nonerythroid spectrin) disruption. Purified membranes from erythrocytes and HEp-2 cells incubated at different times with different Pet toxin concentrations and analyzed by SDS-PAGE showed degradation of both α- and β-spectrin and -fodrin chains. During the spectrin degradation, a 120-kDa subproduct of it was identified using specific anti α- and β-spectrin antibodies (Fig. 3), indicated that α-spectrin is cleavage in the middle of the calmodulin binding domain.[70] Other work using transfectated cells whit the gene of fodrin corroborate this results.[28]

The spectrin and fodrin disruption induced by Pet was inhibited by polyclonal anti-Pet antibodies. The participation of the serine protease motif was confirmed using PMSF and the Pet S260I mutant. HEp-2 cultured cells in suspension were incubated and analyzed from 3 to 36 hours to evaluate the *in vitro* Pet effects. SDS-PAGE and Western blot analysis of HEp-2 protein membranes showed that α- and β-fodrin chain degradation starts at 12 hours, and have a total effect at 36 hours.[182] These last results have been confirmed in a recent study.[28]

The degradation effects induced by Pet toxin on fodrin, in suspension cells visualized by immunofluorescence assay performed with anti-α- and β-fodrin antibodies, showed alterations characterized by cellular swelling and irregular distribution of fluorescence (Fig. 4). These observations suggest that the fodrin fragmentation induces a disarrangement of the cell membrane.[182]

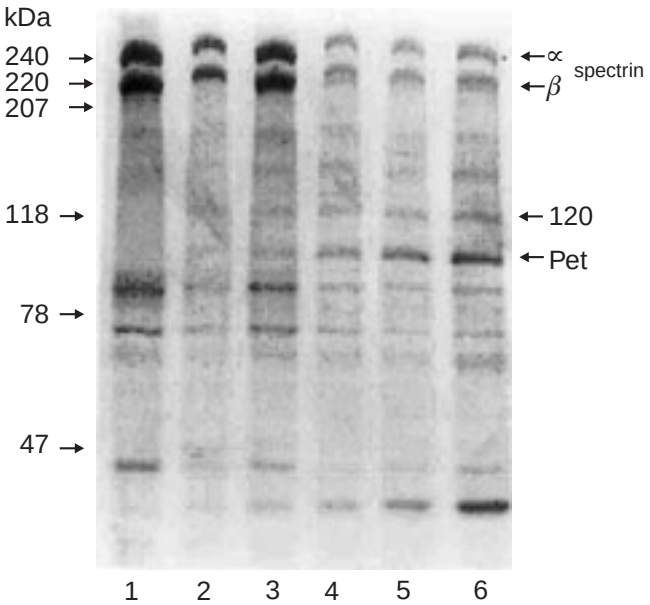

Figure 3. *SDS-PAGE of membrane proteins from horse red blood cells. Spectrin degradation induced by Pet toxin from EAEC. The membrane proteins (10 µg/well) were incubated with different Pet concentrations. Line:* ***1****, Non treated membrane proteins; Lines:* ***2-6****, protein membranes treated with different Pet concentrations;* ***2****, 0.312 µg,* ***3****, 0.625 µg,* ***4****, 1.25 µg,* ***5****, 2.5 µg, and* ***6****, 5 µg. Pet induce degradation of α- and β-spectrin chains producing a 120 kDa spectrin subproduct, the effect is concentration dependent.*

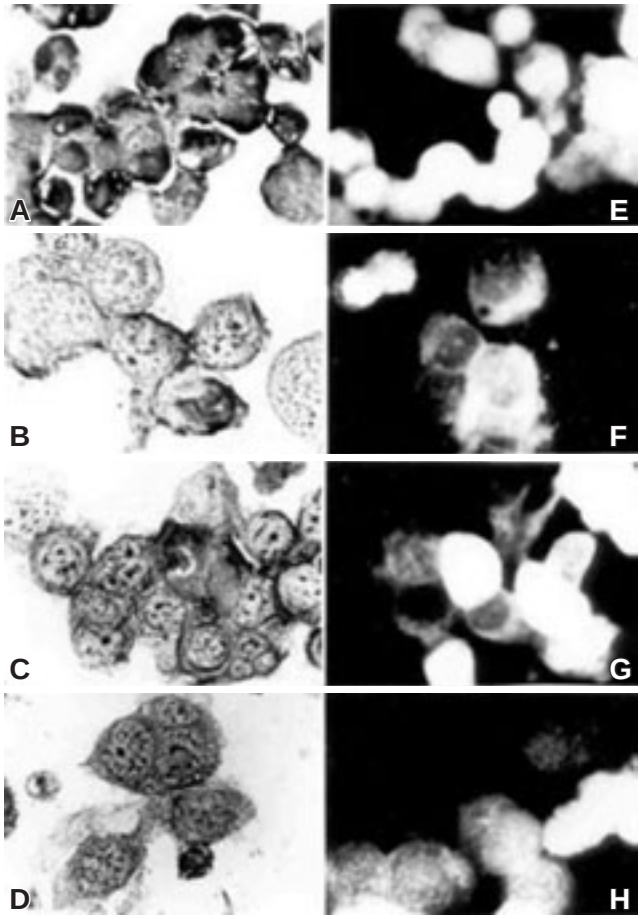

Figure 4. *Light (****A-D****) and fluorescent (****E-H****) microscopy of HEp-2 cells treated with Pet toxin during 36 h.* ***A*** *and* ***E****, untreated HEp-2 cells.* ***B*** *and* ***F****, cells treated with 10 µg/ml of Pet protein.* ***C*** *and* ***G****, 50 µg/ml.* ***D*** *and* ***H****, 100 µg/ml.* ***A****,* ***B****,* ***C****, and* ***D****, Coomassie blue stained preparations.* ***E****,* ***F****,* ***G*** *and* ***H****, preparations incubated with anti-α- and anti-β-fodrin antibodies developed with anti-rabbit antibodies fluorescein labeled. Fodrin degradation induced by Pet toxin cause cellular alterations characterized by swelling and irregular distribution of fluorescence. Fodrin fragmentation causes disarrangement of the cell membrane, leading to epithelial cell destruction. Magnification X 100.*

Previous data suggest that Pet must bind to the HEp-2 cell surface, be internalized, and exert a proteolytic attack on an intracellular host protein. Brefeldin A inhibits the cytopathic effects of Pet by disrupting its trafficking within HEp-2 cells, which suggests that Pet may employ retrograde transport via the Golgi apparatus, however, no endoplasmic reticulum retrieval signature is apparent in Pet.[40] Expression of mature Pet toxin (Passenger domain) in the cytoplasm of HEp-2 transfectated cells showed condensation of spectrin cytoskeleton.[171] Dutta *et al*.,[41] analyzed the structure and function of Pet toxin using scanning linker mutagenesis and showed the existence of separate functional domain mediating proteolysis, secretion, and cell interaction.

This has been considered as a new system of cellular damage identified in bacterial toxins which includes the internalization of the protease,[121] induction of some unknown intermediate signaling steps,[183] and finally fodrin degradation leading to epithelial cell destruction.[103]

PROTEIN INVOLVED IN INTESTINAL COLONIZATION

Using SDS-PAGE electrophoresis Eslava *et al*.,[48] showed the presence of a high molecular weight protein in the culture supernatant of EAEC strains of approximately 116-kDa. Westernblot assays showed that this protein was recognized by serum samples from the children infected with EAEC[48] and serum samples from infected human volunteers.[114] (Fig. 2). The same results were obtained in a study performed with infected children in Brazil.[14] Henderson *et al*.,[71] identified on the chromosome of EAEC strain 042 and *Shigella flexneri* 2457T (wild-type *Shigella flexneri* 2a) the gene that encodes the 116-kDa EAEC secreted protein. The chromosomal locus has been designated *pic/set*, because it encodes at least two putative virulence fac-

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tors. The Pic sequence is identical to that of the protein product of the *she* gene from *S. flexneri* (Gen Bank accession no. U35656).[140] The product of *pic* gene is synthesized as a 146.5-kDa precursor molecule which is processed at the N and C termini during secretion, allowing the release of a mature protein into the culture supernatant. The deduced amino acid sequence of Pic shows that is another member of the autotransporter protein family, included in the SPATEs subgroup also. The molecular weight of this protein, deduced from the gene sequence is of 110-kDa.[71,72]

Functional analysis of Pic protein implicates it in serum resistance, hemagglutination and mucinase activity. Considering these properties the protein was named protein involved in colonization (Pic). The chromosomal locus *pic/set* of *S. flexneri* is encoded on a pathogenicity island called SHI-1.[7] In both EAEC and *S. flexneri* 2a strains, an oligomeric enterotoxin called *Shigella* enterotoxin 1 (ShET1) can be found. It is encoded completely on the opposite strand but within the *pic* gene. ShET1 comprises of a single 20-kDa catalytic A subunit (encoded by the *setA* gene) and five 7-kDa B subunits (encoded by the *setB* gene). The ShET1 mode of action has not been defined, but it does not appear to act through the traditional mediators of toxin-induced intestinal secretion, such as cAMP and cGMP. EAEC strains possess specialized iron acquisition systems. Recently, in our laboratory we demonstrated that Pic is capable of cleavage the human hemoglobin and bound to the hemine may be for iron acquisition.[77] EAEC strains contain more than one iron transport system and the iron utilization may be important for pathogenesis.[129]

S. flexneri and EAEC have different pathogenic strategies. *S. flexneri* is a large-bowel pathogen which causes invasive and inflammatory colitis. Many shigellosis patients experience a watery prodromal phase, which may be a manifestation of early small-bowel involvement and to which ShET1 may contribute. In contrast, EAEC are thought to be distal small-bowel and/or colonic pathogens, which typically cause watery diarrhea without evidence of invasion. However, different studies show that EAEC may induce mild inflammatory enteritis.[16]

SHIGA TOXIN

Eight *E. coli* O111:H2 strains (designated RD1 to RD8) isolated in France during an outbreak of HUS showed aggregative adhesion (AA), instead of the typical localized adhesion, to HEp-2 cells and possessed the genetic markers of EAEC.[106] Accordingly, they agglutinated rat erythrocytes in the presence of 0.5% mannose and gave positive PCR amplification with the primer pairs which amplify a 630-bp region of the EAEC probe and the *astA* determinant of EAST1. Moreover, they hybridized with the EAEC and *astA* probes. The Vero cell assay and the PCR analyses confirmed that all the strains produced Stx2 only. Nucleotide sequence of the B-subunit toxin gene showed 100% identity with the nucleotide sequence of the *stx*2 B gene from the O157:H7 strain EDL933.

All the *E. coli* O111:H2 strains harbored two plasmids of approximately 100 and 7 kb, respectively. Southern analysis showed that the large plasmids hybridized with the EAEC probe but not with the *stx*2 probe, which reacted only with the total cellular DNAs. These results indicated that the EAEC gene cluster was located on the large plasmid, as in the EAEC strain 17-2, and that the *stx*2 gene was present within the chromosome, as in the EHEC strain EDL933. Stx genes are usually phage encoded in both O157 and non-O157 EHEC, and a λ phage regulatory gene, designated *p*, is usually located near both the *stx*1 and *stx*2 genes.[106]

The *E. coli* O111:H2 isolates were negative in a PCR assay performed with a primer pair complementary to *p* but hybridized with a probe produced by PCR amplification of the *p* gene present in strain EDL933. An attempt to induce phages was performed by UV light treatment. Lysates of the *E. coli* O111:H2 strains did not contain infectious phages, while strain EDL933, included as a control in all the experiments, consistently yielded lysates containing 104 PFU/ml. The absence of inducible phages, however, does not exclude the possibility that the *stx*2 determinant is associated with a defective phage, and further work is needed to clarify this issue. Stx production and enteroaggregative adhesion ability might be as pathogenic to human as the classic EHEC.[106]

INVASIVENESS

Benjamin *et al*.,[15] showed that some EAEC strains might invade intestinal epithelial cells *in vitro*. However, using the human intestinal explants model infected with EAEC strains, cell internalization of the bacteria is not revealed. Abe *et al*.,[1] using polarized Caco-2 and T84 human cultured cells and human jejunal and colonic mucosal, showed that EAEC strain 236 (O111:H12) was adherent to both polarized cell lines. In addition the two intestinal tissues also showed severe damage and invasiveness on T84 cells and colonic mucosa. The EAEC 042 strain, although also adhere to the cultured intestinal cells lines, but did not adhere to or invade jejunal or colonic tissue.

THE *YERSINIA* HIGH PATHOGENICITY ISLAND

Y. pestis have the *pgm* (pigmentation) locus of 102-kb of chromosomal DNA, which is composed of two distinct

parts; the pigmentation segment *hms* (hemin storage) of 68 kb, which confers a pigmented phenotype on colonies grown on Congo red-agar plates and the other one of approximately 35 kb named "high-pathogenicity island" (HPI). The HPI, is absolutely necessary for expression of the trait of virulence to mice of *Y. pestis*, *Y. pseudotuberculosis* serotype O1, and *Y. enterocolitica* biotype 1B strains.[159]

The HPI have virulence genes involved in iron uptake, of these the *irp1* and *irp2* genes coding for the iron-repressible high-molecular-weight proteins HMWP1 and HMWP2, which presumably are involved in the production of yersiniabactin; and the *fyuA* or *psn* gene (for ferric yersiniabactin uptake or pesticin sensitivity) encoding for yersiniabactin receptor FyuA that also serves as a receptor for pesticin.[159]

Schubert *et al*.,[159] demonstrated that the HPI of the *Y. pestis* evolutionary group is disseminated among species of the family *Enterobacteriaceae* which are pathogenic to humans. In their study Schubert *et al*. showed that the *irp2* and *fyuA* sequences in EAEC and *E. coli* blood culture isolates were presents in 93 and 80% respectively and only in 27% of EIEC strains, 5% of ETEC and EPEC strains, but in none of the EHEC, *Shigella*, or *Salmonella* strains investigated. The HPI in *Yersinia* and most other enterobacteria is integrated in an *asn tRNA* locus and carries a P4-like integrase gene, suggesting that the island spread among enterobacteria by horizontal transfer, and that this transfer was mediated by a P4-like bacteriophage.[9]

When the nucleotide sequences of *fyuA* gene from EAEC 17-2 strain, *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica* were compared an identity of 99.6, 99.8, and 98.8% respectively was observed. The *fyuA* genes from EAEC and *Y. pestis*, showed that only five of nine nucleotide substitutions caused amino acid mismatches. The G+C content of the *fyuA* gene was 56.2 mol%, which is much higher than the overall G+C content of *E. coli* (48 to 52 mol%) or *Y. enterocolitica* (46 to 50 mol%) chromosomes. The sequences of the EAEC 17-2 and EIEC E12860 *irp2* fragments are identical and have 99.7, 99.7 and 98.6% identity with those of *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*, respectively. The *fyuA-irp* gene cluster of *Yersinia* spp is largely conserved among different HPI-positive *E. coli* strains and the *fyuA-irp* gene cluster of *E. coli* strains is more closely related to the HPIs of *Y. pestis* and *Y. pseudotuberculosis* than to the HPI of *Y. enterocolitica* serotype O8.[159]

In a study by Xu *et al*.,[190] was observed that HPI-harboring *E. coli* was the third most frequently isolated enteric pathogen from patients with diarrhea in China. In this study also was observed that the clinical symptoms differ from those caused by enteroadhesive *E. coli*. Vomiting and abdominal pain were recorded for 33.33 and 66.67% of the patients, respectively. Stools with blood were observed for 9.52% of the patients. Twenty-four of 42 (57%) patients experienced a temperature over 37.4°C.

Recently Okeke *et al*.,[129] in a phenotypic assay showed that EAEC strains posses a specialized iron acquisition system. The genes *fyuA*, *pic*, and the gene *iucA* (for the synthesis of siderophore) were detected in 85.7, 61.9%, and 76.2 of the EAEC strains, respectively.

Although the aggregative adherence pattern on cultured HEp-2 cells of EAEC is the gold standard for the identification of this group of *E. coli* strains, only some virulent clones are responsibles to cause diarrheal disease. Have been show that the HPI contribute to the virulence of *Y. pestis* evolutionary group, the existance of HPI in EAEC strains isolated from patients with diarrhea suggest its participation as a factor implicated in the evolution of the diarrheogenic EAEC strains.

INTESTINAL INFLAMMATION

EAEC can bind to human colonic mucosa, with formation of a thick mucus layer and production of intestinal inflammation.[99,168,191] A mild inflammatory response with edema and mononuclear infiltration of the submucosa was observed during EAEC infection of animals.[48,180] The intestinal alterations observed in a child who died as a consequence of EAEC infection, showed an intense inflammatory response with lymphocytes, plasma cells and eosinophiles.[48]

During a study of infant diarrhea in Fortaleza, Brazil, children with persistent diarrhea associated with EAEC infection showed intestinal inflammation determined by significative elevations of fecal lactoferrin (stable neutrophil product and sensitive marker for fecal neutrophils) and interleukin-8 (IL-8), a neutrophil chemokine.[52,94,168,186] Detection of high concentration of fecal cytokines in patients with EAEC diarrhea suggests increased production and secretion from an inflamed bowel. In inflammatory bowel disease it has been confirmed that levels of fecal cytokines interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), and IL-8 are elevated.[26,83,170] Jiang *et al*.,[86] have showed that individuals with a homozygous genotype -251 AA single-nucleotide polymorphismo (SNP), in the promotor region of IL-8 gene, are associated with EAEC diarrhea.

Bouckenooghe *et al*.,[22] demonstrated that naturally occurring EAEC diarrhea in travelers was associated with fecal lactoferrin. Greenberg *et al*.,[67] studied the intestinal inflammatory response of traveler's diarrhea acquired in Goa, India, and Guadalajara, Mexico. Fecal lactoferrin also was found in stool samples in which EAEC strains were isolated. Travelers to India who had EAEC-associated diarrhea showed elevated levels of fecal IL–8, IL-1β, and IL-1

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receptor antagonist (IL-1ra). Patients with EAEC associated diarrhea from Mexico, 2 of 15 produced IL-8, and 5 of 16 produced detectable levels of IL-1β.

Jiang *et al*.,[85] showed an association between the presence of EAEC plamid-borne virulence factors *aggA*, *aggR*, *aap* (*aspU*), and *aafA*, and the presence of fecal cytokines. The presence of *aggR* or *aafA*, or more virulence factors, in EAEC from patients with diarrhea was associated with a statistically increased concentration of IL-8 and INF-γ in feces, compared with that in EAEC negative for these factors. Symptomatic infection with EAEC isolates positive for *aafA* were associated with elevated levels of fecal IL-1β, and symptomatic infection with EAEC isolates positive for *aap* (*aspU*) were associated with elevated levels of fecal IL-1ra.

Huang *et al*.,[79] showed that 72% of EAEC that carrying the virulence factors *aggR*, *aggA*, and *aap* (*aspU*) isolates from patients with traveler's diarrhea elicit IL-8 production, when was examined on intestinal adenocarcinoma HCT-8 line. However, 40% of those EAEC not carrying virulence factors also elicit IL-8 production. EAEC strains tested on Caco-2 cells were seen to induce release of IL-8, apparently via a novel heat-stable, high molecular weight, chromosomally encoded protein.[168] The cloning, sequencing and expression of this biologically active IL-8–releasing factor from EAEC, has been identified as a flagellin (FliC-EAEC), principal component of EAEC flagella.[39] Flagella purified from EAEC 042 and several other EAEC isolates potently release IL-8 from Caco-2 cells; an engineered aflagellar mutant of 042 does not induce IL-8 release. FliC-EAEC-induced IL-8 secretion requires TLR5-dependent p38 mitogen-activated protein kinase (MAPK) activation.[89] The IL-8 released from epithelial cells, in response to EAEC, could act as the first step in a secretory cascade by recruiting neutrophils in the intestinal epithelium. Epithelial cells release 5'-adenosine monophosphate, which in turn is converted by a 5'-nucleotidase on the apical surface of enterocytes to adenosine, an agonist for chloride secretion.[169]

Partially purified Pet and Pic proteins inoculated in the rat ileal loop model caused shortening of the villi, hemorrhage, necrotic alterations, ulceration of the upper epithelium and an intense inflammatory response characterized by mononuclear and polymorphonuclear phagocytes.[48] Rico *et al*.,[145] showed than Pet protein induce an increase in leucocytes quimiotaxis and raising in neutrophil respiratory burst.

These findings show new mechanisms by which EAEC may cause intestinal inflammation, leading to absorption disturbances that contribute to persistent diarrhea, and as a consequence, growth impairment that is characteristic of human infection by this organism.

A volunteer challenge study has confirmed that EAEC strain JM221 caused diarrhea in 5 of 16 volunteers.[98] Bacterial enteropathogens can induce an intestinal immune response after an episode of diarrhea.[64,189] Gomez *et al*.,[64] studied the secretory immunoglobulin A (sIgA) responses to EAEC JM221 of volunteers. sIgA was extracted from stools obtained prechallenge and 7 days postchallenge. Specific sIgA titers were determined with JM221 antigens (water-extractable surface antigens, whole cells, lipopolysaccharides, and outer membrane proteins). Five subjects who became ill had fourfold or greater rises in titers against each of the four antigens. Five subjects who remained healthy following challenge did not exhibit significant rises in titers to any JM221 antigens, but their mean titers were significantly higher than the mean prechallenge titers of the volunteers with diarrhea, suggesting that high intestinal sIgA titers may be protective. Sutjita *et al*.,[172] studied the intestinal immune response to EAEC strains among travelers with diarrhea. The study population consisted of U.S. adults travelers with acute diarrhea acquired during a stay in Guadalajara, Mexico (June to September 1998). Paired stool samples from patients with diarrhea (caused by EAEC) were sIgA positives to outer membrane proteins antigen from EAEC. Fernandes *et al*.,[53] have showed that IgA from human colostrum inhibits adhesion to HEp-2 cells, and that these antibodies reactive to 15-kDa protein (compatible with the subunits of AAF/II) and others antigens and may protected against EAEC infection.

EAEC STRAINS IDENTIFICATION METHODS

EAEC can be identified using different procedures. Metabolic properties are determined by biochemical tests. Immunological assays are used to identify different bacterial antigens. Molecular biology procedures can be used to determine the virulence associated genes and the clonal relationship of the microorganisms. Nataro J. P.,[119] have suggests that the identification of AggR o members of the AggR regulon may identify pathogenic EAEC strains (typical EAEC). Finally, i*n vitro* systems allow gene expression to be determined, whilsh *in vivo* studies such as animal models or human volunteers are used to identify the clinical symptoms and histological alterations induced by microorganisms.

EAEC strains form clumps visible as a scum at the surface of a Mueller-Hinton broth shaker culture. The sensitivity and specificity of clump-formation test were each about 90%. The identification of EAEC by scum formation is a simple, rapid and inexpensive test.[5,82] Wakimoto *et al*.,[185] have showed that a quantitative biofilm assay is convenient and useful to screen EAEC, although HEp-2 cell adherence assay is need for confirmation.

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HEP-2 CELLS ADHERENCE ASSAY

The aggregative phenotype of *E. coli* strains is determined by testing for attachment in HEp-2 cells at 3 h assay. The HEp-2 cells adherence assay described by Cravioto *et al*.,[35] remains the "gold standard" for identifying EAEC strains. Although reproducible, differences in interpretation associated with lector experiences are common. Recently Miqdady *et al*.,[102] described an adherence test using formalin fixed HEp-2 cells. This assay modification is important as it may be possible to use the cells to 28 days after initial preparation, sensitivity from 94 to 98% in determining the enteroaggregative adherence pattern of *E. coli* was achieved.

Currently, in many laboratories, EAEC is detected from mixed cultures by analyzing individual colonies with the adherence test. However, this technique is cumbersome and inefficient if large numbers of colonies from stool samples must be analyzed.

MOLECULAR ASSAYS

The identification of virulence properties is important for epidemiological studies. The specific genes associated with virulence can be identified using different molecular procedures. Genetic techniques have been shown to be highly effective in detecting and identifying EAEC. Some studies have compared the presence of EAEC markers in strains isolated from children and adults with and without diarrhea in different geographic regions.[46,56,126,127,136,152]

The DNA hybridization test using the EAEC probe is an alternative method for identification and has been found to be both sensitive and specific. Specific DNA probes have enormous advantages over *in vitro* assays, with a greater number of bacterial colonies being tested rapidly and easily, and an interpretation that is generally more objective.

EAEC plasmids are closely linked to their virulence mechanism. DNA probes derived from adherence and toxin-related sequences have been constructed and used in hybridization assays for the detection of EAEC in epidemiological studies.[13,49,111,112,144,154]

In addition to their putative role in the pathogenesis of diarrheal disease, fragments from the large plasmid have been used as DNA probes to diagnose EAEC infections.[13] The EAEC probe currently used for diagnosis consist of a fragment from the plasmid of the EAEC strain 17-2, EAEC probe (a 1-kb *Eco*RI-*Pst*I fragment of pCVD432 from EAEC adherence plasmid). Baudry *et al*.,[13] found that only 56 of 63 strains with the aggregative adherence pattern hybridized with the EAEC probe. Although this probe was not obtained from a virulence region, it has been shown to be highly specific identifying *E. coli* strains with the aggregative phenotype.[47,158,174]

Gomes *et al*.,[62] evaluated the relationship between aggregative adherence pattern and EAEC probe. In *E. coli* isolates from 1- to 4-year-old children with and without acute diarrhea, in Sao Paulo, Brazil, in 1,801 isolates obtained from 200 patients and 200 age-matched controls. The adherence patterns found were classified as aggregative (typical AA), aggregative in a 6-h assay (AA that could be clearly discerned only in the 6-h assay, AA6h), and aggregative predominantly in coverslips (AAcs). The results showed than 256 isolates were typical AA, 27 AA6h, and 132 AAcs. The EAEC probe detected 190 of the 256 isolates with typical AA and 5 of the 132 isolates with AAcs (sensitivity, 47.0%), none of the isolates that showed AA6h reacted with EAEC probe and only 29 isolates don not showing reaction with different AA types (specificity, 97.9%). The association between the aggregative adherence pattern and patients with acute diarrhea and controls was of 21.5% and 19%, respectably ($p = 0.89$). The association between EAEC probe and acute diarrhea was of 13.0% in patients and 17% in the controls ($p = 0.19$). The prevalence of aggregative adherence pattern combined with related EAEC probe was 10.5% of isolates of patients and 13% in the controls ($p = 0.4$). In this study the aggregative adherence pattern and EAEC probe does not were associated with acute diarrhea.

In a prospective study, carried out in two urban centers in northeastern Brazil,[158] also was evaluated the relationship between aggregative adherence pattern and EAEC probe. Forty children with acute diarrhea (16.9%) and 38 children without diarrhea (16.4%) were infected with EAEC; thus, there was no correlation between EAEC carriage and diarrhea ($p = 1.00$). Similarly, there was no age-related association of diarrhea with the presence of EAEC in feces. However, in a group aged 0 to 5 months, the frequency of EAEC isolation was twofold higher for children with diarrhea. EAEC failed to hybridize with the EAEC probe. Twenty-one (8.9%) of EAEC isolates from children with diarrhea hybridize with the EAEC probe, while 27 (11.75%) hybridize with EAEC isolates from control group ($p = 0.39$). Eslava *et al*.,[49] showed that 15% of EAEC strains from various epidemiological studies hybridized with the *pet* gene.

Okeke *et al*.,[126] showed that only 26% of EAEC isolates from Nigeria hybridized with the conventional EAEC probe, 63.4% hybridized with the probe for AAF/I, 35.1% for AAF/II, 22.1% EAST1, 45.8% Pet, 8.4% Aap (AspU), 37.4% pAA replicon, 100% with the AggR probe, and < 5% hybridized with probes for the chromosomally encoded alpha hemolysin and the Pic/ShET1. However, only association of AAF/II with diarrhea was observed ($p < 0.002$).

Other studies have showed different results. Elias *et al*.,[43] detected AAF/I and AAF/II in 19.2% and 7.7%, re-

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spectively, of EAEC strains from São Paulo, Brazil. Czeczulin *et al*.,[38] found that 32% of EAEC strains from various epidemiologic studies hybridized with the AAF/I probe and 18% hybridized with the AAF/II.

The reason why not all EAEC strains hybridize with the probe is unknown. However, it has been hypothesized that different categories of EAEC strains exist.[13,160] It would be interesting to assess whether heterogeneity of the strains with respect to probe hybridization is correlated with differences in their virulence.

PCR technique is an assist in the detection of EAEC.[76] Schmidt *et al*.,[162] developed a PCR with primers complementary to the EAEC probe. A primer pair complementary of the EAEC probe was designed for PCR amplification of a 630-bp region. The analysis of the EAEC probe sequence revealed no significant similarity to any known bacterial gene. *E. coli* strains which demonstrated aggregative adherence to HEp-2 cells, 86% were positive with the EAEC PCR and all these strains reacted with the EAEC probe. In contrast, only 0.96% of the strains representing other categories of diarrheagenic *E. coli* demonstrated a positive PCR result. The PCR was also successful in screening for the presence of EAEC in enriched cultures grown from stool specimens. PCR assay was more rapid, simple, and highly sensitive that cell culture assays and colony hybridization, and can be recommended as a screening method for EAEC detection.

Scaletsky *et al*.,[158] studied the correlation of the aggregative adherence pattern with DNA probe and EAEC PCR assays for the identification of EAEC from children, less than 2 years of age with or without diarrhea, from different urban centers of Brazil. A total of 1,428 isolates obtained from 338 patients and 322 controls children were studied. The presence of bacterial clusters in an attacked-brink configuration was used to identify the EAEC strains and two AA pattern types were detected: 162 typical AA and 27 AA6h. The EAEC probe detected 88 of the 162 with AA, 8 de 27 isolated with AA6h (sensitivity, 50.2%), which is in accordance with the levels of sensitivity found in other studies.[13,74,188] The EAEC probe reacted with only 33 isolates showing none of the different AA types (specificity, 97.3%). Of 162 isolated with AA, 86 were positive with the EAEC PCR and all of these strains reacted with the EAEC probe. EAEC PCR was sensitivity and specificity similar to those of the EAEC probe. EAEC PCR assay could be used instead of the DNA probes as a screening method for typical EAEC carrying the EAEC probe sequence in the clinical laboratory. In this study although EAEC was very frequently found in these children, this do not were associated with acute diarrhea. Ruttler *et al*.,[149] determinate 94.4% sensitivity and 78.26% specificity for AAF/I- and EAST1-PCR, in comparation with the cell culture assay, in *E. coli* strains isolates from patients uless than 2 years of age with acute diarrhea in Mendoza, Argentina.

Vila *et al*.,[181] studied the prevalence of genes encoding putative virulence factors in EAEC traveler's diarrhea strains, using EAEC PCR assays. The results showed a low prevalence of genes for Pet (4.3%), ShET2 (4.3%), and AAF/II (8.7%), and AAF/I was not detected in any EAEC isolates. However, ShET1 and Pic (She) were present in most EAEC strains (56.5%), a rate similar (57%) to that found by Czeczulin *et al*.,[38] using Pic/ShET1 probe. Furthermore, Suzart *et al*.,[174] observed the Pic sequence in 100% of typical EAEC pCVD432 probe positive. Pic/ShET1 may be an important virulence factor in traveler's diarrhea.

The presence of EAEC plamid-borne genes *aggA*, *aggR*, *aap* (*aspU*), and *aafA* among *E. coli* isolates from 176 patients with traveler's diarrhea was studied by Jiang *et al*.,[85] using PCR assays. The rate of occurrence of one or more putative EAEC virulence factors was of 60 (70%) of 86 EAEC isolates from travelers with diarrhea, and only 7 (8%) of 90 in patients with diarrhea who were infected with nonadherent *E. coli*. The two most prevalent virulence factors were *aggR* and *aggA*, in 41% and 32%, respectably, of EAEC strains. The 10% of EAEC strains showed in combination the virulence factors *aggR* and *aap* (*aspU*). The combination of virulence factors *aggA*, *aggR*, and *aap* (*aspU*) in EAEC strains was 9%. In others studies using PCR technique, Tsai *et al*.,[176] detected that the *aggA* gene is in EAEC isolated from diarrhea patients in Taiwan. Studies in Tokyo, Japan, Ogata *et al*.,[125] have showed that *aggR*-positive EAEC is a significant causative agent in traveler's diarrhea, and Yamazaki *et al*,[192] showed that *pet* gene was detected in 23%, among enteropathogenic *E. coli* *aggR* positives isolates from sporadic diarrhea cases. Kahali *et al*.,[87] detected two or more of these virulence genes in 90.1% of EAEC isolates from hospitalized patients in Kolkata, India.

Suzart *et al*.,[173] reported the existence of *aap* gene in all the EAEC strains with the pCVD432 probe positive but only in 12.5% of the probe negative strains. Similar results were obtained by Sheikh *et al*.,[164] observing a 80% of positive strains for the *aap* gene.

A novel PCR multiplex assay was developed by Cerna *et al*.,[29] This has been used for EAEC detection. Three plasmid-borne genes AA probe, *aap* (*aspU*) and *aagR* were analyzed. One or more of the genes were detected in 86% of EAEC strains.

EAEC PCR showed some disagreement. It is possible that *E. coli* strains, which hybridized with the EAEC probe, exhibits sequence variation in the primer binding sites and

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gave a negative PCR result. Mutations in the genes necessary for adherence may induce that EAEC do not adhere to HEp-2 cells and show EAEC PCR and EAEC probe positive. In this study EAEC PCR- and EAEC probe-positive strains did not show the expected aggregative pattern but exhibited diffuse adherence. Also, *E. coli* strains attached to HEp-2 cells in the characteristic aggregative pattern but EAEC probe and PCR negative were detected. However, Baudry *et al*.,[13] in a previous study showed than strains with diffuse adherence were EAEC probe negative. Furthermore, others studies have also shown that not all EAEC strains hybridize with the EAEC probe.[13,160]

The EAEC probe or the adherence test must still supplement the PCR to identify the disease-causing strains. A three-step schedule for EAEC identification may be practical for the clinical laboratory.[162] In the first step, overnight cultures from stools are screened by the PCR method as described above. The PCR may also be directly performed with stool samples, but such a procedure will need to be standardized. In the second step, only samples which are PCR-positive should be analyzed for strain identification. This must be performed for single colonies with either the cell culture tests or the EAEC probe. Because one EAEC probe-positive strain was found to be negative in the cell culture assay, the typical aggregative pattern can only be determined by cell culture. Therefore, the adhesion test appears to have the highest level of specificity and is recommended to be used as a definitive confirmation test. The third step should include species identification by biochemical testing of positive colonies; this can be followed by O and H antigen determination. For epidemiological purposes, molecular typing by DNA fingerprinting may be helpful to determine the clonal relatedness of strains.[162] Efforts to identify a DNA sequence that correlates with the AA phenotype represent a challenging avenue of investigation and could lead to a more practical assay that allows definitive epidemiological studies in the pathogenesis of diarrhea.

DISCUSSION AND CONCLUSIONS

EAEC strains are considered as an emergent pathogen associated with different types of diarrheal disease. Although EAEC strains are recognized by their characteristic aggregative adherence or "stacked-brick" pattern to epithelial cells, this feature is observed both in virulent (diarrhea associated) and non virulent (environmental and non diarrhea associated) strains.

The serotyping of EAEC strains show the existence of many different serotypes associated principally with the adherence pattern, but not with clinical or pathogenic properties. On the other hand the molecular analysis of EAEC pathogenesis, show that the virulence factors of these strains are encoded on mobile genetic elements such as pathogenicity islands, transposons, bacteriophages and plasmids. This observations support the horizontal transference of these virulence factors and as consequence the emergence of different clones, some virulent strains that cause diarrhea and some other non virulent strains.

The clinical and epidemiological importance of EAEC, support the necessity of guide studies on virulence mechanisms, tissue damage and immune response of the infected host. The search on cellular adhesion and toxins receptors, the cell signal transduction events and the characterization of new chromosomal and plasmidic genes, between other studies could be contribute to a better understanding of the basic mechanisms and pathophysiology of prolonged and acute diarrhea in EAEC infected patients.

On the base of these new research, could be possible outline strategies for the development of diagnosis, treatment, and prevention of EAEC infections. Finally a clonality relationships study of EAEC, may to clarify on the evolution of this *E. coli* diarrheogenic group.

We thank Guadalupe Estrada for valuable assistance. Results referred in this work were supported by grants from CONACYT (41073-M) and DGAPA (IN215202-2).

REFERENCES

1. Abe C.M., S. Knutton, P. Margareth, E. Freymuller & T.A.T. Gomes. 2001. An enteroaggregative *Escherichia coli* of serotype O111:H12 damages and invades cultured T84 cells and human colonic mucosa. FEMS Microbiol. Lett. 203:199-205.
2. Adachi J.A., Z.D. Jiang, J.J. Matewson, P. Verenkar, S. Thompson, F. Martinez-Sandoval, R. Steffen, D. Ericsson & DuPont. 2001. Enteroaggregative *Escherichia coli* as a major etiologic agent in traveler's diarrhea in 3 regions of the world. Clin. Infect. Dis. 32:1706-1709.
3. Adachi J.A., J.J. Mathewson, Z.D. Jiang, C.D. Ericsson & H.L. DuPont. 2002. Enteric pathogens in Mexican sauces of popular restaurants in Guadalajara, Mexico, and Houston, Texas. Ann. Intern. Med. 136:884–887.
4. Adachi J.A., C.D. Ericsson, Z.D. Jiang, M.W. DuPont, S.R. Pallegar & H.L. DuPont. 2002. Natural history of enteroaggregative and enterotoxigenic *Escherichia coli* infection among U.S. travelers to Guadalajara, Mexico. J. Infect. Dis. 185:1681–1683.
5. Albert M.J., F. Qadri, A. Haque & N.A. Bhuiyan. 1993. Bacterial clump formation at the surface of liquid culture as a rapid test for identification of enteroaggregative *Escherichia coli*. J. Clin. Microbiol. 31:1397-1399.
6. Albert M.J., S.M. Faruque, A.S. Faruque, P.K. Neogi, M. Ansaruzzaman, N.A. Bhuiyan, K. Alam & M.S. Akbar. 1995. Controlled study of *Escherichia coli* diarrheal infections in Bangladeshi children. J. Clin. Microbiol. 33:973-977.
7. Al-Hasani K., K. Rajakumar, D. Bulach, R. Robins-Browne, B. Adler & H. Sakellaris. 2001. Genetic organization of the *she* pathogenicity island in *Shigella flexneri* 2a. Microb. Pathog. 30:1–8.
8. Arthur J.D., L. Bodhidatta, P. Echeverria, S. Phuphaisan & P. Sakti. 1992. Diarrheal disease in Cambodian children at a camp in Thailand. Am. J. Epidemiol. 135:541-551.
9. Bach S., A. de Almeida & E. Carniel. 2000. The *Yersinia* high-pathogenicity island is present in different members of the family *Enterobacteriaceae*. FEMS Microbiol. Lett. 183:289–294.

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10. Baldwin T.J., S. Knutton, L. Sellers, H.A.M. Hernandez, A. Aitken & P.H. Williams. 1992. Enteroaggregative *Escherichia coli* strains secrete a heat-labile toxin antigenically related to *E. coli* hemolysin. Infect. Immun. 60:2092–2095.
11. Bardhan P.K., M.J. Albert, N.H. Alam, S. M. Faruque, P. K. Neogy & D. Mahalanabis. 1998. Small bowel and fecal microbiology in children suffering from persistent diarrhea in Bangladesh. J. Pediatr. Gastroenterol. Nutr. 26:9-15.
12. Baqui A.H., R.B. Sack, R.E. Black, K. Haider, A. Hossain, A.R. Alim, M. Yunus, H.R. Chowdhury & A.K. Siddique. 1992. Enteropathogens associated with acute and persistent diarrhea in Bangladeshi children less than 5 years of age. J. Infect. Dis. 166:792-796.
13. Baudry B., S.J. Savarino, P. Vial, J. B. Kaper & M.M. Levine. 1990. A sensitive and specific DNA probe to identify enteroaggregative *Escherichia coli*, a recently discovered diarrheal pathogen. J. Infect. Dis. 161:1249-1251.
14. Bellini E.M., W.P. Elias, T.A.T. Gomes, T.L. Tanaka, C.R. Taddei, R. Huerta, F. Navarro & M.B. Martínez. 2005. Antibody response against plasmid-encoded-toxin (Pet) and the protein involved in intestinal colonization (Pic) in children with diarrhea produced by enteroaggregative *Escherichia coli*. FEMS Immunol. Med. Microbiol. 43:259-264.
15. Benjamin P., M. A. Federman & C. A. Wanke. 1995. Characterization of an invasive phenotype associated with enteroaggregative *Escherichia coli*. Infect. Immun. 63:3417-3421.
16. Behrensn M., J. Sheik & J.P. Nataro. 2002. Regulation of the Overlapping pic/set locus in *Shigella flexneri* and enteroaggregative *Escherichia coli*. Infect. Immun. 70:2915-2925.
17. Bernier C., P. Gounon & C.L. Bouguenec. 2002. Identification of an aggregative fimbria (AAF) type III-encoding operon in Enteroaggregative *Escherichia coli* as a sensitive probe for detecting the AAF-encoding operon family. Infect. Immun. 70:4302-4311.
18. Bhan M.K., P. Raj, M.M. Levine, J.B. Kaper, N. Bhandari & R. Srivastava. 1989a. Enteroaggregative *Escherichia coli* associated with persistent diarrhea in a cohort of rural children in India. J. Infect. Dis. 159:1061-1064.
19. Bhan M.K., N. Bhandari, S. Sazawal, J. Clemens, P. Raj, M.M. Levine & J.B. Kaper. 1989b. Descriptive epidemiology of persistent diarrhea among young children in rural northern India. Bull World Health Organ. 67:281-288.
20. Bhan M.K., V. Khoshoo, H. Sommerfelt, P. Raj, S. Sazawal & R. Srivastava. 1989c. Enteroaggregative *Escherichia coli* and *Salmonella* associated with nondysenteric persistent diarrhea. Pediatr. Infect. Dis. J. 8:499-502.
21. Bhatnagar S., M.K. Bhan, H. Sommerfelt, S. Sazawal, R. Kumar & S. Saini. 1993. Enteroaggregative *Escherichia coli* may be a new pathogen causing acute and persistent diarrhea. Scand. J. Infect. Dis. 25:579-583.
22. Bouckenooghe A.R., H.L. DuPont, Z.D. Jiang, J. Adachi, J.J. Mathewson, M.P. Verenkar, S. Rodrigues & R. Steffen. 2000. Markers of enteric inflammation in enteroaggregative *Escherichia coli* diarrhea in travelers. Am. J. Trop. Med. Hyg. 62:711–713.
23. Boudalliez B., P. Berquin, P. Mariani-Kurkdjian, D. D. Ilef, B. Cuvelier, I. Capek, B. Tribout, E. Bingen & C. Piussan. 1997. Possible person-to-person transmission of *Escherichia coli* O111 - associated hemolytic uremic syndrome. Pediatr. Nephrol. 11:36 – 39.
24. Bouzari S., A. Jafari, A.A. Farhoudi-Moghaddam, F. Shokouhi & M. Parsi. 1994. Adherence of non-enteropathogenic *Escherichia coli* to HeLa cells. J. Med. Microbiol. 40:95-97.
25. Bouzari S., A. Jafari, A. Azizi, M. Oloomi & J.P. Nataro. 2001. Short report: characterization of enteroaggregative *Escherichia coli* isolates from Iranian children. Am. J. Trop. Med. Hyg. 65:13-14
26. Brynskov J., N. Tvede, C. B. Anderson & M. Vilien. 1992. Increased concentrations of interleukin-1β, interleukin-2, and soluble interleukin-2 receptors in endoscopical mucosal biopsy specimens with active inflammatory bowel disease. Gut 33:55–58.
27. Campos L.C., T.S. Whittam, T.A.T. Gomes, J.R.C. Andrade & L.R. Trabulsi. 1994. *Escherichia coli* serogroup O111 includes several clones of diarrheagenic strains with different virulence properties. Infect. Immun. 62:3282–3288.
28. Canizales-Roman A. & F. Navarro-García. 2003. Fodrin CaM-binding domain cleavage by Pet from enteroaggregative *Escherichia coli* leads to actin cytoskeleton disruption. Mol. Microbiol. 48:947-958.
29. Cerna J.F., J.P. Nataro & T. Estrada. 2003. Multiplex PCR for detection of three plasmid-borne genes of enteroaggregative *Escherichia coli*. J. Clin. Microbiol. 41:2138-2140.
30. Chan K.N., A.D. Phillips, S. Knutton, H.R. Smith & J.A. Walker-Smith. 1994. Enteroaggregative *Escherichia coli*: another cause of acute and chronic diarrhea in England? J. Pediatric Gastroenterol Nutr. 18:87-91.
31. Chart H., J. Spencer, H.R. Smith & B. Rowe. 1997. Magnesium ions are required for HEp-2 cell adhesion by enteroaggregative strains of *Escherichia coli* O126:H27 and O44:H18. FEMS Microbiol. Lett. 148:49-52.
32. Clarke S. C. 2001. Diarrhoeagenic *Escherichia coli*—an emerging problem? Diagn. Microbiol. Infect. Dis. 41:93-98.
33. Cobeljic M., B. Miljkovic-Selimovie, D. Savic, R. Ilic, S. Konstantinovic, B. Jovanovic & V. Kostic. 1996. Enteroaggregative *Escherichia coli* association with an outbreak of diarrhoea in a neonatal nursery ward. Epidemiol. Infect. 117:11-16.
34. Cohen M.B., J.A. Hawkins, L.S. Weckbach, J.L. Staneck, M.M. Levine & J. E. Heck. 1993. Colonization by enteroaggregative *Escherichia coli* in travelers with and without diarrhea. J. Clin. Microbiol. 31:351–353.
35. Cravioto A., R.J. Gross, S.M. Scotland & B. Rowe. 1979. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile Enteropathogenic serotypes. Curr. Microbiol. 3:95-99.
36. Cravioto A., A. Tello, A. Navarro, J. Ruiz, H. Villafan, F. Uribe & C. Eslava. 1991. Association of *Escherichia coli* HEp-2 adherence patterns with type and duration af diarrhea. Lancet 337:262-264.
37. Czenczuli J.R., S. Balepur, S. Hicks, A. Phillips, R. Hall, M.H. Kothary, F. Navarro-Garcia & J.P. Nataro. 1997. Aggregative adherence fimbria II, a second fimbrial antigen medating aggregative adherence in enteroaggregative *Escherichia coli*. Infect. Immun. 65:4135-4145
38. Czeczulin J.R., T.S. Whittam, I.R. Herderson, F. Navarro-Garcia & J.P. Nataro. 1999. Phylogenetic analysis of enteroaggregative and diffusely adherent *Escherichia coli*. Infect. Immun. 67: 2692-2699.
39. Donnelly M.A., T.S. Steiner. 2002. Two nonadjacents regions in enteroaggregative *Escherichia coli* flagellin are required for activation of toll-like receptor 5. J. Biol. Chem. 277:40456-40461.
40. Dutta P.R., R. Capello, F. Navarro-García & J.P. Nataro. 2002. Functional comparation of serine protease autotransporter of *Enterobacteriaceae*. Infect. Immun. 70:7105-7113.
41. Dutta P.R., B.Q. Sui & J.P. Nataro. 2003. Structure-function analysis of the enteroaggregative *Escherichia coli* Plasmid-encoded toxin autotransporter using scanning linker mutagenesis. J. Biol. Chem. 278:39912-39920.
42. Durrer P., R. Zbinden, F. Fleish, M. Altwegg, B. Ledergerber, H. Karch & R. Weber. 2000. Intestinal infection due to enteroaggregative *Escherichia coli* among human immunodeficiency virus-infected persons. J. Infect. Dis. 182:1540-1544.
43. Elias W.P., S. Suzart, L.R. Trabulsi, J.P. Nataro & T.A.T. Gomes. 1999. Distribution of *aggA* and *aafA* gene sequences among *Escherichia coli* isolates with genotypic or phenotypic characteristic, or broth, of enteroaggregative *E. coli*. J. Med. Microbiol. 48:597-599.
44. Elias Jr. W.P., J.R. Czeczulin, I.R. Henderson, L.R. Trabulsi & J.P. Nataro. 1999. Organization of biogenesis genes for aggregative adherence fimbria II defines a virulence gene cluster in enteroaggregative *Escherichia coli*. J. Bacteriol. 181:1779-1785.
45. Elias W., S.F. Barros, C.G. Moreira & L. R. Trabulsi. 2002. Enteroaggregative *Escherichia coli* strains among classical Enteropathogenic *Escherichia coli* O serogroups J. Clin. Microbiol. 40: 3540-3541.

46. Elias W.P., A.P. Uber, S.K. Tomita, L.R. Trabulsi & T.A. Gomes. 2002. Combinations of putative virulence markers in typical and variant enteroaggregative *Escherichia coli* strains from children with and without diarrhoea. Epidemiol. Infect. 129:49-55.
47. Elliott S.J. & J.P. Nataro. 1995. Enteroaggregative and diffusely adherent *Escherichia coli*. Rev. Med. Microbiol. 6:196–206.
48. Eslava C., J. Villaseca, R. Morales, A. Navarro & A. Cravioto. 1993. Identification of a protein with toxigenic activity produced by enteroaggregative *Escherichia coli*, abstr. B-105. In Abstracts of the 93rd General Meeting of the American Society for Microbiology 1993. American Society for Microbiology, Washington, D. C.
49. Eslava C., F. Navarro-Garcia, J.R. Czeczulin, I.R. Henderson, A. Cravioto & J.P. Nataro. 1998. Pet, an autotransporter enterotoxin from enteroaggregative *Escherichia coli*. Infect. Immun. 66:3155–3163.
50. Falcao J.P., D.P. Falcao & T.A. Gomes. 2004. Ice as a vehicle for diarrheagenic *Escherichia coli*. Int. J. Food Microbiol. 91:99-103.
51. Fang G.D., A.A. M. Lima, C.C. Martin, L.J. Barrett, J.P. Nataro & R.L. Guerrant. 1992. Aggregative *Escherichia coli* and *Cryptosporidium*: important pathogens in hospitalized children with persistent diarrhea in Northeast Brazil, abstrac. 686. In Abstracts of the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy 1992. American Society for Microbiology, Washington, D. C.
52. Fang G.D., A.A. M. Lima, C.V. Martins, J.P. Nataro & R.L. Guerrant. 1995. Etiology and epidemiology of persistent diarrhea in northeastern Brazil: a hospital-based, prospective, case-control study. J. Pediatr. Gastroenterol. Nutr. 21:137-144.
53. Fernandes R.M., S.B. Carbonare, M.M.S. Carneiro-Sampaio & R.L. Trabulsi. 2001. Inhibition of enteroaggregative *Escherichia coli* adhesion to HEp-2 cells by secretory immunoglobulin A from human colostrum. Pediatr. Infect. Dis. J. 20:672-678.
54. Fernandez-Prada C., B.D. Tall, S.E. Elliot, D.L. Hoover, J.P. Nataro & M.M. Venkatesan. 1998. Hemolysin-positive enteroaggregative and cell-detaching *Escherichia coli* strains cause oncosis of human monocyte-derived macrophages and apoptosis of murine J774 cells. Infect. Immun. 66:3918-3924.
55. Freser D., R. Dagan, N. Porat, J. El-On, S. Alkrinawi, R.J. Deckelbaum & L. Naggan. 1998. Persistent diarrhea in a cohort of israeli bedouin infants: role of enteric pathogens and family and environmental factors. J. Infect. Dis. 178:1081-1088.
56. Gascon J., M. Vargas, L. Quinto, M. Corachan, M.T. Jiménez de Anta & J. Vila. 1998. Enteroaggregative *Escherichia coli* strains as a cause of traveler´s diarrhea: A case-control study. J. Infect. Dis. 177:1409-1412.
57. Germani Y., P. Minssart, M. Vohito, S. Yassibanda, P. Glaziou, D. Hocquet, P. Berthelemy & J. Morvan. 1998. Etiologies of acute, persistent, and dysenteric diarrheas in adults in Bangui, Central African Republic, in relation to human immunodeficiency virus serostatus. Am. J. Trop. Med. Hyg. 59:1008-1014.
58. Geyid A., O. Olsvik & A. Ljungh. 1998. Virulence properties of *Escherichia coli* isolated from Ethiopian patients with acute or persistent diarrhea. Ethiop. Med. J. 36:123-139.
59. Gioppo N.M.R., W.P. Elias Jr., M.C. Vidoto, R.E. Lindares, H.O. Saridakis, T.A.T. Gomes, L.R. Trabulsi & J.S. Pelayo. 2000. Prevalence of HEp-2 cell-adherent *Escherichia coli* and characterization of enteroaggregative *E. coli* and chain-like adherent *E. coli* isolated from children with and without diarrhoea, in Londrina, Brazil. FEMS Microbiol. Lett. 190:293-298.
60. Giron J. A., T. Jones, F. Millan Velasco, E. Castro Munoz, L. Zarate, J. Fry, G. Frankel, S.L. Moseley, B. Baudry, J.B. Kaper, G.K. Schoolnik & L.W. Riley. 1991. Diffuse adhering *Escherichia coli* (DAEC) as a putative cause of diarrhea in Mayan children in Mexico. J. Infect. Dis. 163:507-513.
61. Glandt M., J.A. Adachi, J.J. Mathewson, Z.D. Jiang, D. DiCesare, D. Ashley, C.D. Ericsson & H.L. Dupont. 1999. Enteroaggregative *Escherichia coli* as a cause of traveler's diarrhea: clinical response to ciprofloxacin. Clin. Infect. Dis. 29:335-338.
62. Gomes T.A.T., M.A.M. Vieira, C.M. be, D. Rodriges, P.M. Griffin & S.R.T. S. Ramos. 1988. Adherence patterns and adherence related DNA sequence in *Escherichia coli* isolated from children with and without diarrhea in Sao Paoulo City, Brazil. J. Clin. Microbiol. 36:3609-3613.
63. Gomes T.A.T., S. Suzart, B.E.C. Guth, C.M. Abe & M.Z. Pedroso. 1998. Distinctive pattern of adherence to HeLa cells, abstr. D-114, In Abstract of the 98th General Meeting of the American Society for Microbiology 1998. American Society for Microbiology, Atlanta, GA.
64. Gomez H.F., J.J. Mathewson, P.C. Johnson & H. L. DuPont. 1995. Intestinal immune response of volunteers ingesting strain of enteroadherent (HEp-2 cell-adherent) *Escherichia coli*. Clin. Diagn. Lab. Immun. 2:10–13.
65. Gonzalez R., C. Diaz, M. Marino, R. Cloralt, M. Pequeneze & I. Perez-Schael. 1997. Age –specific prevalence of *Escherichia coli* with localized and aggregative adherence in Venezuelan infants with acute diarrhea. J. Clin. Microbiol. 35:1103-1107.
66. Gray L.D. 1995. *Escherichia*, *Salmonella*, *Shigella*, and *Yersinia*, pp. 450-456. In: P.R. Murray P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover, & R.H. Yolken (Eds). Manual of clinical microbiology, American Society for Microbiology, Washinton D. C.
67. Greenberg D., Z.D. Jiang, R. Steffen, M.P. Verenkar & H.L. DuPont. 2002. Markers of inflammation in bacterial diarrhea among travelers, with a focus on enteroaggregative *Escherichia coli* pathogenicity. J. Infect. Dis. 185:944–949.
68. Grover V., S. Ghosh, N. Sharma, A. Chakraborti, S. Majumdar & N.K. Ganguly. 2001. Characterization of a galactose specific adhesin of Enteroaggregative *Escherichia coli*. Arch. Biochem Biophys. 390:109-118.
69. Haque M.A., K. Ohki, M. Kikuchi & O. Kohashi. 1994. Contact hemolysin production by strains of enteroaggregative *Escherichia coli* isolated from children with diarrhea. 32:1109-1111.
70. Harris, A.S. & J.S. Morrow. 1990. Calmodulin and calcium-dependent protease I coordinately regulate the interaction of fodrin with actin. Proc. Natl. Acad. Sci. USA 87:3009-3013.
71. Henderson I.R., J. Czeczulin, C. Eslava, F. Noriega & J.P. Nataro. 1999. Characterization of Pic, a secreted protease of *Shigella flexneri* and enteroaggregative *Escherichia coli*. Infect. Immun. 67:5587–5596.
72. Henderson I.R., R. Capello & J.P. Nataro. 2000. Autotransporter proteins, evolution and redefining protein secretion. Trends Microbiol. 8:529-532.
73. Henderson I.R. & J.P. Nataro. 2001. Virulence functions of autotransporter proteins. Infect. Immun. 69:1231-1243.
74. Henry F.J., A.S. Udoy, C.A. Wanke & K.M.A. Aziz. 1996. Epidemiology of persistent diarrhea and etiologic agents in Mirzapur, Bangladesh. Acta Pediatr. Suppl. 381:27-31.
75. Hicks S., D.C. Candy & A.D. Phillips. 1996. Adhesion of enteroaggregative *Escherichia coli* to pediatric intestinal mucosa *in vitro*. Infect. Immun. 64:4751–4760.
76. Hernández U., J.M. Villaseca, A. Navarro, L.M. Perea, A.E. Inzunza, G. Garcia, A. Cravioto & C. Eslava. 1998. Estandarizacion de un sistema de PCR para la deteccion de la enterotoxina termo labil (Pet) de *Escherichia coli* enteroagregativa, resum. E14. En Resumenes del XXIII Congreso Anual de la Asociación Mexicana de Infectología y Microbiología Clínica. Asociación Mexicana de Infectología y Microbiología Clínica, San Luis Potosí, México.
77. Hernandez U., J.M. Villaseca, J. Molina & C. Eslava. 2002. Pic protease from enteroaggregative *Escherichia coli* induce hemoglobin disruption, abstr. B-13. In: Abstracts of the 102TH of the General Meeting of the American Society for Microbiology 2002. American Society for Microbiology, Salat Lake City, Utah, USA.

Rev Latinoam Microbiol 2005; 47 (3-4): 140-159

78. Huppertz H.I., S. Rutkowski, S. Aleksic & H. Karch. 1997. Acute and chronic diarrhea and abdominal colic associated with enteroaggregative *Escherichia coli* in young children living in western Europe. Lancet. 349:1660-1662.
79. Huang D.B., H.L. DuPont, Z.D. Jiang, L. Carlin & P.C. Okhuysen. 2004. Interleukin-8 response in an intestinal HCT-8 cell line infected with enteroaggregative and enterotoxigenic *Escherichia coli*. Clin. Diagn. Lab. Immunol. 11:548-551.
80. Infante R.M., C.D. Ericsson, Z.D. Jiang, S. Ke, R. Steffen, L. Riopel, D.A. Sack & H.L. DuPont. 2004. Enteroaggregative *Escherichia coli* diarrhea in travelers: response to rifaximin therapy. Clin. Gastroenterol. Hepatol. 2:135-138.
81. Itho Y., I. Nagano, M. Kunishima & T. Ezaki. 1997. Laboratory investigation of enteroaggregative *Escherichia coli* O untypeable:H10 associated with a massive outbreak of gastrointestinal illness. J. Clin. Microbiol. 35:2546-2550.
82. Iwanaga M., T. Song, N. Higa, S. Kakinohana, C. Toma & N. Nakasone. 2002. Enteroaggregative *Escherichia coli*: incidence in Japan and usefulness of the clump-formation test. J. Infect. Chemother. 8:345-348.
83. Izzo R.S., K. Witkon, A.I. Chen, C. Hadjiane, M.I. Weinstein & C. Pellecchi. 1992. Interleukin-8 and neutrophil markers in colonic mucosa from patients with ulcerative colitis. Am. J. Gastroenterol. 87:1447–1452.
84. Jalaluddin S., P. de Mol, W. Hemelhof, N. Bauma, D. Brasseur, P. Hennart, R. E. Lomoyo, B. Rowe & J. P. Butzler. 1998. Isolation and characterization of enteroaggregative *Escherichia coli* (EAggEC) by genotypic and phenotypic markers, isolated from diarrheal children in Congo. Clin. Microbiol. Infect. 4:213-219.
85. Jiang Z.D., D. Greenberg, J.P. Nataro, R. Steffen & H.L. DuPont. 2002. Rate of Occurrence and pathogenic effect of enteroaggregative *Escherichia coli* virulence factors in international travelers. J. Clin. Microbiol. 40:4185-4190.
86. Jiang Z.D., P.C. Okhuysen, D.C. Guo, R. He, T.M. King, H.L. Dupont & D.M. Milewicz. 2003. Genetic susceptibility to enteroaggregative *Escherichia coli* diarrhea: Polymorphism in the interleukin-8 promotor region. J. Infect. Dis. 188:506-511.
87. Kahali S., B. Sarkar, K. Rajendran, J. Khanam, S. Yamasaki, R.K. Nandy, S.K. Brattacharya & T. Ramamurthy. 2004. Virulence characteristics and molecular epidemiology of enteroaggregative *Escherichia coli* isolates from hospitalized diarrheal patients in Kolkata, India J. Clin. Microbiol. 42:4111-4120.
88. Kang C., M.M. Mathan & V.I. Mathan. 1995. Evaluation of a simplified, HEp-2 cell adherence assay for *Escherichia coli* isolated from South Indian children with acute diarrhea and controls. J. Clin. Microbiol. 33:2204-2205.
89. Khan M.A., J. Kang & T.S. Steiner. 2004. Enteroaggregative *Escherichia coli* flagellin-induced interleukin-8 secretion requires Toll-like receptor 5-dependent p38 MAP kinase activation. Immunology. 112:651-660.
90. Knutton S., R.K. Shaw, M.K. Bhan, H.R. Smith, M.M. McConnell, T. Cheasty, P.H. Williams & T.J. Baldwin. 1992. Ability of enteroaggregative *Escherichia coli* strains to adhere in vitro to human intestinal mucosa. Infect. Immun. 60:2083–2091.
91. Knutton S., R. Shaw, A.D. Phillips, H.R. Smith, G.A. Willshaw, P. Watson & E. Price. 2001. Phenotypic and genetic analysis of diarrhea-associated *Escherichia coli* from children in the United Kingdom. J. Pediatr. Gastroenterol. Nutr. 33:32-40.
92. Lanata C.F., R.E. Black, D. Maúrtua, A. Gil, A. Gabilondo, A Yi et al. 1992. Etiologic agents in acute *vs* persistent diarrhea in children under three years of age in periurban Lima, Perú. Acta Paediatr. 381:23-38.
93. Levine M.M. 1987. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J. Infect. Dis. 155:377–389.
94. Lima A.A., G. Fang, J.B. Schorling, L. de Albuquerque, J.F. McAuliffe, S. Mota, R. Leite & R.L. Guerrant. 1992. Persistent diarrhea in northeast Brazil: Etiologies and interactions with malnutrition. Acta Pediatr. Suppl. 381:39-44.
95. Lior H. 1996. Classification of *Escherichia coli*, pp 31-72. In: C.L. Gyles (Eds). *Escherichia coli* in domestic animals and humans. CAB International, Wallingford, United Kingdom.
96. Mathewson J.J., H.L. DuPont, D.R. Morgan, S.A. Thornton & C.D. Ericsson. 1983. Enteroadherent *Escherichia coli* associated with travellers' diarrhoea. Lancet i:1048.
97. Mathewson J.J., P.C. Jhonson, H.L. DuPont, D.R. Morgan, S.A. Thornton & L.V. Wood. 1985. A new recognized cause of traveler's diarrhea: enteroadherent *Escherichia coli*. J. Infect. Dis. 154:471-475.
98. Mathewson J.J., P.C. Johnson, H.L. DuPont, T.K. Satterwhite & D.K. Winsor. 1986. Pathogenicity of enteroadherent *Escherichia coli* in adult volunteers. J. Infect. Dis. 151:524–527.
99. Mathewson J.J., R.A. Oberhelman, H.L. Dupont, J. Cabada, & E.V. Garibay. 1987. Enteroadherent *Escherichia coli* as a causa of diarrhea among children in Mexico. J. Clin. Microbiol. 25:1917-1919.
100. Mathewson J.J., B.M. Salameh, H.L. DuPont, Z.D. Jiang, A.C. Nelson, R. Arduino, M.A. Smith & N. Masozera. 1998. HEp-2 cell-adherent *Escherichia coli* and intestinal secretory immune response to human immunodeficiency virus (HIV) in outpatients with HIV-associated diarrhea. Clin. Diagn. Lab. Immunol. 5:87-90.
101. Mayer H.B. & C.A. Wanke. 1995. Enteropaggragtive *Escherichia coli* as a posible cause of diarrhea in an HIV-infected patient. N. Engl. J. Med. 332:273-274.
102. Miqdady M.S., Z.D. Jiang, J.P. Nataro & H.L. DuPont. 2002. Detection of enteroaggregative *Escherichia coli* with formalin preserved HEp-2 cells. J. Clin. Microbiol. 40:3066-3067.
103. Montecucco C., E. Papini & G. Schiavo. 1994. Bacterial protein toxins penetrate cells via a four step mechanism, *FEBS Lett.* **346:**92–98.
104. Monteiro-Neto V., L.C. Campos, A.J.P. Ferreira, T.A.T. Gomes & L.R. Trabulsi. 1997. Virulence properties of *Escherichia coli* O111:H12 strains. FEMS Microbiol. Lett. 146:123–128.
105. Moreira C.G., S.M. Carneiro, J.P. Nataro, L.R. Trabulsi & W.P. Elias. 2003. Role of type I fimbriae in the aggregative adhesion pattern of enteroaggregative *Escherichia coli*. FEMS Microbiol. Lett. 226:79-85.
106. Morobito S., H. Karch, P.M. Mariani-Kurkdjian, H. Schmidt, F. Minelli, E. Bingen & A. Caprioli. 1998. Enteroaggregative, shiga toxin-producing *Escherichia coli* O111:H2 associated with an outbreak of hemolytic-uremic syndrome. J. Clin. Microbiol. 36:840–842.
107. Mossoro C., P. Glaziou, S. Yassibanda, N.T.P. Lan, C. Bekondi, P. Minssart, C. Bernier, C.L. Bouguénec & Y. Germani. 2002. Chronic diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome associated with HEp-2 adherent *Escherichia coli* in adults infected with human immunodeficiency virus in Bangui, Central African Republic. J. Clin. Microbiol. 40:3086-3088.
108. Mwachari C., I. Batchelor, J. Paul, P.G. Waiyaki & C.F. Gilks. 1998. Chronic diarrhoea among HIV-infected adults patients in Nairobi, Kenya. J. Infect. 37:48-53.
109. Nataro J.P., M.M. Baldini, J.B. Kaper, R.E. Black, N. Bravo & M.M. Levine. 1985. Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. J. Infect. Dis. 152:560-565.
110. Nataro, J.P., J.B. Kaper, R. Robins-Browne, V. Prado, P. Vial & M. M. Levine. 1987. Patterns of adherence of diarrheagenic *Escherichia coli* to HEp-2 cells. Pediatr. Infect. Dis. J. 6:829–831.
111. Nataro, J.P., Y. Deng, D.R. Maneval, A.L. German, W.C. Martin, & M. M. Levine. 1992. Aggregative adherence fimbriae I of enteroaggregative *Escherichia coli* mediate adherence to HEp-2 cells and hemagglutination of human erythrocytes. Infect. Immun. 60:2297–2304.
112. Nataro J.P., D. Yikang, J.A. Giron, S.J. Savarino, M.H. Kothary & R. Hall. 1993. Aggregative adherence fimbria I expression in en-

teroaggregative *Escherichia coli* requires two unlinked plasmid regions. Infect. Immun. 61:1126–1131.
113. Nataro J.P., D. Yikang, D. Yingkang & K. Walker. 1994. AggR, a transcriptional activator of aggregative adherence fimbria I expression in enteroaggregative *Escherichia coli.* J. Bacteriol. 176:4691–4699.
114. Nataro J.P., Y. Deng, S. Cookson, A. Cravioto, S.J. Savarino, L.D. Guers, M.M. Levine & C.O. Tacket. 1995. Heterogeneity of enteroaggregative *Escherichia coli* virulence demonstrated in volunteers. J. Infect. Dis. 171:465–468.
115. Nataro J.P., S. Hicks, A.D. Phillips, P.A. Vial & C.L. Sears. 1996. T84 cells in culture as a model for enteroaggregative *Escherichia coli* pathogenesis. Infect. Immun. 64:4761–4768.
116. Nataro J.P. & J.B. Kaper. 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 11:142–201.
117. Nataro J.P., T. Steiner & R.L. Guerrant. 1998. Enteroaggregative *Escherichia coli*. Emerg. Infect. Dis. 4:251–261.
118. Nataro J.P. 2002. Diarrhoeagenic *Escherichia coli,* pp. 1463-1504. In M. Sussman (Eds). Molecular medical microbiology, Academic Press, San Diego, California.
119. Nataro J.P. 2005. Enteroaggregative *Escherichia coli* pathogenesis. Curr. Opin. Gastroenterol. 21:4-8.
120. Navarro-Garcia F., C. Sears, C. Eslava, A. Cravioto & J.P. Nataro. 1999. Cytoskeletal effects induced by Pet, the serine protease enterotoxin of enteroaggregative *Escherichia coli*. Infect. Immun. 67:2184–2192.
121. Navarro-García F., A. Canizalez-Roman, J. Luna, C. Sears & J.P. Nataro. 2001. Plasmid-encoded toxin of enteroaggregative *Escherichia coli* is internalized by epithelial cells. Infect. Immun. 69:1053-1060.
122. Navarro-Garcia F., C. Eslava, J. M. Villaseca, R. López-Revilla, J.R. Czec-zulin, S. Srinivas, J.P. Nataro & A. Cravioto. 1998. *In vitro* effects of a high-molecular-weight heat-labile enterotoxin from enteroaggregative *Escherichia coli.* Infect. Immun. 66:3149–3154.
123. Neill, M.A., P.I. Tarr, D.N. Taylor & A.F. Trofa. 1994. *Escherichia coli,* pp. 169-213. In: Y.H. Hui, J.R. Gorham, K.D. Murell & D.O. Cliver (Eds). Foodborne Disease Handbook, Marcel Dekker, Inc. New York, NY.
124. Nishi J., J. Sheikh, K. Mizuguchi, B. Luisi, V. Burland, A. Boutin, D.J. Rose, F.R. Blattner & J.P. Nataro. 2003. The export of coat protein from enteroaggregative *Escherichia coli* by a specific ATP-binding cassette transporter system. J. Biol. Chem. 278:45680-45689.
125. Ogata K., R. Kato, K. Ito & S. Yamada. 2002. Prevalence of *Escherichia coli* possessing the *eaeA* gene of enteropathogenic *E. coli* (EPEC) or *aggR* gene of enteroaggregative (EAggEC) in traveler´s diarrhea diagnosed in those returning to Tama, Tokyo from other Asian countries. Jpn. J. Infect. Dis. 55:14-18.
126. Okeke I.N., A. Lamikanra, J. Czeczullin, F. Dubovsky, J.B. Kaper & J.P. Nataro. 2000. Heterogeneous virulence of enteroaggregative *Escherichia coli* strains isolated from children in southwest Nigeria. J. Infect. Dis. 181:252-260.
127. Okeke I.N., A. Lamikanra, H. Steinruck & J.B. Kaper. 2000. Characterization of *Escherichia coli* strains from cases of childhood diarrhea in provincial southwestern Nigeria. J. Clin. Microbiol. 38:7-12.
128. Okeke I.N., & J.P. Nataro. 2001. Enteroaggregative *Escherichia coli*. Lancet Infect. Dis. 1:304-313.
129. Okeke I.N., I.C.A. Scaletsky, E.H, Soars, L.R. Macfarlane & A.G. Torres. 2004. Molecular epidemiology of the iron utilization genes of enteroaggregative *Escherichia coli*. J. Clin. Microbiol. 42:36-44.
130. Old D.C., A. Tavendale & D.E. Yakubu. 1989. Some strains of *Escherichia coli* of putative enteroadherent-aggregative serotypes produce an unusual fibrillar haemagglutinin. FEMS Microbiol. Lett. 59:87-92.
131. Orskov I., F. Orskov, A. Birth-Anderson, M. Kanamore & C. Svanborg-Eden. 1982. O, K, H and fimbrial antigen in *Escherichia coli* serotypes associated with pyelonephritis and cystitis. Scand. J. Infect. Dis. Suppl. 33:18-25.
132. Pabst W.L., M. Altwegg, C. Kind, S. Mirjanic, D. Hardegger & D. Nadal. 2003. Prevalence of enteroaggregative *Escherichia coli* among children with and without diarrhea in Switzerland. J. Clin. Microbiol. 41:2289-2293.
133. Pai M., G. Kang, B.S. Ramakrishna, A. Venkataraman & J. Muliyil. 1997. An epidemic of diarrhoea in south India caused by enteroaggregative *Escherichia coli*. Indian J. Med. Res. 106:7-12.
134. Pakyz A.L. 2005. Rifaximin: a new treatment for traveler´s diarrhea. Ann. Pharmacother. 39:284-289.
135. Paul M., T. Tsukamoto, A.R. Ghosh, S.K. Bhattacharya, B. Manna, S. Chakrabarti, G.B. Nair, D.A. Sack, D. Sen & Y. Takeda. 1994. The significance of enteroaggregative *Escherichia coli* in the etiology hospitalized diarhea in Calcuta, India and the demonstration of a new honey-combed pattern of aggregative adherence. FEMS Microbiol. Let 117:319-325.
136. Piva I.C., A.L. Pereira, L.R. Ferraz, R.S.N. Silva, A.C. Vieira, J.E. Blanco, M. Blanco, J. Blanco & L.G. Giugliano. 2003. Virulence markers of enteroaggregative *Escherichia coli* isolated from children and adults with diarrea in Brasilia, Brazil. J. Clin. Microbiol. 41:1827-1832.
137. Polotsky Y., J.P. Nataro, D.P. Kotler & J.M. Orestein. 1997. Adherence patterns of bacterial diarrhea agents in AIDS. Adv. Exp. Med. Biol. 412:367-371.
138. Presterl E., R. Nadrchal, D. Wolf, M. Rotter & A.M. Hirschl. 1999. Enteroaggregative and enterotoxigenic *Escherichia coli* among isolates from patients with diarrhea in Austria. Eur. J. Clin. Microbiol. Infect. Dis. 18:209-212.
139. Qadri F., A. Haque, S.M. Faruque, K.A. Bettelheim, R. Robins-Browne & M.J. Albert. 1994. Hemagglutinating properties of Enteroaggregative *Escherichia coli.* J. Clin. Microbiol. 32:510-514.
140. Rajakumar K., C. Sasakawa & B. Adler. 1997. Use of a novel approach, termed island probing, identifies the *Shigella flexneri she* pathogenicity island wich encoded a homolog of the immunoglobulin A prorease-like family of protein. Infect. Immun. 65: 4606-4614
141. Ramzan N.N. 2001. Traveler´s diarrhea. Gastroenterol Clin. 30:1-16.
142. Ratchtrachenchai O.A., S. Subpasu, H. Hayashi & W. Ba-Thein. 2004. Prevalence of childrenood diarrhoea-associated *Escherichia coli* in Thailand. J. Med. Microbiol. 53:237-243.
143. Regua-Mangia A.H., T.A.T. Gomes, M.A.M. Vieira, J.R.C. Andrade, K. Irino & L. M. Teixeira. 2003. Frequency and characteristics of diarrhogenic *Escherichia coli* strains isolated from children with and without diarrhoea in Rio de Janeiro, Brazil. J. Infect. 48:161-167.
144. Rich C., S. Favre-Bonte, F. Sapena, B. Joly & Ch. Forestier. Characterization of enteroaggregative *Escherichia coli* isolated. 1999. FEMS Microbiol Let 173:55-61.
145. Rico G., J. Villaseca, R. Kretschmer, A. Cravioto & C. Eslava. 2001. Pet-serine-proteasa de *Escherichia coli* aumenta la quimiotaxis y el estallido respiratorio de leucocitos humanos, resum. E35. En: Resúmenes del XXVI Congreso de la Asociación Mexicana de Microbiología Clínica 2001. Asociación Mexicana de Microbiología Clínica. Guadalajara, Jalisco, México.
146. Robins-Browne R.M. & E.L. Hartland. 2002. *Escherichia coli* as a cause of diarrhea. J. Gastroenterol. Hepatol. 17:467-475.
147. Rodrigues J., V.C. Acosta, J.M. Candeias, L.O. Souza & F.J. Filho. 2002. Prevalence of diarrheogenic *Escherichia coli* and rotavirus among children from Botucatu, Sao Paulo State, Brazil.
148. Ruiz-Perez F., J. Sheikh, S. Davis, E. C. Boedeker & J. P. Nataro. 2004. Use of a continuous-flow anaerobic culture to characterize enteric virulence gene expression. Infect Immun. 72:3793-3802.
149. Ruttler M.E., N.F. Renna, L. Balbi, B. Garcia, L. Guidone, R. Fernandez, O. Puig & A. Ortiz. 2002. Characterization of enteroaggregative *Escherichia coli* strains from children with acute diarrhea,

Rev Latinoam Microbiol 2005; 47 (3-4): 140-159

in Mendoza, Argentina. Rev. Argent. Microbiol. 34:167-170.
150. Sainz T., J. Perez, M.C. Fresan, V. Flores, L. Jimenez, U. Hernandez, I. Herrera & C. Eslava. 2002. Histological alterations and immune response induced by Pet toxin during colonization with enteroaggregative *Escherichia coli* (EAEC) in a mouse model infection. J. Microbiol. 40:91-94.
151. Sang W.K., J.O. Oundo, J.K. Mwituri, P.G. Waiyaki, M. Yoh, T. Lidia & T. Hond. 1997. Multidrug/resistant enteroaggregative *Escherichia coli* associated with persistent diarrhea in Kenyan children. Emerg Infect. Dis. 3:373-374.
152. Sarantuya J., J. Nishi, N. Wakimoto, S. Erdene, J.P. Nataro, J. Sheikh, M. Iwashita, K. Manago, K. Tokuda, Ma. Yoshinaga, K. Miyata & Y. Kawano. 2004. Typical enteroaggregative *Escherichia coli* is the most prevalent pathotype among *E. coli* strains causing diarrhea in mongolian children. J. Clin. Microbiol. 42:133-139.
153. Savarino S.J., A. Fasano, D.C. Robertson & M. M. Levine. 1991. Enteroaggregative *Escherichia coli* elaborate a heat-stable enterotoxin demonstrable in an *in vitro* rabbit intestinal model. J. Clin. Invest. 87:1450–1455.
154. Savarino S.J., A. Fasano, J. Watson, B.M. Martin, M.M. Levine, S. Guandalini & P. Guerry. 1993. Enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 represents another subfamily of *E. coli* heat-stable toxin. Proc. Natl. Acad. Sci. USA 90:3093–3097.
155. Savarino S.J., P. Fox, Y. Deng & J.P. Nataro. 1994. Identification and characterization of a gene cluster mediating enteroaggregative *Escherichia coli* aggregative adherence fimbria I biogenesis. J. Bacteriol. 176:4949-4957.
156. Savarino S.J., A. McVeigh, J. Watson, A. Cravioto, J. Molina, P. Echeverria, M.K. Bhan, M.M. Levine & A. Fasano. 1996. Enteroaggregative *Escherichia coli* heat-stable enterotoxin is not restricted to enteroaggregative *E. col*i. J. Infect. Dis. 173:1019–1022.
157. Scaletsky I.C.A., S.H. Fabbricotti, S.O.G. Silva, M.B. Morais & U. Fagundes-Nieto. 2002. HEp-2-adherence *Escherichia coli* strains associated with acute infantile diarrhea, Sao Paulo, Brazil. Emerg. Infect. Dis. 8:855-858.
158. Scaletsky I., S. Fabbricotti, K. Aranda, M. Morais & U. Fagundes-Neto. 2002. Comparison of DNA hybridization and PCR assays for detection of putative pathogenic enteroadherent *Escherichia coli*. J. Clin. Microbiol. 40:1254-1258.
159. Schubert S., A. Rakin, H. Karch, E. Carniel & J. Heesemann. 1998. Prevalence of the "high-pathogenicity island" of *Yersinia* species among *Escherichia coli* strains that are pathogenic to humans. Infect. Immun. 66:480-485.
160. Scotland S.M., H.R. Smith, B. Said, G.A. Willshaw, T. Cheasty & B. Rowe. 1991. Identification of enterophatogenic *Escherichia coli* isolated in Britain as enteroaggregative or as members of a subclass of attachingand effacing *E. coli* not hybridizing with the EPEC adherence factor probe. J. Med. Microbiol. 35:278-283.
161. Scotland S.M., H.R. Smith, T. Cheasty, B. Said, G.A. Willshaw, N. Stokes & B. Rowe. 1996. Use of gene probes and adhesion tests to characterize *Escherichia coli* belonging to enteropathogenic serogroups isolated in the United Kingdom. J. Med. Microbiol. 44:438–443.
162. Shcmidt H., C. Knop, S. Franke, S. Aleksic, J. Heesemann & H. Karch. 1995. Development of PCR for screening of enteroaggregative *Escherichia coli*. J. Clin. Microbiol. 33:701-705.
163. Sheikh J., S. Hicks, M. Dall'Agnol, A.D. Phillips & J.P. Nataro. 2001. Roles for Fis and YafK in biofilm formation by enteroaggregative *Escherichia coli.* Mol. Microbiol. 41:983-997.
164. Sheikh J., J. Czeczulin, S. Harrington, S. Hicks, I. Herderson, C. Bouguenec, P. Gounon, A. Phillips & J. P. Nataro. 2002. A novel dispersin protein in enteroaggregative *Escherichia coli*. J. Clin. Invest. 110:1329-1337.
165. Smith H.R., T. Cheasty & B. Rowe. 1997. Enteroaggregative *Escherichia coli* and outbreaks of gastroenteritis in UK. Lancet. 350:814-815.
166. Smith H.R., S.M. Scotland, G.A. Willshaw, B. Rowe, A. Cravioto & C. Eslava. 1994. Isolated of *Escherichia coli* O44:H18 of diverse origin are enteroaggregative. J. Infect. Dis. 170:1610-1613.
167. Spencer J., H.R. Smith & H. Chart. 1999. Characterization of enteroaggregative *Escherichia coli* isolated from outbreaks of diarrheal disease in England. Epidemiol. Infect. 123:413-421.
168. Steiner T.S., A.A. Lima, J.P. Nataro & R. L. Guerrant. 1998. Enteroaggregative *Escherichia coli* produce intestinal inflammation and growth impairment and cause interleukin-8 release from intestinal epithelial cells. J. Infect. Dis. 177:88–96.
169. Steiner T.S., J.P. Nataro, C.E. Poteet-Smith, J.A. Smith & R.L. Guerrant. 2000. Enteroaggregative *Escherichia coli* expresses a novel flagellin that causes IL-8 release from intestinal epithelial cells J. Clin. Invest. 105:1769–1777.
170. Stevens C., G. Walz, C. Singaram, M.L. Lipman, B. Zanker, A. Muggia, D. Antonioli, M.A. Peppercorn & T. B. Strom. 1992. Tumor necrosis-α, interleukin-1β, and interleukin-6 expression in inflammatory bowel disease. Dig. Dis. Sci. 37:818–826.
171. Sui B.Q., P.R. Dutta & J.P. Nataro. 2003. Intracellular expression of the Plasmid-encoded toxin from enteroaggregative *Escherichia coli*. Infect. Immun. 71:5364-5370.
172. Sutjita M., A.R. Bouckenooghe, J.A. Adachi, Z.D. Jiang, J.J. Matewson, Ch.D. Ericsson & H. DuPont. 2000. Intestinal secretory immunoglobulin A response to enteroaggregative *Escherichia coli* in traveler´s diarrhea. Clin. Diag. Lab. Immunol. 7:501-503.
173. Suzart S., T. Aparecida, T. Gomes & B. E. Guth. 1999. Characterization of serotypes and outer membrane protein profiles in enteroaggregative *Escherichia coli*. Microbiol. Immunol. 43:201-205.
174. Suzart S., B. Guth, M. Pedroso, U. Okafor & T. Gomes. 2001. Diversity of surface structures and virulence genetic markers among enteroaggregative *Escherichia coli* (EAEC) strains with and without the EAEC DNA probe squence. FEMS Microbiol. Lett. 201:163-168.
175. Tickoo S.K., M.K. Bham, R. Srivastava, B.K. Dass, M. Shariff, S. Saini & R. Kumar. 1992. Intestinal colonization and production of diarrhea by enteroadherent-aggregative *Escherichia coli*. Indian J. Med. Res. 95:278-283.
176. Tsai C.C., S.Y. Chen & H.Y. Tsen. 2003. Screening the enteroaggregative *Escherichia coli* activity and detection of the *aggA*, *aafA*, and *astA* genes with novel PCR primers for the *Escherichia coli* from diarrhea cases in Taiwan. Diagn. Microbiol. Infect. Dis. 46:159-165.
177. Tzipori S., J. Montanaro, R.M. Robins-Browne, P. Vial, R. Gibson & M.M. Levine. 1992. Studies with enteroaggregative *Escherichia coli* in the gnotobiotic piglet gastroenteritis model. Infect. Immun. 60:5302–5306.
178. Urbina F., E.L. Nordmark, Z. Yang, A. Weintraub, F. Scheutz & G. Widmalm. 2005. Structural elucidation of the O-antigenic polysaccharide from the enteroaggregative *Escherichia coli* strain 180/C3 and its immunochemical relationship with *E. coli* O5 and O65. Carbohydr. Res. 340:645-650.
179. Valle G.R.F., T.A.T. Gomes, K. Irino & L.R. Trabulsi. 1997. The traditional enteropathogenic *Escherichia coli* (EPEC) serogroup O125 comprises serotypes which are mainly associated with the category of enteroaggregative *E. coli*. FEMS Microbiol. Lett. 152:95–100.
180. Vial P.A., R. Robins-Browne, H. Lior, V. Prado, J.B. Kaper, J.P. Nataro, D. Maneval, A. Elsayed & M.M. Levine. 1988. Characterization of enteroadherent-aggregative *Escherichia coli*, a putative agent of diarrheal disease. J. Infect. Dis. 158:70–79.
181. Vila J., M. Vargas, I.R. Henderson, J. Gascon & J.P. Nataro. 2000. Enteroaggregative *Escherichia coli* virulence factors in traveler´s diarrhea strains. J. Infect. Dis. 182:1780-1783.
182. Villaseca J.M., F. Navarro-Garcia, G. Mendoza-Hernandez, J.P. Nataro, A. Cravioto & C. Eslava. 2000. Pet toxin from enteroaggregative *Escherichia coli* produces cellular damage associated with fo-

drin disruption. Infect. Immun. 68:5920–5927.
183. Villaseca J.M., M.E. Torres-Marquez, U. Hernandez, J. Molina, S. Vazquez, A. Cravioto & C. Eslava. 2002. MAPK activation correlates with cytoskeletal alterations induced by Pet toxin from enteroaggregative *Escherichia coli*, abstrac. HSP 03. In Abstracts of the 3rd International EPEC symposium 2002. International EPEC symposium, Puerto Vallarta, Mexico.
184. Wai S.N., A. Takade & K. Amako. 1996. The hydrophobic surface protein layer of enteroaggregative *Escherichia coli* strains. FEMS Microbiol. Lett. 135:17-22.
185. Wakimoto N., J. Nishi, J. Sheikn, J.P. Nataro, J. Saratuya, M. Iwashita, K. Manago, K. Tokuda, M Yoshinaga & Y Kawano. 2004. Quantitative biofilm assay using a microtiter plate to screen for enteroaggregative *Escherichia coli*. Am. J. Trop. Med. Hyg. 71:687-690.
186. Wanke C.A., J.B. Schorling, L.J. Barrett, M.A. Desouza & R.L. Guerrant. 1991. Potencial role of adherence traits of *Escherichia coli* in persistent diarrhea in a urban Brazillian slum. Pediatr. Infect. Dis. J. 10:746-751.
187. Wanke C.A., H. Mayer, R. Weber, R. Zbinden, D.A. Watson & D. Acheson. 1998. Enteroaggregative *Escherichia coli* as a potential cause of diarrheal disease in adults infected with human immunodeficiency virus. J. Infect. Dis. 178:185-190.
188. Wanke C.A., J. Gerrior, V. Blais, H. Mayer & D. Acheson. 1998. Successful treatment of diarrheal associated with enteroaggregative *Escherichia coli* in adults infected with human immunodeficiency virus. J. Infect. Dis. 178:1369-1372.
189. Winsor Jr.D.K., J.J. Mathewson & H.L. DuPont. 1986. Western blot analysis of intestinal secretory immunoglobulin. A response to *Campylobacter jejuni* antigens in patients with naturally acquired *Campylobacter* enteritis. Gastroenterology. 90:1217–1222.
190. Xu J.G., B.Q. Cheng, Y.P. Wu, L.B. Huang, X.H. Lai, B.Y. Liu, X.Z. Lo & H.F. Li. 1996. Adherence patterns and DNA probe types of *Escherichia coli* isolated from diarrheal patients in China. Microbiol. Immunol. 40:89-79.
191. Yamamoto T., S. Endo, T. Yokota & P. Echeverria. 1991. Characteristics of adherence of enteroaggregative *Escherichia coli* to human and animal mucosa. Infect. Immun. 59:3722–3739.
192. Yamazaki M., K. Inuzuka, H. Matsui, K. Sakae, Y. Suzuki, Y. Miyazaki & K. Ito. 2000. Plasmid encoged enterotoxin (Pet) gene in enteroaggregative *Escherichia coli* isolated from sporadic diarrea cases. Jpn. J. Infect. Dis. 53:248-249.
193. Yatsuyanagi J., S. Saito, H. Sato, Y. Miyajima, K. Amano & K. Enomoto. 2002. Characterization of enteropathogenic and enteroaggregative *Escherichia coli* isolated from diarrheal outbreaks. J. Clin. Microbiol. 40:294-297.
194. Zhou Z., J. Ogasawara, Y. Nishikawa, Y. Seto, A. Helander, A. Hase, N. Iritani, H. Nakamura, K. Arikawa, A. Kai, Y. Kamata, H. Hoshi & K. Haruki. 2002. An outbreak of gastroenteritis in Osaka, Japan due to *Escherichia coli* serogroup O166:H15 that had a coding gene for enteroaggregative *E. coli* heat-stable enterotoxin 1 (EAST1). Epidemiol. Infect. 128:363-371.

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