Studies on the Expression and Regulation of Enterotoxins and Colonization Factors in Enterotoxigenic *Escherichia coli* (ETEC)

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Sahlgrenska Akademin vid Göteborgs Universitet kommer att försvaras offentligt i hörsal "Arvid Carlsson", Academicum, Medicinaregatan 3, Göteborg

Torsdagen den 31 januari 2008, kl 9.00

av

Matilda Nicklasson

Fakultetsopponent: Docent Sun Nyunt Wai, Umeå Universitet

Avhandlingen baseras på följande delarbeten:

I Sjöling Å, Qadri F, **Nicklasson M**, Ara Begum Y, Wiklund G, Svennerholm AM
In vivo expression of the heat stable (*estA*) and heat labile (*eltB*) toxin genes of enterotoxigenic *Escherichia coli* (ETEC).
Microbes and Infection 8 (2006) *2797-2802*

II Sjöling Å, **Nicklasson M**, Stenberg J, Eriksson S
Gene expression, translation and secretion of the heat stable (ST) and heat labile (LT) toxins of enterotoxigenic *Escherichia coli* (ETEC) are regulated in response to different external stimuli present in the gastrointestinal tract.
Submitted for publication

III **Nicklasson M**, Sjöling Å, Qadri F, Svennerholm AM
Gene and protein expression of colonization factors CS5 and CS6 in enterotoxigenic *Escherichia coli* (ETEC) after growth under different conditions *in vitro* and *in vivo*.
In manuscript

IV **Nicklasson M**, Sjöling Å, Lebens M, Tobias J, Janzon A, Brive L, Svennerholm AM
Mutations in the periplasmic chaperone leading to loss of surface expression of the colonization factor CS6 in enterotoxigenic *Escherichia coli* (ETEC) clinical isolates.
Accepted for publication in Microbial Pathogenesis

V **Nicklasson M**, Klena J, Rodas C, Bourgeois A, Torres O, Svennerholm AM, Sjöling Å
Genetic relationship of enterotoxigenic *Escherichia coli* ST/CS6 strains isolated from children living in Guatemala and adult visitors to Central America.
Submitted for publication

Studies on the Expression and Regulation of Enterotoxins and Colonization Factors in Enterotoxigenic *Escherichia coli* (ETEC)

Matilda Nicklasson

Department of Microbiology and Immunology, Institute of Biomedicine, Göteborg University, Sweden

Abstract

Enterotoxigenic *Escherichia coli* (ETEC) is one of the most common causes of acute watery diarrhoea in developing countries, particularly among local children less than five years and is also the most common cause of diarrhoea in travellers to ETEC endemic areas. The infection is transmitted by ingestion of contaminated food and water and the disease is established in the small intestine. Colonization factors (CFs) on the bacterial surface mediate adhesion to the intestinal epithelium and diarrhoea is manifested by the actions of a heat-stable (ST) and / or a heat-labile (LT) enterotoxin. Two of the most common CFs in strains isolated world-wide are coli surface antigens 5 (CS5) and 6 (CS6). In this thesis the expression and regulation of these important virulence factors as well as the genetic variability among ETEC strains have been studied.

Using ETEC strains isolated directly from diarrhoeal stool specimens of Bangladeshi patients without sub-culturing the gene expression of the two enterotoxins as well as the two CFs were studied *in vivo*. By also quantifying the transcription levels of the respective genes after *in vitro* culture we found that there was no significant up- or down-regulation of transcription of the genes encoding ST (*estA*) or LT (*eltB*) *in vivo* as compared to *in vitro*; however, the CS5 operon was up-regulated 100-fold and CS6 operon 10-fold *in vivo*.

By culturing clinical strains under various conditions *in vitro*, ST, LT, CS5 and CS6 were shown to be differentially regulated by certain environmental factors, *i.e.* the presence of bile salts, lack of oxygen and different carbon sources (glycerol, glucose and amino acids). Thus, secretion of ST was down-regulated by glucose as carbon source under certain conditions but up-regulated by casamino acids, LT was only secreted in complex media in the absence of bile salts and presence of oxygen, phenotypic expression of CS5 on the bacterial surface was induced by bile salts and down-regulated by lack of oxygen, and expression of CS6 was up-regulated by lack of oxygen. An important finding was that the regulation of expression of these virulence factors does not seem to occur at the transcriptional level of the virulence operons.

A majority of wild-type LT-only ETEC strains that were genotypically positive for CS6, but that did not express CS6 on the bacterial surface, were shown to contain truncating mutations within the functional chaperone subunit. This mutation was predicted to severely affect the capacity of the chaperone to bind to the structural subunits, thus indicating a requirement for a functional chaperone for surface expression of CS6. In addition, a single-point mutation was identified in the non-coding region up-stream of the chaperone-encoding gene in these strains; this mutation was found in strains isolated in diverse geographical areas and belonging to different clonal groups.

By investigating the genetic relationship between ST-only CS6 positive strains isolated from children in a region highly endemic for ETEC, *i.e.* Guatemala, and adult travellers to the same region we found that these two groups may be infected by strains of the same genetic background and that ST-only CS6 positive strains belonging to several clonal complexes circulate in this area. We suggest that an ST-only CS6 positive ETEC strain belonging to the most common clonal complex, which was present during several years and found in strains isolated both from children and adults, may be considered as a candidate vaccine strain.

Keywords: ETEC, heat-stable enterotoxin, heat-labile enterotoxin, colonization factors, CS5, CS6, virulence gene expression, *in vivo* and *in vitro*, genetic variability.

ISBN 978-91-628-7389-9 **Göteborg 2008**