VIRULENCE FACTORS AND CLONAL RELATEDNESS OF ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) ISOLATED FROM CHILDREN WITH DIARRHOEA IN BOLIVIA

AKADEMISK AVHANDLING

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av

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Avhandlingen baseras på följande arbeten:

I. **Rodas C**., Iniguez V., Qadri F., Wiklund G., Svennerholm A-M. and Sjöling Å. Development of Multiplex PCR assays for detection of enterotoxigenic Escherichia coli (ETEC) colonisation factors and toxins.
J Clin Microbiol 2009; 47:1218-20

II **Rodas C.,** Mamani R., Blanco J., Blanco J.E., Wiklund G., Svennerholm A-M. Sjöling Å. and Iniguez V. Enterotoxins, colonisation factors, serotypes and antimicrobial resistance of enterotoxigenic *Escherichia coli* (ETEC) strains isolated from hospitalized children with diarrhoea in Bolivia.
Accepted for publication in Brazilian Journal of Infectious Diseases

III Nicklasson M., Klena J., **Rodas C.,** Torres O., Bourgeouis AL., Svennerholm A-M. and Sjöling Å. (2010). Enterotoxigenic *Escherichia coli* Multilocus Sequence Types in Guatemala and Mexico.
Emerging Infectious Diseases, 16:143-6.

IV **Rodas C.,** Klena J., Nicklasson M., Iniguez V., and Sjöling Å. Clonal relatedness of enterotoxigenic Escherichia coli (ETEC) strains expressing LT and CS17 isolated from children with diarrhoea in La Paz, Bolivia.
Submitted

V **Rodas C.**, Iniguez V., Svennerholm A-M., and Sjöling Å. 2010. Clinical isolates of enterotoxigenic *Escherichia coli* (ETEC) from children in Bolivia cause severe diarrhoea but produce comparatively low levels of the heat labile (LT) and heat stable (ST) enterotoxins.
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Virulence factors and clonal relatedness of enterotoxigenic *Escherichia coli* (ETEC) isolated from children with diarrhoea in Bolivia

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Enterotoxigenic *Escherichia coli* (ETEC) is one of the major causes of diarrhoea in children under five years of age in developing countries and travellers to these settings. To cause disease, ETEC must be able to colonise the small intestine and produce heat-stable enterotoxin (ST) or heat-labile toxin (LT) or both toxins. The attachment of ETEC is mediated by different fimbrial antigens, known as colonisation factors (CFs); at least 22 CFs have been identified to date. Besides determination of the toxins and CFs, serotyping has been used to identify and characterize ETEC. However, there is a lack of rapid and simple standardized methods to detect ETEC in many laboratories. In recent years, PCR-based assays have been increasingly common and due to their simplicity and commercially available reagents for diagnostic purposes with the ability to obtain good specificity and sensitivity.

In order to speed up the detection of CFs by PCR, we have established a multiplex PCR assay for detection of ETEC CFs, using previously established CF primers. The published CF primers were assembled into four panels designed to amplify 19 CFs in four PCR reactions. This test was used to amplify on two ETEC strain collections from Bolivia and Bangladesh isolates from children with diarrhoea.

We have also determined the relation between enterotoxins, CFs and serotypes as well as the antimicrobial resistance patterns in 43 ETEC strains isolated from hospitalized children with acute diarrhoea in Bolivia during the period 2002 to 2006. Among the ETEC isolates tested, 30 were positive for LT, 3 for STh and 10 for LT/STh. Sixty-five percent of the strains expressed one or more of the CFs; the most common ones were CS17 (n=8) and CFA/I (n=8). The most common serotypes were O8:H9 LT/CS17 (n=6) and O78:HNM LT/ST CFA/I (n= 4); 67% of the strains were resistant to one or several of the antimicrobial agents tested for.

Phylogenetic analyses are used to localize outbreaks of ETEC disease and to trace disease over time in different geographic regions. Multilocus sequencing Typing (MLST) method has recently been used to determine the genetic variations and clonal lineages of this pathogen. We have investigated 24 ETEC strains isolated from US adult travellers and infected resident children in Mexico and Guatemala that were infected with ETEC strains. These strains expressed ST/CS6 and 7 MLST sequence types (ST), being the most common: ST-398 (n=10), ST-182 (n=6) and ST-278 (n=4) expressing STp and carrying genetically identical CS6 sequences the cause of disease.

We also determined the genetic relatedness of ETEC strains obtained from a cohort of children with diarrhoea in Bolivia. We found 2 different LT/CS17 clones in ETEC strains; one of them had a specific molecular signature and persisted in La Paz from 2002 to 2005. By using the MLST method and sequencing the CS17 operon, we compared the Bolivian LT/CS17 isolates to Bangladeshi LT/CS17 ETEC strains isolated from children with diarrhoea. A common clone was identified which was identical to LT/CS17 strains isolated in subsequent studies in 2007 and 2009 in Bolivia indicating that this is a persistent clone that circulated in Bolivia for at least eight years.

Finally, we analysed toxin production in 54 ETEC strains collected during 2 summer periods in Bolivia and compared these results to Bangladeshi, Egyptian and Guatemalan ETEC strains. Findings showed a high production of toxins in Bangladeshi and Egyptian ETEC strains, followed by the Guatemalan and a low production in Bolivian strains. No association between severity of disease and toxin production was found among Bolivian ETEC strains.

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