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Title of the Thesis: Studies on colonization factors of enterotoxigenic *Escherichia coli*: Prevalence, Expression and Regulation during pathogenesis.

ABSTRACT

In the developing countries, enterotoxigenic *Escherichia coli* (ETEC) infection is the most prevalent cause of diarrhoea in children under the age of five years and adults. ETEC has been identified as the bacterial agent responsible for roughly 20% of instances of acute watery diarrhoea and important etiologic agent for traveler's diarrhea. The interaction of colonization factors (CFs) with their associated receptors on the intestinal epithelium initiates ETEC pathogenesis, and release of either both or any one of the enterotoxins, heat labile (LT) and heat stable (ST), inducing the disease. The pathogenicity of ETEC is also influenced by the presence of non-classical virulence factors. It has been observed that the prevalent colonization factors changes from time to time and with geographical region. This thesis aims to better understand the most prevalent combination of classical and non-classical virulence factors among clinical isolates of ETEC in our settings.

In this study, we focus on the expression of prevalent colonization factors in terms of its identification, expression, distribution and regulation during pathogenesis. Archived ETEC strains between the years 2008-2016, 350 ETEC isolates were used for genetic screening for identifying classical and non-classical virulence factors. Presence of the virulence genes, multiplex-PCR followed by singleplex-PCR using specific primer sets were done. For expression study, RNA isolation followed by quantitative real time PCR was used. Detection and quantification of CS6, CS5, and EatA under different host and environmental conditions were done by Real time q-RT PCR and ELISA. Isogenic mutants of CS5 and EatA were prepared by lambda Red recombinase mutagenesis system. Cell infection studies were done in HT-29. Animal experiments were done in rabbit ileal loop assay and mice colonization model. CS6 promoter was deleted and analysed by β-galactosidase assay.

Among the screened ETEC strains, *est+elt* genes were possessed by 61% strains followed by 25% *est* and 14% *elt*. CS21 (37%) and CS6 (36%) were the prevalent CFs. Among the screened ETEC isolates the most common NCVF was EatA (65 %), followed by EtpA (51 %). There were 100 (29%) isolates that tested negative for CFs or NCVFs. Among the 196 CFs positive strains, 148 strains expressed their respective classical genes. Out of the 71 strains having CS6, 60 strains express CS6. The majority of wild-type ETEC strains did not express CS6 on the bacterial surface though they were CS6 genotypically positive. CS21 expression is 46 out of 71 while that of CS5 is 38 out of 48. For non-classical virulence factors genes 135 from 205 strains expressed RNA. 80 strains express EatA from 134 strains.

Under various host and environmental attributes i.e. in the availability of bile acid salts and its components, mucin, iron, pH, osmolarity, temperature, glucose under *in vitro* conditions LT, ST, CS6, CS5 and EatA were shown to be selectively monitored. CS6 expression was upregulated in presence of components of bile, Iron salts with respect to untreated ETEC. CS6 expression was higher at pH 6 and pH 9. The expression of CS6 was most at 37°C. Expression of CS5 was upregulated in presence of crude bile, bile components, iron salts, NaCl and mucin. CS5 expression were most at pH 6.0. The EatA expression was upregulated in presence components of bile, iron salts and glucose. LT secretion was induced in presence of TCA, NaCDC, NaDC, NaCl while the secretion of ST was up-regulated in presence of NaCH, NaGCH. Maximum secretion of LT and ST toxin was at pH 8. LT and ST secretion was also maximum at 37°C. Minimum ETEC-WT inoculum required for infection is 10^7 cfu/ml. The mutants and becomes less pathogenic in terms of adherence, fluid accumulation and histological damage when compared to ETEC-WT. The minimum region of CS6 promoter lies between -254 and +1. Important region(s) lies between -350 bp to -255 bp upstream in the promoter which might have important elements needed to control CS6 gene expression.

By investigating the genetic relationship between the virulence factors isolated from strains endemic for this region we discovered an association between CS6, CS5, EatA, and toxins. In sole strains most genes were expressed while in mixed strains less genes showed expression. ETEC activated in response to conditions similar to those in the intestinal environment plays a critical role in initial attachment to the intestinal epithelium, including the establishment of microcolonies and the release of toxins, according to our findings.

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