Index No. 78/19/Life Sc./26

Thesis Title: Effect of different regulatory factors on prevalent virulence factors expressed by enterotoxigenic *Escherichia coli* - a comprehensive study

ABSTRACT

Enterotoxigenic *Escherichia coli* (ETEC) is an enteric pathogen isolated from diarrheal patients and accounts for nearly 1,00,000 deaths annually in recent years around the globe. The peak of incidence of ETEC mostly occurs in children below the age of 5 years. In developed nations, like The United States, ETEC is acknowledged as a major cause of foodborne disease. According to Global Burden of Diseases (GBD) 2015, in India, children aged less than 5 years, around 6% of all diarrheal deaths were due to ETEC. Pathogenesis of ETEC causes the release of electrolytes, water and finally watery diarrhea which occur due to the release of plasmid-encoded enterotoxins – heat-labile (LT) and/or heat-stable (ST). To release the enterotoxins, the bacteria first attach to the epithelium of the small intestine via antigenic fimbriae called colonization factors (CFs), a major virulence determinant for initiating pathogenesis. Besides this CFs and some Non- Classical Virulence factors (NCVFs) also aid to the pathogenesis.

With this aim in mind this thesis is focused on comprehending the distribution of virulence determinants as well as the pattern of expression of the ETEC strains of this region. Additionally, we also investigated to find any correlation with the antimicrobial response pattern. In this study we finally focused on effect of different regulatory factors on commonly expressed CFs.

Archived ETEC strains of total of 379 samples isolated between 2015 -2019 used for this study (Isolated fron stool specimens of diarrheal patients admitted at the Infectious Diseases and Beliaghata General Hospital (ID & BG Hospital, Kolkata) and Dr. B.C.Roy Post Graduate Institute of Pediatric Sciences during surveillance study).

Multiplex PCR were employed to identify virulence determinants followed by confirmatory singleplex PCR. For expression study, RNA isolation followed by quantitative real time PCR was used. To observe response of different antibiotics, the Kirby-Bauer method was used. For the detection and quantification of CS6, CS5, and EatA Real time q-RT PCR and ELISA was performed. To prepare isogenic mutants of HNS lambda Red recombinase mutagenesis system was followed. Promoter assay was performed by β-galactosidase assay. Cell adhesion assay was done on intestinal cell-line HT-29. In vivo study was done with Rabbit ileal loop assay recovered from New Zealand White Rabbit.

Out of the tested 379 strains, 46% strains harboured both the enterotoxins ST and LT, whereas 15% were LT only. Among the major colonization factors, CS6 (41%) was the most prevalent followed by CFA/I (35%) and CFA/III was the lowest (3%). Among the NCVFs, EatA (69%) was predominant. However expanding the panel of detection by including most of the discovered CFs in this study revealed 97% positivity. Still 3%(n=350) isolates did not tests for any virulence determinants, indicating that there is still same factor responsible for ETEC colonization that are yet to be discovered. The prevalent combination of virulence determinants was CS6+CS5 and EatA along with elt and or esth. Though CFA/I had emerged as another predominant CF but it had diverse CFs and NCVFs along with it. In our study we observed that not all ETEC strains expressed their respective virulence factors that they possess within their genome and plasmid. Our study also investigated the response of ETEC strains against different antibiotics and surprisingly we found that there were multidrug resistant (MDR) strains. Most common antibiotic combination against which resistance was found is Am+Azm+E+S+NA. This thesis focused on the effect of regulatory factors on prevalent colonisation factors taking multiple parameters into consideration in order to gain a better understanding of the pathogenic mechanisms of this heterogeneous enteropathogen. We also expect that our results will provide a better insight to understand the differential expression of the virulence genes in ETEC and how they relate during pathogenesis. This will help as a foundation for developing various intervention strategies in future.

ETEC encounters different signals within host intestine and it can trigger linked genetic and metabolic pathways. Our experiments with addition of different compounds in the growth media maybe indicative of change in the intestinal environment which sometimes acts advantageous as well as sometime deleterious to the bacteria. It is also important to remember that virulence regulation conditions examined in the lab may differ during human infection as we cannot totally mimic the gut microbiome and the physiological conditions.

We strongly believe that the only way to making an effective vaccine with broader efficacy should contain toxins as well as CS6, CS5 and EatA for this region as this is the only combination found prevalent for a long time in our years of study. Molecular details of expression studies will help in better understanding of the ETEC pathogenesis and this knowledge could be translated for effective and safe drug for disrupting regulators of ETEC pathogenesis. This study should reduce the existing knowledge gap in minimising ETEC infection.

नवेन्दु शेखर चटर्जी / Nabendu Sekhar Chatterjee
वैज्ञानिक - एफ / Scientist F)
आई.सी.एम.आर राष्ट्रीय कॉलरा और आंत्र रोग संस्थान
ICMR National Institute of Cholera & Enteric Diseases
पी-33, सी.आई.टी. रोड, स्कीम-9एम, बेलियाघाटा
P-33, CIT Road, Scheme-XM, Beliaghata
कोलकाता / Kolkata-700010