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Title of Thesis: Molecular And Serological Characterization Of Enteroaggregative *E. coli* (EAEC) Isolated from Diarrhoeic and Non Diarrhoeic Children

Abstract

Keywords: Enteroaggregative *Escherichia coli*; Diarrhoeagenic *E. coli*, Multiplex PCR; Antibiotic Susceptibility, Serotyping.

Despite reductions in mortality worldwide, diarrhoea still accounts for more than 2 million deaths annually and is associated with impaired physical and cognitive development in resource limited countries. There are six well-described categories: Enteroaggregative *E. coli* (EAEC), Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC).

Enteroaggregative *Escherichia coli* (EAEC) strains have been implicated as emerging etiological agents of diarrhoea worldwide. In reality EAEC is probably the most common bacterial cause of diarrhoea and an increasingly recognized cause of acute diarrhoea among children, adults and HIV-infected persons, in both developing and developed countries.

The aim of this study was to investigate the frequency, virulence markers and antibiotic resistance patterns of diarrhoeagenic *E. coli* (DEC) especially Enteroaggregative *E. coli* isolated in New Delhi, India by serotyping, Virulence genes, and antimicrobial resistance typing. Serotypes, virulence genes, and resistance patterns of strains from cases were then compared with those from the control group.

Clinical stool specimens from 925 children with and without diarrhoea (825 children due to diarrhoea and 100 healthy controls of similar age and gender), with ages ranging from birth to <5 years were collected over a period of 30 months (May 2010 to December 2012).

A total of 649 *E. coli* strains from children with diarrhoea and 74 *E. coli* strains from healthy children were investigated by using conventional culture techniques, recently designed Multiplex PCR for 10 virulence genes in two different reactions: Enteropathogenic *E. coli* (EPEC) *eae* and *bfpA*; Enterotoxigenic *E. coli* (ETEC) *Elt* and *Stx*; Enterohemorrhagic *E. coli* *stx1* and *stx2*; and *uidA* gene. Three plasmid-borne genes for Enteroaggregative *E. coli* (EAEC) *AggR*, *aap* and AA probe to detect these pathotypes simultaneously in a two different multiplex PCR and serotyping. Enteroaggregative *E. coli* strains were serotyped and their susceptibility to 12 antimicrobial agents was determined by the disk diffusion method. In order to distinguish Typical and atypical EAEC strains, these were tested for the presence of *AggR* gene.

At least one pathogen was detected in 80.8% of the 575 patients and in 24.3% of the 74 controls. The most active agents for the full isolate collection were Imipenem (97.6%); Colistin (54.9%); Amikacin (43.5%); Nitrofurantoin (26.7%); Cefotaxime (19.7%); Kanamycin (15.3%); Cefepime (10.5%); Aztreonam (8.2%); Amoxycillin (7.3%) and Cefpodoxime (0.3%).

In the present study, 43 EAEC strains were serotyped. We identified 18 isolates as serotype O14; and 9 isolates as O104 and 16 strains as O-untypable and 39 O-typable strains and 10 H-untypable and 20 H-typable strains. In case of H serotypes also H10 which detect in 4 strains were most frequent. Those arranged into 13 O:H combinations, the most frequent combination of O and H serotype in pediatric samples were O104:H10 and in case of control was O14:H30. The study showed that 649/925 children (70.2%) had diarrhoea due to diarrhoeagenic *E. coli*. The most prevalent was Enteroaggregative *E. coli* (EAEC) isolated from 227/649 pediatric patients (34.98%), followed by Enteropathogenic *E. coli* (EPEC) isolated from 140/649 (21.57%), Enterohemorrhagic *E. coli* (EHEC) from 80/649 (12.33%) and Enterotoxigenic *E. coli* (ETEC) from 36/649 patients (5.55%).