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Title of the Thesis: Molecular characterization of Extended Spectrum β - Lactamases in *Escherichia coli* isolates from diarrhoeic and non diarrhoeic children

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Abstract

The Extended Spectrum β-lactamases (ESBL) are by far the most important resistance mechanism among the Gram Negative bacteria. These enzymes inactivate the most widely used antimicrobial group of antibiotics, the β-lactams which include the penicillins, cephalosporins, monobactams and the carbapenems by hydrolysing the β-lactam ring rendering the antibiotic inactive. The *E. coli* strains are known to harbour ESBL genes and express this resistance mechanism. This study focussed on the ESBLs among the Diarrhoegenic *E. coli* from paediatric patients.

The presence of ESBL among the paediatric patients has a higher implication due to narrower therapeutic options for bacterial infections. It was found 32.26% of the *E. coli* isolates carried the *bla* genes for the expression of ESBL. The presence of ESBL in the gut of non-diarrhoeic children was appalling. Compared to the adults the prevalence of ESBL in children is alarming. The gut microflora of these children is still in its infancy and yet harboured antimicrobial resistant genotypes.

As a repercussion the beta lactam drugs which dominate the treatment of diarrhoeal infections caused by Gram negative bacteria can now be treated with limited antibiotics. This was proved by the drug susceptibility results with high resistance was seen against Cephalosporins (97.43%) followed by Penicillins (77.85%) and Monobactams (54.89%). Carbapenems being the drug of last resort was most sensitive but in the samples it showed a 0.96% resistance. This study also focussed to provide information for laboratories and policy makers which could enable them to make informed decisions about the best methods available for detecting newly emergent strains of Diarrhoeagenic *E. coli* and their Antibiotic Susceptibility pattern.

E. coli is the first among the colonisers of the gut of an infant. Acquisition of the virulence determinants of diarrhoea along with the ESBL genes in the commensal *E. coli* raises alarming risk among children. 507(81.38%) of *E. coli* isolates were Multidrug Resistant. The exposure of the antimicrobial agents in the gut and also in the environment has been known to exert pressure on the *E. coli* strains to develop resistance. 7.3% of the samples among diarrhoea showed a lower zone of inhibition of the cephalosporins when used in combination with the beta lactam inhibitor compared to when used alone, due to the development of resistance to β-lactamase inhibitors. The clinical laboratories only rely on the drug sensitivity data without any analysis of the bacterial virulence; clinicians cannot estimate the toxicity of *E. coli*. This study has revealed that the virulence of the ESBL-*E. coli* is essential for determining the therapeutic measures for clinicians. 7 virulence factors were studied and the maximum virulence score observed was 5. EAEC was the most prevalent pathotype among diarrhoea in children followed by EPEC. Molecular data of the virulence genes and the ESBL is not sufficient and serotyping needs to be carried out to assess the antigenic determinants in case of an epidemic and in order to embark upon the production of vaccines. O26 and O111 were the most prominent serotypes among the ESBL positive EPEC, EHEC and EAEC strains and commonly found in all three categories.

The non-diarrhoeal children whose *E. coli* isolates were the commensals, harboured genetic determinants for virulence factors of diarrhoea and also the genetic elements of ESBL. The irrepressible spread of ESBL genes among the commensal strains raises an alarming risk to the use of Antimicrobials. High resistance to β-lactamase inhibitor combinations proves to be a setback to the therapeutics in use and urges the need for rapid development of more effective alternatives with broader spectrum. The clonal dissemination of genetic elements can lead to a rampant epidemic, thus serological identification was carried out being an effective tool for diagnosis of *E .coli* strains and help in assessment and containment of an infection.

Furthermore, the ability of *E. coli* virulence factors to influence a wide range of cellular functions has resulted in an unexpected high level of complexity in order to develop an effective vaccine. The acquisition of different virulence traits, ESBL mediated resistance genes, the continuous exchange of genetic elements and the expression of virulence genes generally regulated by environmental factors probably will reveal different strategies shared by *E. coli* strains. These infections such as diarrhoea acquire epidemic proportions and need to be attended with high priority, especially in India.