Name : **Asiya Afshan**

Name of the supervisor : **Prof. Dr. Arif Ali**

Name of the department : **Department of Biotechnology**

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ABSTRACT OF Ph.D. THESIS

Diarrheal diseases are second only to cardiovascular diseases as a cause of death worldwide (WHO, 1990). In India, diarrheal disease is a major public health problem among children. Among diarrheagenic pathogens, Shiga toxin-producing (Stx), enterohaemorrhagic *Escherichia coli* (EHEC) are described as a heterogeneous group of highly pathogenic bacteria with very low infective dose; even 1-100 cells are capable of causing disease (Griffin 1998, Jaeger and Acheson 2000). Diarrheagenic *E. coli* were among the first pathogens for which molecular diagnostic methods were developed.

The purpose of the study was to characterize isolates of EHEC causing diarrhea in Indian children and identify them rapidly by using different microbiological and molecular biology techniques.

Most of the *E. coli* isolates were resistant the antibiotics used in the study. Our study demonstrated that imipenem is an effective antibiotic that can be used in the treatment of EHEC infection since none of our isolates was found to be resistant to this antibiotic. Since ampicillin resistance was shown by significant number of strains, it indicates that a considerable proportion of the resistance might be derived from plasmid-associated β-lactamases which may result from the general acquisition of resistance genes in sensitive bacteria. These results clearly indicate that the incidence of antimicrobial resistance was widespread and probably resulted from either the intensive use of antibiotics or the uncontrolled availability of them.

Alkaline lysis method was adopted for isolation of plasmid DNA from wild of *E. coli* strains. The plasmid DNA profile of these isolates was analyzed which showed the presence of more than one plasmid numbering from 1 to 3.

Conjugation was carried out for the confirmation of transfer of drug resistance of respective wild strains to recipient *E. coli* K12 strain. Our results showed that all the antibiotic resistance markers were transferable and the transfer frequency varied from 10^{-8} to 10^{-7}. Among the transconjugants obtained, all five strains were showing resistance against ampicillin, kanamycin, tetracycline, chloremphenicol and Streptomycin. Our results from conjugation test thus, clearly showed the presence of most of the antibiotic markers on the transferred (mobile) plasmids.

In order to check the presence of antibiotic resistance genes on the plasmids, transformation experiment was performed. *E. coli DH5α* cells were used as transferring hosts. Thus, it was demonstrated that all the antibiotic genetic markers were plasmid borne. All the selected strains were able to grow on Luria Agar plates amended with chloremphenicol, ampicillin, kanamycin, tetracycline and streptomycin. This refers that plasmids of all the selected strains could transfer multi drug resistance to recipient strains.

In this study *eae* gene sequence polymorphism between different EHEC strains was investigated, to investigate molecular evolution. The *eae* gene sequences were aligned with the nucleotide sequences present in the nucleotide database, and we found that all the sequences were showing high level similarity (up to 98 %) with the intimin gene of EHEC and with other variants of *eae* gene of EHEC.

During sequence alignment, phylogram tree was also generated from results of multiple alignments. The phylogram clearly indicated that most of the bacteria have a different origin.

Our results of multiple sequence alignment of nucleotides of all the 16 strains clearly indicated that there is a major divergence between the sequences of *eae* gene fragment of the isolates used in this study. But few of the sequences were very similar. It was seen that all the products of eae gene fragments were showing similar characteristics means they all had a nearly similar molecular weight, amino acid composition, theoretical pI vaules, instability index etc.

Hence in the present study molecular characterization of EHEC was performed, through multiplex PCR and conventional PCR; and detection of these genes indicated various virulence associated potential of the isolates as indicated by the presence of *eae* along with verotoxigenicity (stx_1 and stx_2). Intimin (*eae*) mediates the intimate attachment of the bacteria to the host cell surface and is required for formation of the attaching and effacing lesions (A/E). Intimin has also been reported to be associated with unique form of pathogenic mechanism by secreting a protein by a bacterial cell which is translocated into host cell membrane which acts as receptor Tir (Translocated intimin receptor) for the attachment of the intimin. Hence further characterization of the intimin may help in understanding the pathogenic mechanism and tropism of bacterial cell.