Molecular Characterization of the Indian Isolate of Enteropathogenic Escherichia Coli Diarrhoea

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Diarrhoeal diseases are second only to cardiovascular diseases as a cause of death worldwide (WHO, 1990). In India, diarrhoeal disease is a major public health problem among children. The enteropathogenic *Escherichia coli* (EPEC) have traditionally been identified on the basis of their belonging to certain stereotypes, which have been classed as the EPEC stereotypes. The definition of a strain belonging to an EPEC stereotype is based on it having both the '0' and 'H' antigens that have been established over the years as associated with these EPEC types. Diarrhoeagenic *E.coli* were among the first pathogens for which molecular diagnostic methods were developed. Molecular methods, especially polymerise chain reaction (PCR), are nowadays considered the most reliable techniques for differentiating diarrhoeagenic *E.coli*strains from non-pathogenic members of the stool flora and for distinguishing one *E.coli* pathogen from another (Nataro and Kaper, 1998).

The purpose of this study was to characterize different stereotypes of Indian isolates of EPEC causing diarrhoea in infants and identify them rapidly by using different molecular biology techniques. The clinical samples for this study were collected from the diarrhoeagenic infants admitted to the Paediatrics Department of All India Institute of Medical Sciences (AIIMS), New Delhi. The non-pathogenic *E.coli* isolates which were used as control strains in this study were obtained from the Gene Expression Laboratory, Jamia Millia Islamia, New Delhi.

Indiscriminate use of antimicrobial agents in humans during infections has resulted in the development of resistance amongst bacteria, including *E. coli,* hence these clinical and non-clinical samples were analyzed for their antibiotic resistance pattern. Their antibiograms were determined using the disc diffusion method. Prevalence of antibiotic resistance amongst these *E.coli* isolates was significantly high for ampicillin, chloramphenicol and streptomycin (100%) followed by kanamycin (83.3%) and relatively low for tetracycline (33.3%) however, all of these isolates were sensitive for nalidixic acid.

The plasmid DNA profile of these isolates was analyzed which showed the presence of more than one plasmid in the EPEC strains including the megaplasmid. The non-pathogenic *E.coli* strain was devoid of this megaplasmid but it harbored a plasmid in the molecular weight range of 24 kb. Our results showed that all the antibiotic resistance markers were transferable and the transfer frequency varied from 10-9 to 10-7. An analysis of the plasmid profile of the transconjugants revealed the presence of plasmids similar to that observed in the wild type strains. By transformation assay, it was

demonstrated that all the antibiotic genetic markers were present on the 24 kb plasmid while the megaplasmid was devoid of these genetic markers. These results imply that the mega plasmid does not playa significant role in imparting drug resistance among the EPEC strains.

As far as the conventional culturing methods for analyzing the pathogenic bacteria are concerned, they are time consuming and laborious too. Therefore there is an immediate need for a rapid, reliable and inexpensive detection and typing method required for the fast detection of microbes. DNA based detection methods provide fast and reliable detection of the desired organisms. Among these are the PCR-based techniques that are nowadays considered to be the most reliable and sensitive techniques for differentiating the pathogenic and the non-pathogenic bacterial species. In our study, *E.coli* strains showed 90% polymorphism with different primers used in arbitrary primer PCR (AP-PCR), indicating a divergence of these isolates from each other at DNA level. Hence suggesting these *E. coli* isolates might be different. Similarly, the phylogenetic tree constructed demonstrated the variation between the pathogenic and the non-pathogenic strains, indicating genetic drifting of these strains from each other. However, one pathogenic strain, ZQA5, demonstrated close similarity with the non-pathogenic strain, ZQA6 suggesting the former to be a modem version of the ZQA6 strain by recently acquiring its virulent nature.

In this study emphasis was laid on using a DNA probe which relies on a proven virulence factor for the pathogenesis of EPEC strains rather than a DNA probe which comprises sequences of undetermined importance. Thus *eaeA* gene that codes for a 94 kDa outer membrane protein, intimin responsible for the intimate attachment of the bacterium with the host was amplified by PCR and used as a DNA probe. Our hybridization results clearly demonstrated that all the strains except the control strain, ZQA6, hybridized with it, thereby showing the gene to be present in the EPEC isolates. Hence it might be suggested that the presence of *eaeA* gene in these strains confirm their virulence. Thus it can be concluded that the PCR amplified fragment of the *eaeA* gene can be used as a DNA probe for the fast detection of the epidemiologically important pathogenic strains.

Protein profiling by SDS-PAGE is also a very reliable and reproducible molecular technique that has been used by many workers to type various microorganisms of epidemiological interest. This technique was utilized in this study for differentiating between the pathogenic and the non-pathogenic strains. Intimins are unique outer membrane proteins expressed by enteric bacterial pathogens capable of inducing intestinal attachment and effacement (*A/E)* lesions. In our study, two types of intimins (a and b) were used. The strains belonging to stereotype Ol42:H6, Ol27:H6, O55:H6 and Ol26:H6 reacted with only an intimin but have shown no signal with the J3 intimin. At the same time, stereotype O86:H34 and the non-pathogenic strain, ZQA6 did not hybridize with any of the intimins. Thus confirming that the intimin protein is expressed by the pathogenic strains only and not by the non-pathogenic ones. The absence of signal in the ZQA1 is because this stereotype expresses a non-typable intimin that is not detected by either a or b anti-intimin antibodies. Since intimin is highly immunogenic, it might be an important component to be considered in the development of EPEC vaccines in future.