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Title: "Molecular Screening and Categorization of Extendedspectrum beta-lactamase (ESBL) *in E. coli* isolates from UTI Patients in Delhi."

Abstract

Urinary tract infection (UTI) is generally defined as the occurrence of pathogenic microbes in the urinary tract with associated symptoms. UTI is one of the most common infectious diseases that rank in its prevalence next to pneumonia infections. Women are more prone to UTIs as compare to men, at a ratio of 8:1, and anatomically it is attributed to women's short urethra, and certain behavioral factors such as delay in micturition, obesity, diabetes, vaginal infection, sexual activity, genetic susceptibility, use of mechanical instrumentation like catheterization, and the use of spermicides and diaphragms which promote growth of the periurethral area with coliform bacteria.

Significant part of the work done in clinical microbiology laboratories still hovers around UTI and uropathogenic *Escherichia coli* (UPEC) which is the most predominant causative pathogen for UTIs.

Most of the UTI pathogens have acquired antibiotic resistance over a period of time. There are many types of resistance mechanism operating in these pathogens. One of the most prevalent mechanisms of resistance among Gram-negative bacteria is the production of β-lactamase enzyme.

ESBL is the extended form of β-lactamase it can also provide resistance against third generation cephalosporins. ESBLs are plasmid mediated or chromosomally mediated β-lactamases with broad activity against penicillins, cephalosporins and monobactam. They inactivate β-lactam antibiotic function by breaking amide bond of the β-lactam ring. An infection with ESBL-producing pathogenic bacteria is related to a worse clinical course that entails deferred clinical and microbiological response, longer hospitalizations, higher costs, and higher death toll.

In the light of all these facts and assumptions, we carried out the study and an abstract of the accomplishments of the study are as follows:

A major observation that needs to be emphasized in this study is that most of the isolated strains showed resistance to 3-5 antibiotics which included the first and second generation of cephalosporins. The most effective antimicrobial agents found to be the carbapenems. Phylagenetic analysis of *bla*TEM sequences with reported varients shows that a novel *bla*TEM-1 like sequence. Among the CTX-M gene, *bla*CTX-M-15 variant was found to be the most prevalent and constituted 85% of it.

A novel variant similar to CTX-M-15 was reported in our study. The *bla*CTX-M novel variant identified in *E. coli* ASF 275 isolate was obtain from urine sample collected from 29 year old female.This variant (CTX-M-novel variant) diverged from CTX-M-15 by amino acid substitution A227T.Threonine is bulky amino acid compare to Alanine, this substitution effect protein folding. This is the first report of any CTX-M variant holding Alanine substituted with Threonine at position 227 in active protein. In view of the above, we carried out *in-silico* studies of the novel CTX-M variant to get an insight into the catalytically important residues. Ligand (Cefotaxime) showed a binding energy of -8.0 Kcal/mol with CTX-M novel variant, in comparison to CTX-M-15 and CTX-M-25 showing a binding energy of -7.9 Kcal/mol and -7.3 Kcal/mol respectively suggesting a more stable and effective interaction between our CTX-M and cefotaxime for facilitating better enzyme activity. The novel CTX-M variant was cloned in expression vector (pET28a) and its subsequent expression in *E. coli* BL2l DE3 strain was studies. The expressed CTX-M variant showed high MIC values against cefotaxime in combination with clavulanic acid.