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Topic: **Studies on the expression of acquired multidrug resistant genes - Extended Spectrum β-Lactamases (ESBLs) among environmental isolates of *E. coli* in heavily polluted Delhi stretch of river Yamuna**

In present day world antibiotic resistant is becoming an extremely serious clinical and public health concern. A dramatic increase has been recorded in resistance to third generation cephalosporins, regularly used for treatment of infections caused by members of enterobacteriaceae. Extended Spectrum β-Lactamases (ESBLs) enzymes are known to confer resistance to nearly all β-lactam antibiotics. There is increasing volume of evidence indicating involvement of ESBLs with co-resistance to other classes of antibiotics like carbapenems, aminoglycosides and quinolones as well. Extensive endeavor in development of new treatment options against the ESBL producing organisms has not yielded any fundamental breakthrough. It is therefore imperative to develop strategies to minimize the spread of ESBLs and explore better options for their control. Having stepped outside hospital doors and infiltrated the community, there is an ever increasing list of multidrug resistant bacteria from natural environment especially polluted water bodies.

Taken the scenario into consideration, present study was undertaken to determine the prevalence of ESBLs among *E.coli* isolates of anthropogenically influenced Delhi stretch of river Yamuna, India. An effort was made to unravel the molecular mechanisms contributing enhancement in β-lactamase mediated resistance and co-resistance to other class of antibiotics. Furthermore, impact of environmental factors in resistance co-selection among different bacterial isolates was also studied.

In order to achieve the said objectives, we carried out the study and an abstract of the accomplishments are as follows:

- Delhi stretch of river Yamuna was surveyed and altogether 13 sites were selected downstream of major drains discharging into the river. A total of 227 non-duplicate bacterial isolates were obtained on nutrient agar. Selective isolation using specific media and biochemical tests revealed 75 isolates as putative *E.coli*.
- Heavily influenced by anthropogenic activities, forty ESBL producing *E.coli* isolates obtained were showing high resistance to frequently used antibiotics including third generation cephalosporins (74%), monobactams (55%) and penicillin + inhibitor (40%).
- Co-resistance of β-lactam and non-β-Lactam classes of antibiotics was observed among high proportion of $ESBL^{+}$ isolates. 55% isolates conferred resistance to ciprofloxacin from fluoroquinolone class, generally preferred for treatment of UTI infections.

- ❖ Least resistance was observed for aminoglycoside class of antibiotic. Resistance to multiple antibiotics was also observed in ESBL$^-$ isolates but to a lesser extent.
- ❖ Conferring extended spectrum activity, *bla*TEM gene (*bla*TEM-116) was prevalent among 58% isolates, whereas *bla*SHV gene was found having low prevalence (7.5%).
- ❖ Widespread β-lactamase gene, *bla*CTX-M was present among 68% of isolates harbouring five different variants (*bla*CTX-M-15, -3, -32, 55 and -71) from CTX-M-group-1.
- ❖ Identification of *bla*CTX-M-152 in *Kluyvera georgiana* MRB7 as part of this study is the first report of identification of any CTX-M-group-25 member from India.
- ❖ With small catalytic pocket and no Asp240Gly like substitution associated with increased catalytic efficacy of β-lactamases, CTX-M-152 was exceptionally observed to have high substrate affinity and catalytic efficiency similar to *bla*CTX-M-9.
- ❖ Additionally, this is the first report of any CTX-M variant that has histidine substituted with glutamine at position 26. The genetic relatedness of CTX-M-152 to members of group-25 differ possibly due to Q26H, T154A, G89D, P99S, and D146G substitutions.
- ❖ Higher tolerance to mercuric chloride among ESBL$^+$ isolates was attributed to amplification of *mer*P and *mer*T genes encoding membrane transport proteins and *mer*B encoding organomercurial lyase among 73%, 70% and 43% of isolates respectively.
- ❖ Co-resistance was demonstrated by co-existence of both ESBL (*bla*TEM, *bla*CTX-M) and *mer* operon (*mer*P, *mer*T, *mer*B) genes in seven isolates, and at least one gene of ESBL (*bla*TEM, *bla*CTX-M) and one gene of *mer* operon (*mer*P, *mer*T, *mer*B), simultaneously, in thirty three isolates.
- ❖ High conjugation frequency among multidrug resistant *E.coli* isolates was observed at neutral pH (~7), with maximum transconjugants obtained at a temperature of 40°C under *in vitro* conditions.
- ❖ Addition of mercuric chloride at sub-inhibitory conc. significantly increased the biofilm formation in ESBL$^+$ isolates, whereas cefotaxime alone was not equally effective.
- ❖ In combined toxicity test, chromium (III) oxide at a concentration of 30mg/L was found to have a synergistic effect with amikacin, ciprofloxacin, cefoxitin and imipenem increasing the sensitivity of ESBL$^+$ *E.coli* isolates.

Results of the study will substantially help to frame policies and measures for affirmative action to prevent selection and spread of multi drug resistance. To this, advanced drug resistance surveillance and molecular characterization of ESBL producing isolates on larger scale is required to guide the appropriate and judicious antibiotic use for the control of emergence and spread of resistance.