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“Studies on molecular, structural and functional aspects of CTX-M type β -lactamase”

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Widespread usage of antibiotics has imposed a strong selection pressure for resistance development that has taken a strong grip over the health care system worldwide. Infections caused by Extended spectrum β -lactamase (ESBL) found mostly in gram negative bacteria pushing us back to pre-antibiotic era. ESBLs enzymes are known to confer resistance to nearly all β -lactam antibiotics. The emergence of ESBL producing bacteria is a point of concern for the development of therapies against bacterial infection. ESBLs consist of 3 major genetic groups: TEM, SHV, and CTX-M types. The mechanisms for β -lactam resistance among gram-negative bacteria are due to production of β -lactamases. As ESBL enzymes are plasmid mediated, the genes encoding these enzymes are easily transferable among different bacteria. In India mortality rate caused by the infectious disease 416.75 per 100,000 persons is twice the rate prevailing in the United States at the time of introduction of antibiotics (roughly 200 per 100,000 persons). Water samples for Present study was carried out to evaluate the occurrence of CTX-M type of ESBLs producing bacterial isolates in anthropogenically influenced lake, pond, treatment plant and drain of Delhi-NCR, India. Five different sites were chosen from Delhi-NCR i.e. Ghazipur slaughterhouse, Lodhi garden pond, Hauz Khas lake, Jasola sewage treatment plant and Shaheen Bagh drain.

Findings of the above mention tasks of this study can be drawn as follows:

- A total of 457 isolates were obtained from 5 different selected sites in this study.
- 32, 23, 20, 23 and 43% isolates from Ghazipur slaughterhouse, Lodhi garden pond, Hauz Khas lake, Jasola sewage treatment plant and Shaheen Bagh drain respectively were found to be ESBL producers.
- Among the ESBL producing isolates three genes *bla*CTX-M, *bla*SHV, and *bla*TEM were studied. The prevalence of *bla*CTX-M was found to be 65.21%. *bla*TEM and *bla*SHV gene were prevalent among 41.33% and 17.33% isolates respectively.
- Three variants of both ESBL genes *bla*CTX-M (CTX-M-15, CTX-M-152 and CTX-M-205) and *bla*TEM (*bla*TEM-116, *bla*TEM-1, *bla*TEM-166) were detected among ESBL positive isolates. A total of 55 different variants of *bla*CTX-M and 21 *bla*TEM gene sequences have been submitted to NCBI Genbank.

- Multidrug resistance phenotype observed among clinically important isolates of bacteria such as *Escherichia coli*, *Enterobacter sp.*, *Acinetobacter sp.*, *Citrobacter sp.*, *Klebsiella pneumoniae*, from aforesaid these sites of Delhi-NCR, India is worrisome.
- Co-resistance of β -lactam and non- β -Lactam classes of antibiotics observed among high proportion of ESBL positive bacterial isolates. Highest resistance was observed for cefotaxime (73%). Most of the isolates (62%) exhibited multidrug resistance phenotype.
- The MIC values of antibiotics against tested isolates varied from <2 to ≥ 1024 $\mu\text{g/ml}$ Highest MIC values were observed for the antibiotic cefotaxime, ceftazidime, and trimethoprim against almost all tested isolates.
- The MAR index value for all five selected sites was in the range of 0.2 to 0.53.
- In the present study, predicted CTX-M enzyme models i. e. Model-1, 2 and 3 showed highest homology with CTX-M-15, CTX-M-9, Toho-1 enzyme from PDB database respectively.
- Outcome of the conjugation studies indicates the high possibility of horizontal gene transfer between different bacteria in aquatic environment.
- Model-1 showed maximum stability with drug cefoxitin complex while Model-2 and Model-3 with ceftaroline and cefazoline complex respectively which depicts a more stable and effective interaction for facilitating enzyme activity among all docked cephalosporins.
- Based on docking result, tazobactam is the most effective against targeted enzyme as compared to clavulanate, sulbactam and NXL104.

Some of the new reports of this study include:

- ❖ This is the first study on prevalence and diversity of antibiotic resistance among bacterial isolates obtained from these sampling sites.
- ❖ Occurrence of ESBL genes *bla*TEM, *bla*SHV and *bla*CTX-M from *Exiguobacterium mexicanum* is the first report.
- ❖ Furthermore, *bla*CTX-M gene from environmental isolate of *Aeromonas caviae* has not been reported earlier.

A more comprehensive genetic study of diverse microorganisms and their extra-chromosomal elements that promote dissemination of multiple resistance determinants are required. Improved quality of antimicrobial prescription and proper treatment of sewage before discharge, extensive surveillance of community environment for resistant determinants and resistance bacteria are key to limiting emergence and dissemination of resistance in natural aquatic environment. Further research needs to be aimed at establishing both the exposure risk associated with surface waters specially used for recreational activities and irrigation, and the relative contributions of different types of contamination sources and factors influencing variation in the prevalence of AMR bacteria in surface water.