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## ABSTRACT

Mercury is the 6th most abundant toxic element on earth. Its levels in many parts of the world including India has reached at the verge of exceeding threshold level in soil as well as in water bodies and is still increasing because of agricultural runoff and sludge produced by industries and population centers. Stringent cleanup standards are being directed to protect ecological systems and to protect contamination of natural water resources of mercury pollutants. Basic concept behind any remediation technology is to reduce or eliminate deleterious effects of mercury pollutants in the environment. Investigations carried out for this study were based on three main objectives. The primary objective of this study was to isolate and screen mercury-resistant bacteria and to compare their occurrence and distribution spatially and temporally in polluted water bodies. The hypothesis put forth for this study that bacterial strains capable of mercury resistance can also show tolerance to different antibiotics was also examined. As water bodies experience adverse effects of a variety of toxic chemicals that may play a role in the development of resistance mechanism in mercury-resistant bacteria, investigations were carried out to examine genetic make-up MRB in terms of expression of different genes of mer operon either singly or in combination to deal with toxic mercury. In order to achieve the said objectives, we performed many experiments and the final summary of the accomplishments of the study are as follows:

- In order to screen mercury tolerant isolates, water samples were collected from eight geographically distinct polluted sites along with pristine Dal lake (J&K) India. Samples from Yamuna river and Najafgarh drain were found to have highest bacterial load as compared to Hooghly and least from Dal lake.
- Mercury content in all the samples collected was found variable. As revealed by Cold Vapor Atomic Absorption Spectroscopy (CV-AAS), highest content of mercury i.e., 9.39 µg/lt was present in the samples from Yamuna river, Delhi and Agra, followed by Hooghly river, Kolkatta i.e., 7.66 µg/lt and least beyond detection limit in samples collected from pristine Dal lake.
- Out of 80 isolates, only 18 were found tolerating greater concentration of mercury among which ARY1, ND1, ND3 and ND6 isolates and to some extent *Pseudomonas aeruginosa*

ATCC 9027 were found tolerating 1000 µM concentration of phenylmercuric acetate; while as MIC for the rest of isolates was 100 µM. Isolates ARY1, ND3, ND6 along with ARY4, ARY7, ARR4, ARTK3 and ND5 were found tolerating 1000 µM concentration of mercuric chloride. Only three isolates ARY1 (Yamuna river, Delhi), ND3 along with ND6 (both from Najafgarh drain, Delhi) were tolerating higher (1000µM) concentration of both phenylmercuric acetate and mercuric chloride.

- Growth inhibition study in presence of varied concentration of phenylmercuric acetate revealed that most of our isolates except ND1, ND3 and ND6 showed an extended lag phase that is interpreted as the period during which cells acclimatize to increase in concentration of phenylmercuric acetate. Bacterial metabolism supposed to be diverted in presence of toxic phenylmercuric acetate results in suppressed and delayed exponential phases.
- During studies on antibiotic resistance among the bacteria, it was observed that all the isolates with in a particular group follow almost same pattern of growth inhibition, but vary with isolates of different group. Among screened organisms, isolates ARY1, ARY7 (Yamuna river, Delhi) along with ND1 (Najafgarh drain, Delhi) showed multiple resistances against the tested antimicrobial agents.
- 16S rRNA gene based identification is a gold standard for microbial identification and in inference of deep evolutionary relationships. 16S rRNA gene of all the isolates under study showing mercury tolerance were cloned and sequenced. Based on sequence data analysis, we have successfully assigned correct annotation.
- Out of 18 isolates studied for genes involved in the transport, 13 were found positive for the presence of *mer* P and *mer* T genes. Sequence data analysis revealed more than 90% homology with the other reported sequences of *mer* P and *mer* T gene. Sequences of *mer* P gene were found to be more conserved than *mer* T.
- Despite being positive for *mer* P and *mer* T genes, only 3 out of 18 isolates screened, were found positive for *mer* B gene encoding organomercurial lyase. Successful amplification of *mer* B gene of *mer* operon from ARY1 (*Pseudomonas aeruginosa)* along with ND3 (*Klebsiella* sp.) and ND6 (*Klebsiella pneumonia* sp.) suggest that organomercurial lyase encoded by *mer* B gene might be involved in assigning greater tolerability to phenylmercuric acetate and its transformation. Based on these results, we can infer that most of the isolates were bearing genes of narrow spectrum mercury operon. Phylogram based on sequence data of *mer* B gene revealed close relationship between our isolate ARY1 (*Pseudomonas aeruginosa*) from Yamuna river, Delhi and *Pseudomonas* sp. K-12, the only isolate reported to tolerate highest concentration of mercury.