Abstract

The development and spread of antibiotic-resistant bacteria are now recognised as a key threat to our community, public health and domestic animal. A small number of antibiotics imipenem and meropenem are considered to be the drugs of 'last resort' in the treatment of bacterial infection. Now a day's MBLs producing bacteria easily hydrolyse carbapenem drug-like imipenem and meropenem and bacterial cells become resistant against of these antibiotic groups. In this study, we have investigated the incidence of metallo-β-lactamase (MBL) producing bacteria (specially *bla*$_{NDM}$ producing strains) within abundantly occurring carbapenem-resistant bacterial population in two river water samples, collected in the pre-monsoon (March and April) and post-monsoon (November and December) months of two significant rivers, Mahananda and Karala, bisecting two most populous town, Siliguri and Jalpaiguri respectively, in northern West Bengal, India. Before this study, the presence and significance of MBL producing eubacterial isolates in Mahananda and Karala River remained elusive. MBL-producing bacteria were screened out from imipenem resistant bacteria isolates by the phenotypic (Carba NP test and EDTA inhibition test) and genotypic (multiplex PCR) methods. Overall, 7/1,237 (0.56%) and 8/1,593 (0.50%) MBL -producing bacteria were detected in Mahananda and Karla river water samples respectively. Remarkably, all MBL producing isolates were obtained only from mid-stream (city-based sampling point) sampling point of both the river water samples and conspicuously absent in isolates of up and downstream of both the river. In antimicrobial susceptibility testing, all 15 MBL producing isolates have shown resistance against the 10 different groups of antibiotics and the highest MIC values were observed in all MBL positive isolates against ampicillin molecules. The MDR pattern was detected in all 15 MBL-producing bacterial isolates of both rivers. The metallo-β-lactamase activity shown that the MBLs producing gene appears to be the main reason for carbapenem-resistance in all bacterial isolates. Based on 16S rRNA gene sequences, three different genera among MBL-producers, *Pseudomonas*, *Myroides* (pathogenic organism) and *Acinetobacter* were identified in Mahananda River water samples, and in Karala River water samples, four different genera among MBL-producers, *Pseudomonas*, *Proteus*, *Escherichia* and *Acinetobacter* were detected. Overall, the *Acinetobacter* is the most abundant and common MBL producing genus in both river water samples. PCR products of the MBL-producing

gene were generated and sequenced using primer targeting New Delhi Metallo-β-lactamase (*bla*$_{NDM}$) genes. The complete ORF sequence analysis of *bla*$_{NDM}$ genes showed that the most common MBLs are *bla*$_{NDM-1}$ 10/15 (73.34%) observed in both river water samples and *bla*$_{NDM-7}$ was observed only in the genus, *Escherichia* , of Karala River isolates 3/15 (20%). The result suggests that *bla*$_{NDM-1}$ types of MBLs are common for carbapenem resistance within MBL-producing bacteria present in both the rivers. Class 1 integrons with the frequent presence of *aad*A and *aac*(6´)-Ib gene cassettes in 50 % of NDM-1 bearing isolate is indicative of selective pressures generated out of unregulated use of streptomycin, in agriculture field, owned by the tea cultivators living in locales, drained by these two rivers, in their up and downstream; and amikacin remains as one of the most often prescribed drugs in the highly-crowded government-sponsored 'Sadar' and district hospitals of Siliguri and Jalpaiguri. The isolate, *Pseudomonas* sp. MR 02 was used as a model strain. Whole genome sequence of MR 02 was explored to understand why it was resistant to most of the antibiotic groups. Genome sequence analyses revealed the carriage of 100 distinct genes contributing to antibiotic resistance that made MR 02 a pandrug resistant organism (PDR). Among these 100 genes, 64 genes were identified as an antibiotic-related efflux pump, 14 genes related to OprD porin, 18 genes related to antibiotics hydrolysis and modification, and 4 genes were related to target site modification. Three chromosomally located genes coding for β-lactamases belonging to class A extended-spectrum β-lactamase *bla*$_{PME-1}$, subclass B1 Metallo-β-lactamase *bla*$_{NDM-1}$ and class C β-lactamase *bla*$_{PDC}$ respectively were identified in MR 02 strain. Hence, MR 02 was established as a PDR strain. The antibiotic susceptibility profile of MR 02 correlated with the analyses of antibiotic-resistance genes present in its genome. Another significant attribute of the NDM-producing strain, *Pseudomonas* sp. MR 02, not reported earlier for any PDR or MDR bacterial strain, is its ability to utilize ampicillin molecule as a sole source of carbon, nitrogen and energy source. It was hypothesized that the elevated catalytic efficiency of NDM-1 towards ampicillin leading to very high minimum inhibitory concentration (MIC) might render the host bacterium an opportunity to utilize the drug as a sole source of carbon, nitrogen, and energy. Despite having a 6-aminopenicillanic acid core containing nitrogen in all β-lactam antibiotics, ampicillin possesses a side chain with an additional amino group. From the results derived out of this study, it was revealed that

ampicillin is also being used by MR 02 as a nitrogen source. When MR 02 confronts a new environment where no easily metabolizable carbon and energy source like glucose is available, it confronts a survival challenge and takes an extended lag phase to focus and synthesize enzymes for the utilization of inactivated-ampicillin resource disposed of with the aid of NDM-1. This study has demonstrated that only by the expression of $bla_{\text{NDM-1}}$ in *E. coli* DH5α strain, it was capable of utilizing ampicillin as a carbon source to grow in a minimal medium for β-lactam catabolism. RNA-Seq was used to generate a differential gene expression profile in ampicillin and glucose, grown MR 02 cells and it revealed that the expression of $bla_{\text{NDM-1}}$ was significantly higher [upregulated (log2 fold change 2.00088)]. For ampicillin catabolism to take place, three genetic components are required: (i) New Delhi Metallo-β-lactamase genes (bla_{NDM}) (ii) amidase gene and (iii) PAA pathway-related genes; and absence of any one of the three components would not support bacteria to utilize β-lactam antibiotics as carbon and energy source.