# General Introduction

***"..... For men may come and men may go, but I go on for ever......"***

**-Tennyson.**

The facts of history, vis-à-vis the plight of most mankind today are sufficient evidences that the problems of man and his environment are not problems of the men of individual nations. They are problems of all men and all nations. This is especially true of water. The mobility of water is one of its useful properties, but it also gives rise to serious problems, both practical and scientific, international as well as national.

River is not just a large flowing water body, but always remained in the driving seat of all human civilizations. Since the dawn of civilization, increased number of people and proliferation of their activities have depended on surmounting natural environmental restrictions, including the amount and distribution of water. Water development and water policies always have been important, as is evident from the many physical and administrative measures to control its distribution and use, beginning with the Indus valley civilization and becoming even more complex with the passage of time. Even so, water problems are becoming increasingly critical in many regions even where water is relatively abundant. The reason is that in many regions problems are less apt to relate to water quantity than to its quality. Broadly stated water problems are few but basic: distribution in space (too much or too little); distribution in time (too much in some seasons or years and not enough in others); chemical quality (too high mineralized; lacking in desirable minerals; containing deleterious minerals); and pollution.

If the liquid comprising the natural stream were pure water, the river story would differ greatly from its actuality. But, no river water is pure. The impurities of each stream are characteristic of it and for certain periods of observation, constant. Their chemistry is related to climate and terrain. The water of a very large river, composed as it is of tributary contributions from various sources, represent as nearly as possible the average of its region.

The impurities that occur in natural water fall under three groups. The first group comprised of completely dissolved substances present as separate molecules or ions, dissolved gases, dissolved minerals (salts of Na, K, Ca, Mg, Fe, Al, $NH_4$, Mn), heavy metals (Cu, Pb, Hg, Ag, Zn), and various organic substances (eg. phenols, formaldehyde etc.). The second group consist of colloidal substances of mineral origin such as $SiO_2$, $Al(OH)_3$, $Fe(OH)_3$ etc. The third group consists of suspended matters like clay, silt, sand etc. The natural impurities are derived from atmosphere, catchment areas and the soil. They are in very low amounts and normally do not pollute water and it is potable. But the fact remains that less than 0.01% of Worlds water is fresh water and <1% of that is potable water; the rest is polluted fresh water.

Adequate supply of fresh water is the basic need for all human beings on earth, yet it has been observed that millions of people worldwide are being deprived of this. Freshwater resources all over the world are threatened not only by over exploitation and poor management but also by ecological degradation. Population growth, urbanization, unplanned development, land degradation and lack of infrastructure for waste disposals have serious and adverse impacts on fresh water bodies. All these causes have a cumulative effect leading to the rapid deterioration in water quality in majority of rivers, streams and lakes worldwide.

The pollution of rivers and streams with various contaminants has become one of the most crucial environmental problems since the 20th century. International Conference on Primary Health Care held in Alma Ata in 1978 identified the provision of an adequate supply of safe water as one of the eight components of primary health care. In most countries the principal risks to human health are associated with the consumption of polluted water. Water borne transmission is a highly effective means of spreading infectious agents to a larger portion of the population. An estimated 80% of all human diseases and over one third of deaths in developing countries are caused by the consumption of contaminated water and on average as much as one tenth of each person productive time is sacrificed to water related diseases.

In this perspective, the monitoring of river water quality is gaining importance both nationally and internationally. One of the only global attempts at water quality monitoring has been the UNEPGEMS/WATER program that examined data from 82 major river basins worldwide over a period of a decade and a half (www.gemswater.org). Yet the numbers of monitored watersheds were too sparse and the frequency and the type of measurements were too inconsistent to point a comprehensive picture of global water quality trends.

India is bestowed with many rivers and rivulets, which in turn has supported life of Indians in all possible ways. However, in the present time they are also inviting our attention to the threats they are receiving from their benefactors. Today, in India, the most significant environmental problem and threat to public health in both rural and urban, is inadequate access to clean drinking water and sanitary facilities. Of the urban population, 84.9% had access to clean drinking water in 1993 as compared to 69% in 1985, but for the rural population the figure fell from 82% in 1985 to 78.4% in 1993 (World Resource Institute, 1995) (www.wri.org). According to water demand projections, the domestic and industrial sectors are expected to experience a 3 fold and 2-fold increase, respectively, in water demand by 2025.

India has had environmental legislation dealing with water pollution in the Indian penal code since 1960. However this law and similar laws over the next 100 years would be dealing with only public springs and reservoirs used for the purpose of drinking. The water quality was considered legally as an environmental issue after implementation of 1974 water (Prevention and Control of Pollution) Act. This act along with the environmental pollution act (which followed in 1986), empowered the Central Pollution Control Board to lay down and maintain ambient water standards, to demand information regarding effluent emission, to shut down pollution activities and to prevent new discharges of effluent and sewage. The water quality monitoring of rivers is being done under the national water quality-monitoring programme.

The state of West Bengal is geographically networked with a variety of perennial rivers. Broadly speaking, West Bengal has two natural divisions - the magnificent belt of the Himalayas in the northern part and alluvial plain in the southern part. The sub Himalayan Northern West Bengal has variety of renewable and non-renewable resources. A variety of swift flowing rivers along with their tributaries are flowing through this region from time immemorial. Among which four major snow-fed rivers are Teesta, Torsa, Jaldakha and Mahananda. Of late, this repository of natural wealth has been subjected to a virtual plunder. In consequence, the local ecosystems are fast loosing resilience and regenerative capacity. The ever-increasing influx of population, simultaneously by keeping pace with the changes in the land use pattern, has affected the region in a dynamic way. Such natural effectors are serving as the major causes of environmental hazards of this particular region. Since for centuries, rivers are being used as the dumping grounds for all these types of effluents, this naturally has acted as the sole cause to change the original character of the water. These changed characteristics of the river waters, therefore, demand thorough scientific investigations.

Torsa is the second largest river of North Bengal and also an international river. It traverses four countries - China (Tibet), Bhutan, India and Bangladesh. Torsa is called as Amo-Chu in Bhutan. It rises in Tibet, where it drains the Chumbi valley and enters Bhutan, north of the frontier settlement of Pasha. The river flows rapidly and follows a confined valley between precipitous mountains. She cuts across in a southeasterly direction and passes by the market town of Phuntsholing on the Indo-Bhutan border. In the plains of North Bengal it is familiar as Torsa. As it leaves the foothills of Bhutan and enters undulating Dooars plain in the northern part of West Bengal, it widens into a braided channel. Phuntsholing, the southern terminus of the Indo-Bhutan road, lies on the left bank of the Torsa. To the west of the river, Buxa-dolomites formed striking ridges, which is observable from Phuntsholing. Concentrated urban development and establishment of cement factories in the nearby region must have contributed to the degradation of the water quality of this river. River Torsa and its tributaries are of considerable importance because they drain the Jaldapara Wild Life Sanctuary, as well as the Totopara (where the endangered and anthropologically high valued Toto lives). Torsa flows through human settlements, tea gardens and agricultural lands. As a consequence of pest control practices in the tea gardens, use of nitrogen fertilizers in the agricultural fields and several point and nonpoint sources of sewage disposals, the hazards of persistent pollutants might have increased in the river water. These might have caused serious health problems and there are reports on several outbreak of water borne diseases in this region. It was therefore important to know whether the quality of the water remained satisfactory or not, over the entire length of the river in the Indian province. It was also pertinent to know if the river water is suitable even for drinking purposes by direct surface intake, after a simple clarification, filtration and chlorination. Water quality monitoring coupled with hydrological parameters of rivers of North Bengal has generally been overlooked for various reasons. Those are mainly resource and manpower constraints, institutional inertia and public apathy due to lack of awareness. Water quality directly influences many issues in this part of West Bengal. Human health, agriculture, aquaculture, industry and tourism, all are vital to regional economy as well as national development. So a comprehensive monitoring and water quality control strategy is needed to serve an explicit purpose, be it for environmental impact assessment, project planning and design, project evaluation, or for regulatory requirements.

"Water quality" is not to be defined objectively. It should be defined socially, as to the desired use of water. Water quality monitoring has evolved along two different lines. One of which is a river basin approach. Another approach is an integration of chemical and biological parameters to measure condition. One potential problem with river basin level approaches is that such basins often cross-state and national boundaries necessitating the need for a high level of governmental and intergovernmental coordination and monitoring activities.

Monitoring of the chemical quality is usually undertaken in two different directions, one includes the routine monitoring of known problem substances while the other includes periodic quality assessment. The later type of monitoring is either routine or non-routine assessment of water quality done on a relatively infrequent basis (annual or greater). Such assessments are likely to include a wider analytical range. They are used to provide regular comprehensive assessments of water quality. It also assists in long-term water source and supply management, and long-term trend analysis.

In certain instances, monitoring of microbiological quality of water is much more important than monitoring of chemical quality and chemical testing. Because health hazards caused by chemicals are chronic rather acute. Changes in water chemistry tend to be long term unless a specific pollution event has occurred. On the other hand, microbiological monitoring is a practical method to determine the potential health risk of water exposure.

Natural waters are rich in bacteria, algae, protozoa, worms and other organisms. The greater the amounts of organic nutrients in water, the faster the biological contamination of water. Bacteria are the most frequently occurred microorganisms. They take an active part in the formation of all aquatic populations. The composition of the micro flora or fauna of a stream or a river serves as a good indicator to determine the extent of pollution. Along with their indicative property, such natural microbial population, in turn, function as the vectors, causing rapid and widespread dissemination of the water borne diseases leading to high rates of morbidity and mortality.

Since their first isolation from feces in the late 19$^{th}$ century the coliform group of bacteria has been used as an indicator of the bacteriological safety of water (APHA, 1989). Total coliforms as a general group are not particularly useful in terms of estimating human health risks because they can also be found in soil and plants naturally. The fecal coliform group comprises the genera *Escherichia* and to a lesser extent, *Klebsiella* and *Enterobacter*. High levels of fecal indicator bacteria in rivers and streams can indicate the possible presence of pathogenic (disease causing) microorganisms. Cholera, typhoid fever, bacterial dysentery, infectious hepatitis and cryptosporidiosis are some of the well known waterborne diseases that spread through water contaminated with fecal matter. However, in many fresh water systems, fecal bacteria are of little numerical significance despite the fact that they are discharged into almost all inland waters.

A number of bacterial enteropathogens, namely *Campylobacter jejuni/coli, Salmonella, Shigella, Plesiomonas, Aeromonas, Vibrio cholerae* and *Escherichia coli,* were isolated from river water sources (Obi *et al.* 2002). These enteric bacterial pathogens are variously incriminated in cases of diarrhoea, which accounts for a substantial degree of morbidity and mortality in different age groups world wide (Black *et al.* 1993, Nath *et al.* 1993, Prado and O'Ryan 1994, Obi *et al.* 1997, 1998, El-Sheikh and El Assouli 2001). Isolation of pathogens from water sources connotes a serious public health risk for consumers. To further compound this problem, enteric pathogens have been widely reported to demonstrate resistance to several antibiotics (Cooke 1976, Kelch and Lee 1978, Hoge *et al.* 1998, Obi *et al.* 1998, Boon and Cattanach 1999, McArthur and Tuckfield 2000, Engberg *et al.* 2001, Ash *et al.* 2002, Lin *et al.* 2004). For example, in 1984, 82% of *Campylobacter* strains from Lagos, Nigeria were sensitive to erythromycin and 10 years later 20.8% were sensitive (Coker and Adefoso 1994). In Thailand ciprofloxacin resistance among *Campylobacter* species increased from 0% before 1991 to 84% in 1995 (Hoge *et al.* 1998). Strains of *Salmonella typhi* with multiple resistances to chloramphenicol, ampicillin and trimethoprim have led to several outbreaks (Rowe *et al.* 1997). Presence of antibiotics and the bacteria resistant to them in various environmental compartments like surface water, ground water, soils etc. may pose a serious threat to public health in that more and more infections may no longer be treatable with known antibiotics. A recent study (Ash *et al.* 2002) showed that several rivers in the United States of America have become a major reservoir for antibiotic resistant microbes.

Antibiotic pollution has been a growing concern as it has the potential for several human and environmental health impacts. The source from where the antibiotic resistant microorganisms are originating is of less importance. But it is alarming that gradually the rivers are becoming reservoirs of antibiotic resistant bacterial population. As rivers are one of the major sources of water, directly or indirectly, for human and animal consumption, this pollution may contribute to the maintenance and even spread of bacterial antibiotic resistance. Since these antibiotic resistant bacteria are significant environmental contaminants, calls have been made for antibiotic resistance to be considered when establishing bacteriological water quality criteria. The results of the studies that have been carried out on the different rivers all over the world, deduce that monitoring of river waters on the ground of microbiological analysis by analyzing the antimicrobial resistance pattern of the native bacterial population is gaining importance day by day. The relevance of information obtained on the resistance of bacteria to antibiotics is to appreciate the magnitude of the problem and establish baseline for taking action. The survey of antibiotic resistances in the microbial flora of fresh waters allows detection of hidden uses that contribute to the increase of bacterial resistances and thus limit the efficacy of these drugs in the treatment of human and animal infections. Simultaneously, monitoring resistance patterns in commensal bacteria could also provide valuable clues about non-antibiotic selection pressures.

The study of the phenomenon of antibiotic resistance is an active area of investigation that covers many aspects of gene transfer mechanism, including the biology of plasmids. Thus, there is interest in, not only the biochemical mechanism by which the determinants express their resistance, but also in the distribution, origin and dissemination of resistance mechanisms. Plasmid mediated resistance to antibiotic was discovered in Japan because of the unexpected appearance of multiple drug resistance during an outbreak of bacillary dysentery. Ever since this time, unexpected appearance of a new or unusual drug resistance marker or unusual pattern of multiple drug resistance has been a clue that plasmids might be involved as carriers of the resistance genes. Most studies on genetic recombination in bacteria have been conducted *in vitro* and there are few data showing that gene transfer occurs *in situ*. Antibiotic resistant bacteria containing conjugative R plasmids have been isolated from sewage-impacted waters in the US (eg. in Hudson river, the New York Bight and in Chesapeake Bay). Many of these strains contained plasmids that conferred resistance not only to antibiotics but also to heavy metals and to other antibacterial agents such as the algal product, chlorellin. It has been suggested by several authors that the selective pressure exerted upon the bacterial flora of animals by antibiotics, gives rise to large populations of resistant microorganisms. The organisms are then postulated to enter the human population either through agricultural practice or via the food chain. Once in contact with man, the resistant bacteria could presumably cause disease directly or transfer their resistance to organisms more pathogenic for humans.

The study relating to the distribution of antibiotic resistance bacteria in river water is not only important, at the same time it is also very much essential to identify the nature of the elements responsible for the development and spread of antimicrobial resistance to far and wide. In the event, that antibiotic resistance is spread from nonpathogenic to pathogenic bacteria, epidemics may also result. This spread represents an elegant, if not desirable, example of molecular evolution over a very short time scale. Recent studies have demonstrated that the majority of these multiple antimicrobial resistant phenotypes are obtained by the acquisition of external genes that may provide resistance to entire class of antimicrobials. A number of these resistance genes have been associated with various gene transfer elements namely plasmids, transposons, integrons and so on, which take a very active part in the development of multi-drug resistance and rapid dissemination of resistance genes among different bacterial genera and species. Loss of efficacy through the emergence and transfer of bacterial antibiotic resistance is an increasing reality. Bacteria have been observed to transfer resistance in laboratory settings as well as in the natural environment. In this context, several authors have emphasized the need to review water quality standards as they relate to the spread of antibiotic resistance genes in water borne bacteria carrying transmissible R factors. Gene transfer elements can- and often do- hunt as a pack, by interacting with each other in a variety of ways that enhance their collective ability to transfer resistance genes. This interactive capacity needs to be taken into account when considering the potential for horizontal transfer of resistance genes. Although the acquisition of new resistance genes is an important factor in the increasing incidence of resistant strains, it is only part of the resistance story.

A critical but often under appreciated feature of resistance gene transfer elements is their stability. The widespread existence of plasmids in natural isolates and their apparent stability, even where antibiotics are not present argue against the widely held belief that plasmids and other gene transfer elements are readily lost in the absence of antibiotics. Moreover the abundance of antibiotic resistant strains in environmental settings where bacteria presumably do not come into contact with antibiotics suggests that resistance genes can also be stably maintained in the absence of antibiotic selection. Much is known about the fact that plasmids carrying multiple resistance genes can be held in bacterial strains by selection for any one of the resistance genes on the plasmid but remarkably little is known about the reasons for stability of gene transfer elements and resistance genes in the absence of any known selection pressure.

Horizontal gene transfer event permits movements of alleles among bacterial lineages, increasing the opportunities for the spread of antibiotic resistance. Although the actual contribution and consequences of horizontal gene transfer remain highly debated for a long time, its importance is immense as it leads to the "evolution in quantum leaps" (Jain *et al.* 2003). During bacterial evolution, the ability of bacteria to exploit new environments and to respond new selective pressures can often be more readily explained by the acquisition of new genes by horizontal transfer rather than by sequential modification of gene function by the accumulation of point mutations. Especially conjugal transfer itself can contribute to stable maintenance of antibiotic resistance genes in a bacterial population by continually reseeding the members of a population that have lost a resistance gene transfer element.

Keeping all these facts in mind, the assessment of the fluidity of the antibiotic resistance genes in the aquatic environment of Torsa river was done by thorough analysis of antimicrobial resistance patterns of the copiotrophic bacterial flora followed by investigating the elements and the mechanisms responsible for the development as well as spread of the antimicrobial resistance genes. The major objectives that had been set forth were:

1. To provide detailed descriptive information about the antibiotic resistances of culturable copiotrophic bacteria isolated from water samples of river Torsa.
2. To explore whether antibiotic resistance patterns among isolates from different sampling sites and seasons vary in a systematic manner.
3. To understand the molecular mechanism of spread and persistence of easy to get and hard to loose antibiotic resistance genes.
4. To explore the molecular diversity of antibiotic resistance gene cassettes.

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