Chapter 1

Antibiotic resistance patterns of culturable copiotrophic bacteria from river Torsa

1.1 Introduction

The antibiotic era began with the discovery of the first three significant antibiotics-tyrothricin, penicillin and actinomycin, in 1939 and 1940. Within the first 18 years of the antibiotic era, about 30 antimicrobial agents had come into use (Swartz 2000). Since their discovery, the use of manufactured antibiotics to control diseases has revolutionized medicine. It has also greatly reduced the threat of many once fatal illnesses. The use of these wonder drugs, combined with improvements in sanitation, housing and nutrition and the advent of widespread immunization programme, has led to a dramatic drop in deaths from diseases that were previously widespread, untreatable and frequently fatal. By helping to bring many infectious diseases under control, these drugs have also contributed to the major gains in life expectancy experienced during the latter part of the last century. These gains are now seriously jeopardized by another recent development: the emergence and spread of resistant microbes. Antibiotic resistance has been called one of the world's most pressing public health problems as it creates complications due to the propensity to distribute multiple antimicrobial resistance genes to susceptible bacterial genera and species.

1.1.1 Antimicrobial resistance in the environment

The bacterial resistance to multiple antibiotics characterizes the present decade. In a report by the UK House of Lords, it is stated: "Resistance to antibiotics and other anti-infective agents constitutes a major threat to public health and ought to be recognized as such more widely than it is at present"(House of Lords) (Kummerer 2004). The percent occurrence of multiple antibiotic resistant bacteria (MAR) in different environmental compartments such as wastewater, surface water, ground water, sediments and soils, has been a growing concern. Resistance genes as well as resistant bacteria in the environment are increasingly seen as an ecological problem (Davison 1999). It has been evidenced that the selective pressure exerted following the widespread use and misuse of antibiotics in both medical and agricultural fields does select those bacterial strains possessing antibiotic resistance genes. These resistant bacteria, which develop, find their way into lakes and rivers. The potential for antibiotic contamination is just one part of the larger problem, an issue with both medical and environmental components.

1.1.1.1 Drinking water

Antibiotic resistant bacteria were detected in drinking water as early as the 1980s (Armstrong *et al.* 1981) and later in the 1990s (Kolwzan *et al.* 1991). These authors found the occurrence of resistant bacteria within the distribution network of drinking water supply systems. It was reported that the percentage of multiple antibiotic resistant (MAR) bacteria was significantly greater among isolates from distribution water samples than that of bacteria in corresponding untreated source waters (Armstrong *et al.* 1981). The study conducted by Diab *et al.* 2000 has proved the presence of antibiotic resistant gram-negative bacteria in several drinking water samples in Ismalia city of Pakistan. In agreement with these data, increased resistance rates were also detected in the drinking water from different sampling points in another study by Schwartz *et al.* (2003).

1.1.1.2 Ground water

Antibiotics are rarely found in ground water (Kummerer 2004). The presence of antibiotic resistant bacteria in ground water has been documented by several authors (McKeon *et al.* 1995). Sacher and coworkers (2001) analyzed 105 ground water wells in Baden-Wuettemberg, Germany. Among 60 pharmaceuticals tested, erythromycin-HO and sulfamethoxazole, which were the only antibiotics out of eight compounds, were detected in at least three ground water samples. Though microbial contamination of ground water sources, revealing the presence of coliforms above the permissible limits, has been recorded by Indian workers (Box: 1.1), no attempts have been made to assess the antibiotic resistance profile of the isolates.

Box : 1.1 Investigation on microbiological status of ground water in India: Classified Abstracts

Dayal, G.1992. Groundwater qualities of rural and urban settlements at Agra. J. Nature. Conserv. **4** : 89-93

A detailed investigation was carried out to ascertain the degree of contamination of groundwater in Agra, both urban and rural areas. The investigations indicated a high degree of pollution in ground waters of Agra city. Though much of the variables were within the standard limit of potable water, a few heavy metals recorded a concentration much beyond the permissible limits set by the WHO (1984). The water sources around septic tanks and sewage channels showed a high contamination of coliforms.

Somasekhara, R. K., R. L. Venkateswara., D. Padmavathy, and C. Rambabu, 1992. Groundwater quality in Challapalli Mandalam. Indian. J. Environ. Prot. **12** : 341-347.

Physicochemical and bacterial parameters of 23 bore wells and dug wells of 23 villages of Challapalli Mandal were monitored. The quality of well waters was assessed by comparing with existing standards for important parameters. Correlation coefficients among various water quality parameters were determined. It was found that there is high incidence of fluoride.

Raja Sekhar, C. R., C. Vasudeva Reddy, and B. Kotaiah .1994. Ground water pollution from unsewered sanitation-a case study in Tirupati. Indian. J. Environ. Prot. **14** : 845-847.

The pollution potential of septic tank effluents and their impact on ground water quality is assessed in an unsewered area of Tirupati. The results indicate that the septic tank effluents contain carbonaceous and nitrogenous matters in addition to phosphorous and high bacterial population. The ground water quality analysis data indicates that there is wide spread variations in the ground water.

Sharma, S, and R. Mathur. 1994. Bacteriological quality of groundwater in Gwalior. Indian. J. Environ. Prot. **14** : 905-907.

The study was carried out on the ground waters sources adjacent to the Swarna Rekha Sewage channel in Gwalior for their microbiological quality. The seasonal survey of 51 potable raw water sources revealed that the hand pumps and the bore wells are comparatively safer sources as compared to the dug wells. Unhygienic practices of the population and unsanitary conditions in the area are the reasons for poor microbial quality in the dug wells.

Narain Rai, J. P, and H. C. Sharma 1995. Bacterial contamination of ground water in rural areas of north west Uttar Pradesh. Indian. J. Environ. Hlth. **37** : 37-41.

Total aerobic heterotrophic bacteria (THB). total coliforms (TC), fecal coliforms (FC) and *Escherichia coli* Type-I (ECI) were estimated in fifteen well water samples collected from rural areas of Bareilly and Nainital districts. Maximum THB, TC, FC and ECI were 28,000/mL, 4460, 1480 and 305 per 100 mL of water respectively while few samples were free from ECI. However, the presence of FC and ECI revealed the unsanitary conditions of the wells.

Mitra, A, and S. K. Gupta, 1997. Assessment of groundwater quality from sewage fed farming area of east Calcutta. Indian. J. Environ. Prot. **17** : 447-447.

Groundwater of shallow and deep tube wells were collected from raw sewage irrigated farm areas of eastern fringe of the Calcutta city. At all the locations groundwater have been contaminated due to presence of high amounts of calcium, magnesium, sodium, chloride and phenolic compound. Heavy metals, like iron and manganese were also present at toxic level. Groundwater from shallow aquifer contained total and fecal coliform. Irrespective of depth the groundwater from all the locations are unsafe for drinking purpose.

1.1.1.3 Surface water

Presence of antibiotic resistant bacteria in the aquatic environment has been studied in different parts of the world. A detailed descriptive information about the antibiotic resistances of gram-negative bacteria isolated from four tributaries which enter Tillamook bay, Oregon and the bay itself, has been provided by Kelch and Lee (1978). They have also explored the correlation between the antibiotic resistances patterns exhibited by different genera involved in the study. In another study, the distribution of resistance to antimicrobial drugs among fecal coliforms in sewage, surface waters and sea water was investigated by paying attention to the effect of the species composition of the sample on the incidence of resistance and resistance patterns (Niemi *et al.* 1983). Several others have demonstrated the wide spread occurrence of such organisms in many rivers and streams (Jones 1986, Sokari *et al.* 1988, Magee *et al.* 1991, Ogan *et al.* 1993, Leff *et al.* 1993). Polluted water samples collected from the River Tigris in the vicinity of a raw sewage outfall were examined for the incidence of antibiotic resistance among coliform bacteria on three occasions during 1983. The result of the said study revealed high incidence of antibiotic resistant bacteria in natural waters that could be related to the widespread use of antibiotics in that locality (Al-Jebouri *et al.*1985). Boon *et al.* (1999) have studied the antibiotic resistance of native and faecal bacteria isolated from rivers, reservoirs and sewage treatment facilities in Victoria, southeastern Australia. The occurrence of several representatives from the main group of antibiotics in wastewater treatment plant effluents and in river water was investigated by Hirsch *et al.* (1999). They described the analysis of various water samples for 18 antibiotic substances, representing macrolid antibiotics, sulfonamides, penicillins and tetracyclines. The study conducted by McArthur and Tuckfield (2000) have demonstrated the spatial distribution of antibiotic resistance to streptomycin and kanamycin in natural bacterial communities of two streams. The proportion of resistant bacteria was substantially higher ($P < 0.05$) in the midreaches of an industrially perturbed stream but no such pattern was apparent in an undisturbed reference stream. The results of the said study implied that heavy metal pollution might contribute to increased antibiotic resistance through indirect selection. Goni-Urizza *et al.* (2000) have found a correlation between resistant bacteria in rivers and urban output.

According to a report (1999-2000) of the toxic substances hydrology program at the U. S. Geological Survey (USGS), antibiotics were found to be present in many fresh water sources throughout the United States. The scientists of USGS have detected at least one antibiotic in nearly 50% of water samples collected across 30 different states between 1999 and 2000. Four or five different antibiotic residues, out of 22 antibiotics assayed, were present in 139 water samples collected from different streams and rivers. The most frequently detected antibiotics were erythromycin-H20 (22%), lincomycin (19%), trimethoprim (27%) and sulfamethoxazole (19%). The other nine antibiotics detected were: tetracycline, chlortetracycline, oxytetracycline, ciprofloxacin, norfloxacin, roxithromycin, sulfadimethoxine, sulfamethazine and sulfamethizole (Kolpin *et al.* 2002). It has been pointed out that antibiotics used in livestock production have made their way,

via animal waste products, into the nations waterways. Studies conducted on 16 U. S. rivers revealed that rivers have started to turn into the reservoirs of antibiotic resistance genes (Ash *et al.* 2002).

Another study on Mhlathuze River (Lin *et al.* 2004) has shown that the river has become a medium for the spread of antibiotic resistance genes as well as the reservoir of resistance genes. A study on an Indian River Mahananda, has also revealed the abundance of multiple antibiotic resistant (MAR) bacteria (Mukherjee *et al.* 2005). The occurrence of strains that are resistant to oxolinic acid, oxytetracycline, sulfamethoxazole-trimethoprim and nitrofurantoin among heterotrophic bacteria, including human and fish pathogens, in two fresh water eel farms has been reported (Alcaide *et al.* 2005). High levels of single and multiple-drug-resistant bacteria were detected, although sampling events were not correlated with clinical outbreaks and drug therapy. Antimicrobial resistance was also found to occur in marine and estuarine bacteria (Cohen *et al.* 1986, Barkay *et al.* 1995). Microbiological analyses of coastal waters polluted with sewage has revealed the presence of gentamicin resistance genes in members of Enterobacteriaceae, *Acinetobacter* spp., *Pseudomonas* spp., as well as in phylogenetically distant bacterial members of alpha and beta proteobacteria. (Heuer *et al.* 2002).

1.1.2. River water quality monitoring in the light of bacterial antibiotic resistance

For centuries, rivers have been used as the dumping grounds for the sewage of urban effluents, agricultural wastes, industrial wastes that contain substances varying from simple nutrients to highly toxic chemicals (including heavy metals). The obvious consequence therefore would be that the river water received different types of chemicals, organic and inorganic compounds as it flowed through human settlements. These dissolved compounds brought about major changes in river water quality by inducing quantitative variation in certain minerals. In addition to heavy metals, the contamination of antimicrobial agents in river water bodies has become a major threat to public health. The presence of antibiotic residues and the occurrence of bacteria resistant to them in environment are rapidly changing the nature of commensal and nonclinical bacterial flora. Moreover, investigations on antimicrobial resistance of river microflora have led to a new dimension in water pollution studies. 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. In this perspective calls have been made for antibiotic resistance to be considered when establishing bacteriological water quality criteria (Grabow *et al.* 1974).

1.1.3. Review of water quality monitoring programs in India

Water quality monitoring of different water sources in India was largely restricted to physico-chemical analysis (summaries have been presented in Box: 1.2). Not withstanding the fact that measurement of physico-chemical characteristics is an easy way to examine the changes in water quality, but monitoring of microbiological quality of water is much more relevant in the Indian context. It is a practical method to determine the potential health risk associated with water exposure. Microbiological monitoring of different

rivers of India included mainly the determination of total and fecal coliform counts (MPN determination) (Box: 1.2). It leaves behind a paucity of information regarding antibiotic susceptibilities of native bacterial population of Indian rivers. This gap demanded a thorough study of river water bacteria in the light of antibiotic resistance profile (antibiogram) determination.

Box 1.2 Classified abstracts on water quality monitoring studies in India

A.Physico-chemical monitoring of river water

Khan Asif, A., N .Haque., A Siddiqui Intisar, and K. A Narayanan 1994. Comprehensive study on water quality parameters in the river Ganga between Narora and Kannauj, UP. Physico chemical characteristics. J. Freshwater. Bio. **6** : 295-304.

Paper presents a seasonal profile of the physicochemical and biological parameters over a period from October 1987 to March 1990 in the selected stretch of river Ganga. The physicochemical characteristics did not show marked change over the period of study. The biological features however represented no definite pattern. The utility of various parameters as water quality indices has been discussed.

Murugesan, A. G., K. M. S. A Abdul Hameed, and N. Sukumaran. 1994. Water quality profile of the perennial river Tampraparani. Indian. J. Environ. Prot. **14** : 567-572 .

River Tampraparni is the principal water source in Tirunelveli and Chidambaranar districts of southern Tamilnadu. Paper deals with monthly estimations of water quality parameters of this fluvial ecosystem with special reference to the aspects of pollution at various stations for one year. The result of the base flow quality analysis delineates variation in several physicochemical characteristics. The level of pollution is discussed with possible reasons.

Ramana Murthy, G. V., S. Venkata Mohan., P. Harishchandra, and J. Karthikeyan. 1994. A preliminary study on water quality of river Tungabhadra at Kurnool town. Indian J. Environ. Prot. **14** : 604-607.

Physicochemical characteristics of Tungabhadra river water over a stretch of 8 km near Kurnool town was studied for a period of 4 months to assess the suitability of river water for public consumption. Except at one sampling station, the water qualities of the river examined were within the permissible limit for human consumption. WQI calculated suggest treatment of river water before supplying to public.

Sharma. D., G. Chetri., J .Kalita, and A Dutta. 1994. Pollution status of the Bharalu river with special reference to physico chemical parameters. J. Freshwater. Bio. **6** : 209-213.

Paper deals with studies on the physicochemical parameters of Bharalu river water flowing through Guwahati city. The results showed high pollution status with values beyond the permissible limits DO (1.0 mg/l), Alkalinity (430 mg/l), BOD (12.2 mg/l) and COD (62.0 mg/l) etc. It has been observed that the water of Bharalu iS highly deteriorated due to various types of industrial effluents, domestic.

Srivastava, A. K, and D. K. Sinha. 1994. Water quality index for river Sai at Rae Bareli for the premonsoon period and after the onset of monsoon. Indian. J. Environ. Prot. **14** : 340-345.

Water quality index (WQI) for river Sai water at Rae Bareli at 10 different sites for the premonsoon period as well as after the onset of monsoon has been calculated to evaluate the water quality. Sixteen water quality physicochemical parameters were selected to calculate WQI. Values of WQI have been

found to be very high as compared to drinking water standard. It is suggested that discharge of wastewater and effluents play important role in determining the quality of water of river Sai. The water quality shows improvement after the onset of monsoon.

Mitra, A. K. 1995. Water quality of some tributaries of Mahanadi. Indian. J. Environ. Hlth. **37** : 26-36

Samples at five stations in streams Seonath, Jonk and Hasdeo, tributaries of Mahanadi river were analyzed at monthly intervals and the data presented. The samples were mostly alkaline, low in solute content and contained calcium, sodium and magnesium as major cations, and bicarbonate, sulphate, chloride as the major anions.

Chetana Suvarna, A, and R. K. Somasekhar. 1997. Ecological study on the riverine ecosystem of Karnataka. I. Physico- chemical Characteristics of river Cauvery. J. Env. Polln. **4** : 57-63.

The physico-chemical characteristics of the river water at three stations stretched over a distance of 20 km were studied at monthly intervals. The dissolved constituents fluctuated temporally and decreased with high flow. Turbidity and pH along with phosphates showed an increase during periods of high flow. The interrelationships between various physico- chemical characteristics are elucidated which assist in understanding the nature of intricate interactions occurring in this ecosystem.

Murthi Krishna, and S. G. Bharati. 1997 . A study on concentration of chloride of the river Kali near Dandeli, Karnataka (India). J. Env. Polln. **4** : 9-15.

Study reveals that chloride increased with the pollution load due to domestic and industrial wastes. The increase in chloride is accompanied by an increase in ammonical nitrogen and organic matter at all the sampling stations. The values of chloride were low during winter and high during monsoon. Further, the relationship of chloride with pH, major cations and anions are also discussed.

Nair. J, and S. Ganapathi 1997. Water quality of the Bhadar river basin. Indian. J. Environ. Hlth. **39** : 197-206.

EC and SAR values were determined for the surface and subsurface water of the Bhader river basin, Gujarat during pre-monsoon and two, three postmonsoon seasons. The analytical results show erratic EC and SAR values from Atkot to Navibandar, the variation mainly due to tidal ingression. The point source effluents from the dyeing and printing units show higher values, which decrease with river water dilution.

Shinde, R. S., D. G.Thorat., P.S. Gunjal, and S. R. Kuchekar. 1997. Studies on water quality of river Godavari at Nasik, Maharastra state India. J. Aquatih. Bio. **12** : 85-86.

Water samples collected from sampling stations along the stretch of river passing though Nasik city, were analyzed for a number of water quality parameters. The results reveal that most of the physic-chemical parameters were in permissible limits as recommended by ISI and in general the water is suitable for human consumption after disinfections.

Jain Praveen., S. Telang, and J. A. Khan 1998. Pollution status of Parbati river, Sehore. Eco. Env. Conerv. **4** : 71-72.

Attempt has been made to evaluate water quality of Parbati river flowing through Sehore for a period of 4 months to assess the suitability of dam water for irrigation use. The parameters observed for this study were electric conductance, percent sodium and sodium absorption ratio. These observations confirm that the dam water is suitable for irrigation.

Prasad, V. K, and R. N. Trivedi 1998. Water quality of the river basin. Int. J. Mendel. **15** : 39-40.

EC and SAR values were determined for the surface and subsurface water of the Ganga river basin, Bihar during three premonsoon and two postmonsoon seasons. The analytical results show erratic EC and SAR values from every station. The point source effluents from the dyeing and printing units show higher values, which decrease with river water dilution. However, when used for irrigation, it may affect the soil, crops, human life and the cattle. The utility of basin water for irrigation has been discussed.

Hussain, M. F, and I. Ahmad. 2002. Variability in physico-chemical parameters of Pachin river (Itanagar). Indian. J. Environ. Hlth. **44** : 329-336.

The concentration of water quality parameters in river and heavy metals in the bed sediment were measured for Pachin river for the three major flow periods. The variability in the physico-chemical parameters for different flow periods may be assigned to dilution of river water by direct runoff, human activities and organic load. The correlation study of physico-chemical parameters shows that their source of entering the river system is the same whether it may be a natural or anthropogenic or both.

Kumar Adarsh, and M. Shukla. 2002. Water Quality Index (WQI) of river Sai water at Raebareli city, U.P. J. Ecophysio. Occupl. Hlth. **2** : 163-172.

Water Quality Index (WQI) of River Sai water at six sampling stations at Raebareli city in a stretch of about 20 kms has been calculated to evaluate the water quality. Nine water quality physico-chemical parameters were selected. Values of WQI have been found to be very high as compared to drinking water standard and the river was found to be severely polluted.

Kumar Neeraj, and R .C. Sharma. 2002. Studies on the self-purification and allowable BOD load in river Krishni. Aquacult. **3** : 215-218.

The self-purification capacity of river Krishni has been calculated on the basis of dissolved oxygen and biochemical oxygen demand in the different stretch of the river at different sampling points. Along with this, allowable BOD load to be discharge in different flow of water have also been calculated.

Pathani, S. S., K. K Upadhyay, and S. K. Joshi. 2002. Some physico-chemical parameters and primary productivity of river west Ramganga (Uttaranchal). Himalayan. J. Env. Zoo. **16** : 151-158.

Paper describes physico-chemical characteristics and primary productivity of river west Ramganga at two stations Chakuhatiya and Masi Almora, Uttaranchal. DO is higher in the month of October and minimum in the month of May. Free CO_2 has been recorded higher in rainy and summer season due to high percentage of organic compounds and absence of free CO_2 in winter season. The productivity values are recorded maximum in summer and minimum in rainy season (July) due to low transparency and high velocity of the water current.

Gopalswami P.M., P. E. Kumar, and A. R. Kulandaivelu. 2003. Study on the quality of water in the Bhavani river. Asian. J. Chem. **15**: 306-310.

The Bhavani river water is being highly polluted by letting out industrial effluents, industrial wastewater, agricultural run off and sewage into the stream. The presence of inorganic ions such as hexavalent chromium, sulphate ions, etc., and biological waste has contributed to the pollution of the river water. As a result water borne diseases have become common in this area and the raw water cannot be used as such for industrial purposes. The Bhavani River water should be treated properly and disinfected before being supplied for industrial purposes and human consumption.

B. Investigation on bacteriology of river water:

Pathak, S. P., S. Kumar., P. W. Ramteke., R. C. Murthy., K. P. Singh., J. W. Bhattacharjee, and P. K. Ray. 1992. Riverine pollution in some northern and north eastern states in India. Environ. Monit. Assessment. **22** : 227-236.

Water samples from 30 rivers in northern and northeastern hilly states of India were analyzed for bacteriological and physicochemical parameters along with metals and pesticide residues. It was found that 34% of samples had > 50 coliforms/ 100 ml. while 24% of samples demonstrated > 50 thermo tolerant (fecal) coliforms/100 ml. Among the metals, iron was found to be above maximum permissible limits in the rivers of all the states, while manganese was found to be above the maximum permissible limits in the rivers of Tripura and some northern states.

Shukla, S., C., B. D. Tripathi., B. P. Mishra, and S. S. Chaturvedi. 1992. Physicochemical and bacteriological properties of the water of river Ganga at Ghazipur. Comp. Physio. Eco. **17** :92-96.

The bacteriological and physicochemical properties of the water of River Ganga were studied at four sampling sites at Ghazipur, U.P., from May 1987 to April 1988. For bacteriological analysis, samples were tested for standard plate count (SPC) and total coliform (TC) bacteria. Depletion in the dissolved oxygen, and increase in ECE, BOD, COD, pH, nitrate N, phosphate P, sodium, potassium and calcium contents of Ganga water was recorded near the area affected with sewage and industrial effluents.

Haniffa, M. A, P. Martin, and J. Jeevaraj. 1994. Hydrobiological studies on the channels of river Tambaraparani for the assessment of water quality. Indian. J. Environ. Prot. **14** : 821-828.

The channels of river Tambaraparani are polluted by both point sources and nonpoint sources of pollutants. All the channels are facially contaminated by coliforms. Generally the most probable number (MPN) of coliform and total place count of bacteria (TPC) was very high in sediments compared to that of water. The MPN index of coliform in water and sediment was high in North Kodamelagian channel due to sewage contamination. But the MPN index was low in Kodagan channel where the fecal and sewage entries were less. The TPC was higher in south Kodamelagian channel whereas the count was less in the Kodagan channel both in water and in sediment.

Raiyani, C. V., P. B. Doctor., Y. Verma., N. M. Desai., P. K. Kulkarni., S. G. Ruparelia, and S. K. Ghosh. 1994. Magnitude of pollution of dyecontaminated river water-its physicochemical and microbial analysis. Indian. J. Environ. Prot. **14** : 252-255.

Bacteriological and physicochemical analysis of water samples of river Bhadar between Jetpur and Dhoraji were carried out. Results of analysis showed a good correlation between MPN, BOD and COD. Other parameters included pH, sulphate, nitrate, total hardness, etc. Nitrate content of the river water was always above the permissible level (l0 mg/l) as suggested by WHO.

Doctor, P. V., C. V. Ranjani., Y. Verma., N. M. Desai., P. K. Kulkarni., S. G. Ruparalia, and S. K. Ghosh. 1998. Physico-chemical and microbial analysis of dye-contaminated river water. Indian. J. Environ. Hlth. **40**: 7-14.

Magnitude and degree of pollution in river Bhadar caused by azo dye containing effluents discharge from printing cotton textile industries has been studied, both by micorbiological and physco-chemical analysis. Nitrate concentrations were above the permissible level. BOD and COD values correlated well with MPN and heterotrophic plate count. Among all the isolates *E. coli* found to be site-specific dominant microflora.

Bhadra, B., S. Mukherjee., R. Chakraborty, and A. K. Nanda 2003. Physico-chemical and bacteriological investigation on the river Torsa of North Bengal. J. Environ. Bio. **24** :125-133.

A few physico-chemical and bacteriological parameters on certain locations of the river Torsa were studied. The major characteristics of Torsa river water were high alkalinity, high concentration of free ammonia with respect to albuminoid ammonia and the presence of bacteria of fecal origin. Marked seasonal variations of the parameters were also observed.

C. Investigation on metal contamination:

Mishra, A., J. S. Datta Munshi, and M. Singh. 1994. Heavy metal pollution of river Subarnarekha in Bihar. Part I: Industrial effluents. J. Fresh Water. Bio. **6** : 197-199.

Effluents, discharged into the river Subarnarekha, Bihar during different seasons of the three-year period from five major industries, have been analyzed for Cu, Zn, Pb, Fe, Cr, and Cd. The average concentration data have been made the basis of pollution consideration. Industry wise and placewise gradations of heavy metal discharge have been made. Industries situated at Ghatsila appear to be making the highest flux of heavy metals into the river.

Shyama Sundar P. S., G. Madhu., K. Srinivasa Murthy, and V. Mangathyaramma 1994. River Krishna estimation of trace metals and their distribution. Indian. J. Environ. Prot. **14** : 654-663.

Krishna river is an important east flowing peninsular river in South India which con-flounces at Divi point in Andhra Pradesh. It has tributaries, which traverse through areas rich in industries, agricultural run off and major towns/cities in this area pointing to possible pollution. A sample base line data has been collected and reasonable interpretations were made. The biogeochemical and anthropogenic mechanisms were discussed with the data collected in this basin.

Krishnamurthy, S. R, and S. G. Bharati. 1995. Distribution of manganese in the surface water of the polluted river Kali, around Dandeli area, North Kanara district, Karnataka. Env. Eco. **13** :132-135.

The concentrations of manganese were determined at four sampling stations of the polluted river Kali near Kandeli. The values of manganese increased from unpolluted stations to polluted stations. The manganese value showed direct relationship with pH, chloride, total hardness, phosphates and dissolved organic matter content of the river water.

Krishnamurthy, S. R, S. G. Bharati. 1995. Distribution of copper in the surface waters of the polluted river Kali, around Dandeli area, Karnatakan India. Env. Eco. **13** : 192-197.

The monthly variations and yearly concentrations of copper ion at four different sampling stations of the river Kali were studied. The variations of copper ions showed inverse relationship with total hardness, total dissolved residue and alkalinity.

Krishnamurthy, S. R, and S. G. Bharati. 1995. Distribution of zinc in the surface waters of the polluted river Kali, around Dandeli area, Karnataka, India. Env. Eco. **13**: 253-257.

The distribution of zinc in the surface waters of the river Kali was investigated between June 1987 and May 1988. The average value of zinc was higher than the world mean stream concentration. Further, at all the sampling stations of the river yearly average values of zinc, chloride, and sulphate corresponded with one another.

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Dwivedi, S, and I. C. Tewari. 1997. Seasonal variation in heavy metal content of river Ganga at Varanasi. Indian. J. Environ. Prot. **17** : 281-286.

The concentrations of five heavy metals namely Cu, Cd, Cr, Fe and Pb were studied in the river Ganga at Varanasi from the University ghat (the most upstream point) to Rajghat (the most downstream point). The data has been discussed with reference to flow characteristics and other hydrological aspects and the values observed have been compared with standards prescribed by various river authorities. Levels of all the heavy metals were highest during the summer season and lowest during the rainy season.

Prbha S, Selvapathy. 1998. Heavy metal pollution in Indian rivers. Indian. J. Environ. Prot. **17** : 641-649.

Paper reviews the status and trends of heavy metal pollution in the major Indian rivers. A brief review of the analytical procedures for the determination of heavy metals in water sediments is also included.

Koshy Mathew, and Vasudevan N. T. 2002. Trace metals in the sediments of river Pamba. Polln. Res. **21** : 235-242.

The trace metals in the sediments of river Pamba in the state of Kerala from ten stations were analysed for a period of one year by taking monthly samples. Copper, zinc, iron and manganese showed low concentrations as compared to other rivers. The enhanced values of the metals during pre-monsoon and post-monsoon period is attributed to the seasonal accumulation of allochthonous organic residues.

Bhosle, A. B. 2003. The iron content in the river Godavari at Nanded and its impact on river ecology. J. Ecotoxico. Environ. Monit. **12** : 193-199 .

Natural waters can be very heterogeneous vertically, horizontally and with time. This is not only to man-made pollution, but also can be caused by natural phenomena such as erosion, currents, thermo cline and precipitation washout of dust. The total iron content of river Godavari was investigated and the overall study showed the fluctuations in the iron content more than permissible limit prescribed by Indian Council of Medical Research (ICMR). The iron was maximum during November and minimum during June.

Walther, D., S. Prebha., P. Selvapathy, and D. Beck. 2003 Heavy metals from the river Adayar, India : Infiltration into the adjacent groundwater aquifer. Ambio. **32** :153-157.

The mobilization of heavy metals into greater depths and their probable effects on the groundwater body are discussed. The high concentration of heavy metals and the influent character of the river Adayar allow the mobilization of metal ions and their transport into the deeper layers of the sediment. A changing environment due to effects such as saltwater intrusion and monsoon floods is the driving force for this phenomenon.

D. Investigation on pollution of river water with urban and industrial effluents:

Siddiqi, Z. M., R.S. Panesar, and S. Rani. 1994.Biochemical effect of few sewerage disposals on the water quality of Sutlej river. *Indian J Environ Prot,* **14** : 740-743.

Due to disposing off the sewerage and industrial wastes directly into the Sutlej river, the quality of water has the high BOD and enhanced turbidity. The presence of *Escherichia coli* suggests its pathological effect on human consumption and hence its removal from water body.

Trivedy, R.K, and S. S. Nakate. 1994. Pollution of clusters of industries in the Krishna river basin. J. Indl. Polln. Contl. **10** : 119-126.

Paper reports pollution load caused by a cluster of industries. Two clusters of industries, Satara and Wai M.I.D.Cs, Maharashtra Industrial Development Corporations' Industrial Estates have been studied. It was found that majority of industries have not installed effluent treatment plants. While the pollution load generated at Wai M.I.D.C. is minimal, Satara M.I.D.C. presents an ideal case for establishing combined effluent treatment plant.

Chetana Suivarna, A, and R. K. Somasekhar. 1997. Ecological study on the riverine ecosystem of Karnataka. III. physico chemical characterisation of river Vris\tabhavathi. J. Env. Polln. **4** : 71-77.

The study was carried out to comprehend the physico-chemical characteristics of river volume Vrishabhavathi. Large of urban wastes discharged into his river influenced its physico-chemical make-up considerably. Most of the chemical components estimated were in higher concentration with the pH of water being alkaline.

Jain, C.K., K. K. S. Bhatia, and S. M. Seth. 1997. Characterization of waste disposals and their impact on the water quality of river Kali. Indian. J. Environ. Prot. **17** : 287-295.

The physico-chemical characterization of municipal waste of Muzaffarnagar city and composite industrial waste have been carried out with a view to assess the likely impact of these effluents on the quality of water of river Kali. High values of BOD and COD in the waste effluents is an indication of high degree of organic contamination in these wastes. The important characteristics associated with pollution of the river due dot the discharge of these wastes is the heavy depletion of oxygen over a small stretch of the river.

Aher, H. R., D. G. Zinjad., P. S. Gunjal, and S. R. Kuchekar. 2002. Impact of human activities on the quality of water in Pravara river basin and Pravara left bank canal. Cheml. Environ. Res. **11**: 101-104 .

Chemical analysis of water samples from Pravara river basin and Pravara left bank canal shows that the water is characterized by alkaline earth. Water samples from thirteen spots of down stream from Bhandara to Babhaleshwar were collected for analysis at an interval of ten kilometers. The results show that the physico-chemical characteristic of water changes to downstream from Bhandara to Babhaleshwar due to human activities.

Gupta, A. K, and A. K. Raghubanshi. 2002. Comparative study of enrichment of nutrients and heavy metals in river waters Ghaghra and Ganga due to anthrophogenic pressures. Polln. Res. **21** : 261-263.

For a comparative study of enrichment of nutrients in river waters of Ghaghra and Ganga due to anthropogenic activities, two sites were selected at both the river corridors and one at the confluence point of both the rivers. The findings show that, due to different anthropogenic activities, the level of nutrient enrichment varies at different sites. Similarly the heavy metal content also varies with the biotic activities. Due to nutrient and heavy metal's enrichment the water quality is adversely affected.

Kumar Neeraj, and R. C. Sharma. 2002. Water quality of river Krishni [Part-1. Physico-chemical characteristics]. J. Nature. Conservator. **14** : 273-297.

The water quality of river Krishni has been studied at eight sampling points fixed at 70 kilometers stretch. The river received about 50 to 67 cusec. of domestic and industrial waste water via three waste water channels. These waste contents of wastewater have changed the characteristics of the river water to great extent.

Pande, R. K, and A. Mishra. 2002. Impact of paper and pulp industry effluent on the water quality of river Hindon. J. Ecophysio. Occupl. Hlth. **2** : 173-184.

Hindon is a tributary of Yamuna and flows along the western district of U.P. and Uttaranchal and certain physical and chemical parameters have been studied and the effluent stress were observed to understand its possible impact on water quality of river Hindon, where effluent was discharged. It was observed that certain undesired elements had a self reducing tendency along with certain physico-chemical parameters but a few remain contaminated for a longer period of time and for longer distance in stream.

1.1.4. Importance of antibiogram surveillance

Surveillance of bacterial resistance is a key element in understanding the size of the environmental problem and disease management. One promising approach was based on the analysis of differences in antibiotic resistance by using the multiple antibiotic resistance index (Kaspar *et al.* 1990, Pillai *et al.* 1997). Wiggins (1996) used discriminant analysis of patterns of antibiotic resistance in fecal streptococci to differentiate between human and animal sources of fecal pollution in natural waters and to classify unknown isolates from polluted streams on the basis of the patterns of the unknown isolates. Multiple antibiotic resistance (MAR) analysis was used as a method for determining point and nonpoint pollution sources. Parveen *et al.* 1997, examined 765 multiple antibiotic resistant *Escherichia coli* isolates and used their antibiotic resistance profile as a possible tool to differentiate point and nonpoint sources of pollution within the Apalachicola National Estuarine Research Reserve (ANERR). The results of their study reflected the applicability of the method in facilitating management of other estuaries. Although a specific *E. coli* MAR profile may not always correlate with PS (point source) and NPS (nonpoint source) pollution in all estuaries, extensive databases may well be required to develop associations between specific MAR profiles and sources of pollution. The reliability and repeatability of antibiotic resistance analysis as a method of identifying the sources of fecal pollution in surface water and ground water was tested (Wiggins *et al.* 1999) and the study confirmed the measurable and consistent differences in the antibiotic resistance patterns of fecal streptococci isolated from various sources of fecal pollution which could be used to classify and identify the sources. The study of Harwood *et al.* 2000, described the application of antibiotic resistance analysis as a tool to differentiate between animal and human fecal isolates in subtropical surface waters of Florida. The ARPs (antibiotic resistance patterns) of fecal streptococci and fecal coliforms from known animal sources and from human-dominated sources (domestic water) were determined in order to create separate databases (fecal streptococcus and fecal coliform) to which ARPs of isolates from surface waters could be compared and categorized by probable source. The antibiograms varied with time and geographical regions. Periodic monitoring of antibiograms could, therefore, enable to document changes in resistance patterns and characterize the isolates on the basis of antibiogram updates. The multiple resistances of isolates to some antibiotic class are of great public concern and calls for caution in the indiscriminate use of antibiotics on humans and animals. There has been a growing necessity to look at how antibiotics are being used and locate

the residence of resistant strains on a global scale, because of the mobility of these organisms across countries. Good quality local data would be helpful in providing a strong basis for national and international surveillance. Exploring natural reservoirs of resistance genes may predispose the sources of transferable traits for emerging pathogens. Surveillance studies on changing pattern of antibiotic resistance in micro flora of Indian rivers were practically non-existent.

The present study is a maiden study on antibiogram surveillance of copiotrophic bacteria from a river of Northern West Bengal, India. Copiotrophs are those, which compete, well in nutrient rich environments. The Torsa is an international river, which crosses through three countries, China (Tibet), Bhutan and India. As it left the foothills of Bhutan and entered duars plain of West Bengal, it widened into a braided channel. Torsa has on its banks several tea gardens, agricultural fields and hamlets of human habitation. This river also drains Jaldapara Wild Life Sanctuary. Extensive heedless deforestation, concentrated urban development, unscientific mining, uptake of dolomite colloidal water traveling through several streams, inadequate drainage have contributed to the degradation of water quality of Torsa. The major characteristics of the river water were a high concentration of free ammonia with respect to albuminoid ammonia, high alkalinity and the presence of bacteria of both fecal and non-fecal origin (Bhadra *et al.* 2003). The aim of this chapter was to provide detailed descriptive information about the antibiotic resistances of copiotrophic bacteria isolated from this water way and the second was to analyze whether antibiotic resistance patterns vary in a systematic or random manner.

1.2 Materials and Methods

1.2.1 Sampling stations and sample collection from the river water

For successful monitoring of river water quality it is essential to have sufficient knowledge about the morphometric details of the subject river, selection of particular sampling site(s), sample collection methods, and preservation & maintenance of samples for parameter(s) in question. The study of morphometry, i.e., measurement of morphological features of the river basin, always provides valuable information in selecting sample collection site(s). Water quality of the river water also depends on physiographical factors, such as, basin, bank, catchments area, and settlement around the river, as well as annual sedimentation load, water volume, width, and depth of the river.

During analysis of river water quality, assortment of sampling site(s) is very important, and it should be done in the light of environmental monitoring program. The selection of actual sampling location shall depend upon the character of the water body. In case of widened region of the river many sampling sites should be selected at various corners. In monitoring the stream, which is narrow, the rapidly moving water should be thoroughly mixed laterally and vertically, hence only one sampling point needs to be selected at each location along the stream.

In an organically polluted river course at least one site should be selected above the outfall of the wastes and others should be selected downstream representing the zone of recent pollution. In places where the river is polluted by inorganic

substances, one point above and the other point below the actual point of discharge should be selected for sampling.

Water samples collected from the river are of two main types depending on the collection principle: grab-samples, and composite samples. Grab samples are collected at a specific spot in a site over a short period of time, on the other hand, when multiple grab samples are combined and treated as a single sample, it is called composite sample. Samplers and containers should always be thoroughly cleaned before use, and should be rinsed with the sample water before collection. Preferably the amber colored glass containers with polypropylene cap should be used for the collection and preservation of samples.

1.2.1.1 Selection of sampling stations on Torsa River

Sampling sites on Torsa River were selected after scrutinizing topographic map of the river. For the purpose of analysis of water quality, three sampling stations were selected (Figure 1.1). The first sampling station, SS I, was located at Hasimara, Dist., Jalpaiguri of the state West Bengal. The second sampling station, SS II, was located at village- Falakata of Jalpaiguri district. The third sampling station, SS III, was located at Coochbehar Town, district Coochbehar, of the state West Bengal, India.

1.2.1.2 Sample collection

From each sampling site, three grab samples were collected from left, right and middle of the river; all three samples were mixed and, were analyzed as single sample. For sampling, sterilized water bottles were used. The bottles were opened under water, rinsed thoroughly with the sample water even it was pre-cleaned and were half filled by opening and closing the bottles underneath. Samples were collected once in every month from January 2000 to December 2001, leaving the months July and August, because of heavy rainfall and flood during the monsoon which made the condition of river and roadways both equally deplorable so that the task of collecting sample became very difficult. The samples were transported to the laboratory in icebox and analyses were performed within 24 hours.

1.2.2 Preparation of antibiotic stock solutions and antibiotic plates

Antibiotic stock solutions were prepared by dissolving measured amounts of respective antibiotics to its suitable diluents. These concentrated stock solutions were made at least once a month and were stored at -20°C. Tests with known sensitive isolates of *E. coli* indicated adequate storage stability for all antibiotics stored under these conditions. Antibiotic powders were weighed to 0.1mg accuracy; liquids were quantified by micropipette. The antibiotics used were ampicillin (100 μg ml^{-1}), chloramphenicol (100 μg ml^{-1}), kanamycin (50 μg ml^{-1}), streptomycin (100 μg ml^{-1}) and tetracycline (20 μg ml^{-1}). The desired concentrations of the antibiotics (diluted from the stock) were stirred into the melted agar at approximately 45°C and immediately poured into petridishes to minimize the exposure of elevated temperatures. These LB agar plates containing standard concentration of a respective antibiotic were stored at 4°C and were used within seven days of preparation.

1.2.3 Enumeration of total culturable copiotrophic bacteria and the fraction resistant to different antibiotics

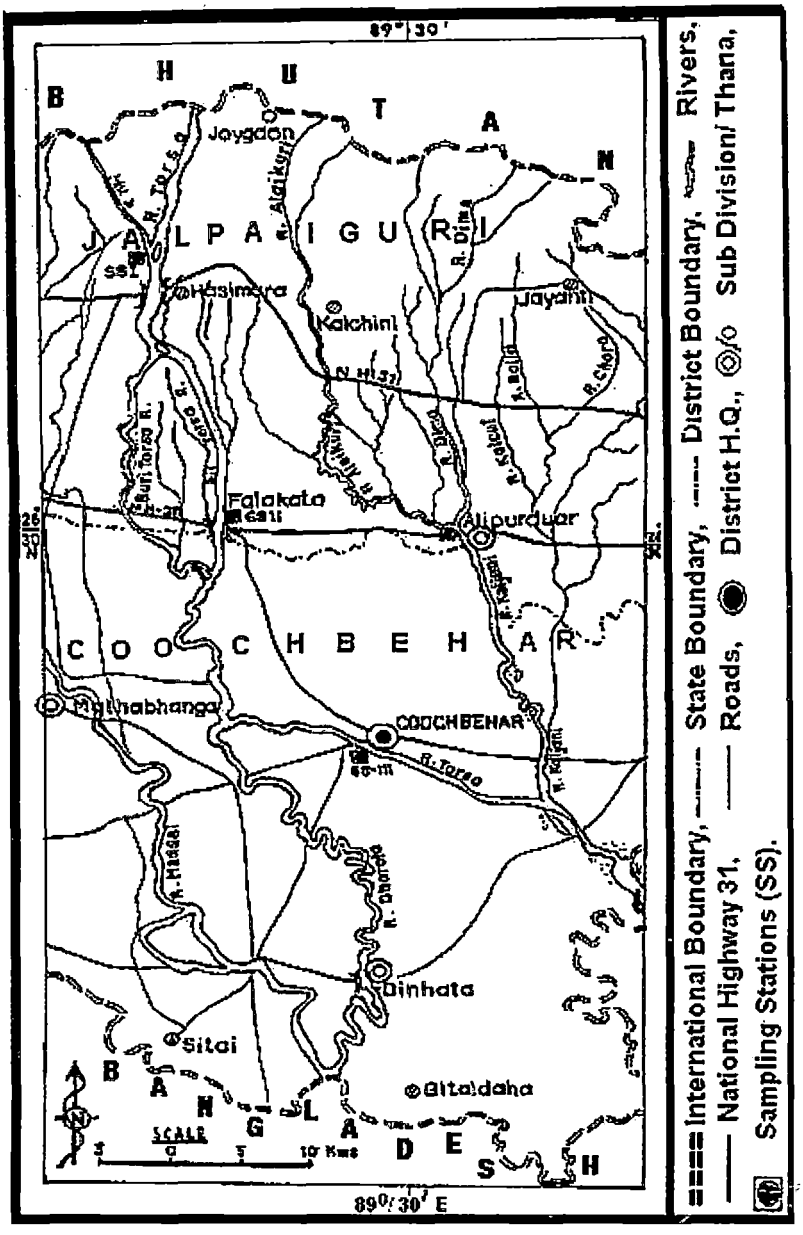


Figure 1.1. Map showing three sampling sites on river

Serial dilutions of river water samples were made in sterile 0.5% NaCl solution, which served as diluents of a known volume. Once diluted, 0.1ml of the suspension was spread uniformly on Luria-Bertani agar plates with a glass spreader. The plates were incubated at 37°C overnight. The total culturable copiotrophic bacterial count of the suspension was obtained by multiplying the number of colonies per plate by reciprocal of the dilution.

0.1ml aliquots from serially diluted tubes of river water samples (from the same dilution series of the samples as described above) was spreaded onto LB agar plates containing standard concentration of a single antibiotic and incubated overnight at 37°C for getting the total count of copiotrophs resistant to a particular antibiotic. The count obtained was finally expressed as the fraction of the total count obtained from the LB agar plates without antibiotic.

1.2.4 Determination of multiple-antibiotic-resistance (MAR) profile of copiotrophic bacterial isolates

Antibiotic resistance was determined by the method described earlier by Armstrong *et al.* (1981). The resistant bacterial colonies that appeared on LB agar plates containing single antibiotic were picked up randomly with sterile tooth picks and transferred to a gridded LB agar plate containing no antibiotic. These plates were considered as the master plates and were incubated for 24 hours at 37°C. These master plates were then replicated onto LB agar plates, each containing a single antibiotic at the concentration indicated in section 1.2.2. The final plate replicated was one of plain LB agar plate. This method allows slight differences in amount of inoculums. However, the control plate was inoculated last to confirm the successful inoculation of the preceding plates and provided a fresh master plate of cultures. The replicated plates were incubated at 37°C for approximately 24 hours and drug resistance was determined. The isolates were considered resistant to multiple antibiotics, only if their growth on the presence of antibiotic were as well developed as their growth on the control plates. Any sign of inhibition or sensitivity was considered to be indicative of nonresistance. This meant that resistance was very strictly defined so that no organism with any sign of sensitivity would be classified as resistant. This strict definition of resistance was necessary to make the interpretation of results easier and consistent.

1.2.5 Statistical analyses

A series of observations on total culturable copiotrophic bacteria and their fractions recovered on nutrient-rich solid medium supplemented singly with five different antibiotics, ampicillin, chloramphenicol, kanamycin, streptomycin, and tetracycline, from water samples collected from three different sampling stations, were recorded in different sampling months. An appropriate type of mathematical equation was selected for trend, and the constants appearing in the trend equation were determined on the basis of the given time series data. SPSS package was used to generate the trend line.

The Arcsine transformation, also known as 'angular transformation', being especially appropriate to percentages and proportions, was applied to the percentages in the original data so that the resulting transformed variates met the assumptions of the analysis.

A random sample consisting of 15 observations (per sampling month) were classified according to two factors- into 3 classes according to a factor, sampling site, and also into 5 classes according to another factor, proportions of copiotrophic bacteria resistant towards five different antibiotics. There was one observation in each of the cells corresponding to a class of factor, sampling site, and simultaneously a class of factor, fraction of copiotrophic bacteria resistant to specific antibiotics. These 15 observations (per sampling month of a particular year) were arranged in the form of a two-way table with 3 rows and 5 columns. Analyses of variance (ANOVA) on these data were performed with the help of SPSS package to determine whether there was any significant difference in the recovery of copiotrophic bacteria resistant towards specific antibiotic or between three sampling sites. It was also tested which pairs of sampling sites or antibiotic-resistant groups differed significantly, if

any. The data on occurrence of fractions of five antibiotic-resistant bacterial groups in different sampling months per site was further classified/distributed into three seasons; pre-monsoon, post-monsoon, and winter. Differences in occurrence of fractions of five antibiotic-resistant bacterial groups in three different sites or seasons as well as interaction between the factors was tested with ANOVA (two way classified data with replication). With the help of ANOVA (two way classified data with replication) it was also tested whether the occurrence of the individual antibiotic-resistant fraction differed significantly in seasons or in sampling sites, and interaction between factors, if any.

The Wilcoxon matched pairs signed ranks test, which make use of the magnitude of the differences between quantified data, was used to compare the proportion and test the significance of the difference in abundance of the five different antibiotic-resistant groups. Correlation coefficient was calculated and test on significance of correlation coefficient was done.

1.3 Results

1.3.1 Quantification of culturable copiotrophic bacteria and their fractions resistant to five different antibiotics, ampicillin, chloramphenicol, kanamycin, streptomycin, and tetracycline, in water samples collected in different months, spread over the years 2000 and 2001, from three sampling stations on river Torsa

Thirty-nine water samples, thirteen from each sampling site, were collected and studied on monthly basis from January 2000 to December 2001. The data on total culturable copiotrophic bacteria of the river Torsa in different sampling months for three sampling sites are shown in tables 1.1, 1.2 and 1.3.

Maximum and minimum occurrences of culturable copiotrophs were recorded in water samples of the month of May 2001 and January 2000 from sampling station I. The concentration of culturable bacteria remained invariant in water samples collected in the month of December of the two successive years 2000 and 2001. The fraction of copiotrophic bacterial population resistant to ampicillin occurred maximally in December 2001 followed by June 2001 and October 2000 and 2001. The lowest occurrence of the said population was recorded in March 2001. The chloramphenicol resistant copiotrophs occurred maximally during March 2001. In May 2000 also, the occurrence of chloramphenicol resistant population was recorded to be quite high. Moderately high percentage of occurrence was recorded in June 2001, November 2000 and December 2000. In September 2001, lowest occurrence for chloramphenicol resistant population was recorded. The fraction of bacterial population resistant to kanamycin was found to be present in only six of the thirteen water samples analyzed. The maximum occurrence of this particular population was observed in March 2001 and then in June and December 2001. Very low percentage of the kanamycin resisting population was found during October 2000 and 2001, as well as in December 2000. The fraction of bacterial population exhibiting resistance to streptomycin was quite low in comparison to the ampicillin and chloramphenicol resistant population. The highest occurrence of this population was recorded in September 2001 and lowest was during

Table 1.1. Total copiotrophic bacterial count (TCBC) and fraction of TCBC resistant to five different antibiotics in water samples collected in different sampling months (January 2000-December 2001) at Hasimara (SS I).

Months	Total copiotrophic bacterial count (TCBC) (c.f.u ml^{-1})	Percent resistant population (taking from TCBC value in plates without antibiotic as 100%)				
		Amp^r	Chl^r	Kan^r	Str^r	Tet^r
Jan.2000	1×10^3	34.0	16.66	0.0	0.0	0.0
May 2000	45×10^3	17.28	67.77	0.0	6.22	1.0
Oct.2000	55×10^3	41.45	22.72	0.03	3.63	1.5
Nov.2000	10×10^3	30.0	25.0	0.0	4.5	0.0
Dec.2000	80×10^3	28.75	20.7	0.84	6.97	0.0
Jan.2001	37×10^3	28.0	17.86	0.0	5.89	0.34
Mar.2001	28×10^3	4.17	89.28	5.0	8.92	4.82
May 2001	90×10^3	7.2	12.2	0.0	3.7	0.77
June 2001	9×10^3	43.47	26.08	4.78	0.02	36.95
Sept.2001	46×10^3	13.04	8.69	0.0	19.56	1.17
Oct.2001	21×10^3	35.23	15.40	0.08	10.72	1.49
Nov.2001	26×10^3	21.02	10.25	0.0	8.65	0.69
Dec.2001	80×10^3	75.0	62.5	4.6	8.75	0.93

Amp, Ampicillin; Chl, Chloramphenicol; Kan, Kanamycin; Str, Streptomycin; Tet, Tetracycline

June 2001. For tetracycline resistant bacteria, maximum occurrence was recorded in June 2001 and their occurrence was not detectable in the months of January, November and December 2000. It is interesting to note that in January 2000, only the fraction resisting ampicillin and chloramphenicol were recovered from the copiotrophic population. It was also observed that in the months where recovery of the chloramphenicol resistant populations were maximum, recovery of the other antibiotic resisting population were quite low as was observed in the month of May 2000 and March 2001.The same phenomenon was observed regarding the abundance of the recovered ampicillin resistant population compared to other antibiotic-resistant bacterial populations.

The maximum and minimum bacterial load in the water samples from sampling station II were recorded in the months of May 2000 and March 2001. The fraction of copiotrophic bacterial population resistant to ampicillin occurred maximally in May 2000 and during October 2000 and 2001. The lowest occurrence of the said population was found in March 2001. Among the recovered bacterial population, the highest fraction resisting chloramphenicol appeared in March 2001. The occurrences of the said population were quite high in the months of May 2000 and December 2001. Moderately high percentage of occurrence was recorded in June 2001, October 2000 and 2001, as well as in November 2001. In September 2001, lowest occurrence for chloramphenicol resistant population was recorded. The fraction of recovered kanamycin resistant bacterial population was lowest among all other antibiotic resisting populations (i.e, ampicillin, chloramphenicol, streptomycin and tetracycline resistant ones). Their occurrences were detected in only six of the thirteen water samples analyzed. The maximum recovery of this population took place in the month of May 2000. Very low percentage of the kanamycin resisting population was found during September 2001, October 2001, as well as in December 2001. The highest occurrence of streptomycin resisting population was recorded in May 2000 and lowest was during November 2000. For tetracycline

Table 1.2. Total copiotrophic bacterial count (TCBC) and fraction of TCBC resistant to five different antibiotics in water samples collected in different sampling months (January 2000-December 2001) at Falakata (SS II).

Months	Total copiotrophic bacterial count (TCBC) (c.f.u ml^{-1})	Percent resistant population (taking from TCBC value in plates without antibiotic as 100%)				
		Amp^r	Chl^r	Kan^r	Str^r	Tet^r
Jan.2000	50×10^3	8.75	16.25	0.0	0.0	0.0
May 2000	500×10^3	61.53	53.84	46.15	84.82	9.03
Oct.2000	40×10^3	47.50	22.50	1.25	0.85	0.375
Nov.2000	30×10^3	12.66	9.0	0.0	0.43	0.23
Dec.2000	70×10^3	9.60	7.54	0.0	0.62	0.37
Jan.2001	11.6×10^3	3.65	10.25	0.0	0.0	0.14
Mar.2001	1.9×10^3	2.5	60.25	0.0	6.71	1.5
May 2001	50×10^3	11.4	16.0	2.6	5.0	1.72
June 2001	180×10^3	22.22	27.77	0.0	0.0	16.66
Sept.2001	21×10^3	10.95	6.19	0.476	1.42	0.03
Oct.2001	150×10^3	43.25	20.9	0.672	0.85	0.47
Nov.2001	110×10^3	14.69	20.6	0.0	0.0	1.24
Dec.2001	60×10^3	21.03	42.60	0.268	0.0	0.04

Amp, Ampicillin; Chl, Chloramphenicol; Kan, Kanamycin; Str, Streptomycin; Tet, Tetracycline

resistant bacteria, maximum occurrence was recorded in June 2001 and their occurrence was not detectable in the month of January 2000. The recovery of the fraction of the bacterial populations exhibiting resistance to each antibiotic was quite high during May 2000. In the months like May 2000 and 2001, September 2001 as well as in October 2000 and 2001, all the five different types of antibiotic resistant bacteria were recovered from the copiotrophic bacterial population. In January 2000, only the fraction resisting ampicillin and chloramphenicol were recovered. Analysis of all the thirteen water samples led to the observation that only one type of antibiotic resistant population that recovered as a fraction resistant population, in a particular month, dominated over the other types. In May 2000, the maximum recovery was noted for streptomycin resistant population. It was also observed that in the months where recovery of the chloramphenicol resistant populations was maximum, recovery of the ampicillin resisting population was quite low and in months where ampicillin resistant populations excelled, the chloramphenicol resistant population declined.

For the sampling station III, the maximum occurrence of culturable copiotrophs was recorded in water sample of the month of May 2001. The concentration of culturable bacteria remained invariant in water samples collected in the months of January (lowest recovered bacterial population), as well as in November of the two successive years 2000 and 2001. The fraction of copiotrophic bacterial population resistant to ampicillin occurred maximally in September 2001. The lowest occurrence of the said population was recorded in January 2000. The chloramphenicol resistant population appeared in highest proportion during March 2001. In December and June 2001 also, the occurrence of chloramphenicol resistant population was recorded to be quite high. In January 2000, lowest occurrence for chloramphenicol resistant population was recorded. It was observed that the fractions of ampicillin and chloramphenicol resistant bacterial populations were quite high compared to the other antibiotic resistant populations among the recovered

Table 1.3. Total copiotrophic bacterial count (TCBC) and fraction of TCBC resistant to five different antibiotics in water samples collected in different sampling months (January 2000-December 2001) at Coochbehar (SS III)

Months	Total copiotrophic bacterial count (TCBC) (c.f.u ml^{-1})	Percent resistant population (taking from TCBC value in plates without antibiotic as 100%)				
		Amp^r	Chl^r	Kan^r	Str^r	Tet^r
Jan.2000	0.51×10^3	0.39	4.31	0.0	0.0	0.0
May 2000	23.6×10^3	26.09	14.68	0.0	0.0	1.52
Oct.2000	19×10^3	23.31	17.56	0.18	1.55	1.60
Nov.2000	270×10^3	16.66	12.22	0.03	2.59	5.18
Dec.2000	196×10^3	15.49	10.04	1.48	1.79	0.68
Jan.2001	0.51×10^3	0.49	15.84	0.79	0.0	0.0
Mar.2001	80×10^3	2.87	35.27	0.64	6.42	0.82
May 2001	650×10^3	5.38	13.85	0.92	0.246	3.38
June 2001	300×10^3	36.36	30.90	2.81	1.09	33.18
Sept.2001	19×10^3	47.36	28.94	0.36	1.57	0.36
Oct.2001	30×10^3	28.75	10.97	0.45	1.76	1.29
Nov.2001	270×10^3	30.59	22.68	0.69	0.79	0.48
Dec.2001	90×10^3	17.68	33.07	0.0	0.0	0.91

Amp, Ampicillin; Chl, Chloramphenicol; Kan, Kanamycin; Str, Streptomycin; Tet, Tetracycline

culturable copiotrophs. Except for three months (January and May 2000 and December 2001), fraction of kanamycin resistant population was recovered from each of the sampling months. The maximum occurrence of this particular population was observed in June 2001 and then in December 2000. In other months, recovery of the said population was quite low. The fractions of bacterial populations exhibiting resistance to streptomycin and tetracycline were quite low in comparison to the ampicillin and chloramphenicol resistant populations. The highest occurrence of streptomycin resisting population was recorded in March 2001 and lowest was during May 2001. For tetracycline resistant bacteria, maximum occurrence was recorded in June 2001 and their occurrence was not detectable in the months of January 2000 and 2001. It is interesting to note that in January 2000, only the fractions resisting ampicillin and chloramphenicol were recovered from the copiotrophic bacterial population. It was also observed that in the months where recovery of the chloramphenicol resistant populations was maximum, recovery of the other antibiotic resisting population were quite low. Similar observation was found in case of data on recovery of ampicillin resistant population.

Copiotrophic bacterial counts (CBCs) that were recorded in different sampling months displayed very wide variations and therefore larger were the dispersion (measured in terms of standard deviation) values (Table 1.4). Geometric mean values were considered because it is less affected by the presence of extremely large or small values. The CBC mean values for the water samples collected from three different sampling stations ranged from 2.64×10^4 to 4.80×10^4. The SPC GM values for SS II and SS III were very close.

1.3.2 Time series analysis: Determination of trend(s) of occurrence(s) of five different antibiotic resistant bacterial fractions of the culturable copiotrophs

The percentage occurrence of ampicillin-resistant bacteria in water samples collected from three different sampling stations, spanning a period from January 2000 to December 2001, have shown an

Table 1.4. Antibiotic resistance of copiotrophic bacteria isolated from three sampling sites of river Torsa of North Bengal

	Average SPC Density (c.f.u. ml^{-1})	Average % resistant population (taking SPC value in plates without antibiotic as 100%)				
		Amp	Chl	Kan	Str	Tet
SS I	2.64×10^4 (2.64×10^4)	22.86 (12.29)	22.96 (23.73)	1.12 (1.80)	3.25 (5.03)	1.39 (9.99)
SS II	4.80×10^4 (1.31×10^5)	14.13 (18.95)	18.79 (16.69)	1.21 (12.65)	1.66 (23.07)	1.67 (4.85)
SS III	4.18×10^4 (1.86×10^5)	10.09 (14.58)	16.44 (9.00)	1.67 (0.76)	1.28 (1.69)	1.47 (8.90)

Geometric mean of 13 samples
The standard deviation are given in the first bracket
Amp, Ampicillin; Chl, Chloramphenicol; Kan, Kanamycin; Str, Streptomycin; Tet, Tetracycline

upward trend in SS I and SS III, while a lower trend was observed in samples from SS II. There was a differential trend in case of occurrence of chloramphenicol resistant bacteria. Analysis of Site I samples revealed a lower trend while Site III showed higher trend of occurrence of the chloramphenicol-resistant bacterial population. There was a fairly constant occurrence or stagnation of the said population in water samples of SS II, over the period of time. Although the fraction of kanamycin resistant populations recovered from the water samples of each site were quite low, time series analysis has revealed an upward trend for occurrence of the said population in SS I and SS III. A lower trend was observed in samples from SS II. In case of populations resistant to streptomycin, an upward trend in SS I and a lower trend in SS II was noted. A fairly constant trend for the same population was observed in SS III. An upward trend was observed for the fraction of populations resisting tetracycline in both SS I and SS III. The said population stagnated in SS II.

1.3.3 Wilcoxon matched pairs signed ranks test

The Wilcoxon matched pairs signed ranks test, which makes use of the magnitude of the differences between quantified data, was used to compare the proportion and test the significance of the difference in abundance of the five different antibiotic-resistant groups. The significant difference in recovery of proportion of matched pairs were kanamycin and ampicillin ($p<0.001$), streptomycin and ampicillin ($p< 0.003$), tetracycline and ampicillin ($p<0.001$), kanamycin and chloramphenicol ($p<0.001$), streptomycin and chloramphenicol ($p <0.002$), tetracycline and chloramphenicol ($p< 0.001$), streptomycin and kanamycin ($p< 0.007$), and tetracycline and streptomycin ($p< 0.023$) resistant bacteria in water samples of SS I. The occurrence of the individual group of the matched pairs that were found significantly different in water samples of SS II were, kanamycin and ampicillin ($p<0.001$), streptomycin and ampicillin ($p< 0.005$), tetracycline and ampicillin ($p<0.001$), kanamycin and chloramphenicol ($p<0.001$), streptomycin and chloramphenicol ($p <0.004$), tetracycline and chloramphenicol ($p< 0.001$), and streptomycin and kanamycin ($p<0.047$) resistant bacteria. The occurrence of the individual group of the matched pairs, in the data derived from water samples of SS III, that were found significantly different were, kanamycin and ampicillin ($p< 0.001$), streptomycin and ampicillin ($p< 0.002$), tetracycline and ampicillin ($p< 0.001$), kanamycin and chloramphenicol ($p< 0.001$), Streptomycin

and chloramphenicol ($p < 0.001$), tetracycline and chloramphenicol ($p < 0.001$), and tetracycline and kanamycin ($p < 0.041$) resistant bacteria. The water samples analyzed from all the three sampling sites, SS I, II, & III, did not have any significant difference in occurrence of the proportion of ampicillin and chloramphenicol resistant fraction of the culturable copiotrophs of river Torsa.

1.3.4 Variance analysis in two-way classification

Analysis of variance (two-way without replication) for testing equality of proportions (sampling site X fraction of copiotrophs resistant to different antibiotics) for monthly data yielded the following results. In the first sampling month, January 2000, there were no significant differences between sampling sites, but the proportions of different antibiotic-resistant bacteria was not equal i.e there were significant differences ($p < 0.0124$) in the occurrences of five different antibiotic resistant fractions. Significant difference ($p < 0.05$) in occurrence of ampicillin-resistant bacteria compared with kanamycin or streptomycin or tetracycline was noted. Also, the occurrence of chloramphenicol-resistant bacteria differed significantly from the proportion of kanamycin or streptomycin or tetracycline resistant bacteria. Similar significant difference in occurrence of ampicillin-resistant/chloramphenicol-resistant bacteria compared with kanamycin or streptomycin or tetracycline was noted in the month of November 2000 ($p < 0.01$), December 2000 ($p < 0.01$), January 2001 ($p < 0.05$), and November 2001 ($p < 0.01$). Differences in the proportions of different antibiotic-resistant bacteria were most significant in the months of October 2000 ($p < 1.02$ e-5), December 2000 ($p < 0.000469$), March 2001 ($p < 0.000557$), May 2001 ($p < 0.000183$), June 2001 ($p < 0.000239$), October 2001 ($p < 0.000327$) and November 2001 ($p < 0.001104$).

In the month of May 2000, there were no differences between fractions of different antibiotic-resistant bacteria but the difference was significant ($p < 0.002$) between sampling sites. There were significant differences between SS I and SS II, and between SS II and SS III ($p < 0.05$). There were similar significant differences between sampling sites in the months of December 2000 ($p < 0.049467$) and March 2001($p < 0.019791$). Significant differences in occurrence of ampicillin-resistant/chloramphenicol-resistant bacteria compared with the all the other four (rest) of the five proportions of antibiotic resistant bacteria were noted in the month of October 2000 ($p < 0.01$).

The data of 13 samplings per site were distributed to three seasons, pre-monsoon, post-monsoon, and winter respectively and analysis of variance (two-way classified data with replication) for testing equality of proportion (Season X fraction of copiotrophs resistant to different antibiotics) site wise was performed. There were significant differences between seasons in the occurrence of fractions of different antibiotic-resistant bacteria in the sampling sites II and III ($p < 0.000835$ and $p < 0.008682$). Significant differences have been noted between winter and pre-monsoon; and pre and post-monsoon ($p < 0.01$) in site II. Similarly, the recovery of different fractions of antibiotic-resistant bacteria in the winter season was significantly different from pre or post monsoon ($p < 0.01$) in site III. Most significant differences between ampicillin-resistant/chloramphenicol-resistant

bacteria compared with kanamycin or streptomycin or tetracycline were noted in all the sampling sites, SS I ($p < 4.19e-8$), SS II ($p < 3.01e-5$), and SS III ($p < 3.5e-11$). There was no significant interaction between the factors.

ANOVA (two-way classified data with replication) for fraction of different antibiotic-resistant-bacteria for testing equality of proportions (Season X Site) was also performed to understand the interaction between factors as well as to reveal significant differences in occurrence of particular antibiotic-resistant in three seasons or sites, if any. The occurrences of particular antibiotic-resistant bacterial population in three different seasons did not differ significantly, except chloramphenicol ($p < 0.001711$) and tetracycline-resistant ($p < 0.003435$) bacterial fractions. In terms of the fractions of chloramphenicol-resistant bacteria or tetracycline-resistant bacteria there were differences in occurrence between seasons ($p < 0.01$). The fraction of chloramphenicol-resistant copiotrophs recovered in winter was significantly different from pre- or post monsoon occurrence. The occurrence of the same population in pre-monsoon significantly varied from post-monsoon. Tetracycline-resistant copiotrophic bacterial population in the winter varied significantly from the pre-monsoon. Again, the pre-monsoon occurrences of tetracycline-resistant bacteria were significantly different from post-monsoon recovery. The occurrences of particular antibiotic-resistant bacterial population in three different sampling sites did not differ significantly. No significant interaction between the factors resulted from this analysis.

1.3.5 Determination of antibiotic-resistance- patterns (ARPs)

1.3.5.1 Analysis of ARPs of the antibiotic resistant bacteria from SS I

A total of 5712 antibiotic resistant copiotrophic bacterial isolates from 13 water samples of SS I were screened for multiple antibiotic resistance phenotype (Table 1.5). Only, 5.04% of the exhibited resistance to single antibiotic and the rest 94.95% (5424) were resistant to two or more of the screening antibiotics (MAR). Among the singly resistant group, resistance to chloramphenicol, ampicillin, tetracycline and streptomycin were 2.31%, 1.31%, 1.24% and 0.17% respectively. Singly resistant bacteria to kanamycin were absent. A total of 22 different combinations of MAR phenotype were observed among the isolates. Within the group of MAR isolates, 34.48% were doubly resistant, 34.10% were triply resistant, 23.94% were quadruply resistant and 2.41% were quintuply resistant to the antibiotics that were used (Figure 1.2). Among the double resistant combinations AT (16.31%) was the most frequent one, followed by AC (13.56%) and CT (2.24%) combination. Only a single isolate exhibited CK resistance phenotype.

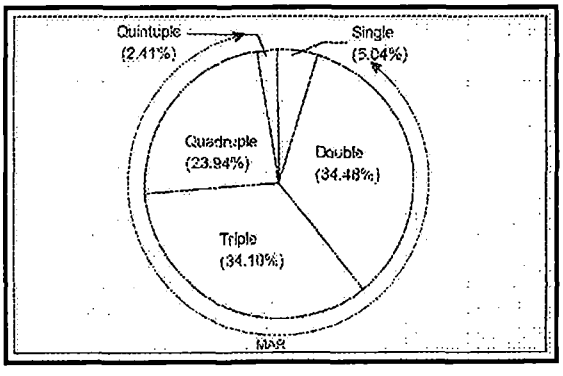


Figure 1.2. Frequency of singly resistant and MAR phenotypes among antibiotic resistant copiotrophic bacterial isolates from SS I.

Table 1.5: Antibiotic resistance patterns (ARPs) of the antibiotic resistant copiotrophic bacterial populations in different sampling months (January 2000 to December 2001) at SS I

	Jan. 2000	May 2000	Oct. 2000	Nov. 2000	Dec. 2000	Jan. 2001	Mar. 2001	May 2001	June 2001	Sep. 2001	Oct. 2001	Nov. 2001	Dec. 2001
A	48.27 (14)	0.75 (7)	1.76 (5)	0.59 (1)		0.33 (1)			0.74 (5)	0.85 (3)	1.39 (10)	2.93 (19)	1.69 (10)
C	10.34 (3)		1.06 (3)	3.55 (6)		27.0 (81)		0.69 (4)	1.18 (8)		0.41 (3)	1.38 (9)	2.54 (15)
K													
S			1.06 (3)	0.59 (1)		0.33 (1)							0.84 (5)
T		1.60 (15)	0.70 (2)	14.20 (24)				1.04 (6)	0.14 (1)	3.14 (11)		1.69 (11)	0.16 (1)
AC	34.48 (10)		1.06 (3)			9.66 (29)		20.0 (115)	12.75 (86)	9.71 (34)	2.09 (15)	39.66 (257)	38.3 (226)
AK		0.10 (1)						4.69 (27)			0.69 (5)		
AS			0.35 (1)	1.18 (2)						2.0 (7)		6.17 (40)	
AT		23.28 (217)	10.60 (30)	1.77 (3)				14.6 (84)	17.80 (120)	15.71 (55)	10.62 (76)	26.38 (171)	29.83 (176)
CK											0.13 (1)		
CS			2.82 (8)								0.41 (3)		
CT		0.53 (5)	5.30 (15)	5.32 (9)	3.58 (8)	1.66 (5)		2.43 (14)	8.16 (55)		1.81 (13)	0.61 (4)	
KS													
KT									1.33 (9)				
ST			3.53 (10)	3.55 (6)					2.22 (15)				
ACK										5.42 (19)	1.25 (9)	1.54 (10)	0.50 (3)
ACS			0.35 (1)	2.95 (5)								0.15 (1)	
ACT		44.74 (417)	36.04 (102)	29.58 (50)	4.03 (9)	51.0 (153)	75.89 (170)	40.0 (230)	27.44 (185)	24.28 (85)	15.38 (110)	17.59 (114)	20.50 (121)
AKT		0.21 (2)						2.60 (15)					
AST		3.54 (33)	2.82 (8)	7.69 (13)						3.42 (12)			
CKT						0.33 (1)		2.08 (12)	1.78 (12)		2.37 (17)		
CST		0.10 (1)	0.70 (2)	3.55 (6)	1.34 (3)	0.66 (2)		1.21 (7)	0.59 (4)		0.55 (4)		
ACKS										2.28 (8)			
ACKT		0.21(2)					4.46 (10)	7.82 (45)	11.57 (78)	22.28 (78)	18.88 (135)		1.18 (7)
ACST		24.35 (227)	31.09 (88)	24.26 (41)	91.03 (203)	9.0 (27)	19.64 (44)		7.56 (51)	3.14 (11)	22.23 (159)	0.77 (5)	1.01 (6)
AKST			0.35 (1)						2.96 (20)		13.42 (96)		
CKST								0.34 (2)			3.35 (24)		
ACKST	6.89 (2)	0.53 (5)	0.35 (1)	1.18 (2)				2.43 (14)	3.70 (25)	7.71 (27)	4.89 (35)	1.08 (7)	3.38 (20)
Total	29	932	283	169	223	300	224	575	674	350	715	648	590

A, Ampicillin; C,Chloramphenicol; K, Kanamycin; S, Streptomycin; T, Tetracycline
The numbers in parenthesis denotes the actual number of isolates

The KS combination was not found among the screened population. ACT (30.56%) and ACST (15.09%) were the most frequent patterns observed within the triply and quadruply resistant group.

In the month of January 2000, a total of 29 antibiotic resistant bacteria from SS I were screened. It was found that the 58.64% of the screened population were single resistant, whereas, 41.37% exhibited resistance to two or more antibiotics (multiple-antibiotic resistant). The population resisting only ampicillin (48.27%) was the most dominant among the singly resistant group. In this month only two different MAR phenotypes – AC (34.48%) and ACKST (6.89%) were observed. A total of three hundred bacterial isolates were screened in January 2001. Among them, 27.66% were single resistant, 11.32% were doubly resistant, 51.99% were triply resistant and 9.0% were quadruply resistant. In both the years, populations resisting only kanamycin or tetracycline were not present among the screened population. The most dominant combination found was ACT (51.0%). The MAR combinations like AK, AS, AT, CK, CS, KS, KT, ST, ACK, ACS, AKT, AST, ACKS, ACKT, AKST and CKST were absent in January 2000 and 2001.

In May 2000, a total of 932 isolates were screened. Only a small fraction (2.35%) of the population exhibited single resistance. Majority of them (97.59%) were multi-resistant. Isolates exhibiting resistance to three different antibiotics were most common among the multi-resistance group (represented by 48.59% of the population). ACT (44.74%) and ACST (24.35%) were the most frequent combination present among triply and quadruply resistant group. Similar MAR combinations were observed among the antibiotic resistant isolates screened during May 2001. None of the isolates exhibited resistance only to ampicillin, kanamycin or streptomycin. AC (20.0%), ACT (40.0%) and ACKT (7.82%) combinations were the dominant ones among the doubly, triply and quadruply resistant group.In May 2000, 0.53% and in May 2001, 2.43% were quintuply resistant. It was found that in both the years, 2000 and 2001, the water samples of the month of May, contained no isolates demonstrating multiple antibiotic resistance combinations like AS, CK, CS, KS, KT, ST, ACK, ACS, CKT, ACKS and AKST.

In two successive years, 2000 and 2001, the numbers of antibiotic resistant bacteria screened for multi-resistance in the month of October were 223 and 715 bacteria respectively. The occurrences of number of different resistance-combinations in the said month, in both the years, exceeded from all other data from other sampling months. Thirteen and fifteen different resistance-combination was noted in the year 2000 and 2001 respectively. In October 2000, 39.91% of the populations were triply resistant, 31.44% were quadruply resistant, 23.66% were doubly resistant, 4.58% were singly resistant and 0.35% was resistant to all the five antibiotics tested. ACT (36.04%) was the most dominant MAR phenotype followed by ACST (31.09%) combination. Somewhat different picture was observed during October 2001 where quadruply-resistant group was most dominant, comprising 57.88% of the total antibiotic-resistant bacteria screened. Among the quadruply resistant group, ACST (22.23%) was the most frequent combination, followed by ACKT, AKST and CKST

patterns. The incidences of other resistance-groups were: 19.55%, triply resistant; 15.75%, doubly resistant; 4.89%, quintuply resistant; and 1.8%, singly resistant. AT (10.62%) and ACT (15.38%) were the most frequent combinations among the doubly and triply resistant groups.

In November 2000, 169 antibiotic resistant bacteria were screened. 81.03% of the population exhibited resistance to two or more antibiotics. Among them 43.77% were triply resistant, 24.26% were quadruply resistant, 11.82% were doubly resistant and only 1.18% were quintuply resistant. 93.95% of the 648 isolates that were screened during November 2001 exhibited multiresistance, of which 72.82% were doubly resistant, 19.28% were triply resistant, 1.08% was quintuply resistant and 0.77% was quadruply resistant. During November 2000 and 2001, populations exhibiting resistance to only ampicillin, chloramphenicol or tetracycline were present. The populations resisting only streptomycin were found during November 2000 but not during 2001. Strikingly, only kanamycin resistant populations were absent in both the years in the month of November. In November 2000, isolates having ACT (29.58%) combination were more frequent followed by the occurrence of ACST (24.26%). The resistance combination, AC (39.66%) and AT (26.38%) constituted the most frequent ones in November 2001.

Cent percent of the screened population exhibited multi-resistant phenotype during December 2000, dominated by the occurrence of ACST (91.03%). A total of 590 antibiotic resistant bacteria were screened during December 2001. Within the population, 68.13% were doubly resistant, 21.0% were triply resistant, 5.23% were single resistant and 3.38% were quadruply resistant. Among the doubly resistant group, AC (38.3%) pattern was most frequent followed by AT (29.83%). Another dominant combination was ACT (20.50%).

In March 2001, single as well as doubly resistant was absent among the screened population from the same site. Three different MAR phenotypes were observed in the said month, of which ACT combination was present among 75.89% of the population screened. Rest of the population exhibited ACKT (4.46%) and ACST (19.64%) phenotype. In the month of June 2001, 674 isolates were screened and 12 different MAR combinations were observed among 97.86% of them. Among the resistance-groups, 42.26% were doubly resistant, 29.81% were triply resistant, 22.09% were quadruply resistant and 3.70% were quintuply resistant. The occurrence of ACT (27.44%) was most dominant among the different MAR combinations. Of the antibiotic resistant bacteria in September 2001, 95.95% exhibited multi-resistance phenotype. Of them 33.12% were triply resistant, 27.7% were quadruply resistant, 27.42% were doubly resistant and 7.71% were quintuply resistant. ACT (24.28%) was the most common ARP found among the multi-resistant isolates.

The maximum occurrence of singly resistant bacteria toward ampicillin was noted in January 2000. The occurrence of bacteria resistant to only chloramphenicol was highest in the month of January 2001 while isolates resisting only ampicillin was least occurring. The number of bacteria exhibiting only tetracycline resistance was highest in November 2000. Although

kanamycin appeared in different multi-resistance combinations, populations exhibiting only kanamycin resistance or kanamycin-streptomycin in combination were completely absent. Among the double resistant combinations, AT was the most frequent one showing highest occurrence in December 2001, followed by November 2001 and May 2000 samplings. During October 2000 and 2001, only 1.06% and 2.09% of the population exhibited this phenotype. The second highest occurrence was noted for AC. ACT represented the maximally occurred ARP among the combinations of three different antibiotics. The highest occurrence of this pattern was noted in March 2001. 44.74 and 40.0% of the population during May 2000 and 2001 exhibited the said phenotype.

1.3.5.2 Analysis of ARPs of the antibiotic resistant bacteria from SS II

From sampling site II, a total of 4429 antibiotic resistant copiotrophic bacterial isolates from 13 water samples were screened (Table 1.6). The distribution of singly, doubly, triply, quadruply and quintiply resistant bacteria have been presented in Figure 1.3. Altogether 9.73% of the screened population exhibited single resistance of which chloramphenicol resistance was most frequent (6.29%), followed by ampicillin (1.80%), tetracycline (0.69%), streptomycin (0.51%) and kanamycin (0.40%) resistance. Among 23 possible different ARPs, combinations like CK, KT and CKST were completely absent. 32.89% of the MAR population exhibited resistance to two different combinations of antibiotics. Within this group, most frequent occurrence was observed for the combination AC (20.61%). The frequencies of occurrence of other doubly resistant combinations were 7.83% for AT and 3.68% for the CT. 29.69% of the populations were triply resistant of which 24.02% exhibited ACT phenotype. ACKT (6.11%) was the most frequent pattern observed within the quadruply resistant group.

During January 2000, 145 antibiotic resistant bacteria were screened. Among the screened population, 40.0% were triply resistant, 39.29% were singly resistant and 20.67% were doubly resistant. Quadruply and quintuply resistant combinations were not found. Singly resistant group was represented by 39.28% of the population, whereas, 60.67% of the population were multi-resistant. In this month four different combinations of MAR phenotypes were observed of which the ARP like ACS (40.0%) was the most dominant. During January 2001, a total of 338 bacteria were screened. In the said month, among the bacterial isolates, ARP of five different antibiotics was not present. Chloramphenicol resisting populations were the sole representatives of the singly resistant group. Among the multi-resistant group, 44.65% were doubly resistant, 32.83% were triply resistant and 2.95%

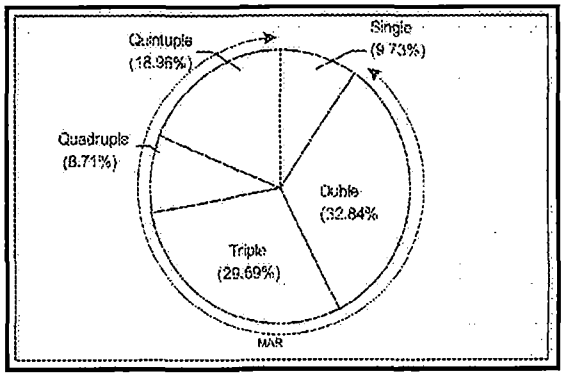


Figure 1.3. Frequency of singly resistant and MAR phenotypes among antibiotic resistant copiotrophic bacterial isolates from SS II.

Table 1.6 Antibiotic resistance patterns (ARPs) of the antibiotic resistant copiotrophic bacterial populations in different sampling months (January 2000 to December 2001) at SS II

	Jan. 2000	May 2000	Oct. 2000	Nov. 2000	Dec. 2000	Jan. 2001	Mar. 2001	May 2001	June 2001	Sep. 2001	Oct. 2001	Nov. 2001	Dec. 2001
A	13.79 (20)	1.47(15)		2.88 (3)			5.20 (14)	2.13 (7)	4.12 (9)	0.63 (2)	1.39 (8)	0.46 (2)	
C	24.82 (36)	0.49(5)		10.57 (11)	100.0 (62)	19.52 (66)				2.22 (7)	4.34 (25)	6.29 (27)	33.33 (40)
K											1.73 (10)	1.86 (8)	
S	0.68 (1)	1.08 (11)					1.48 (4)			1.58 (5)			1.66 (2)
T		0.29 (3)		2.88 (3)				0.60 (2)	2.29 (5)			2.56 (11)	5.83 (7)
AC	14.48 (21)	0.029(3)	73.52 (375)	55.76 (58)		5.91 (20)		29.26 (96)			6.95 (40)	61.7 (265)	29.16 (35)
AK											0.17 (1)		
AS							0.74 (2)				0.52 (3)		
AT		3.63 (37)		3.84 (4)			25.27 (68)	21.03 (69)	40.82 (89)		8.0 (46)	7.92 (34)	
CK													
CS	5.51 (8)	0.09 (1)	0.39 (2)			0.88 (3)							4.16 (5)
CT	0.68 (1)	0.09 (1)		7.69 (8)		37.86 (128)			11.46 (25)				
KS							1.48 (4)						
KT													
ST			0.58 (3)				0.74 (2)						
ACK										7.93 (25)			0.83 (1)
ACS	40.0 (58)		0.78 (4)			12.72 (43)							1.66 (2)
ACT		3.44 (35)	20.58 (105)	11.53 (12)		16.27 (55)	40.14 (108)	41.15 (135)	35.77 (78)	34.92 (110)	57.39 (330)	17.71 (76)	16.66 (20)
AKT		0.29 (3)					5.57 (15)	1.52 (5)					
AST		0.19 (2)	0.19 (1)				9.29 (25)			5.71 (18)			
CKT						0.29 (1)	1.85 (5)						
CST			1.96 (10)			3.55 (12)	7.43 (20)	0.30 (1)					
ACKS		0.09 (1)											
ACKT		12.58 (128)						2.43 (8)		30.47 (96)	6.26 (36)		2.5 (3)
ACST		0.88 (9)	1.37 (7)	1.92 (2)		2.95 (10)			4.58 (10)	11.74 (37)	2.60 (15)	0.46 (2)	
AKST											3.82 (22)		
CKST													
ACKST		74.92 (762)	0.58 (3)	2.88 (3)			0.74 (2)	1.52 (5)	0.91 (2)	4.76 (15)	6.78 (39)	0.93 (4)	4.16 (5)
Total	145	1016	510	104	62	338	269	328	218	315	575	429	120

were quadruply resistant. CT (37.86%) was the most dominant MAR phenotype.

In the month of May 2000, 74.92% of the total screened population (1016 isolates) exhibited resistance to all the five antibiotics tested. Among the other multi-resistance combinations, quadruply resistant group were represented by 13.55% of the population, 3.92% were triply resistant and 3.81% were doubly resistant. Within the singly resistant group (represented by 3.33% of the population), populations exhibiting resistance to only ampicillin, chloramphenicol, streptomycin and tetracycline were present. During May 2001, 328 antibiotic resistant bacterial isolates were screened. Among them 50.29% were doubly resistant, 42.97% were triply resistant, 2.73% were singly resistant, 2.43% were quadruply resistant and only 1.52% were quintuply resistant. The populations exhibiting resistance to ACT (41.45%) in combination were the most frequent ones. The ARPs like AK, AS, CK, KS, KT, ST, ACK, ACS, CKT, AKST and CKST in the MAR population were absent both during May 2000 and 2001.

The numbers of antibiotic resistant bacteria screened for multi-resistance in the month of October of 2000 and 2001 were 510 and 575 respectively. In October 2000, cent percent of the screened population exhibited multi-resistance, dominated by the occurrence of doubly resistant combinations (74.49%). AC (73.52%) represented the most frequent ARP among doubly resistant MAR isolates. Among the other MAR groups, 23.51%, 1.37% and 0.58% were triply, quadruply and quintuply resistant respectively. A different picture was observed in October 2001. The triply resistant combinations (57.39%) dominated the multi-resistance group. 15.64% represented the doubly resistant group, 12.68% were quadruply resistant and 6.78% were quintuply resistant. The isolates having ACT (57.39%) combinations were more frequent. The populations exhibiting resistance to only ampicillin, chloramphenicol and kanamycin were present but only streptomycin and tetracycline resisting populations were not found. During both the years, ARP like CK, CT, KS, KT, ACK, AKT, CKT, ACKS and CKST among MAR bacteria were absent.

In November 2000, 104 isolates were screened. 16.33% of the screened populations were resistant to a single antibiotic. The population resisting only chloramphenicol (10.57%) was the most dominant among the singly resistant group. Incidences of doubly, triply, quadruply and quintuply resistant bacteria were 67.29%, 11.53%, 1.92% and 2.88% respectively of the screened population. AC (55.76%) and ACT (11.53%) were most frequent ARPs among the doubly and triply resistant combinations. In November 2001, 429 bacteria were screened- of which 88.72% were multi-resistant. Among the multiresistant group, 69.62% were doubly resistant, 17.71% were triply resistant, 0.46% was quadruply resistant and 0.93% was quintuply resistant. The AC (61.7%) combination was the most frequent ARP among MAR isolates. In water samples of November 2000 and 2001, populations exhibiting resistance to only ampicillin, chloramphenicol or tetracycline were present. The isolates exhibiting only kanamycin resistance were found in November 2001 but not in 2000. Isolates exclusively resistant to streptomycin were absent in both the years in the month of November.

In December 2000, the entire screened population (a total of 62 isolates) exhibited resistance singly to chloramphenicol. A completely different picture of antibiotic resistance combinations was found during December 2001. In the said month, a total of 120 antibiotic resistant bacteria were screened. Singly resistant isolates was found among 40.82% of the screened population. Although, isolates exhibiting resistance singly to chloramphenicol was the most dominating one (33.33%), small fractions of the population also exhibited resistance singly to streptomycin and tetracycline. Multi-resistance was observed among 59.13% of the screened population. Among them, 33.32% were doubly resistant, 19.15% were triply resistant, 2.5% were quadruply resistant and 4.16% were quintuply resistant. In this month seven different ARPs of MAR isolates were found. The most frequently occurring ARPs were AC (29.16%) and ACT (16.66%).

In the month of March 2001, a total of 269 antibiotic resistant bacterial isolates were screened to check their antibiotic resistance profile. Among the singly resistant population (6.68%), only ampicillin resisting population was the most prevailing one. 64.28% of the screened population exhibited resistance to three different antibiotics, 28.23% were doubly resistant, 6.68% were singly resistant and only 0.74% was resistant to all the five antibiotics. No quintuply resistant populations were recovered. The most dominant ARP among MAR isolates was ACT (40.14%), followed by AT (25.27%). In June 2001, 218 bacteria were screened. Among them 52.28% were doubly resistant, 35.77% were triply resistant, 6.41% were singly resistant, 4.58% were quadruply resistant and 0.91% was quintuply resistant. Among the most populous MAR groups, the frequency of occurrence of AT (40.82%) and ACT (35.77%) were exceedingly higher than the other combinations of the respective groups. In the month of September 2001, a total of 315 bacteria were screened, of which 48.56% were triply resistant, 42.21% were quadruply resistant, 4.76% were quintuply resistant and 4.43% exhibited resistance to any one of the tested antibiotics.

The maximum occurrence of singly resistant bacteria toward ampicillin was noted in January 2000. The occurrence of bacteria resistant to only chloramphenicol was highest in the month of December 2000. In that particular month there was complete absence of other antibiotic resistant phenotypes either singly or in multi combinations. The occurrences of only tetracycline resisting population were highest in December 2001. Very few bacterial isolates, compared to that of ampicillin or chloramphenicol resisting populations, exhibited single resistance to either kanamycin or streptomycin. The most dominated ARP among doubly resistant isolates was AC, showing highest occurrence in October 2000, followed by November 2001 and 2000. During May 2000 only 0.029% of the population exhibited this phenotype. The second highest occurrence was noted for AT (maximum in June 2001). Among the triply resistant group, ACT represented the most dominant ARP. The highest occurrence of this phenotype was noted in October 2001 and lowest in the month of May 2000. ACKT was the most frequent ARP among the quadruply resistant group.

1.3.5.3 Analysis of ARPs of the antibiotic resistant bacteria from SS III

A total of 4254 antibiotic resistant copiotrophic bacterial isolates from 13 water samples of SS III were screened for multiple antibiotic resistance phenotype (Table 1.7). The distribution of singly, doubly, triply, quadruply and quintuply resistant bacteria have been presented in Figure 1.4. Single resistance was exhibited by 304 isolates. Among the singly resistant group, resistance to Chloramphenicol (5.82%) was most frequent, followed by occurrence of isolates resistant to ampicillin (0.68%), tetracycline (0.32%), streptomycin (0.25%) and kanamycin (0.04%). Twenty-one different ARPs among MAR isolates were found. The combinations like CK and KS were absent. Among the most populous MAR group, doubly resistant, of SS III, the frequency of occurrence of AC (23.57%) was exceedingly higher than the other combinations of this group. The frequency of occurrence of AT was 16.12%. Likewise, ACT (20.54%) and ACST (9.44%) were the most frequent ARPs observed within the triply and quadruply resistant group. 5.33% of the population were quintuply resistant.

In the month of January 2000, a total of 75 antibiotic resistant bacteria from SS III were screened. Among the screened population, 54.66% were doubly resistant, 26.66% were singly resistant and 18.66% were triply resistant. The population resisting only chloramphenicol (20.0%) was the most prevailing one among the singly resistant group. Three different ARPs, AC (54.66%), ACT (14.66%) and ACS (4.0%) were found in this month. Hundred isolates were screened during January 2001. All of them were found to exhibit resistance either to a single or to two different antibiotics. The maximum percentage of the population exhibited resistance to only chloramphenicol (50.0%). A single ARP, AC (45.0%), was observed in the said month. It was found that, in January 2000 and 2001, the populations resistant to four or five different antibiotics were completely absent. Moreover, in January 2001, triply resistant populations were also absent.

In May 2000, a total of 364 isolates were screened. Cent percent of the screened population were multi-resistant. Isolates exhibiting resistance to four different antibiotics were most common among the multi-resistance group (represented by the 57.14%) of the population. ACKT (54.12%) and ACKST (32.96%) were the dominant ARPs among quadruply and quintuply resistant group. In May 2001, a total of 330 isolates were screened, of which 3.33% were singly resistant. The doubly resistant isolates constituted 54.22% of the population and 29.99% were triply resistant. The quadruply and quintuply resistant isolates represented 9.69 and 2.72% of the screened population. AC and AT combinations among the doubly resistant group and ACT combination among the triply resistant group were the dominant ones.

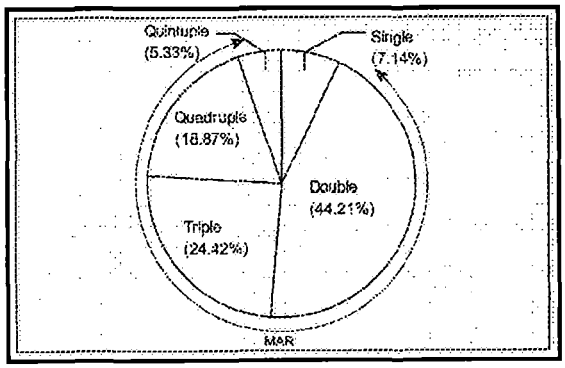


Figure 1.4. Frequency of singly resistant and MAR phenotypes among antibiotic resistant copiotrophic bacterial isolates from SS III.

Table 1.7 Antibiotic resistance patterns (ARPs) of the antibiotic resistant copiotrophic bacterial populations in different sampling months (January 2000 to December 2001) at SS III

	Jan 2000	May 2000	Oct. 2000	Nov. 2000	Dec. 2000	Jan. 2001	March 2001	May 2001	June 2001	Sep. 2001	Oct. 2001	Nov. 2001	Dec. 2001
A	4.0 (3)		0.93 (4)	1.07 (5)	0.44 (2)	5.0 (5)					0.81 (3)	2.02 (7)	
C	20.0 (15)		8.15 (35)	8.35 (39)	19.91 (89)	50.0 (50)		3.03 (10)		1.27 (5)	1.35 (5)		
K												0.28 (1)	0.43 (1)
S	2.66 (2)							0.30 (1)		0.25 (1)	1.08 (4)	0.86 (3)	
T			0.46 (2)						0.5 (2)	1.02 (4)	0.81 (3)	0.86 (3)	
AC	54.66 (41)	1.09 (4)	50.58 (217)	38.3 (179)	7.60 (34)	45.0 (45)		23.93 (79)	14.0 (56)	9.46 (37)	2.43 (9)	54.2 (187)	50.0 (115)
AK								4.84 (16)			0.81 (3)		
AS										4.34 (17)	1.89 (7)	4.34 (15)	
AT				10.06 (47)	7.82 (35)		28.66 (88)	23.03 (76)	24.5 (98)	17.64 (69)	30.60 (113)	18.84 (65)	41.30 (95)
CK													
CS			0.46 (2)	0.21 (1)						0.25 (1)			
CT			0.23 (1)		12.9 (58)			2.42 (8)	9.25 (37)	0.76 (3)	1.89 (7)		0.86 (2)
KS													
KT									1.0 (4)				
ST									2.5 (10)				
ACK		0.54 (2)		0.85 (4)						3.32 (13)		0.57 (2)	
ACS	4.0 (3)		0.23 (1)							0.25 (1)		0.57 (2)	
ACT	14.66 (11)	8.24 (30)	12.58 (54)	16.9 (79)	21.25 (95)		27.36 (84)	29.69 (98)	22.25 (89)	24.55 (96)	51.49 (190)	11.01 (38)	4.34 (10)
AKT							4.56 (14)						
AST				2.78 (13)	6.04 (27)		20.19 (62)						
CKT									4.5 (18)				
CST			0.46 (2)					0.30 (1)					
ACKS										0.51 (2)			
ACKT		54.12 (197)					5.86 (18)	7.27 (24)	8.0 (32)	14.06 (55)	1.62 (6)	3.47 (12)	
ACST		3.02 (11)	25.87 (111)	20.5 (96)	23.93 (107)				7.0 (28)	12.27 (48)	0.27 (1)		
AKST							10.09 (31)		2.0 (8)		1.89 (7)		
CKST								2.42 (8)			0.27 (1)		
ACKST		32.96 (120)		0.85 (4)			3.25 (10)	2.72 (9)	4.5 (18)	9.97 (39)	2.71 (10)	2.89 (10)	3.04 (7)
Total	75	364	429	467	447	100	307	330	400	391	369	345	230

A, Ampicillin; C,Chloramphenicol; K, Kanamycin; S, Streptomycin; T, Tetracycline
The numbers in parenthesis denotes the actual number of isolates

During 2000 and 2001, in the month of May, none of the isolates exhibited resistance to only kanamycin or tetracycline. The ARPs like AS, CK, CS, KS, KT, ST, ACS, AKT, AST, CKT, ACKS and AKST were absent in both the years during the same month. Although in May 2000, only six different ARPs among MAR isolates were found, nine different ARPs were noted in MAR isolates of May 2001.

A total of 429 and 369 isolates were screened for multiple-antibiotic-resistance in the month of October during the years 2000 and 2001 respectively. In October 2000, 51.27% of the populations were doubly resistant. The incidences of other resistance groups were: 25.87%, quadruply resistant; 13.27%, triply resistant; and 9.54% were singly resistant. Quintuply resistant populations were not found. AC (50.58%) and ACST (25.87%) were the most frequently occurring ARPs among MAR isolates. During October 2001, 51.49% of the populations were triply resistant, followed by next higher incidence of the doubly resistant group (represented by 37.62% of the population). Singly resistant isolates comprised 4.05% of the total antibiotic resistant isolates screened. The rest 4.05 and 2.71% of the population were the quadruply and quintuply resistant isolates. The most occurring ARP of the MAR isolates in the said month was ACT (51.49%) followed by AT (30.6%). Eleven different ARPs among MAR isolates were found in October 2001 whereas only seven ARPs were encountered in analysis of water samples of October 2000. Isolates exhibiting resistance to only kanamycin were absent in both October 2000 and 2001. The water samples in the month of October, in both the years 2000 and 2001, contained no isolates demonstrating ARPs like CK, KS, KT, ST, ACK, AKT, AST, CKT and ACKS.

In November 2000, 467 antibiotic resistant bacteria were screened. 90.45% of the populations were MAR. Among the MAR isolates, 48.57% were doubly resistant, 20.53% were triply resistant, 20.5% were quadruply resistant and 0.85% was quintuply resistant. The most frequent occurrences were encountered for the ARPs like AC (38.3%) and ACST (20.5%). A total of 345 isolates were screened during November 2001. Of the population 77.38% were doubly resistant, 12.15% were triply resistant, 4.02% were singly resistant, 3.47% were quadruply resistant and 2.89% were quintuply resistant. AC (54.2%) was the most frequently occurring ARP among the MAR isolates. During November 2000, there was complete absence of singly resistant isolates toward kanamycin, streptomycin or tetracycline. But in 2001, isolates resisting only chloramphenicol were absent. In two successive years, 2000 and 2001, complete absence of ARPs like AK, CK, CT, KS, KT, ST, AKT, CKT, CST, ACKS, AKST and CKST among MAR isolates were noted.

During December 2000, 447 bacteria were screened. Among them, 28.32% were doubly resistant, 27.29% were triply resistant, 23.93% were quadruply resistant and 20.35% were singly resistant. There was complete absence of the quintuply resistant population. The resistance to only chloramphenicol was dominant among the singly resistant group. Among the multi-resistance combinations, ACT (21.25%) and ACST (23.93%) were the most dominant ones. Among the 230 bacterial isolates that were screened during December 2001, 99.54% were MAR bacteria. Five different ARPs

were found among MAR isolates, of which AC (50.0%) followed by AT (41.30%) were the most frequent ones. During December 2000 and 2001, populations resisting only streptomycin or tetracycline were absent. Only ampicillin or chloramphenicol resisting singly resistant isolates were reported in December 2000 but not during December 2001. The isolates exhibiting resistance to only kanamycin were found in December 2001. Some of the ARPs like AK, AS, CK, CS, KS, KT, ST, ACK, ACS, AKT, CKT, CST ACKS, ACKT, AKST and CKST were not found in MAR isolates in water samples of December in two successive years, 2000 and 2001.

In March 2001, 307 bacteria were screened. Within the population, 52.11% were triply resistant, 28.66% were doubly resistant, 15.95% were quadruply resistant and 3.25% were quintuply resistant. The most dominant ARPs among MAR isolates were AT (28.66%), ACT (27.36%) and AST (20.19%). The singly resistant isolates were not present. Of the antibiotic resistant bacteria screened during June 2001, 99.5% were MAR. The MAR population consisted of 51.25% doubly resistant, 26.75% triply resistant, 17.0% quadruply resistant and 4.5% of the quintuply resistant population. AT (24.5%) and ACT (22.25%) were the most common ARPs found among the MAR isolates. A total of 391 antibiotic resistant bacteria were screened in September 2001. Among the MAR population, 32.45% were doubly resistant, 28.12% were triply resistant, 26.84% were quadruply and 9.97% were quintuply resistant. The most common ARP was ACT (24.55%). Only 2.54% of the populations were singly resistant.

The maximum occurrence of singly resistant bacteria toward ampicillin was noted in January 2001 and the lowest in October 2001. The occurrence of bacteria resistant to only chloramphenicol was highest in the month of January 2001 while in the same month isolates resisting only ampicillin was the least. Only 1.02% of the bacteria exhibited resistance singly to tetracycline in September 2001 and that was the highest for only tetracycline resisting population. Although kanamycin appeared in different multi-resistance combinations, isolates exhibiting only kanamycin resistance were too low and found only in the months of November 2001 and December 2001. Among the doubly resistant combinations, AC was the most frequent one. More than 50% of the screened populations, in the months of January 2000, October 2000, November 2001 and December 2001, exhibited this phenotype. The occurrence for AT combination was noted next to AC. Among the doubly resistant isolates, ARPs like CK and KS were absent. ACT was the most frequently occurring ARP among the triply resistant isolates. The highest occurrence of this pattern was found in October 2001. Maximum occurrence of quintuply resistant population was recorded in May 2000. During January 2000 to December 2000, maximum of eight different ARPs were observed among the MAR isolates. An increase in number of different ARPs was recorded from May 2001 to October 2001. Eleven to twelve different ARPs among MAR isolates were found during the said time frame.

1.3.6 Comparison of antibiotic resistant bacteria recovered from three sampling sites on river Torsa

The relative abundance of singly-resistant and multi-resistant (resistant to two or more antibiotics tested) bacteria in the pool of antibiotic-resistant-copiotrophs

(bacterial colonies picked up randomly from plates containing single antibiotic) from water samples collected in the same month of the two successive years, 2000 and 2001, from three different sampling stations (SS I, SS II, & SS III) on River Torsa have been presented in figures (Figure 1.5, 1.6, 1.7, 1.8, 1.9).

In the month of January 2000, in SS I singly resistant populations were dominant while in the next year triply resistant populations was most dominant. In January 2000, triply and quadruply resistant populations were completely absent while January 2001 was marked by the absence of quintuply resistant bacteria among antibiotic resistant bacteria. The water samples collected from SS II in month of January 2000 showed maximum incidence of triply resistant bacteria while in the next year doubly resistant populations was most dominant. In both the years, the SS II water samples were devoid of quintuply resistant bacteria among antibiotic resistant bacterial population. The water samples collected from SS III in month of January 2000 showed maximum incidence of doubly resistant bacteria while in 2001 singly resistant populations was most dominant. In both the years, the SS III water samples were devoid of quadruply as well as quintuply resistant bacteria among antibiotic resistant bacterial population. Though triply resistant bacteria could be isolated from the water samples of the same site in 2000 but it was absent in 2001.

In the month of May of both the years, 2000 & 2001, the water samples collected from SS I contained mostly triply resistant bacteria. The water samples collected from SS II in month of May 2000 showed

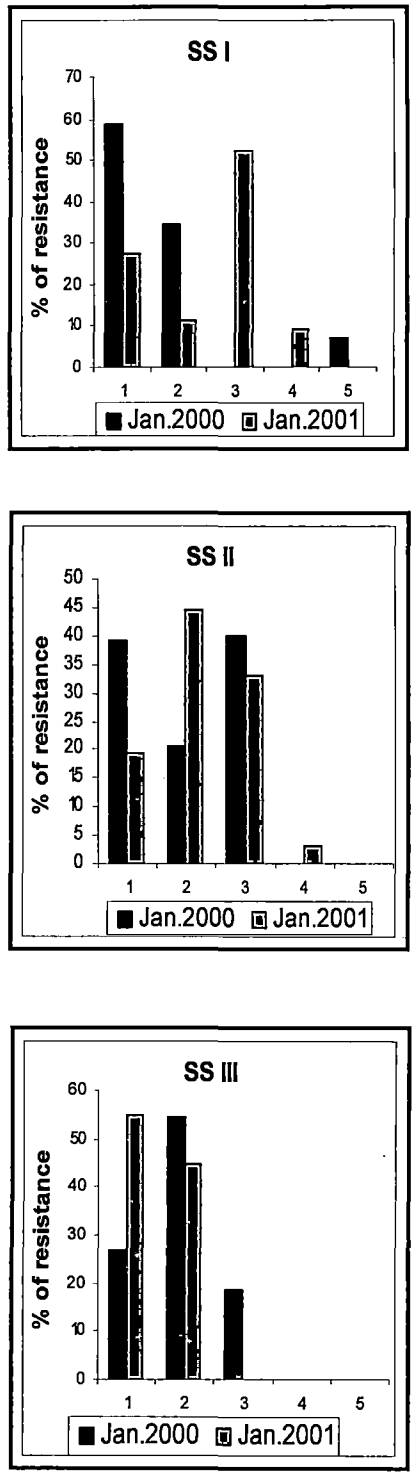


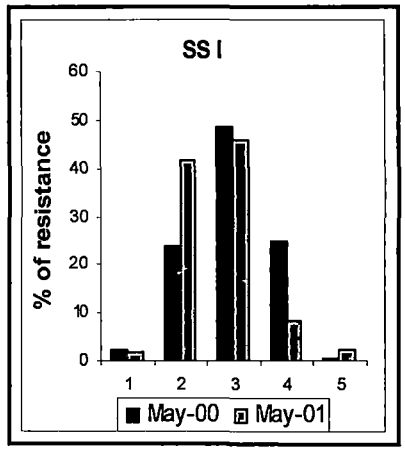
Figure 1.5. Relative abundance of singly resistant and multi-resistant bacteria in the pool of antibiotic- resistant copiotrophs from water samples collected in the month of January of the two successive years, 2000 and 2001, from three sampling stations [SS I, SSII and SS III] on river Torsa.
1, Singly resistant; 2, Doubly resistant; 3, Triply resistant; 4, quadruply resistant; 5, quintuply resistant.

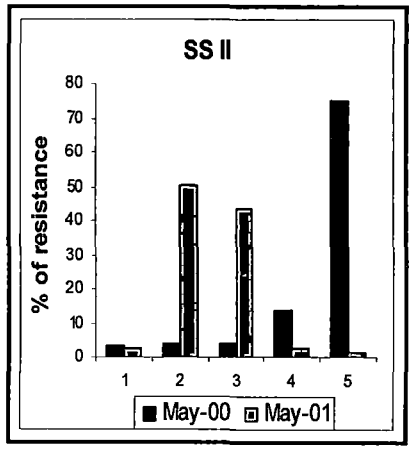
maximum incidence of quintuply resistant bacteria while in the next year doubly resistant populations was most dominant. The water samples collected from SS III in month of May 2000 showed maximum incidence of quadruply resistant bacteria while in 2001 doubly resistant populations was most dominant. In the year, 2000, the SS III water samples were devoid of singly resistant bacteria among antibiotic resistant bacterial population.

In the month of October 2000, in SS I triply resistant populations were dominant while in the next year quadruply resistant populations was most prevalent. The water samples, collected from SS II in month of October 2000, were marked by the absence of singly resistant and abundance of doubly resistant bacteria while in the next year triply resistant populations dominated. In the year 2000, the SS III water samples were devoid of quintuply resistant bacteria but dominated by doubly resistant among antibiotic resistant bacterial population. In the following year, 2001, triply resistant bacteria were most abundant.

In the month of November, triply and doubly resistant bacteria were most abundant in the year 2000 and 2001 respectively in the water samples collected from SS I. In both the years, doubly resistant bacteria were most dominant in water samples collected from both the sampling sites, SS II and SS III.

In the month of December, quadruply and doubly resistant bacteria were most abundant in the year 2000 and 2001 respectively in the water samples collected from SS I. The water samples of SS I in the month of December 2000 were devoid of singly and quintuply resistant bacterial





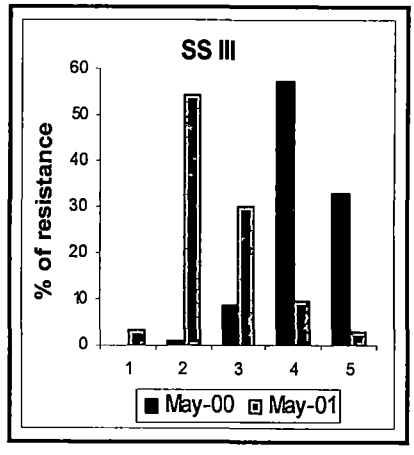


Figure 1.6. Relative abundance of singly resistant and multi-resistant bacteria in the pool of antibiotic- resistant copiotrophs from water samples collected in the month of May of the two successive years, 2000 and 2001, from three sampling stations [SS I, SSII and SS III] on river Torsa.
1, Singly resistant; 2, Doubly resistant; 3, Triply resistant; 4, quadruply resistant; 5, quintuply resistant

population The water samples, collected from SS II in month of December 2000, was marked by the sole presence of singly resistant and complete absence of bacteria resistant to two or more antibiotics, while emergence of multi-resistant bacteria was noted in the next year samples with dominance of singly resistant bacteria. In both the years, the SS III water samples contained maximum number of doubly resistant bacteria. Complete absences of quintuply and quadruply resistant bacteria were noted in the year 2000 and 2001 respectively, in water samples of SS III in the month of December.

1.3.7 Statistical analysis of data on singly and mutiply-antibiotic-resistant (resistance to two or more antibiotics tested) bacteria in the antibiotic-resistant population pooled from water samples collected from three different sampling stations, SS I, SS II, SS III, along the river Torsa

The data presented in the tables 1.5, 1.6 and 1.7 on percentage occurrence of singly, doubly, triply, quadruply and quintuply resistant groups were arcsine transformed before analysis and were rearranged as follows-

a. The percent occurrences of doubly, triply, quadruply and quintuply resistant groups were added together to form a group, tentatively termed as multi-resistant (treated as one variable) while percent occurrence of singly resistant was treated as another variable. Correlation coefficient (r) of the variable pair (singly and multi-resistant) revealed a strong negative correlation from the data of SS I (r= - 0.958) and SS III (r= -1.0). The correlation coefficient obtained for the SS III was - 0.458.

b. The existing data of singly, doubly, triply, quadruply and quintuply resistant

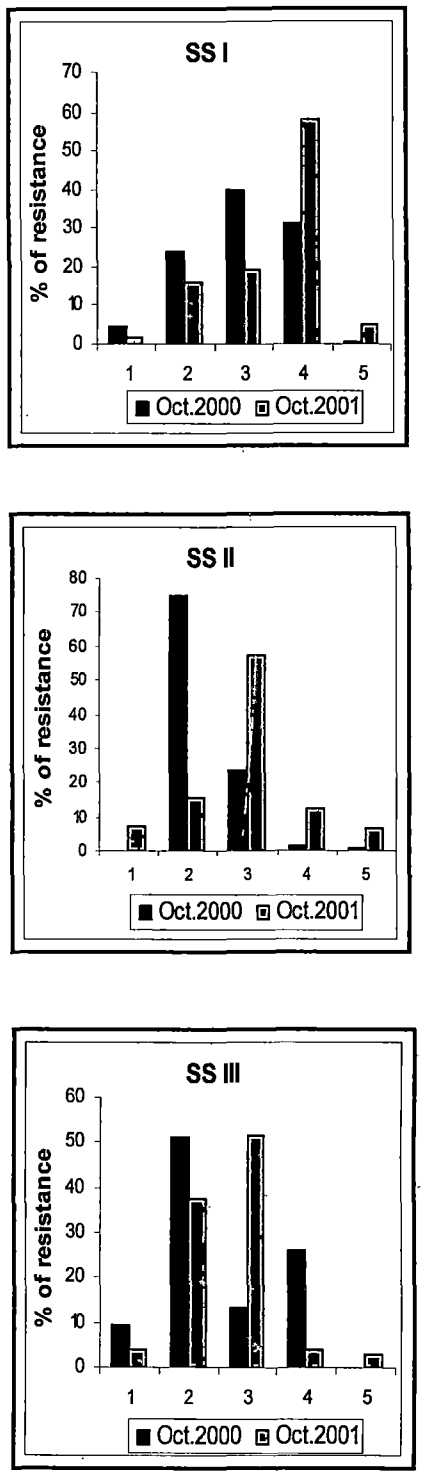
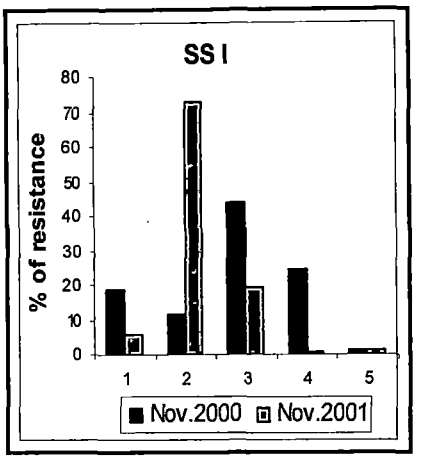


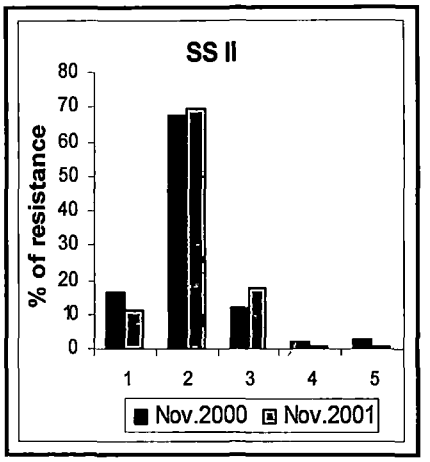
Figure 1.7. Relative abundance of singly resistant and multi-resistant bacteria in the pool of antibiotic- resistant copiotrophs from water samples collected in the month of October of the two successive years, 2000 and 2001, from three sampling stations [SS I, SSII and SS III] on river Torsa.
1, Singly resistant; 2, Doubly resistant; 3, Triply resistant; 4, quadruply resistant; 5, quintuply resistant

groups were treated as separate variables. Significant negative correlation coefficient(s) between occurrences of (i) Singly resistant with quadruply- resistant (r = -0.595), (ii) doubly-resistant and Quadruply-resistant (r = -0.627) proportions of antibiotic resistant bacteria were observed from the data of SS I.

In case of the data derived from water sample analysis of SS II, no significant correlation coefficient was obtained amongst the different proportions of antibiotic resistant bacteria screened for multiple-antibiotic-resistance. The data derived from water sample analyses of SS III revealed significant negative correlation coefficient(s) between the occurrence of doubly and quadruply-resistant (r = - 0.742), and singly and quintuply-resistant (r = - 0.693); while quadruply and quintuply-resistant bacterial proportions showed positive correlation coefficient (r = + 0.589).

The Wilcoxon matched pairs signed ranks test was used to compare the proportion and test the significance of the difference in abundance of the MAR groups. The significant difference in recovery of proportion of matched pairs were doubly and quintuply($p< 0.002$), triply and quintuply ($p< 0.003$), and quadruply and quintuply ($p< 0.007$) resistant bacteria in water samples of SS I. The occurrence of the matched pairs, singly and doubly, singly and triply, doubly and triply, and triply and quadruply resistant bacteria were significantly different with the probability < 0.003, < 0.002, < 0.002, and < 0.004 respectively, in water samples of SS II. There was significant difference in recovery of proportion of singly and doubly ($p< 0.002$), doubly and quintuply ($p< 0.004$), and triply and quintuply ($p< 0.006$)





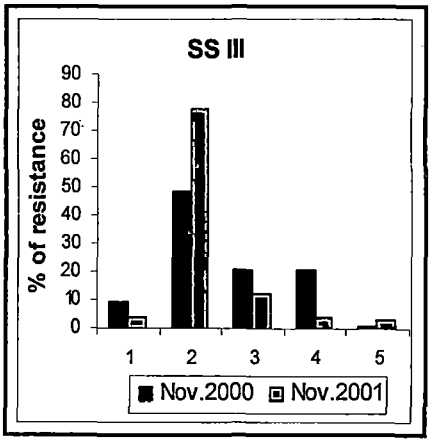


Figure 1.8. Relative abundance of singly resistant and multi-resistant bacteria in the pool of antibiotic- resistant copiotrophs from water samples collected in the month of November of the two successive years, 2000 and 2001, from three sampling stations [SS I, SSII and SS III] on river Torsa.
1, Singly resistant; 2, Doubly resistant; 3, Triply resistant; 4, quadruply resistant; 5, quintuply resistant

resistant bacteria in water samples of SS III.

1.4 Discussion

The reserves of water on the earth are immense, but this is mostly salt water which is unfit for drinking and irrigation purposes. Freshwater is the liquid of life, without which the planet would be a barren wasteland. The amount of fresh water is large as well but its distribution over the globe is uneven. With the increase in population, there is growing demand for fresh water supplies all over the world. Globally between 12.5 and 14 billion cubic meters of water are available for human use on an annual basis. In 1989 this amount equaled about 9000 cubic meters per person per year and by 2000 had dropped to around 7800 cubic meters per person. In 2025 the amount of per capita expected to fall to 5100 cubic meters per person as the worlds population grows from 6 billion to over 8 billion (www.peopleandplanet.net). Even this amount would be enough to meet human needs with the even distribution of fresh water resources. But available fresh water supplies are not distributed evenly around the globe throughout the seasons or from year to year.

Rivers are the main sources of fresh water. There are no national waters in the rivers that travel through several countries. They are constantly moving, while the nations can divide land, they can never divide waters running above it. River waters do not mind political or national boundaries. Demographic explosion, urbanization, unplanned development, land degradation and lack of infrastructure for waste disposal are leading to a rapid deterioration in water quality in the

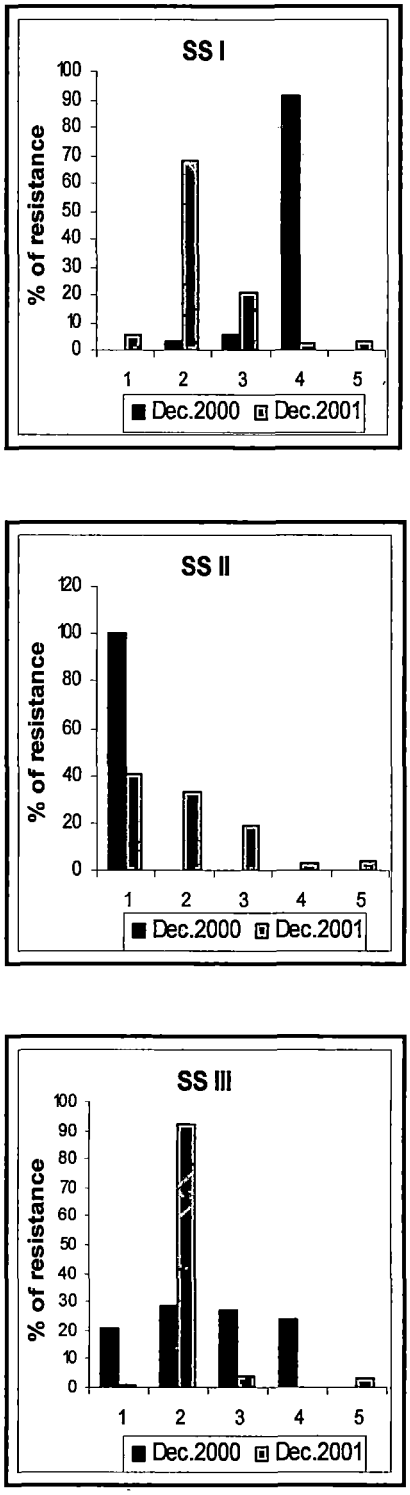


Figure 1.9. Relative abundance of singly resistant and multi-resistant bacteria in the pool of antibiotic- resistant copiotrophs from water samples collected in the month of December of the two successive years, 2000 and 2001, from three sampling stations [SS I, SSII and SS III] on river Torsa.
1, Singly resistant; 2, Doubly resistant; 3, Triply resistant; 4, quadruply resistant; 5, quintuply resistant

majority of rivers all over the world. This poses a threat both to the environment and to the health of the people in the region. Pollution of rivers and lakes reduces accessible fresh water supplies. In developing countries 90% of the sewage is being discharged directly into rivers, lakes, coastal waters without any treatment (World Resource Institute, 1996) (www.wri.org). Each year roughly 450 cubic meters of wastewater are discharged into rivers, streams and lakes. To dilute and transport this dirty water before it can be used again, another 6000 cubic kilometers of clean water are needed – an amount equal to about two thirds of the worlds total annual usable fresh water runoff (www.peopleandplanet.net).

The trends, in the contamination of river water world wide, have changed greatly over time. The fecal and organic pollution from untreated wastewater was the major contamination problem 100 years ago. In most industrialized countries, fecal contamination of water has been largely eliminated, however, in much of the world especially in cities in developing countries, organic pollution is still a problem. New pollution problems, particularly from agricultural runoff and industrial effluents are increasing in both industrialized and developing countries. In rapidly industrializing countries like China, India, Mexico, Brazil untreated sewage and industrial waste create substantial pressures on water quality that are much greater than the problems of the past.

In the recent years, emphasis is on the so-called emerging contaminants including pharmaceuticals such as antibiotics, endocrine disrupters and on various additives. Human use pharmaceuticals enter sewage effluents through improper disposal from private households and from hospitals. Direct inputs into natural waters are also possible during rain events and this normally occurs in less industrialized countries. In wastewater treatment plants the antibiotics are only partially eliminated and residual amounts can reach ambient waters and ground water. Most pharmaceuticals are found in natural waters in only very low concentrations. Antibiotics are of particular interest because we do not know currently whether their presence in natural waters contributes to the spread of antibiotic resistant organisms. Subsequent knowledge regarding the effect of sub-inhibitory concentrations of the antimicrobials on the survival of bacteria in environment is scarce and contradictory. But on the other side voluminous evidences are there which revealed the existence of antibiotic resistant bacteria in nature and horizontal transfer of antibiotic resistance determinants between them. Therefore there is a great dilemma between the choices of the major determinant for the stable maintenance of antibiotic resistant bacterial populations in different environmental compartments. Whatever the reasons for antibiotic resistance, such traits are apparently found in many bacteria and in different environments.

In environmental settings polluted by human and animal waste or both, high frequencies of MAR isolates exist in the coliform and fecal coliform population. These environments include surface waters receiving runoff from lands occupied by livestock, polluted estuaries and contaminated water supplies. Fluvial waters receive human and animal wastewater discharges, which are expected to contain antimicrobial agents

likely to exert a selective pressure, and commensal resistant bacteria, capable of transferring their resistances to autochthonous bacteria. Consequently, the fresh water indigenous flora may become a reservoir for antimicrobial resistance genes, and the reuse of these waters for humans and animals may contribute to the limitation of antimicrobials efficiency. Any body of water that receives human waste products can be studied for its content of antibiotic resistant bacteria. Beyond human use of antibiotics, there are a number of other sources that may shoulder part of the blame for high resistance levels. Resistance can come from the natural production of antibiotics by organisms in the soil. It may also result from antibiotic-contaminated runoff from animal feed or crops, or wastes from farm animals (Ash *et al.* 2002). It was shown in Greece that some resistant bacteria came from the feces of seabirds or warm-blooded mammals that live near the coastal waters (Aravanitidou *et al.* 2001). Studies have also been performed within the animal production industry to show the impact of antibiotic resistance. For example, fish farms routinely treat bacterial infections in the fish with the use of antibiotics. These antibiotics are released into the water. They then can move downstream, unfiltered and untreated by the fish farms (Schmidt *et al.* 2000). Occurrences such as this allow for an increased ability of bacteria to develop a resistance.

The distribution of antibiotic-resistant strains in the aquatic environment has been studied in different parts of the world. Majority of the investigations focused on the antibiotic resistance patterns of the fecal coliform bacteria because of their use as pollution indicators and association with disease causing genera of importance to public health and hygiene. However, in many freshwater systems, fecal bacteria are of little numerical significance in spite of the fact that they are released into almost all inland waters. It is also not uncommon to find standard plate count bacteria (SPC) in drinking water at frequencies more than 10,000 times the frequency of coliforms. Earlier studies have reported the occurrence of high frequencies of antibiotic resistant organisms within the SPC populations (Armstrong *et al.* 1982). There is evidence that SPC bacteria in marine and freshwater environments can possess the same kinds of antibiotic resistance patterns as total and fecal coliform populations. To date, little work has been done to assess the prevalence of drug resistant bacteria in surface waters mainly rivers. Antibiotic resistance of the native bacterial population other than those of fecal origin must be considered for the precise assessment of the environmental pool of bacterial antibiotic resistance. Several groups have considered the whole bacterial populations, gram-negative bacteria, heterotrophic bacteria or viable bacteria and dealt with global antibiotic resistance (the frequency of cells able to grow on antibiotic supplemented media). In the present study, an intensive bacteriological investigation was made of the incidence and abundance of antibiotic resistant copiotrophic bacteria in water samples of the river Torsa.

In this study, water samples were collected from three sampling stations (shown in Figure 1.1). At the first sampling site (SS I) the river Torsa enters into Indian territory from Bhutan, and thereby analysis of water quality at this place throws light on different anthropogenic

activities influencing the water quality of Amo-Chu (synonym of Torsa at Bhutan) and also helps to understand the quality of water that flows within the Jaldapara Wild Life Sanctuary of North Bengal, which is the immediate destination of the river. At this sampling site the river is very turbulent, flows at high speed through pebbles and the river basin is around 700 meter wide. After SS I, the river is alienated into small channels and enters into Jaldapara Wildlife Sanctuary; serving as one of the source of drinking water for the wild life and forest tribes. After leaving the sanctuary, small channels of the river again fuse and form two large and wide branches, at village- Falakata of Jalpaiguri district, named Sil-torsa and Buri-torsa. Siltorsa is the major and wider (890 m) stream and second sampling station (SS II) was located at this point. At this site, the river is wide and less turbulent. The riverbank is made up of dry white sand. The entire hinterland of Torsa catchments between SS I and SS II sustains mainly the agricultural fields including tea gardens and small hamlets of human habitations. After leaving SS II, the tributaries, Siltorsa and Buritorsa, meet at Coochbehar Town; district Coochbehar, of the state of West Bengal, India. Here was the third sample collection site, SS III, where the depth of the river increases and flows slowly. Here the riverbank is made up of clay, sand and humus. Compared with the other sampling sites, the load of urban effluents on river water is quite high at this point as is evidenced from the results of the previous study. It was expected that frequency of resistance in the bacterial assemblages would be greater among the population isolated from the site with the putatively greatest human impact. In a previous study conducted on Yarra river of Australia, seven sites were used which has headwaters in the central highlands of Victoria and flows through forests, rural farmland and finally, through urban and inner city of Melbourne before discharging to the sea. It was anticipated that the incidence of antibiotic resistance in native bacteria isolated from polluted sites (such as river stretches in well developed urban areas, sewage treatment effluents) would be greater than the incidence in native bacteria isolated from pristine sites, such as drinking water catchments closed to public access and upstream reaches of rivers situated in forested uplands or flowing through areas of low intensity agriculture (Boon and Cattanach 1999).

Thirty-nine water samples were collected altogether and analyzed between January 2000 to December 2001 from the three sampling stations on the river Torsa. Standard plate count was made to assess the general copiotrophic bacterial content of the water samples collected from the three sampling stations. For the cultivation of bacteria, Luria Bertani agar plates were used. The recovered bacteria did not represent all the bacterium present in water but only those able to grow copiously and produce visible colonies on the nutrient rich media used and under prescribed condition of temperature and incubation. The cultivable bacteria represent only a small portion of the vast number of bacteria present in the environment and that the number that can be enumerated is higher than can be cultivated. It has been observed that the known and cultivable environmental bacteria add up to only 5-10% of the total number assumed to be present in waste water and waste water treatment plants. Use of classical microbiological methods can lead to the cultivation and Identification of only 1% of the soil

bacteria (Kummerer 2004).

The maximum recovery of the culturable copiotrophic bacterial populations took place mainly in the month of May in both the years from all three sampling sites. The minimum occurrence of the population was recorded during the months of January and March, in all the three sampling stations. The general population of bacteria in surface water may include some genera that could, under special circumstances, contribute a health risk. Although copiotrophic bacterial counts is not a true indicator of potential pathogens that might be present. It seems reasonable to assume, however, that chance occurrences are proportionately greater as the copiotrophic bacterial population increases. A wide range of variation in the occurrence of recovered bacterial population was observed. It is likely that tremendous fluctuations occur within the river water populations as related to season, temperature, turbidity, total organic carbon and chemical content and this probability accounts for much of the variability that have been observed in regard to the recovery of the culturable copiotrophs obtained from three different sampling sites. We found that the culturable antibiotic resistant bacteria were widespread in water samples from three different sampling sites located on river Torsa.

In the second step, the isolates were classified according to their susceptibility to a certain antibiotic. Five different antibiotics, namely ampicillin, chloramphenicol, kanamycin, streptomycin and tetracycline were used singly in nutrient rich solid medium to recover the fraction of the copiotrophs that could withstand the vulnerability of the specific antibiotic and could be termed as resistant to that particular antibiotic. The fractions resisting ampicillin and chloramphenicol were recovered maximally from the water samples of all the three sites. The percent occurrences of kanamycin resisting populations were recorded to be the lowest among all other antibiotic resisting populations. It was observed that in all the water samples analyzed, in the months where maximum recoveries of the ampicillin or chloramphenicol resisting populations were recorded, the occurrences of other four antibiotic resisting populations were quite low.

The data on percent occurrence of the different antibiotic resistant bacterial populations were arcsine transformed before performing different statistical analyses. The arcsine transformation, also known as angular transformation, is especially appropriate to percentages and proportions. The arcsine transformation finds $\theta=\arcsin\sqrt{p}$, where p is a proportion. The term arcsin is synonymous with inverse sin or $\sin^{-1}$, which stands for the angle whose sine is the given quantity. The arcsine transformation stretches out both tails of distribution of percentages or proportions and compresses the middle. When the percentages in the original data fall between 30 and 70%, it is generally not necessary to apply the arcsine transformation. In the present study data fell between 0.03 and 89.28%, and therefore arcsine transformation was applied to stabilize the data set.

Trend is the smooth, regular and long term movement of time series exhibiting the basic tendency of growth, decline or stagnation over a period of time. It was observed that fraction of population exhibiting resistance to ampicillin or

kanamycin or tetracycline have shown similar trends of increase in the sampling sites, SS I and SS III. The water samples analyzed from all the three sampling sites, SS I, II, and III, did not have any significant difference in occurrence of the proportion of ampicillin and chloramphenicol resistant fraction of the culturable copiotrophs of River Torsa. Analyses of Wilcoxon matched pairs signed ranks test revealed significant difference(s) in occurrence, irrespective of the sampling sites, for following pairs: Kanamycin and ampicillin, streptomycin and ampicillin, tetracycline and ampicillin, kanamycin and chloramphenicol, streptomycin and chloramphenicol, and tetracycline and chloramphenicol, antibiotic resistant fractions of copiotrophic bacteria.

Analysis of variance (ANOVA) was conducted to test – 1) relation between sampling site and fraction of copiotrophs resistant to different antibiotics, 2) to test whether seasonal variations in percent occurrences for antibiotic resistant populations were evident and 3) to observe any significant differences in occurrence of particular antibiotic resistant population in three different seasons or sites. Significant differences ($p < 0.05$) between the sampling sites SS I and SS II were noted in the months of May 2000, December 2000 and March 2001. In the other sampling months, no significant differences were scored between the sampling sites. Antibiotic resistant populations exhibited significant differences in occurrence in various sampling months. It was observed from this analysis that percent occurrence of ampicillin and chloramphenicol differed significantly from other antibiotic resistant populations.

This investigation also documents the occurrence of multiple antibiotic resistant (MAR) copiotrophic bacterial isolates in river water of Torsa. Multiple-antibiotic-resistance (MAR) profile of the recovered copiotrophic bacterial population on plates containing single antibiotic was obtained by the replica plating technique against five antibiotics. It was found that more than 90% of the antibiotic resistant bacteria obtained from each sampling station were MAR. The percent occurrence of population exhibiting resistance to only one antibiotic was in the range of 5.04-9.73%. Lin *et al.* (2004) studied the antibiotic resistance profiles of the enteric bacteria isolated from the Mhlathuze catchment and found that 94.7% of these isolates were resistant to at least one class of antibiotic while 75.2% were multi resistant. However, Park *et al.* (2003) showed that 53.6% of coliform isolates of an aquatic environment were resistant to one or more antibiotics tested. Ampicillin as one of the constituent in the ARPs happened to occur in 90.10%, 84.84% and 88.91% of the total MAR bacteria analyzed from SS I, SS II, and SS III respectively. Similarly, occurrence of chloramphenicol in the ARPs of MAR population were 72.98% in SS I, 80.04% in SS II, and 71.22% in SS III water samples. The maximum occurrence of ampicillin and chloramphenicol as a constituent in the ARPs of the MAR bacteria actually supported the insignificant difference in occurrence of ampicillin and chloramphenicol resistant fractions in Wilcoxon matched pairs signed ranks test. Presence of high percentage of ampicillin resistant bacterial populations indicated that beta-lactamase gene might be widely present in the gene pool of microbes in the environment. The analysis of antibiograms revealed that the MAR bacteria were in a

dynamic state of fluctuation within the aquatic environment. An examination of MAR bacteria isolated from three different sampling sites revealed striking differences in types and frequencies. Such observation is comparable to the results of an earlier study (Armstrong *et al.* 1981) where striking differences were observed for the types and frequencies of MAR bacteria isolated from two nearby sites. But the observation was quite different from the previous work conducted by Lin *et al.* (2004) on Mhlathuze river. They found that antibiotic resistance profiles of *E.coli* and non-*E. coli* isolates were similar regardless of the site. There was no clear pattern of antibiotic resistance in the antibiotic resistant bacterial population isolated from water samples of the river Torsa during January 2000 to December 2001. Population fluctuation was also observed at a single site sampled on a month-to-month basis. Variations were observed in the percentage of MAR organisms recovered and the resistance markers selected. Similar observation was made by earlier authors (Boon and Cattanach 1999) who found no distinct pattern to antibiotic resistance in the native bacteria isolated from the various river sites of Yarra river in Australia. It was observed that maximum percent of multiple antibiotic resistant bacteria were isolated from downstream (SS III) which is mainly urban than those from the upstream point (SS I and SS II) that is predominantly rural. The results suggest that urban effluents may have impact on the level of antibiotic resistance in the environment.

Correlation analysis between the singly and multi-resistant groups revealed whether any systematic or nonsystematic variations exist between the different MAR groups. A high correlation indicates systematic variation while a low correlation indicates nonsystematic variations between antibiotic resistance patterns of the bacterial population. Significant negative correlation was scored between the singly and quadruply resistant and doubly and quadruply resistant MAR population of SS I. It means that increase in occurrence of singly or doubly resistant groups will lead to the decrease in occurrence of the quadruply resistant population. Very low correlation was scored between the other antibiotic resistant populations from the same site. No significant correlation was scored between the antibiotic resistant populations recovered from water samples of SS II, which led to conclude that nonsystematic variation took place between the different antibiotic resistant groups. Significant negative correlation between doubly and triply resistant and singly and quadruply resistant groups was scored among the recovered antibiotic resistant population of the SS III. A positive correlation was observed among the quadruply and quintuply resistant groups, meaning that increase in occurrence of one group would be leading to the simultaneous increase of the other group. The values obtained from the correlation matrix revealed that antibiotic resistances of the culturable copiotrophs were random rather systematic in their occurrence. Such results were quite different from the results of the earlier findings where systematic variations were scored among the antibiotic resistances for the bacterial isolates recovered from different environmental sources (Kelch and Lee 1978).

The comparison of the percentage of resistant strains with published work from

other times and places are complicated because different groups have used different numbers and kinds of antibiotics in their studies. It is difficult to provide a single consistent picture and to make a comparison between the results obtained in this study with the results of previous works. Because the factors, that affect the survival and proliferation of antibiotic resistant populations, change with respect to land and water use pattern in different regions of the same country as well as between different countries. If the incidence of resistance to antibiotics and synthetic antimicrobial drugs is to be compared in different areas and at different times, it is necessary to identify the bacteria and to use the same set of antimicrobial drugs in the tests.

1.5 Conclusion

The problem of antibiotic resistance is a global one. Global surveillance are needed to look after antibiotic use and the residence of resistant strains, since genetic fluidity of bacteria allow them to cross national and international boundaries. It is essential to identify the ecological nature of the problem and to concentrate on the kinds of resistances in the so called reservoirs – both commensal and the non-clinical bacteria. Because the data going to generate from this background will forecast about the commencement of the next resistance story and will also identify the site where antibiotic selection pressure for resistance is high. Studies have found that rivers have become major reservoirs of antibiotic resistance genes (Park *et al.* 2003 and Biyela *et al.* 2004). Finding antibiotic resistant bacteria in rivers is hardly novel. What has not been appreciated is the extent of contamination. A noteworthy paper by Chee *et al.* (2001) addressed the critical area of antibiotic resistance and dissemination of resistance genes in the environment. The ground water was shown to be contaminated with genes that were identical to those present in swine farm waste lagoons; therefore, the authors concluded that the contaminants seat into the ground water from lagoons. The potential risk to human health from antibiotic resistance contamination of ground water is several folds. Genes may be mobilized into soil microorganisms and hence into food chain. Ingestion of microbes, whether or not they are pathogenic, can enable acquisition of antibiotic resistance genes by gutflora of humans and animals. Subsequent discharge in fecal material continues the cycle of antibiotic resistance. Moreover, ground water discharged to surface waters, like lakes and rivers, could promote mobilization of resistance genes through the aquatic food chain with consequent exposures to humans. Therefore surveillance programmes are needed to understand the size of the problem and also for the better control of antibiotic resistance. In present days perspective, with alarming rise in antibiotic resistance, reemergence of once fatal illnesses and with the changing nature of the drug resistant pathogens, not only the increased numbers of surveillance programmes are needed but also resistance surveillance programmes are needed to be coordinated and the results made available.

1.6 Summary of chapter 1

A total of thirty-nine water samples, thirteen from each sampling site on river Torsa, were collected and studied on monthly basis from January 2000 to December 2001. Maximum occurrence of culturable copiotrophs was recorded in the month of May in water samples irrespective of the sampling sites. Copiotrophic bacterial counts (CBCs) recorded in different months per sampling site exhibited very wide variations with large dispersion values. The geometric mean of CBCs in water samples ranged from 2.64×10^4 to 4.80×10^4. Five different antibiotics, ampicillin, chloramphenicol, kanamycin, streptomycin and tetracycline, were used singly in Luria-Bertani agar plates to enumerate the recovery of resistant bacteria against each antibiotic. Percent occurrence of ampicillin and chloramphenicol resistant population were much higher than other antibiotic resistant bacteria. The lowest occurrence was recorded for kanamycin resistant population. In the months where recovery of either ampicillin or chloramphenicol resistant population was exceedingly high, the occurrences of other antibiotic resistant populations were quite low. In spite of the least occurrence of kanamycin resistant populations among antibiotic resistant bacteria, an upward trend of occurrence of the said population was recorded in SS I and SS III with passage of time. Wilcoxon matched pairs signed ranks test was used to compare the proportion and test the significance of the difference in abundance of the five antibiotic resistant groups. No significant difference in occurrence of the proportion of ampicillin and chloramphenicol resistant fraction of copiotrophs was found among water samples analyzed. Analysis of variance (ANOVA) (two way classified data with and without replication) was performed to test- 1) relation between sampling site and fraction of copiotrophs resistant to different antibiotics, 2) the effect of seasonal variations on percent occurrences of antibiotic resistant populations and 3) any significant differences in occurrence of particular antibiotic resistant population in three different seasons or sites. Antibiotic resistant populations exhibited significant differences in occurrence in various sampling months. The percent occurrence of ampicillin and chloramphenicol differed significantly from other antibiotic resistant populations. Antibiotic-resistant-patterns (ARPs) of a total of 14,395 antibiotic- resistant isolates were determined. Analyses of ARPs revealed that more than 90% were resistant to two or more antibiotics tested. The occurrence of ampicillin and chloramphenicol as one of the constituent of the ARP combinations of MAR isolates was most frequent. Some ARP combinations like AC, AT, ACT were most dominant ARPs among MAR bacteria. It was found that when singly resistant bacteria dominated in water samples, MAR bacteria were only a few and vice-versa. This observation was supported by negative correlation coefficient value. Among MAR groups, significant negative correlation was observed between the occurrence of doubly and quadruply resistant ($r = -0.742$) and singly and quintuply resistant ($r = -0.693$) bacteria isolated from water samples of SS III. To compare the proportion and test the significance of the difference in abundance of MAR groups, Wilcoxon matched pairs signed ranks test was used. Significant differences in occurrence found among matched pairs were: doubly and quintuply ($p < 0.002$); triply and quintuply ($p < 0.003$); and quadruply and quintuply ($p < 0.007$) resistant bacteria isolated from SS I. Similar significant differences in recovery of proportion of singly and doubly ($p < 0.003$); singly and triply ($p < 0.002$); doubly and triply ($p < 0.002$); triply and quadruply ($p < 0.004$) resistant bacteria were noted in samples from SS II. The occurrences of singly and doubly ($p < 0.002$); doubly and quintuply ($p < 0.004$) and triply and quintuply ($p < 0.006$) resistant bacteria were also significantly different in water samples collected from SS III.

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