Bull. Environ. Contam. Toxicol. (1994) 53:198–203


Environmental Contamination and Toxicology

# Effect of Pollutants on Survival of *Escherichia coli* in Microcosms of River Water

S. P. Pathak,[1] J. W. Bhattacherjee[2]

[1]Aquatic Toxicology, Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Post Box No. 80, Lucknow-226 001, India
[2]Public Health Laboratory, Musgrove Park Hospital, Taunton, Somerset, United Kingdom

Received: 14 December 1992/Accepted: 14 January 1994

Microorganisms contribute significantly to the ecology and biotic population in natural aquatic environments. Microbes are the first among the aquatic biota to be affected by any variation in the quality of water in which they inhabit. The microbial survival is very much dependent upon the trophic state and concentration of various organic and/or inorganic pollutants of municipal, industrial and/or agricultural origin along with other macrobiotic population. It further depends on the ability to tolerate an alien set of biological, physico-chemical and environmental conditions.

With increasing urbanization, the disposal of municipal sewage containing fecal wastes from homeotherms causes fecal contamination of aquatic environments. The prime member of enteric bacteria is Escherchia coli, an opportunistic pathogen with wide distribution in nature. Most of the fecal bacteria are usually genetically manipulated, particularly for metal and antibiotic resistance along with virulence. Hence they are important from public health point of view. Several in vitro and diffusion chamber as well as microcosm studies on effect of various environmental factors and pollutants on the survival of E. coli and other indicator organisms in different natural water have been done (Fliermans 1978; McFeters et al. 1974; Flint 1987; Shannon et al. 1989). However, more studies are required in this field. The present study is an attempt to assess the effect of aquatic pollutants on the survival of resistant as well as susceptible strains of E. coli in the microcosm (micro-environment) of less polluted and highly polluted water of river Gomati in India at hourly intervals.

## MATERIALS AND METHODS

River water samples were collected in glass bottles from upstream (Gaughat) and downstream (Pipraghat) of river Gomati in vicinity of Lucknow city situated at 26°55'N latitude and 80°59'E longitude (Fig. 1). It is a medium sized perennial river in Gangetic plains of Uttar Pradesh, India, passing through Lucknow city.

*Correspondence to:* S. P. Pathak

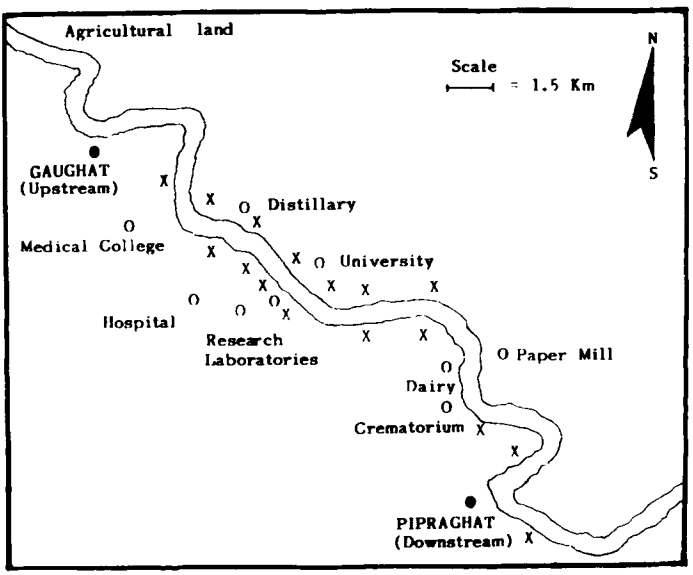


**Figure 1.** Map of river Gomati in vicinity of Lucknow area showing sampling stations (●) and major sources (0 and X) of river pollution. X = Sewage discharge points.

It receives municipal sewage, industrial effluents and agricultural run-off along its course of 940 km before joining with the Ganges near Varanasi.

River water samples from both the stations were sterilized by filtering through membrane filter (Maxflow Ltd., India) with 0.45 μm porosity to avoid denaturing of natural pollutants due to high temperature in autoclave. Sterilized water (100 mL) were aseptically transferred into a sterile Erlenmeyer flasks (250-mL cap.) separately.

Two strains of E. coli isolated from Gomati river water, were taken as test organisms. One strain (No.2) was resistant to ampicillin, chloramphenicol, streptomycin and tetracycline along with tolerance for mercury and chromium, while other strain (No. 28) was susceptible to these antibiotics and metals.

For survival studies, 0.1 mL of fresh culture of both strains in nutrient broth after 18 hr incubation were inoculated separately into the flasks with microcosm,i.e. 100 mL of sterilized river water. After gentle shaking the initial bacterial count was taken by using dropper (50 drops/mL) on MacConkey's agar plate followed by 18 hr incubation at 35°C temperature. The viable bacteria in microcosms of river water were enumerated by drop plating as earlier at 1, 2, 3, 6, 12, 24, 48, 96 and 120 hr with incubation at room temperature (30-

35°C during study). The growth curves were plotted after $10_{10}$ transformation of viable counts of E.coli per mL.

All the bacteriological media and antibiotics were obtained from Hi-Media Pvt. Ltd., Bombay, India. Mercury and chromium were used as mercuric chloride (Glaxo Lab. (India) Ltd, Bombay) and potassium dichromate (IDPL, India), respectively.

The temperature and pH of water samples were measured by using electronic probes with the portable kit (Century Instruments Ltd., Chandigarh, India). BOD was determined by dilution water check method. COD was measured by open reflux method. Chloride was estimated by the argentometric method. Alkalinity, total nitrogen, phosphate, sulfate and hardness were measured titrimetrically by using methyl orange, phenoldisulphonic acid, stannous chloride, barium chloride and ethylene diamine tetra-acetic acid, respectively according to standard methods (APHA, 1989).

For metal analysis 100 mL of each river water sample was acidified with 5 mL of concentrated nitric acid (Glaxo Lab. (India) Ltd. Bombay) and boiled to concentrate upto 20 mL. Levels of zinc, copper and cadmium were determined by direct aspiration of concentrated sample into an air-acetylene flame of Direct Current Plasma (DCP) Spectrophotometer (Beckman Model, Spectroscan V, Geneva) at 206.2, 327.5 and 228.8 μm wave length, respectively. Double distilled deionized water was also processed and read simultaneously as blank. The instrument was caliberated by using appropriate standards (Aldrich Chem. Co. USA).

## RESULTS AND DISCUSSION

Variation in physico-chemical, biological and/or any other qualities in an aquatic environment have a significant effect on survival and phenotypic as well as genotypic characteristics including pathogenicity and resistance phenomenon among the microorganisms. The qualitative observations reveals that there is a considerable difference in most of the physicochemical qualities and metal levels in water from Gaughat and Pipraghat stations of river. This difference in water qualities is obviously due to the discharge of municipal sewage and industrial effluents from urban area in downstream zone of river. As this river mostly receives agricultural land run-off in upstream zone, phosphate, sulfate and total nitrogen were found at higher levels (Table 1).

The viable counts of both the test strains of E.coli in microcosms of upstream and downstream stations at hourly intervals significantly exhibited adverse effect of pollutants on bacterial survival. The initial counts of resistant and sensitive E.coli were $2.8 \times 10^2$ and $1.4 \times 10^2$ / mL, respectively. The variation in viability could be observed only after 6 hr of incubation because the viable counts of resistant and sensitive strains

at this period were $1.8 \times 10^2$ and $1.2 \times 10^2$ /mL, respectively, which was very close to initial counts. The maximum viable counts of resistant strain were $2.7 \times 10^5$ and $1.2 \times 10^4$ /mL and of sensitive strain it was $5.2 \times 10^3$ and $2.8 \times 10^2$ / mL in microcosms of upstream and downstream, respectively after 48 hours incuba-

**Table 1.** Physico-chemical qualities including metal levels in water from upstream and downstream of river Gomati relative to Lucknow city. Each value is mathematical mean of at least three observations. All the physico-chemical parameters, except temperature and pH, have been expressed as mg/L, while metal levels have been expressed as µg/L.

| Parameters | Upstream Mean±SD | Downstream Mean±SD |
|---|---|---|
| Temperature °C | 28.6±4.5 | 29.1±6.4 |
| pH | 8.3±0.2 | 8.5±0.4 |
| DO | 8.2±2.3 | 6.2±1.6 |
| BOD | 7.0±2.6 | 8.2±0.8 |
| COD | 16.6±4.6 | 21.0±5.9 |
| Alkalinity | 204.4±34.7 | 208.9±32.4 |
| Phosphate | 0.1±0.05 | 0.1±0.01 |
| Sulphate | 9.2±0.4 | 8.3±2.3 |
| Chloride | 7.9±3.0 | 10.4±4.4 |
| Total nitrogen | 3.7±1.0 | 3.3±1.3 |
| Hardness | 181.2±22.6 | 185.3±37.1 |
| Cadmium | 18.0±2.6 | 23.0±8.2 |
| Copper | 173.3±145.0 | 350.0±222.7 |
| Zinc | 180.0±42.4 | 385.0±49.5 |

DO = Dissolved OXygen; BOD = Biochemical Oxygen Demand; COD = Chemical Oxygen Demand and SD = Standard Deviation.

tion (Fig. 2). It shows that the survival of sensitive and resistant strains was 18.6 and 22.5 times, respectively, higher in microcosms of upstream than that of downstream. A significant decline of 55.6% and 94.6% in survival of resistant and sensitive strains, respectively has been observed in microcosm of downstream water. Thus, these observations indicate that the effect of pollution is more on survival of the sensitive organisms.

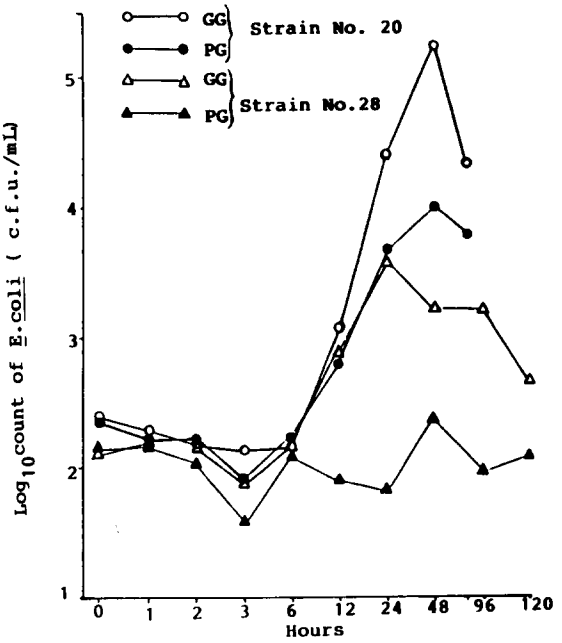


**Figure 2.** Mean $\log_{10}$ counts of two strains of E.coli (No.20 & 28) at hourly intervals in microcosms of river water from upstream - Gaughat (GG) and downstream- Pipraghat (PG). c.f.u. = colony forming units.

Since the microcosms, used in this study, were free from particulate materials and bacteria, the decline in growth rate of E. coli in microcosm of downstream may be attributed to dissolved pollutants of municipal and/or industrial origin. The significant differences could be seen in viable counts of both the strains in microcosms of upstream and downstream during the period of 12 to 96 hr. Flint (1987) also observed a significant decrease in survival of E. coli in sewage polluted river water. Environmental stress due to variations in temperature, pH, trophic state and concentration of various chemicals associated with aquatic pollution have been found to be responsible for the variation in microbial survival in a natural aquatic microcosm (Bissonnette et al. 1975; Gorden and Fliermans 1978; Mancini 1978; Klein and Alexander 1986). McFeters et al. (1974) have realized that coliforms are more susceptible to environmental stress than other aquatic bacteria.

Although Jana and Bhattacherjee (1988) found that heavy metals inhibit the growth of E.coli in natural water, low levels of certain metals such as iron, zinc and potassium in aquatic environments were found to enhance the growth of some natural aquatic microorganisms (States et al. 1985). Variation in quantity of chemical pollutants in water have also been found to affect

the pathogenicity and other plasmid-mediated traits of enteric organisms, eg. Vibrio cholerae (Tamplin and Colwell 1986). The increased survival of antibiotic resistant E.coli may also be due to its R-plasmids (Bennette and Linton 1986).

Therefore, it is obvious from these observations that soluble chemical pollutants above certain concentrations have an adverse effect on natural aquatic bacteria. The growth and survival of suceptible organisms are more affected by pollutants than those resistant to antibiotic, metal and/or other aquatic pollutants. The survival of such resistant and particularly pathogenic organisms in natural aquatic environments with frequent public use, is a serious matter, as antibiotic resistant pathogens may pose risk to aquatic fauna and public health.

**REFERENCES**


APHA (1989) Standard Methods for Examination of Water and Waste water, 17th ed. American Public Health Association, Washington, DC.

Bennett PM, Linton AH (1986) Do plasmids influence the survival of bacteria? J Antimicrob Chemother, 18 Suppl, C:123-126

Bissonnette GK, Jezeski JJ, McFeters GA, Stuart DG (1975) Influence of environmental stress on enumeration of indicator bacteria from natural water. Appl Microbol 29:186-194

Flint KP (1987) The long-term survival of Escherichia coli in river water. J Appl Bacteriol 63:261-270

Gorden RW, Fliermans CB (1978) Survival and viability of Escherichia coli in a thermally altered reservoir. Water Res 12:343-352

Jana S, Bhattacherjee DN (1988) Effect of heavy metals on growth of faecal bacterium, Escherichia coli in aquatic environment. Water Air Soil Pollut 38:251-254

Klein TM, Alexander M (1986) Bacterial inhibitors in lake water. Appl Environ Microbiol 52:114-118

Mancini JL (1989) Numerical estimates of coliform mortality rate under various conditions. J Water Pollut Control Fed 50:2477-2484

McFeters GA, Bissonnette GK, Jezeski JJ, Thomson CA, Stuart DG (1974) Comparative survival of indicator bacteria and enteric pathogens in well water. Appl Microbiol 27:823-829

Shannon LJ, Flum TE, Anderson RL, Yount, JD (1989) Adaptation of mixed flask culture microcosms for testing the survival and effects of introduced microorganisms. In Cowgill UM, William LR (eds) Aquatic Toxicology and Hazard Assessment 12th, ASTM STP 1027, American Society for Testing and Materials, Philadelphia, 224-239

States SJ, Conley LF, Ceraso M, Stephenson TE, Wolford RS, Wadosky RM, McNamara AM, Yee RB (1965) Effect of metals on Legionella pneumophila growth in drinking water plumbing systems. Appl Environ Microbiol 50: 1149-1154

Tamplin ML, Colwell RR (1986) Effect of microcosm salinity and organic substrate concentration on production of Vibrio cholerae enterotoxin. Appl Environ Microbiol 52:297-301