Bull Environ Contam Toxicol (2007) 79:163–167
DOI 10.1007/s00128-007-9128-4

Bacterial Contamination and Antibiotic Resistance in Fecal Coliforms from Glacial Water Runoff

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Published online: 1 June 2007

Glaciers and icecaps constitute about 1.76% of global water resources (WQMD and ILEC, 1995) and contribute to most of the riverine system. Gangotri, in western Himalaya, is the largest valley-type glacier of the Indian continent. Every year, thousands of pilgrims, tourists, students, and explorers visit Gaumukh, Gangotri and Gangetic rivers. Microbial contamination in such an ecologically sensitive stream, with fecal waste from man and animals, is a great risk to public health (Scott et al., 2003). Coliforms, fecal coliforms and fecal streptococci are established ecological indicators of fecal contamination in water, and are used for determination of water suitability for human use (Okawasili and Akujobi, 1996). Among these, *Escherichia coli*, a fecal coliform, occurs most commonly in fecal waste of homeotherms, including man, and has been found to be the best biological indicator of fecal contamination in drinking water (Edberg et al., 2000).

Indiscriminate antibiotic therapy since last few decades has caused emergence of antibiotic resistance among the gut flora of homeotherms, particularly human beings (Bhattacherjee et al., 1988). More often antibiotic resistance among bacterial population is a plasmid-mediated phenomenon and is transferable in nature, which results into its spread among the sensitive aquatic bacterial species (Chen et al., 2005).

Since the stretch of Gangotri glacier and river Ganga we studied has great relevance to geological exploration and tourism, in addition to environment and public health safety, our observations may be useful in monitoring of water quality and conservation of glaciers and the associated riverine system. Recently, it has been realized that in addition to various anthropogenic activities, a changing global climate is also exerting a significant impact on stream flow and water availability, as evidenced by regular regression of the Gangotri glacier snout along with shrinking and thinning of the glacier area (Milly et al., 2005).

This study is an attempt to assess the bacteriological contamination in glacial water runoff from the Gangotri glacier and Gangetic river system (Gaumukh to Rishikesh) by enumerating aerobic heterotrophs, coliforms, fecal coliforms and fecal streptococci. Antibiotic resistance among the fecal coliforms, identified as *E. coli*, was also studied.

Materials and Methods

Gangotri glacier is situated at 30°56’N latitude, 79°04–79°15’E longitude and 3892 meters above sea level in western Himalaya in India. The pre-Gangotri stretch is a U-shaped broad valley with high side walls of mountains covered with thick ice. The post-Gangotri stretch of river (Bhagirathi-Ganga) passes through hilly areas with dense vegetation and scattered habitation. ‘Gaumukh’ the snout of glacier is situated 19 km upstream from Gangotri. Bhagirathi river, originating from Gaumukh, becomes the Ganga river, which flows through the hills up to Rishikesh where it enters the plain (Sangewar et al., 2003).

Sampling stations were selected on the basis of geomorphometry, pollution level and population along the study stretch. Samples of glacial water runoff were collected during summer in sterile borosilicate glass bottles from twenty stations between the ice cave at Gaumukh to Rishikesh. Four water samples were from pre-Gangotri stations, viz., Gaumukh, Bhojwasa, and Bhogalwasa along

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with three samples from Gangotri stations, viz., Devgarha, Gangotri and Dharali, were in the glacier region, directly receiving ice melt water. The remaining thirteen samples were from seven post-Gangotri stations, viz., Gangnani, Bhatwari, Maneri, Uttarkashi, Dunda, Dharasu and Tehari, and six Gangetic stations, viz., Devprayag (upstream), Devprayag (downstream), Chinalisaur, Marine drive, Shivpuri and Rishikesh; these receive tributaries, monsoon runoff, and domestic sewage from along the study stretch (Fig. 1).

Samples were analyzed to enumerate pollution indicator bacteria by determining total viable count as colony forming units (c.f.u.) of aerobic heterotrophs per ml, along with most probable number (MPN) of coliforms, fecal coliforms and fecal streptococci per 100 ml after inoculation of respective culture media with multiple tube method (APHA, 2005). Temperature and pH were determined a portable digital water analysis kit (Century Instruments Ltd., Chandigarh, India).

The proportion of aerobic heterotrophs, coliforms, fecal coliforms and fecal streptococci with respect to total viable count in water samples from all the four stretches were statistically compared by using Chi-square test (Zar, 1984).

Fecal coliforms were isolated from samples of glacial water runoff at different stations and identified by IMViC test on basis of their biochemical characteristics (Chattopadhya and Basu, 1986; Niemi et al., 2003). The facal

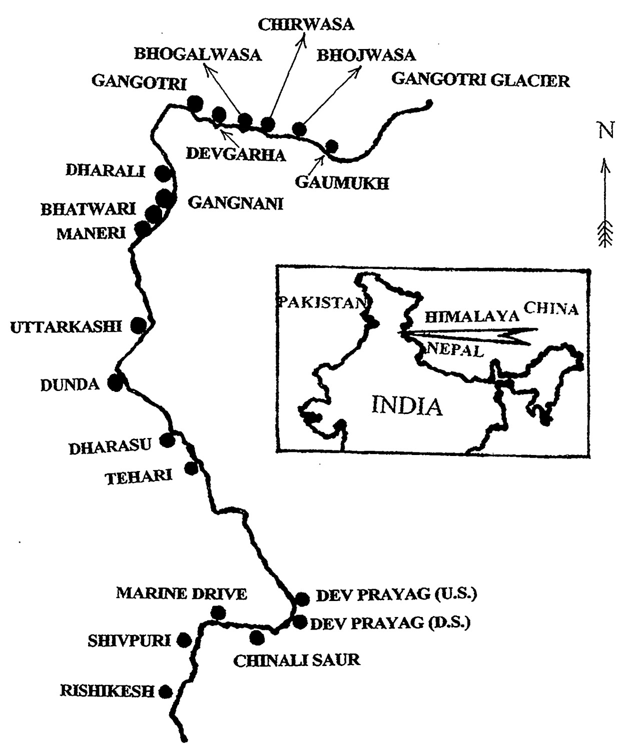

Fig. 1 Sampling sites in the study stretch of the Gangotri glacier and the Gangetic river system in the Himalayan region of India

coliform isolates identified as *E.coli* (n = 14) were subjected to antibiotic susceptibility by disc diffusion method (Bauer et al., 1996). Discs impregnated separately with appropriate concentrations (μg) of ten antibiotics, viz., amoxycillin (10), chloramphenicol (30), ciprofloxacin (10), kanamycin (30), tetracycline (30), gentamycin (10), nalidixic acid (30), norfloxacin (10), streptomycin (10) and trimethoprim (10) were used in this study. Fresh cultures of the test strains were prepared in Nutrient broth after overnight incubation at 37°C. Cultures were separately spread over Mueller Hinton agar plates by sterile cotton swab. Antibiotic-impregnated discs were dispensed on the surface of inoculated agar plate (5 discs per plate) and incubated overnight at 37°C. Antibiotic susceptibility was estimated by measuring their respective zone of inhibition around each disc, as is the standard protocol (NCCLS, 2002). Antibiotic resistance index (ARI) of all test strains was calculated as per formula: ARI = y/nx, where y = number of resistant strains, n = number of test strains and x = number of antibiotics (Hinton and Linton, 1983).

Culture media, and antibiotics discs were all obtained from Hi-Media Laboratories Ltd., Mumbai, India.

Results and Discussion

Fecal contamination in glacial water runoff was evidenced by occurrence of pollution indicator bacteria, the coliforms (2/100ml) and fecal streptococci (4/100 ml) in water samples from 'Gaumukh', origin of Gangotri stream. The total viable count of aerobic heterotrophs ranged from $3.4 \times 10^2 – 8.0 \times 10^3$ c.f.u./ml. Water samples from remaining pre-Gangotri stations were also contaminated with all four indicator bacteria. Gangotri water sample was contaminated with coliform (130/100 ml) and fecal streptococci (7/100ml), but no fecal coliform. Samples from Gangotri were with lower numbers of all Nfour indicator bacterial species. Samples from the post-Gangotri and Gangetic stretches have shown maximum counts (> 1600/100ml) of all the observed indicator bacteria which showed an increasing trend from pre-Gangotri to post-Gangotri and Gangetic stretches (WHO, 2004). Contamination of coliform was observed in all samples, while fecal coliform and fecal streptococci were detected in 17 and 18 samples, respectively (Fig. 2). Thus, bacteriological analysis exhibited maximum contamination in most of the water samples from post-Gangotri and Gangetic stations. The observed increase in the proportion of coliforms and fecal coliforms was statistically significant ($p < 0.001$). The counts of fecal streptococci in all study stretches were too low for statistical comparison.

Glacial water has always been considered as pristine, but due to increasing anti-environmental anthropogenic

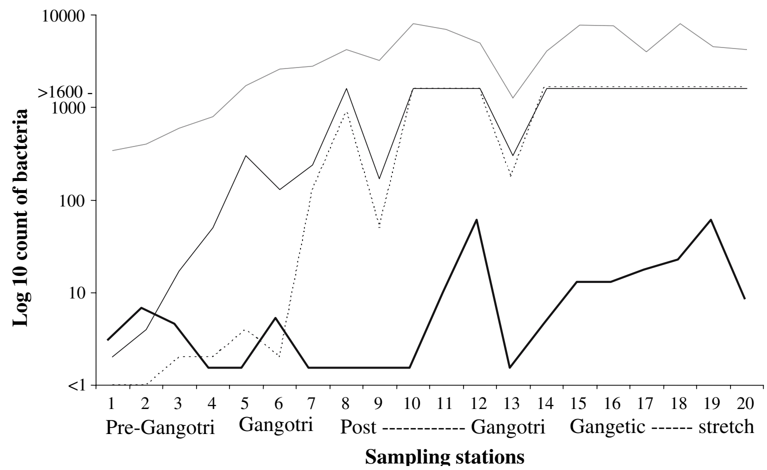

Fig. 2 Population density of pollution indicator bacteria (....... total viable count), (—— coliforms), (········ fecal coliforms) and (—— fecal streptococci) in samples of glacial water runoff from Gangotri glacier and Gangetic river system (Gaumukh-Rishikesh)

activities, the natural qualities of glacial and associated riverine systems are deteriorating. Microbial contamination is mainly due to fecal waste from local residents, camp dwellers, pilgrims, tourists, cattle, and wild animals has resulted into increased numbers of aerobic heterotrophs, coliforms, fecal coliforms and fecal streptococci as ecological indicators of pollution in such natural water sources (Scott et al., 2003). Decaying vegetation and soil erosion also impart water contamination. Increased aerobic heterotroph, coliform and fecal streptococci counts in Gangotri water may be due to the mass bathing of pilgrims, and the huge halt of ponies. The population along the post-Gangotri and Gangetic stretches is also significantly contributing to the deterioration of microbial quality, as evidenced by increased counts of indicator bacteria. Statistical analysis of data exhibited a significant gradual increase in coliform and fecal coliform counts in water from all stretches, but fecal streptococci counts in same samples were too low for comparison and not significant (Table 1).

Temperature and pH of the water samples from study area were observed at 15 ± 5°C and pH 6.5 ± 0.5, respectively. The observed range of temperature and pH was also quite favorable for the survival and growth of mesophilic microbial population in water.

Although, resistance against common antibiotics among the bacterial population in glacial and glacio-fluvial regions with low anthropogenic activities is very unusual, most of the *E. coli* isolates from pre-Gangotri samples exhibited multiple antibiotic resistance (MAR). Maximum resistans (for seven out of ten antibiotics) was observed for trimethoprim followed by kanamycin and ciprofloxacin in the isolates from Rishikesh, while minimum resistance (for one antibiotic) was found in isolates from two stations adjacent to Gangotri. The highest ARI value (0.050) corresponds to the isolates from Rishikesh, and the lowest (0.007) corresponds to isolates from Bhogalwasa (Table 2).

Bacterial antibiotic resistance results from aquatic contamination with fecal waste from homeotherms by coincidental co-selection of resistance (Kaspar et al., 1990). Thus, more use of antibiotics will exert more selective pressure and cause a more frequent incidence of resistant pathogens (MacMillan, 2001). In this regard, WHO has defined a set of core interventions to promote more rational use of antimicrobial medicines (Simonsen et al., 2004). In our earlier studies, MAR coliforms were also isolated from drinking water sources in cold climate and hilly areas (Pathak and Gopal, 1994). Antibiotic resistant enteric bacteria in wetlands indicate the presence of potential

Table 1 Comparison of total viable bacteria, coliforms, fecal coliforms and fecal streptococci counts in water samples from the Gangotri glacier and Gangetic river system

Stretch – No. of stations	Average count of indicator bacteria per 100ml			
	Total viable count	Coliforms	Fecal coliforms	Fecal streptococci
Pre-Gangotri – 4	5.35×10^4	1.80×10^1 (34)*	1.50×10^0 (03)	5.25×10^0 (10) ^{NS}
Gangotri – 3	2.83×10^5	2.23×10^2 (94)*	4.50×10^1 (231)*	3.67×10^0 (02) ^{NS}
Post-Gangotri –7	4.68×10^5	1.24×10^3 (266)*	1.08×10^3 (231)*	1.53×10^1 (03) ^{NS}
Gangetic stretch – 6	6.02×10^5	1.60×10^3 (266)*	1.60×10^3 (266)*	2.97×10^1 (05) ^{NS}

* $p < 0.001$; NS = not significant

Figures in parentheses are number of bacteria/10^5 total viable count

Table 2 Resistance to selected concentration (μg) of antibiotics among fecal coliforms in samples of glacial water runoff from different stations along study stretch of the Gangotri glacier and Gangetic river system

Sl.No	Stretches & Stations	Amoxycillin (10 μg)	Chloramphenicol (30 μg)	Ciprofloxacin (10 μg)	Kanamycin (30 μg)	Gentamycin (10 μg)	Nalidixic acid (30 μg)	Norflo-xacin 10 μg	Strepto-mycin 10 μg	Tetra-cycline 30 μg	Trime-thoprim 05 μg	ARI
	Pre-Gangotri											
1.	Chirwasa	-	-	-	R	-	R	-	-	-	R	0.021
2.	Bhogalwasa	-	-	-	R	-	-	-	-	-	-	0.007
	Gangotri											
3.	Devgarha	-	-	-	R	-	-	-	-	-	R	0.014
4.	Gangotri	-	R	-	R	-	R	-	-	-	R	0.029
5.	Dharali	-	R	-	-	-	-	-	-	-	-	0.007
	Post-Gangotri											
6.	Gangnani	-	R	-	R	-	R	R	-	-	R	0.036
7.	Bhatwari	R	R	-	R	-	R	-	-	R	R	0.043
8.	Maneri	-	R	-	R	-	R	R	-	-	R	0.036
9.	Dharasu	-	R	-	R	-	R	-	-	-	R	0.029
10.	Tehari	-	R	-	-	-	-	R	-	R	R	0.029
	Gangetic stretch											
11.	Devprayag	-	R	-	R	-	R	-	R	-	R	0.036
12.	Marine drive	-	R	R	R	-	R	R	-	-	R	0.043
13.	Shivpuri	-	R	-	R	-	R	-	-	-	R	0.029
14.	Rishikesh	-	R	R	-	-	R	R	R	-	R	0.050

R, resistant; ARI, antibiotic resistance index pathogens that are able to transmit virulent factors to sensitive ones (Halda-Alija, 2004). Jones et al. (1986a) observed higher numbers of antibiotic resistant bacteria from upland tarns (receiving no sewage/effluent). They noticed variation in bacterial species in different aquatic habitats and growth in oligotrophic environments contribute to the incidence of antibiotic resistance. Antibiotic susceptibility patterns compared with ribotyping groups are used for source identification and fecal pollution tracking (Samadpour et al., 2005). Vantarakis et al. (2006) have suggested the MAR analysis as a tool to differentiate human and animal sources of *E. coli*.

Significant rise in bacterial contamination exhibited by pollution indicator organisms is a risk to public health, particularly due to the emergence of MAR and microbial diversity in the Gangotri and Gangetic stretch. This study may be relevant and useful in conservation of glacial as well as riverine systems for the safety of the aquatic environment and human health.

Acknowledgements We are thankful to Department of Science & Technology, Government of India for financial support, Director of Industrial Toxicology Research Centre, Lucknow for guidance and Dr. Neeraj Mathur for his cooperation in statistical analysis.

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