SALMONELLA AS AN INDEX OF POLLUTION OF FRESH-WATER ENVIRONMENTS

ANJANA SHARMA and SUSHMA RAJPUT
Bacteriological Laboratory, Bio-Science Department, R.D. University, Jabalpur 482001, India

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Abstract. An environmental study was done to examine the prevalence of *Salmonella* in some aquatic environments of Jabalpur. Environmental factors in the fresh-water environment exert an influence on the distribution and behaviour of pathogenic bacteria. During the period from January 1991 to December 1992, a total number of 103 isolates of *Salmonella* were tested for their resistance against antibiotics. Among different isolates, *Salmonella paratyphii* showed 100% multiple resistance against antibiotics, i.e. Ampicillin, Carbenicillin, Cephalexin, Chloramphenicol, Gentamicin, Penicillin, Streptomycin, Tetracycline, Norfloxacine and Cloxacine. The density of *Salmonella* correlated with the densities of total coliforms, faecal coliforms and faecal streptococci. The increased survival, possible indigenous nature and behaviour of *Salmonella* further emphasize the need for direct enumeration, reformation of standards and health risk assessments for underdeveloped countries, where waterborne disease exert a horrible toll.

1. Introduction

Natural lakes and impoundments of small streams are attractive environmental resources that create a demand for shared uses ranging from water supply to community development of shorelines for housing and recreational activities. The increase of pollution in natural water has intensified the frequency and persistence of pathogenic microorganisms mainly *Salmonella* spp. in areas affected by sewage discharge[1,2] with the subsequent hazards of public health.

The detection of pathogens from natural water has still to be established, due to their low concentration[3] and to the fact that their minimum dose has not been determined[4,5]. For these reasons, several indicator microorganisms are commonly used to evaluate the degree of faecal pollution of the water. However, the indicator analysis presents some problems; for example, pathogens have been detected which have previously been considered safe on the basis of pollution indicator bacteria[6–8]. Thus, determining the relationship between different indicator bacteria and pathogens may provide some information about the degree of pollution.

The development of resistance to antibiotics is a natural genetic process attributable to many factors. In recent years, the wide-spread addition of sublethal concentrations of antibiotics to animal feed and the abuse of these substances in medical practice can led to an increase in the number of resistant bacteria from environmental sources[9].

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2. Material and Methods

Two freshwater lakes, Ganga Sagar and Adhartal, are subjected to enormous anthropogenic stress and receive heavy runoff from domestic sewage and industrial waste. These lakes are used for recreational activities by surrounding people.

2.1. Water samples

Four different and opposite sites in each lake were selected to estimate the average pollution condition far enough downstream to ensure complete mixing of pollutant and water. The water samples were collected by holding the glass-stoppered, sterile bottle near its base in the hand and plugging it (necked downward below the surface) and transporting to the laboratory in an ice box to avoid unpredictable changes in physico-chemical as well as bateriological characteristics. The pH, temperature, chloride, dissolved oxygen (DO), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were analysed [3, 10, 11] at monthly intervals up to 24 months.

2.2. Indicator bacteria

Quantitative estimation of total coliforms, faecal coliforms and faecal streptococci were made using the MPN technique[3].

2.3. Salmonella

Membrane filtration techniques were employed for detection of *Salmonella* using an 0.45 μm pore size of Sartorius filter membrane[3, 12].

2.4. Qualitative analysis

Presumptive *Salmonelle* colonies were maintained on nutrient agar slants. Further identification and probable confirmation was made on the basis of biochemical tests[13] and with the help of a PIB computer kit[14] and *Bergey's Manual of Determinative Bacteriology*[15].

2.5. Antibiotic sensitivity

To assess the antibiotic resistance/sensitivity pattern of the isolates, the disk diffusion method was followed. The organisms which showed resistance against more than one antibiotic were considered as multiple resistant bacteria. Antibiotics, i.e. Ampicillin, Carbenicillin, Cephalexin, Chloramphenicol, Gentamicin, Penicillin, Streptomycin, Tetracycline, Norfloxacine and Cloxacine, from Span Diagnostic (Bombay, India) were used.

TABLE I

Physico-chemical characteristics of fresh water environments at Jabalpur (from Jan. 1991 to Dec. 1992)

Sampling		Physico-chemical variables								
Months	Sites	pH (units)	Temperature (°C)	Total alkalinity (mg/l)	Chloride (mg/l)	Nitrate (mg/l)	Phosphate (mg/l)	Dissolved oxygen (mg/l)	Bio-chemical oxygen demand (mg/l)	Chemical oxygen demand (mg/l)
Jan. 1991	a	8.2	19.2	303	160	6.75	0.07	2.6	18	43
	b	7.6	17.6	237	108	0.70	0.12	3.8	12	36
Feb. 1991	a	8.4	20.5	286	135	7.25	0.12	2.4	19	82
	b	8.2	18.5	228	125	1.40	0.14	2.4	12	46
March 1991	a	8.6	22.8	259	185	2.65	0.07	1.4	28	69
	b	7.6	21.5	230	124	1.20	0.03	1.8	20	67
April 1991	a	8.4	23.9	235	220	12.70	0.14	1.6	27	52
	b	7.6	22.5	248	132	1.6	0.12	2.0	19	32
May 1991	a	8.9	30.2	204	252	8.85	0.06	1.7	28	58
	b	7.6	29.5	306	186	0.90	0.09	2.2	18	36
June 1991	a	8.2	28.5	268	238	11.80	0.10	1.7	24	27
	b	7.2	30.5	118	121	5.60	0.12	2.4	18	28
July 1991	a	7.8	25.5	192	228	6.45	0.26	2.0	22	75
	b	7.3	26.4	96	192	6.80	0.29	2.3	15	20
Aug. 1991	a	7.7	25.6	234	235	8.45	0.41	2.1	19	27
	b	7.4	24.3	120	138	10.80	0.15	2.4	16	38
Sept. 1991	a	7.5	25.2	260	168	9.25	0.26	2.2	17	38
	b	7.4	23.3	139	128	6.80	0.06	2.3	13	44
Oct. 1991	a	7.8	23.6	307	206	9.45	0.41	2.5	16	52
	b	7.3	20.1	138	132	6.20	0.13	2.4	14	63
Nov. 1991	a	8.6	22.8	259	185	1.65	0.07	1.4	28	69
	b	7.6	21.5	230	124	1.20	0.03	1.8	20	67
Dec. 1991	a	8.2	20.2	118	87	6.40	0.11	2.2	17	63
	b	7.6	19.7	226	142	4.20	3.80	3.0	13	56
Jan. 1992	a	8.8	18.6	269	169	0.40	0.29	2.4	18	96
	b	7.8	18.4	228	112	0.60	0.14	3.1	13	94
Feb. 1992	a	8.1	19.5	227	178	0.90	0.11	2.4	19	56
	b	7.6	18.1	208	78	0.80	0.16	2.8	17	79
March 1992	a	8.4	22.3	227	112	1.20	0.13	1.8	25	36
	b	7.8	23.6	196	125	0.90	0.06	3.1	23	46
April 1992	a	8.4	23.0	227	118	13.40	0.09	1.4	28	36
	b	7.8	27.0	216	192	1.20	0.05	1.9	25	37
May 1992	a	7.2	28.0	272	180	8.60	0.04	1.0	25	39
	b	7.5	28.0	226	222	0.40	0.05	1.8	22	34
June 1992	a	7.5	29.0	225	220	5.80	0.09	1.9	25	28
	b	7.6	30.0	196	88	2.20	0.04	1.8	21	32

TABLE I

Continued.

Sampling		Physico-chemical variables								
Months	Sites	pH (units)	Temperature (°C)	Total alkalinity (mg/l)	Chloride (mg/l)	Nitrate (mg/l)	Phosphate (mg/l)	Dissolved oxygen (mg/l)	Bio-chemical oxygen demand (mg/l)	Chemical oxygen demand (mg/l)
July 1992	a	7.2	26.0	131	232	5.80	0.09	1.9	20	32
	b	7.1	28.0	95	98	3.90	0.11	1.6	14	18
Aug. 1992	a	7.3	25.0	96	182	9.20	0.08	1.6	21	27
	b	7.2	26.0	144	149	3.20	0.19	2.0	13	31
Sept. 1992	a	8.2	24.0	129	119	4.60	0.27	1.8	19	35
	b	7.4	23.0	122	132	6.20	0.12	1.9	12	32
Oct. 1992	a	8.1	25.0	145	305	8.60	0.20	1.2	23	69
	b	7.2	23.0	128	118	6.90	0.30	2.0	17	58
Nov. 1992	a	6.2	21.0	310	146	7.20	0.15	2.1	16	89
	b	7.4	20.0	122	127	7.80	0.12	2.8	15	75
Dec. 1992	a	8.4	26.0	269	168	6.90	0.09	2.4	12	86
	b	7.4	18.0	194	87	8.20	0.07	3.4	14	92

a, Ganga Sagar Lake; b, Adhartal Lake.

3. Results and Discussion

The physico-chemical characteristics of the water sample revealed the pollution status of these lakes (Table I). The occurrence of *Salmonella* ranges from 2043 to 1105 MPN/1000 ml in Ganga Sagar Lake and 188 to 1518 MPN/1000 ml in Adhartal lake with the highest density during the summer season (Figure 1). This may be due to the excessive discharge generally associated with storm water runoff, human and animal waste. The occurrence of *Salmonella* during the rest of the months in both the lakes showed that the habitat of *Salmonella* is not only the intestinal tract of animals, but these bacteria may also exist as free-living organisms multiplying in a natural condition even at a low temperature, i.e. 10 °C[8,12,16]. However, cattle and animal pets may also be involved in the perpetuation of this pathogen in fresh-water systems[17]. Discharge of human and animal excreta is sufficient for faecal coliform persistence, regrowth and the increased rate of *Salmonella* in fresh-water systems. Birds and waterfowl can also contribute *Salmonella* by their faecal dropping into fresh water systems[18–20].

Water temperature appears to play an important role in the distribution of *Salmonella* as can be concluded from the usually clear seasonal variation during the present study. Thus, out of all the environmental factors studied, only temperature, BOD and COD might have had an influence on the seasonal variation of *Salmonella*;

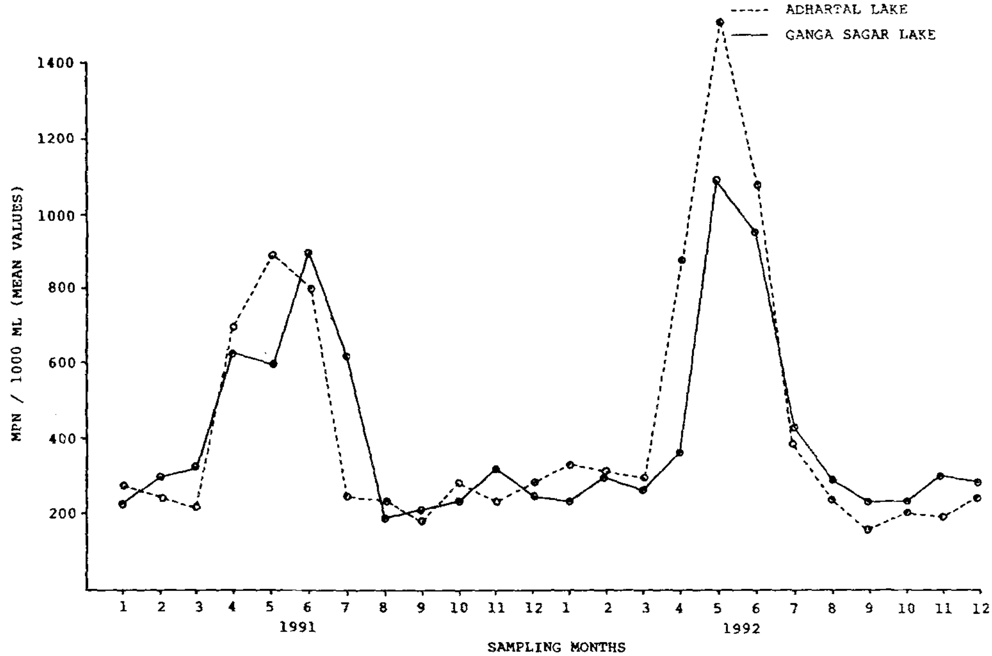

Fig. 1. Concentrations of *Salmonella* in two recreational lakes at Jabalpur during January 1991 to December 1992.

when these values were high, the *Salmonella* density was also high in these lakes. The result is in accordance with WHO and UNEP[21], who suggested that higher BOD and COD values are better indicators of faecal coliforms and *Salmonella* regrowth in aquatic systems.

The density of *Salmonella* also correlated with the densities of total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS). The densities of FC and FS were found to be directly proportional to the *Salmonella* throughout the investigation period in both the lakes (Table II). This may be because these lakes received sufficient organic matter which directly increases the *Salmonella* densities of FC and FS, and thus, indirectly, increases the *Salmonella* density. Similar positive correlation has also been observed by several authors[16,22–24]. However, the insignificant correlation observed with TC may be due to the fact that the TC can also originate from non-faecal sources as well[23] and in these lakes the origin of polluted discharge is not exlusively faecal, especially during the rainy season when TC were maximum and *Salmonella* was minimum. Results are in agreement with other publications[18,19–23].

Out of 136 isolates, 40 isolates were presumptively identified as *Salmonella typhii*, 43 were as *S. paratyphii*, 29 were as *S. gallinarum* and 24 isolates were confirmed as *S. cholerasuis* on the basis of differential biochemical tests (Table III).

TABLE II

Indicator bacteria (MPN/100 ml) in fresh water environments of Jabalpur (January 1991 to december 1992)

Sampling Months	Sites	Total coliforms	Faecal coliforms	F. streptococci
Jan. 1991	a	1085	412	142
	b	985	357	107
Feb. 1991	a	1075	390	125
	b	800	372	117
March 1991	a	1225	667	312
	b	1080	495	375
April 1991	a	1425	905	550
	b	1075	615	462
May 1991	a	1600	1425	645
	b	1600	1225	512
June 1991	a	1800	1600	650
	b	2000	1600	600
July 1991	a	2000	1800	337
	b	1800	1425	220
Aug. 1991	a	1800	1430	210
	b	1600	985	125
Sept. 1991	a	1425	1250	175
	b	1250	720	120
Oct. 1991	a	1110	655	147
	b	990	622	127
Nov. 1991	a	900	367	145
	b	1075	362	210
Dec. 1991	a	710	385	160
	b	1085	360	127
Jan. 1992	a	1025	427	162
	b	985	435	147
Feb. 1992	a	910	500	182
	b	810	495	175
March 1992	a	1080	610	495
	b	900	612	375
April 1992	a	1260	810	522
	b	1250	517	500
May 1992	a	1600	1255	662
	b	1800	975	575
June 1992	a	2000	1800	475
	b	1885	1425	312
July 1992	a	2035	1800	320
	b	2000	1430	271
Aug. 1992	a	2000	1075	255
	b	1425	1425	255

TABLE II

Continued.

Sampling Months	Sites	Total coliforms	Faecal coliforms	F. streptococci
Sept. 1992	a	1250	985	215
	b	1200	805	202
Oct. 1992	a	1430	800	187
	b	905	715	147
Nov. 1992	a	1255	500	320
	b	1080	422	270
Dec. 1992	a	1085	485	182
	b	985	397	197

a, Ganga Sagar lake; b, Adhartal Lake.

TABLE III

Biochemical characteristic of *Samonella* species obtained from fresh water environment at Jabalpur (January 1991 to December 1992)

Biochemical characteristics	Isolates			
	1–40	1–43	1–29	1–24
1. Glucose Fermentation	–	AG	A	AG
2. Lactose	–	–	–	–
3. Sucrose	+	–	+	–
4. Inositol	–	+	–	–
5. Arabinose	–	–	+	–
6. Motility test	+	+	–	+
7. Indole production	+	–	–	+
8. Methyl red test	+	+	+	+
9. Voges Proskaur test	–	–	–	–
10. Citrate utilization	–	–	–	–
11. Triple Sugar Iron test	+	+	+	+
12. H_2S production	+	+	+	–
13. Gelatinase test	–	–	–	–
14. Malonate utilization	–	–	–	–
15. Gluconate utilization	–	–	–	–
16. ONPG test	–	–	+	–
Identified species	*S. typhii*	*S. paratyphii*	*S. gallinarum*	*S. cholerasuis*

AG, acid and gas production; A, acid production.

TABLE IV

Incidence of antibiotic resistance among *Salmonella* species obtained from fresh-water environment at Jabalpur (January 1991 to December 1992)

Isolates	No. of isolates	No. of isolates resistant	Isolate resistant (%)	Percentage of isolates resistant to										Multiple resistance (%)
				I	CB	CP	C	J	P	S	T	NF	V	
Salmonella typhii	48	18	25	100	25	100	100	100	100	100	100	37	87	85.0
S. paratyphii	43	43	100	100	100	100	100	100	100	100	100	100	100	37.0
S. gallinarum	29	0	0	100	44	100	100	11.1	0	100	11.5	0	100	57.0
S. cholerasuis	24	0	0	100	100	100	100	100	100	100	0	0	75	77.5

I, Ampicillin; CD, Carbenicillin; CP, Cephalexin; C, Chloramphenicol; J, Gentamicin; P, Penicillin; S, Streptomycin; T, Tetracycline; NF, Norfloxacine; V, Cloxacillin.

The antibiotic resistance/susceptibility pattern of all the isolates revealed that there are wide differences among bacterial species. (Table IV) in relation to sensitivity against particular antibiotics. During the present study *S. gallinarum* and *S. cholerasuis* showed 57% and 77.5% multiple resistance against the antibiotics studies respectively. *S. typhii* showed 85% and *S. paratyphii* showed 100% multiple resistance against the antibiotics studied. These high figures of resistance are very similar to those reported by several authors[22,23]. Our results, however, showed a more resistance pattern than that obtained in other studies[25,26].

Thus, the data obtained on pollution indicators and pathogenic densities in these fresh water lakes throughout the investigation period concluded that the pollution indicators as well as enteropathogenic bacteria are indigenous inhabitants of fresh-water environments.

The frequent occurrence of multiple resistant bacteria in both the lakes throughout the investigation period constitutes a public health problem, the magnitude of which is obscured by inadequate diagnosis and under-reporting of diarrhoeal disease.

Comprehensive epidemiologic studies relating disease exposure to polluted water are needed. The simpler means for detecting these organisms and their significance as pathogens may make them superior to coliforms as indexes of water quality from the viewpoint of public health.

Monitoring water quality is an important aspect related to both microbiological and chemical health effects. Investigation suggests that without careful management of these water resources, desirable features may quickly be lost and the public health risk increased.

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