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Population dynamics of *Vibrio* species in the river Narmada at Jabalpur

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Abstract: *Several studies on the presence and ecology of various Vibrio sp have been reported in coastal and estuarine waters throughout the world, but there is trifling information available on the distribution of this organism of colossal pathogenic potential in the fresh water riverine environment. Thus, we conducted a multiyear environmental study to scrutinize the occurrence of members of genus Vibrio in the largest west flowing river of the Indian subcontinent, which is also the largest river of central India, the Narmada. Statistical analysis was done to reveal major environmental factors controlling the presence of Vibrio sp in the river Narmada. Monthly field samplings were conducted between January 2002 and December 2003 at four different sites in Jabalpur (MP), India. At each site, water samples were taken and physicochemical and bacteriological parameters were measured. The identity of the isolates was confirmed by employing 16S rRNA analysis. The organisms were found to be widely distributed in the river with regular seasonal variations. The density of Vibrio was found to be correlated with temperature, coliforms and other heterotrophic bacteria. Water temperature accounted for most of the variability in the concentration of Vibrio sp As typical fecal pollution indicators may not access public health risk from potential pathogens such as vibrios, hence special monitoring programme for vibrios may adequately be included in the water quality management.*

Key words: *Vibrio sp, River Narmada, Population dynamics*
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Introduction

Vibrios are gram-negative bacteria, which often cause disease in humans. Infections by *V. cholerae* (Finkelstein, 1973), *V. mimicus* (Davis *et al.*, 1981), *V. vulnificus* (Chakraborty *et al.*, 1997) and *V. parahaemolyticus* (Joseph *et al.*, 1982; Paramasivan *et al.*, 2004) are acquired through consumption of contaminated water and lead to excessive watery diarrhea (*V. cholerae* and *V. mimicus*), acute gastroenteritis (*V. parahaemolyticus*) and septicemia (*V. vulnificus*).

The World Health Organization (WHO) estimates that about 1.1 billion people globally drink unsafe water (Kindhauser, 2003) and the vast majority of diarrheal disease in the world (88%) is attributable to unsafe water, sanitation and hygiene (WHO, 2003). Approximately 3.1% of annual deaths (1.7 million) and 3.7% of the annual health burden (Disability adjusted life years [DALYs]) worldwide (54.2 million) are attributable to unsafe water, sanitation and hygiene. Due to interactions between exposure to enteric pathogens *via* poor quality water, lack of sanitation and derisory hygiene, data resolving the waterborne component is not generally available (Ashbolt, 2004).

Fresh water environment represents a critical reservoir of *Vibrio* species (Caldini *et al.*, 1997). The pervasiveness of pathogenic vibrios appears to be influenced by the physico-chemical features of the environment (West, 1989; Epstein, 1993; Radha Krishnan *et al.*, 2007), whereas factors influencing the production and the activity of their toxins remain to be defined (Barbieri *et al.*, 1999). Previously, this laboratory has described the occurrence of enteropathogenic vibrios in fresh water lakes at Jabalpur (MP), India (Sharma and Rajput, 1992, 1998) and in the river Narmada (Sharma and Rajput, 1995).

Although it is palpable that infections caused by *Vibrio* sp are frequently of environmental origin, the seasonal distribution, ecology and difficulties associated with isolation and identification of the organisms have not been well described.

Mostly indicator organisms are used to assess the degree of fecal pollution of fresh water since river, lakes and ground waters are common sources of community water supplies. However, indicator analysis presents some problems in addition to public health risk associated with infectious microbes transmitted by water. It has become evident that there is also a risk of diseases caused by organisms, which occur naturally in the aquatic environment. Thus, determining the relationship between different indicator bacteria and pathogen may provide some information about the degree of pollution.

The present investigation was undertaken on the vibrios isolated from the largest west flowing river of Indian subcontinent. The total length of the river is 1,312 km, which before draining off into the gulf of Gambay passes through two states of India *viz.,* Madhya Pradesh (MP) and Gujarat. River Narmada is an important source of fresh water supply for Jabalpur (MP), where the sampling was carried out. The present study was undertaken for the isolation, identification and enumeration of *Vibrio* sp through all seasons during two years, from fresh water environment of river Narmada at Jabalpur (MP), India.

Materials and Methods

Sampling sites and survey methods: The study included the largest river of central India, the Narmada. The sampling sites were selected as the site where the river enters the city. The site where the

river leaves and two other sites which are extensively used for various recreational purposes, besides being used for fishing, irrigation, potable water and dumping of industrial and domestic wastes. Four sampling sites *viz.* Gwarighat, Jelharighat, Tilwaraghat and Lamhetaghat were selected for the isolation of *Vibrio* sp .

Physico-chemical and pollution indicator parameter measurement: During the 20 months period between January 2002 and December 2003, 1,000 ml water sample were collected monthly for 20 months (no samples were collected during August and September for both the year) from four sites. The water samples were collected by holding the glass stoppered, sterile bottle near its base in the hand and plugging it (neck downwards below the surface) and transporting to the laboratory in an icebox to avoid unpredictable changes in physico-chemical as well as bacteriological characteristics.

Environmental parameters measured at each site included water temperature, alkalinity, pH, chloride, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD) and levels of nitrate and phosphate. Water temperature was measured with a waterproof thermometer on the site. Standard methods (APHA, 1985; NEERI, 1988) were followed to determine the physico-chemical parameters.

Quantitative estimation of heterotrophic bacteria, total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) were made following standard techniques (APHA, 1985).

Enumeration of *Vibrio* sp: Enrichment for *Vibrio* species and viable bacterial counting were performed by the membrane filtration technique. Water volumes of 0.1, 1.0, 10 and 100 ml (for counting) and 500 ml (for enrichment) were filtered through 0.45 μm-poresize filters (Sartorius). All filters except those used for the enrichment were placed on thiosulfate-citrate-bile salts- sucrose (TCBS; Himedia) agar plates and incubated at 37°C for 16-18 hr. The number of viable *Vibrio* isolates was estimated as CFU/100 ml of water. The primary and secondary enrichments into alkaline peptone water (APW) for *Vibrio* detection were performed as described previously (Baumann *et al*., 1984; Kaper *et al*., 1995).

Identification: The cells from TCBS agar were identified on the basis of biochemical tests (Alsina and Blanch, 1994 a, b) and with the help of Bergey's manual of systematic bacteriology (1984) and PIB (Probabilistic Identification of Bacteria) computer kit (Bryant, 1993).

The identity of *Vibrio* sp was confirmed by using 16S rRNA analysis (Koeleman *et al*., 1998).

Statistical analysis: Correlation coefficient 'r' for vibrios with different bacteriological and physico-chemical characteristics was determined using Karl Pearson coefficient (Misra and Misra, 1989).

Results and Discussion

One hundred fifteen isolates of *Vibrio* sp were isolated during the study period. On the basis of biochemical tests, the isolated vibrios were differentiated into seven species (Table 1). The isolates were confirmed as *Vibrio* sp on the basis of ARDRA (data not shown). Analysis of our data with correlation coefficient (r) revealed that the isolation of *Vibrio* sp was positively correlated with water temperature (r = 0.93), alkalinity (r = 0.109), chloride (r = 0.18), COD (r = 0.75), phosphate (r = 0.66), total coliforms (r = 0.24), fecal streptococci (r = 0.085), the presence of heterotrophic bacteria (r = 0.63), dissolved oxygen(r = 0.33) and negatively correlated with pH (- 0.114), BOD (r = -0.24), nitrate (r = - 0.21), fecal coliforms (r = - 0.07). A significant correlation was observed between water temperature and *Vibrio* densities, whereas alkalinity was not strongly correlated with the distribution of *Vibrio* sp.

In continuance with previous studies by Sharma and Rajput (1998), Barbieri *et al.* (1999) and Pfeffer *et al.* (2003), we found water temperature to be the most highly correlated parameter with the isolation of *Vibrio* sp (Fig. 1). A regular seasonal variation of *Vibrio* was observed at all sampling stations throughout the study period. The *Vibrio* sp was found more frequently at higher concentrations in the river water during summers, which is in agreement with an earlier study by Kaper *et al.* (1979) .The overall reduction in *Vibrio* count during winter was noted at all the stations, this can be due to the fact that organism may enter viable but non-culturable (VBNC) state, a survival strategy used to counter temperature stress (Oliver *et al*., 1995). Repetitive attempts to isolate *Vibrio* sp during winters were not very successful when compared to the isolation during summers, indicating that these organisms are present in low numbers during winter or are present in VBNC state.

In agreement with the results of an earlier study from our laboratory (Sharma and Rajput, 1998), we found the frequencies of isolation of *Vibrio* sp and total coliforms to vary together (Fig. 2), statistical analysis also indicated a positive correlation between these two groups. This finding is very significant for the river Narmada since discharge of human and animal waste is sufficient for total coliforms persistence, regrowth and the spread of *Vibrio* in fresh water. The public health significance of *V. cholerae* non-O1 in Indian aquatic environments remains to be determined, because the studies generally carried out are not systematic and in India recovery and identification of this microorganism is not included in routine laboratory analysis. Table 2 shows correlation of *Vibrio* sp with different physico-chemical parameters.

While use of bacterial indicators to measure water quality is wide spread, there is no universal agreement on which indicator organism is most useful, nor are there governmental regulations mandating a single standard for bacterial indicators. Thus, different indicators and different indicator levels identified as standards are used by water quality programs in different countries and regions. Koh *et al.* (1994) reported that correlations between vibrios and indicator bacteria, such as total and fecal coliforms, enterococci and *E. coli*, were either negative or not present at two test sites in Apalachicola Bay, Florida.

The diversity of vibrios was not correlated with different

Table - 1: Biochemical characteristics of *Vibrio* sp isolated from the river Narmada at Jabalpur (MP)

Biochemical characteristics	Isolate no.						
	BGCC 58-82	BGCC 83-93	BGCC 94-103	BGCC 104-113	BGCC 114-139	BGCC 140-153	BGCC 154-173
1	-	-	-	-	-	+	-
2	+	+	-	+	+	+	+
3	+	+	-	+	+	-	+
4	+	-	-	+	-	-	-
5	+	+	+	+	+	+	+
6	-	+	+	+	+	+	+
7	-	-	+	-	+	+	-
8	-	-	+	-	-	+	-
9	-	-	-	-	-	-	-
10	+	+	-	+	+	+	+
11	+	-	-	+	+	+	+
12	-	-	-	+	+	+	+
13	+	+	+	+	+	+	+
14	-	+	-	-	+	+	-
15	+	-	-	+	-	+	-
16	-	-	+	-	+	-	-
17	-	-	-	-	-	-	-
18	+	+	-	+	+	-	+
19	-	+	-	-	-	-	+
20	-	-	-	-	-	+	-
21	+	+	-	-	-	-	+
Identified sp	*V. cholerae* non-O1	*V. fischeri*	*V. hollisae*	*V. mimicus*	*V. parahaem-olyticus*	*V. proteolyticus*	*V. vulnificus*

1 = Arginine dihydrolase (Moeller), 2 = Lysine decarboxylase (Moeller), 3 = Ornithine decarboxylase (Moeller), 4 = Growth at 0% NaCl, 5 = 3% NaCl, 6 = 6% NaCl, 7 = 8% NaCl, 8 = 10% NaCl, 9 = Aesculin hydrolysis, 10 = Citrate, 11 = Gelatinase, 12 = Indole, 13 = Oxidase, 14 = Urease, 15 = VP, 16 = Acid from arabinose, 17 = Acid from inositol, 18 = Acid from mannitol, 19 = Acid from salicin, 20 = Acid from sorbitol, 21 = Acid from sucrose
BGCC = Bacterial Germplasm Collection Centre, Bacteriology laboratory, R.D. University, Jabalpur (MP)

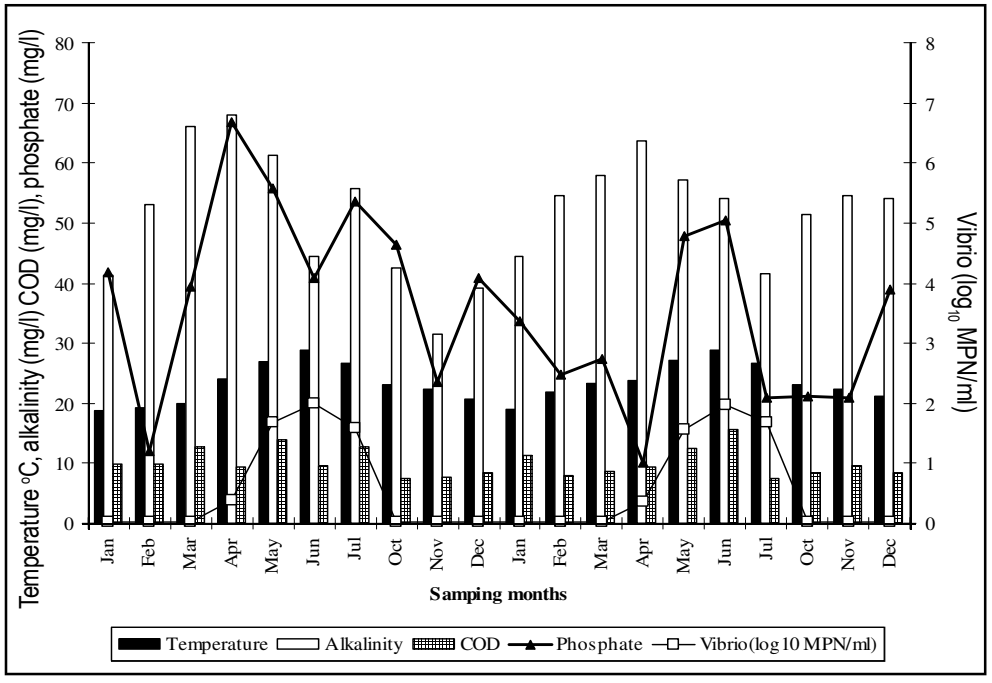

Fig. 1: Seasonal distribution of *Vibrio* sp in the river Narmada during 2002-03 (mean values of temperature, alkalinity, COD and phosphate)

Table - 2: Relation of environmental parameters with the occurrence of *Vibrio* in the river Narmada at Jabalpur

Sampling months	Environmental parameters							*Vibrio* (log_{10} MPN/ml)
	pH	Chloride	DO	BOD	Nitrate	FC	FS	
Jan. 02	7.79	21.75	8.775	5.25	7.53	665	750.5	0.020
Feb. 02	7.85	18.5	7.475	8.55	7.97	997.5	978.12	0.021
Mar. 02	8.85	16.5	6.2	7.6	9.36	1033.75	781.75	0.294
Apr. 02	8.27	19.0	6.7	5.35	3.23	1450	1071.3	0.374
May 02	7.98	16.25	8.95	4.05	4.81	1305	1353	1.673
Jun. 02	8.25	23.25	6.45	4.875	4.975	1500	1062	1.987
Jul. 02	8.02	21.5	8.275	5.875	3.275	1600	1595.5	1.584
Oct. 02	8.1	20.75	4.825	4.3	5.48	2400	2400	0.033
Nov. 02	7.45	24.75	5.475	3.95	5.58	2400	2387.5	0.03
Dec. 02	8.15	18.25	6.275	2.725	3.975	2400	2400	0.03
Jan. 03	8.12	21.0	8.0	5.275	6.0	2400	750.5	0.020
Feb. 03	8.64	19.5	7.075	3.95	2.49	2400	1506.8	0.025
Mar. 03	9.22	22.0	5.55	2.825	4.90	2481.25	2400	0.028
Apr. 03	7.87	18.25	9.3	2.775	3.61	1305	1062.5	0.37
May 03	8.14	20.0	8.875	4.3	3.327	2400	2387.5	1.553
Jun. 03	8.1	20.75	6.75	3.025	8.02	2400	2400	1.98
Jul. 03	7.45	22.75	6.225	4.975	5.58	281.25	2400	1.67
Oct. 03	7.6	24.75	7.2	5.35	8.08	2400	2400	0.034
Nov. 03	7.45	17.25	6.725	3.95	7.18	2400	2387.5	0.03
Dec. 03	7.9	18.0	6.2	3.8	4.1	2400	2400	0.029

* Sampling was not carried out in the months of August and September for both the years due to heavy rains

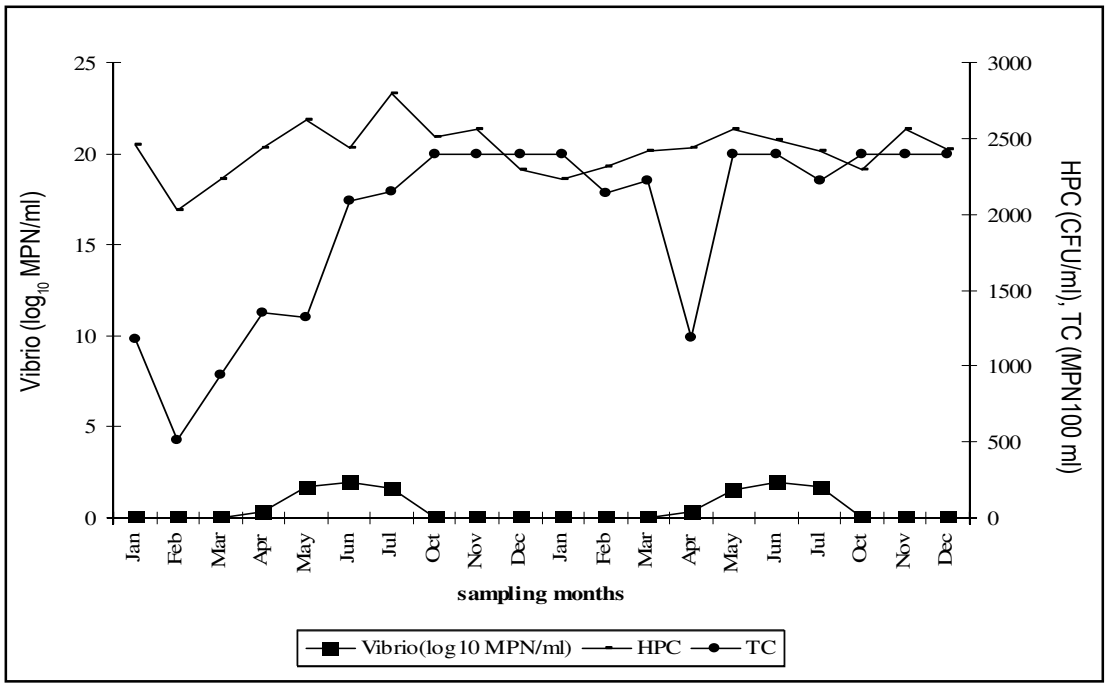

Fig.2: Seasonal distribution of *Vibrio* sp in the river Narmada during 2002-03) (mean values of HPC and TC)

sampling sites, since identical clones were isolated from different locations across the river in Jabalpur at the same time of sampling. We interpret that the vibrios in the river Narmada are being transported by water currents or wind driven water movements or responding similarly to environmental conditions at various sites.

Water borne diseases are one of the major causes of increased morbidity and mortality in the world being known as a carrier of human disease-causing organisms that can pose health risks to people if it is not properly treated. The current major obstacle to human health in developing regions is well understood and a large component relates to unsafe water, poor sanitation and inappropriate hygiene. Associated with increased human activity in the eutrophication of waterways and the resultant increases in diseases. For example, cholera outbreaks are well known to be associated with phytoplanktons blooms in nutrient rich waters. Climate change too is now seen as a reality, with not only change in the distribution of rainfall, but one also of greater extremes in weather patterns. The level of seasonal contamination followed the order summer > rainy season > winter.

Since the river Narmada is a very significant source of fresh water for Jabalpur (MP) sustaining millions of populace and also used for recreational purposes, the occurrence of toxigenic *V. cholerae* raises a question regarding potential risk of human exposure; hence it's indispensable to monitor the river water recurrently to check the possibility of any epidemic.

Despite all the efforts, vibrios will always be a major issue for human health and particularly so in developing countries. In India, the lack of epidemiological studies aimed at assessing the presence of *V.cholerae* non-O1 does not allow evaluation of the risk of exposure to this microorganism to be evaluated. Further studies will be necessary to understand the presence, spread and public health importance of *V. cholerae* non-O1 and other *Vibrio* sp in India.

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