Microbial contamination of various water sources in Delhi

Water-borne diseases constitute one of the major public health hazards in developing countries. Worldwide, in 1995, contaminated water and food caused more than 3 million deaths, of which more than 80% were among children under age five[1]. Besides the conventional pathogens which are transmitted by water, several emerging water-borne pathogens have become increasingly important during the last decade or so. These include *Vibrio cholerae* O139, *Cryptosporidium parvum*, shiga toxin producing *E. coli* especially enterohaemorrhagic *E. coli* (EHEC), *Yersinia enterocolitica*, *Campylobacter jejuni*, Calciviruses and *Microsporidia*[2].

In India, more than 70% of the epidemic emergencies are either water-borne or are water related[3]. Although a substantial amount of work has been carried out on common water-borne pathogens in India, unfortunately only a little information is available on the emerging water-borne pathogens. A regular surveillance of resource and drinking water is one of the major mainstays of containing dreaded and often fatal water-borne diseases. It was in this context that an assessment of microbial load of different types of water and the prevalence of emerging water-borne pathogens, viz. *Vibrio cholerae* O139 and enterohaemorrhagic *E. coli* (EHEC, serotype O157 : H7) was undertaken in the national capital of Delhi.

Samples of waste water (29), surface (10), ground (100) and drinking (100) waters were collected from the entire region of the city of Delhi. Waste water samples were collected from the stretch of river Yamuna traversing through the city and downstream (*ca.* 100 km). Surface water samples were collected from the river Yamuna upstream (*ca.* 30 km) of Delhi. Ground and drinking water samples were collected from hand pumps and municipal water supplies respectively. Samples were collected in sterilized screw-capped bottles making sure there was no additive, transported to the laboratory in cold and processed within 6 h of their collection.

To assess the microbial load, indicator parameters, viz. heterotrophic plate count (HPC), total coliform count (TCC), faecal coliform count (FCC) and faecal streptococcal count (FSC) were studied by standard methodologies recommended by the American Public Health Association[4]. Briefly, HPC was enumerated on tryptone glucose yeast extract (TGYE) agar by standard plate count; TCC, FCC, and FSC were determined by the most probable number (MPN) technique. Isolation of emerging water-borne pathogens, viz. *Vibrio cholerae* O139 and enterohaemorrhagic *E. coli* (serotype O157 : H7) was carried out as described below.

For *Vibrio cholerae* O139, 100 ml of water sample was enriched overnight in 200 ml of double strength alkaline peptone water (pH 8.6) at 37℃, and streaked on thiosulphate citrate bile sucrose (TCBS) agar. From each sample, four typical yellow coloured colonies were subjected to confirmation using *Vibrio cholerae* medium[5]. Isolates showing alkaline slant/acid butt (K/A), negative for H_2S and gas production, and positive for indole production and oxidase reaction, were further serotyped using O1 polyvalent (Span Diagnostics) and specific O139 antisera by slide agglutination. The specific antiserum to *Vibrio cholerae* O139 was procured from WHO, SEARO, New Delhi.

For *E. coli* O157 : H7, 100 ml of sample was enriched overnight in 50 ml of triple strength lauryl tryptose broth at 37℃, and streaked on sorbitol McConkey (SMAC) agar containing potassium tellurite[6]. The sorbitol negative strains ($SMAC^-$) were tested for enzyme β-D-glucuronidase using MUG (4-methylumbelliferyl-β-D-glucuronide) as the substrate[7]. A colony of the test isolate was applied to MUG impregnated filter paper, moistened with a drop of saline and incubated at 37℃ for 20 min. The absence of enzyme was indicated by the lack of fluorescence when the filter paper was examined under UV light. The MUG negative colonies were further subjected to serotyping using specific O157 and H7 antisera (DIFCO) separately.

Table 1 summarizes microbial load of different types of water as assessed by the indicator parameters and isolation of *Vibrio cholerae* and enterohaemorrhagic *E. coli*.

In comparison to surface water (collected from the river Yamuna upstream of Delhi), the waste water (collected from the river Yamuna as it traversed through the city) showed a 100- to 1000-fold increase in all the indicator parameters. This data can be used to assess the impact of various pollution remediation programmes currently being undertaken by the Delhi Government and the Ministry of Environment and Forests, such as installation of a series of combined effluent treatment plants along the

Table 1. Microbial contamination of different types of water in Delhi

		Counts (Geometric mean)				*Vibrio cholerae*		*E. coli*	
Water samples	*n*	FCC (counts/100 ml)	TCC (counts/100 ml)	FSC (counts/100 ml)	HPC (counts/ml)	O1 (%)	O139 (%)	$SMAC^-$ and MUG^- (%)	O157 : H7 (%)
Surface water*	10	4.2×10^3	7.8×10^3	2.6×10^3	9.4×10^4	100 ($n = 20$)	0	30 ($n = 20$)	0
Waste water**	29	3.2×10^6	6.4×10^6	6.6×10^5	2.9×10^6	100	0	51.7	0
Groundwater	100	0.7×10^2	1.4×10^2	1.2×10^2	1.0×10^3	5	0	25	0
Drinking water	100	0.02×10^2	0.03×10^2	0.8×10^2	1.8×10^2	ND	ND	ND	ND

*Yamuna water upstream of Delhi.
**Yamuna water traversing through Delhi and downstream.
FCC, Faecal coliform count; TCC, Total coliform count; FSC, Faecal streptococcal count; HPC, Heterotrophic plate count; $SMAC^-$, non-fermentative on sorbitol McConkey agar; MUG^-, methylumbelliferyl-β-D-glucuronidase negative; *n*, number of the samples analysed; ND, Not determined.

river Yamuna. The data presented here may serve as a baseline to which all future data may be compared. All groundwater samples, whether collected from shallow or deep bore pumps, showed the presence of coliforms. The presence of coliforms of faecal origin in a majority of these samples showed that microbial contamination in groundwater was widespread and even deeper layers of groundwater may not be regarded as free from disease-causing micro-organisms. However, except for some samples, most drinking water samples were found to be devoid of any coliform count.

Vibrio cholerae O1 was isolated from all surface and waste water samples while only 5% of the groundwater samples showed the presence of *Vibrio cholerae* O1. *Vibrio cholerae* O139 however, could not be isolated from any of the water samples tested. Although outbreaks of cholera due to *Vibrio cholerae* O139 have been reported from Delhi in the past[8], there has been no information on the presence of this organism in aquatic environments. *Esherichia coli* O157 : H7 while being primarily associated with food-borne outbreaks, has also become an important public health concern as a water-borne pathogen. Though generally considered to be of concern to developing countries, a large-scale outbreak of haemorrhagic colitis due to water-borne *E. coli* O157 : H7 was reported from Africa[9]. In India, *E. coli* O157 : H7 has recently been isolated from dairy cattle and beef in Kolkata[10,11]. In our study, although a number of isolates which were non-fermentative on sorbitol McConkey agar ($SMAC^-$) and also lacked the enzyme methylumbelliferyl-β-D-glucuronidase (MUG^-) were obtained, none of these proved to be enterohaemorrhagic *E. coli* when tested with specific O157 and H7 antisera. Our inability to isolate *E. coli* O157 : H7 from waste water, surface and groundwater may be attributed to the absence of this organism in this geographical region, or its presence in extremely low numbers necessitating the use of more efficient isolation methods such as immunogenetic separation or direct PCR amplification of *stx* genes using specific primers[11]. Clearly, more rigorous surveillance is required that would help to clarify the public health significance of water-borne *E. coli* O157 : H7 in India. Earlier, *Yersinia enterocolitica*, another emerging food- and water-borne pathogen has been isolated from waste water collected from sewage treatment plants (STPs) located in various parts of the national capital of Delhi[12]. As regards other emerging water-borne pathogens, viz. *Cryptosporidium*, *Campylobacter jejuni* and *Microsporidia*, there is no information on the prevalence of these in the aquatic environment of India. Studies in this respect are warranted to safeguard ourselves against emerging water-borne pathogens.

1. The World Health Report. *Fighting Diseases, Fostering development*, WHO, Geneva, 1996.
2. Szewzyk, U., Szewzyk, R., Manz, W. and Schleifer, K.-H., *Annu. Rev. Microbiol.*, 2000, **54**, 81–127.
3. Khera, A. K., Jain, D. C. and Dutta, K. K., *J. Commun. Dis.*, 1996, **28**, 129–138.
4. *Standard method for examination of water and waste water*, American Public Health Association (APHA), Washington DC, USA, 19th edn, 1995.
5. Nair, G. B., Sarkar, B. L., De, S. P., Chakrabarti, M. K., Bhandra, R. K. and Pal, S. C., *Microb. Ecol.*, 1988, **15**, 203–215.
6. Zadic, P. M., Chapman, P. A. and Siddons, C. A., *J. Med. Microbiol.*, 1993, **39**, 155–158.
7. *Mackie & McCartney, Practical Medical Microbiology* (eds Collee, J. G., Fraser, A. G., Marmion, B. P. and Simmons, A.), Churchill Livingstone, New York, USA, 14th edn, 1996.
8. *CD Alert, Cholera: need for a constant and continuous vigil*, **4**, Directorate of General Health Services, New Delhi, 2000, pp. 1–8.
9. Effler, P. *et al.*, *Emerg. Infect. Dis.*, 2001, **7**, 812–818.
10. Dutta, S. C., Deb, A., Chattopadhyay, U. K. and Tsukamoto, T., *J. Med. Microbiol.*, 2000, **49**, 765–767.
11. Khan, A. *et al.*, *Emerg. Infect. Dis.*, 2002, **8**, 54–62.
12. Sinha, I., Choudhary, I. and Virdi, J. S., *Curr. Sci.*, 2000, **79**, 510–513.

ACKNOWLEDGEMENT. Financial support from Department of Science and Technology (project no. SP/SO/B-34/96) to JSV is gratefully acknowledged.

Received 30 November 2002; revised accepted 3 March 2003

SACHIN SHARMA
I. SINGH
J. S. VIRDI*

Microbial Pathogenicity Laboratory,
Department of Microbiology,
University of Delhi South Campus,
Benito Juarez Road,
New Delhi 110 021, India
**For correspondence.*
e-mail: virdi_dusc@rediffmail.com

A potential *Aspergillus* species for biodegradation of polymeric materials

In recent years, considerable attention has been focused on biodegradability of polymeric materials mainly because pollution of the environment by waste polymers has become a worldwide problem. Such materials need to be resistant to degradation both prior to and during use and should be capable of being degraded, if discarded after use, without causing any environmental problems. Two possible approaches to reduce the 'vices of polymeric materials' are (a) to develop biodegradable commodity plastic[1] and (b) to identify potential micro-organisms and develop protocol to effectively biodegrade the polymeric materials[2].

The present study is an attempt to assess the potential of *Aspergillus foetidus* for polymer degradation. This fungal culture was isolated from a polymeric sheet under degradation due to fungal colonization. The sheet was used as a greenhouse covering at Pithoragarh, India. The fungus obtained from degraded polymeric sheet was cultured, brought in pure form and was identified as *Aspergillus foetidus* with the help of the Indian Institute of Microbial Technology, Chandigarh. The culture has not yet been reported to degrade polymer in the available literature.