Detection and occurrence of waterborne bacterial and viral pathogens

E. Kathleen Black, Gordon R. Finch

INTRODUCTION

Waterborne disease plays a significant role in world health, as shown by the cholera epidemic, which began in Peru in 1991 but has now spread east and north, affecting persons in most South and Central American countries, as well as the United States and parts of the Caribbean (Centers for Disease Control). Although bacterial waterborne diseases, such as cholera, play a significant role in human health worldwide, many waterborne disease outbreaks are caused by organisms that are not bacterial and are often equally as debilitating as bacterial infections. For example, during 1989 and 1990 a major cause of waterborne disease in the United States was the protozoan parasite *Giardia lamblia* (Herwaldt et al., 1991). In the same period, viral infection was the source of four outbreaks of waterborne disease, three from water intended for drinking and one from recreational water use.

MONOGRAPHS

The 18th edition of *Standard Methods for the Examination of Water and Wastewater* was published during 1992. Changes in the microbiological examination section include modifications in the proposed detection of staphylococci in recreational waters, the total coliform multiple-tube fermentation technique and enumeration, virus concentration methodology, as well as a proposed chromogenic substrate test for coliforms. *Environmental Microbiology* was a monograph published in 1992 featuring a broad range of topics related to environmental microbiology, including detection of several waterborne bacterial, protozoan, and viral pathogens (Singh and McFeters).

OCCURRENCE OF BACTERIAL PATHOGENS

The occurrence of waterborne bacterial pathogens is summarized in Table 1. Water types included in this table are drinking water (including bottled water), surface water, groundwater, and wastewater. The most frequently reported bacteria were *Escherichia coli*, followed by the coliform group. These indicator bacteria are included in this review because of their use as indicators of pathogens. Attempts were made to correlate the presence of coliform bacteria with pathogens, such as *Salmonella*, *Vibrio parahaemolyticus*, and *Aeromonas hydrophila* (Martinez-Manzanares et al.; O'Shea and Field; Stelzer and Jacob; and Townsend). The correlation between salmonella and coliform bacteria or enterococci was unclear in a survey of two pools in the Australian wet-dry tropics (Townsend), in an estuary of a river in Spain (Martinez-Manzanares et al.), and in storm water runoff (O’Shea and Field). *V. parahaemolyticus* and *A. hydrophila* in shellfish were correlated with total coliforms (Martinez-Manzanares et al.), and aeromonads increased relative to coliforms in treated drinking water (Stelzer et al.).

Studies on the persistence of pathogens and indicators in water were reported. The survival of *E. coli* (Gauthier et al.; Korhonen and Martikainen, 1991; Prabu and Mahadevan; Rice et al.; Roberts; and Terzieva and McFeters, 1991), *Salmonella* species (Townsend), *Campylobacter* species (Korhonen and Martikainen, and Terzieva and McFeters), *Legionella pneumophila* (Cargill et al., and Paszko-Kolva et al., 1991), and *Helicobacter pylori* (West et al.) was examined in surface water, groundwater, seawater, and under varying saline conditions.

Surveys of the incidence of *Vibrio vulnificus* indicated that this organism shows seasonal variance in numbers and in habitat (O'Neill et al., Tamplin and Capers; and Vanoy et al., increasing in numbers only when the temperature of the water is greater than 20°-23°C. During the cold weather months, *V. vulnificus* was detected only in sediments, whereas at higher water temperatures, such as found during the summer months, the organism was detected in oysters, sediment, and seawater (Vanoy et al.).

A survey of pools in the Australian wet-dry tropics revealed that the levels of *Salmonella*, coliforms, and enterococci varied throughout the year, being lowest during the dry season (Townsend). The concentration of salmonellas varied by as much as two log cycles, and the concentration of coliforms and enterococci varied by as much as three log cycles. The high reported levels of *Salmonella* were attributed to the high carrier rate in reptiles and marsupials and the high water temperatures.

OCCURRENCE OF VIRAL PATHOGENS

The Norwalk agent and hepatitis A were implicated in several outbreaks of waterborne illness in North America during 1989–1991 (Health and Welfare Canada, and Herwaldt et al., 1991). The occurrence of viruses in water are summarized in Table 2. Surveys of river water and recreational water showed that virus levels varied throughout the year (Hughes et al., and Tani et al.). Poliovirus presence in river water was found to be associated with the application of the polio vaccine, however, the presence of echoviruses, reoviruses, coxsackieviruses, and adenoviruses did not vary throughout the year (Tani et al.). In coastal and inland seawater, coxsackieviruses were most frequently isolated. The other viruses that were identified were polioviruses (Hughes et al.), possibly derived from immunized children. The authors noted, however, that identification and isolation of viruses in their study were limited by the cell culture used and the number of plaque forming units seeded.

The infectivity of viruses from lawns irrigated with wastewater was examined using an animal model (Deming et al.). Fewer piglets exposed for 2 hours to lawns irrigated with 4 × 10^3 50% cell-culture infectious dose (CCID_{50}) virus particles became positive than piglets inoculated with 100 CCID_{50} virus particles.
A survey of municipal wastewater reported that the survival of human immunodeficiency viruses in wastewater was less than that of polioviruses (Casson et al.).

OTHER PATHOGENS

The occurrence of G. lambia and two other protozoa, Cryptosporidium parvum and Acanthamoeba, is summarized in Table 3. The survival of C. parvum oocysts was reported under various environmental conditions and it was found to be a robust organism in all of the water types examined (Robertson et al.). Acanthamoeba is distributed widely in the freshwater environment. Studies have shown that the detection of L. pneumophila in water is more probable if Acanthamoeba species are present also (Sanden et al.).

DETECTION

Representative literature from the past year related to the detection of waterborne pathogens are summarized in Table 4. Several articles investigated differences in recovery of waterborne pathogen detection methods, particularly with respect to quantity and speed of analysis (Barrell; Covert et al.; Foster et al.; Schets and Havelaar, 1991; Warburton et al.; and Whipple et al.). Visible but nonculturable organisms present a problem when detecting organisms in water (McCarty et al.; McKay; and Singh and McFeters). Prenrichment and selective enrichment of samples, as well as the newer technologies of gene probes and immunoassays, improve the detection of injured and stressed organisms. Methods for quantifying pathogens continue to improve, resulting in improved detection of stressed and injured organisms and reducing the time committed to identification (Bifulco and Schaefer; Covert et al.; Jacob and Stelzer; Oliver et al.; and Warburton et al.).

Developments in gene probe and immunoassay technologies are making these detection methods more accessible to routine water analysis laboratories (Atlas et al.; Kerr et al.; Sano et al.; and Yamamoto, K. et al.). Gene probes are rapid, sensitive, and specific, however, the probes do not always detect viable cells, and in some cases the technology requires special equipment. An extensive overview of gene probe technology was published during 1992 (Atlas et al.). Immunoassays can detect toxins produced by organisms or the organisms themselves, however, viability determination of the detected cells is not reliable.

Table 1—Occurrence of bacterial pathogens in drinking water (DW), groundwater (GW), surface water (SW), and wastewater (WW).

| Organism                        | DW | GW | SW | WW | References                                                                 |
|---------------------------------|----|----|----|----|----------------------------------------------------------------------------|
| Aeromonas sp.                   | √  | ×  | ×  | ×  | (Leung et al.; Martinez-Manzanares et al.; Moyer et al.; Stelzer et al.)    |
| Campylobacter sp.               | √  | ×  | ×  | ×  | (Korhonen and Martikainen, 1991; Stelzer and Jacob; Terzieva and McFeters, 1991) |
| Clostridium perfringens         | √  | ×  | ×  | ×  | (Martinez-Manzanares et al.)                                                |
| Coliform bacteria               | √  | √  | √  | √  | (Doyle et al.; Leung et al.; Martinez-Manzanares et al.; O'Shea and Field; Stelzer and Jacob; Stelzer et al.; Townsend) |
| Cyanobacteria                   | √  | ×  | ×  | ×  | (Carmichael; Harding; Li, W. K. et al.)                                     |
| Enterococci                     | ×  | √  | ×  | √  | (O'Shea and Field; Townsend)                                                 |
| Escherichia coli                | ×  | ×  | ×  | ×  | (Barrell; Gauthier et al.; Martinez-Manzanares et al.; Prabu and Mahadevan, Rice et al.; Shadix and Rice, 1991; Townsend) |
| Helicobacter sp.                | √  | ×  | ×  | ×  | (West et al.)                                                               |
| Heterotrophic plate count bacteria | √  | √  | √  | √  | (Lye and Dufour, 1991; Martinez-Manzanares et al.; Yamamoto et al.)           |
| Legionella sp.                  | √  | ×  | ×  | ×  | (Cargil et al.; Paszko-Kolva et al., 1991; Sanden et al.; Yamamoto, H. et al.) |
| Pseudomonas sp.                 | √  | ×  | ×  | √  | (Gennari and Dragotto; Leung et al.; O'Shea and Field)                       |
| Salmonella sp.                  | √  | ×  | ×  | ×  | (Martinez-Manzanares et al.; O'Shea and Field; Townsend)                     |
| Staphylococcus sp.              | √  | ×  | ×  | √  | (Martinez-Manzanares et al.; O'Shea and Field; Stelz and Zschock)            |
| Vibrio sp.                      | √  | ×  | ×  | √  | (Martinez-Manzanares et al.; O'Neil et al.; Vanoy et al.)                     |
| Yersinia enterocolitica         | √  | ×  | ×  | √  | (Terzieva and McFeters, 1991)                                               |

Table 2—Occurrence of viral pathogens in drinking water (DW), groundwater (GW), surface water (SW), and wastewater (WW).

| Organism                        | DW | GW | SW | WW | References                  |
|---------------------------------|----|----|----|----|-----------------------------|
| Adenovirus                      | √  | ×  | ×  | ×  | (Tani et al.)               |
| Coxackievirus                   | √  | ×  | ×  | ×  | (Hughes et al.; Tani et al.) |
| Echovirus                       | √  | ×  | ×  | ×  | (Botero et al.; Tani et al.) |
| Enterovirus                     | √  | ×  | ×  | ×  | (Botero et al.; Deming et al.; Hughes et al.) |
| Hepatitis                       | ×  | ×  | ×  | ×  | (Williams and Fout)         |
| Human immunodeficiency virus    | √  | ×  | ×  | ×  | (Casson et al.)             |
| Norwalk agent                   | ×  | ×  | ×  | ×  | (Williams and Fout)         |
| Poliovirus                      | ×  | ×  | ×  | ×  | (Botero et al.; Hughes et al.; Tani et al.) |
| Reovirus                        | ×  | ×  | ×  | ×  | (Tani et al.)               |
Table 3—Occurrence of other waterborne pathogens in drinking water (DW), groundwater (GW), surface water (SW), and wastewater (WW).

| Organism            | DW | GW | SW | WW | References                     |
|---------------------|----|----|----|----|--------------------------------|
| Acanthamoeba        | X  |    |    |    | (Sanden et al.)                |
| Cryptosporidium     | X  |    |    |    | (Robertson et al.)             |
| Giardia lamblia     | X  |    |    |    | (Brown et al.)                 |

A new technology that shows promise is the combined use of conductance and immunology (Parmar et al.). Beads coated with the antigen for a specific pathogen are exposed to the organism. After a short incubation, the beads are separated, washed, and resuspended in broth. The change in conductance of the broth is measured, and the resulting curve is specific for the organism. Salmonella typhimurium can be detected from a preenrichment broth within 14 hours using this method (Parmar et al.).

The sensitivity of the immunobead assay is yet to be determined, specialized equipment is necessary, and competition between the Salmonella species studied and some species of Citrobacter was reported. Characteristic substances produced by Mycobacterium species have been detected by gas chromatography-mass spectrometry (Alugupalli et al.). Although this method is extremely sensitive and considerably faster than current recovery methods, the cost of the detection equipment is beyond the capabilities of most laboratories.

E. Kathleen Black is a Research Associate in the Environmental Engineering and Science Program and Gordon R. Finch is Associate Professor of Environmental Engineering, Department of Civil Engineering, University of Alberta, Edmonton, Alberta, Canada, T6G 2G7. Correspondence should be directed to Gordon R. Finch.

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Table 4—Detection of waterborne pathogens.

| Organism                  | Recovery methods and viability techniques | Gene probes | Immunoassay | Reference |
|---------------------------|-------------------------------------------|-------------|-------------|-----------|
| Bacteria                  |                                           |             |             |           |
| Aeromonas sp.             | X                                         | X           |             | (Moyer et al.) |
| Campylobacter sp.         | X                                         | X           |             | (Burnens and Nicolet; Jacob and Stelzer) |
| Coliform bacteria         | X                                         | X           |             | (Barrell; Covert et al.; Kuhn et al., 1991; Martínez-Manzanares et al.; Rusin et al.; Schets and Havelaar, 1991; Shadix and Rice, 1991) |
| Cyanobacteria             | X                                         |             |             | (Carmichael; Li, W. K. W. et al.) |
| Enteric bacteria          |                                           | X           | X           | (Bej et al., 1991; Hubner et al.) |
| Enterobacter sp.          | X                                         | X           |             | (Bej et al., 1991; Covert et al.; Hubner et al.; Martínez-Manzanares et al.; Martins et al.; Meyer et al., 1991; Sano et al.; Woodward et al.) |
| Escherichia coli          | X                                         | X           |             | (Bej et al., 1991) |
| Klebsiella pneumoniae     |                                           | X           |             | (Bej et al., 1991; Sanden et al.) |
| Legionella sp.            | X                                         | X           |             | (Bej et al., 1991; Oladipo et al.; Peterkin et al.; Rossen et al., 1991; Wang et al.; Warburton et al.) |
| Listeria monocytogenes    | X                                         | X           | X           | (Alugupalli et al.; Whipple et al.) |
| Mycobacterium sp.         | X                                         |             |             | (Alonso et al.; Baule and Cox; Cano et al.; Enlis and Boleszkuk, 1991; Foster et al.; Kerr et al.; Martínez-Manzanares et al.; Parmar et al.; Todd et al., 1991; Townsend; Tseng et al., 1991; Tseng et al., 1991) |
| Salmoneilla sp.           | X                                         | X           | X           | (Bej et al., 1991) |
| Shigella flexneri         | X                                         | X           |             | (Jaulhac et al.; Martínez-Manzanares et al.; Park et al.; Ruzickova, 1991; Shigawawa et al., 1991; Steil and Zschock; Tseng et al., 1991) |
| Staphylococcus sp.        | X                                         | X           | X           | (Lee and Ruby; Martínez-Manzanares et al.; Oliver et al.; Vanoy et al.; Via et al.; Yamamoto, K. et al.) |
| Vibrio sp.                | X                                         |             |             | (Li, Q. Z. et al.) |
| Yersinia enterocolitica   |                                           | X           |             | (Li, Q. Z. et al.) |
| Viruses                   |                                           |             |             |           |
| General                   | X                                         | X           | X           | (Tani et al.; Williams and Foul) |
| Adenovirus                |                                           |             |             | (Rusvai and Belak; Tani et al.) |
| Echovirus                 |                                           |             |             | (Peige-Lafouille et al.) |
| Enteric viruses           | X                                         |             |             | (Hughes et al.) |
| Rotavirus                 | X                                         |             |             | (Fernández et al.; Parwani et al.; Soares et al.; Thorns et al.) |
| Other pathogens           |                                           |             |             |           |
| Cryptosporidum parvum     | X                                         | X           |             | (Campbell et al.; Siddons et al.) |
| Giardia lamblia           | X                                         | X           |             | (Hastie et al.; Isaacrenton et al.; Mahbubani et al., 1991; Mahbubani et al.) |
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Monitoring, sampling, and automated analysis

Reginald Goo

MONITORING

Effluent monitoring data collected under the National Pollutant Discharge Elimination System (NPDES) Program were used to estimate Priority Pollutant loads to the San Francisco Estuary (Davis et al.). Chromium, copper, nickel, and zinc from four municipal wastewater treatment facilities accounted for approximately 50% of the total loads to these elements to the Estuary. Silver concentrations were measured in coastal surface waters in the Southern California Bight (Samuel-Wilhelmy and Flegal). Based on mass balance calculations, anthropogenic silver inputs from the Point Loma discharge off San Diego, Calif., account for essentially all of the silver in coastal waters along the United States-Mexico border during summer conditions. Grimalt et al. reported a study of a sediment sampling network encompassing six coastline perpendicular transects, each situated in front of a river mouth. Variance analysis has shown this type of sampling scheme can recognize the main coastal pollution discharge sites, but is not useful for tracing their area of influence.

Aichinger et al. used electrolytic respirometry to measure the biodegradability of six phthalate esters and four polynuclear aromatic hydrocarbons. Dissolution appeared to govern the degradation rate when these compounds were present in amounts exceeding their solubility. Alexander and Fu found microbial mineralization of styrene rapid in wastewater and certain types of soil. They suggested that styrene will be rapidly destroyed by biodegradation in most aerobic environments and the rate may be slow in environments of low pH. Chaudhuri et al. studied the effect of temperature on the incubation time in biochemical oxygen demand (BOD) tests. They concluded that BODs, is equivalent to BODs,27 and should be used in tropical countries.

Fendlinger et al. published an analytical method for the determination of alkyl sulfate surfactants in natural waters. Their monitoring results indicated that alkyl sulfate surfactants removal is similar to BOD removal and was at least 90% at two trickling filter wastewater treatment plants sampled. Field et al. published a method for the determination of sulfonated aliphatic and aromatic surfactants in wastewater sludge. Concentrations of secondary alkanesulfonate and linear alkylbenzene sulfonate surfactants in wastewater sludge ranged from 0.27 to 0.80 and from 3.83 to 7.51 g/kg, respectively.

Comprehensive air emission tests were conducted on two multiple-hearth incinerators (Hentz et al.). Lead and cadmium control efficiencies were significantly higher than those assumed in proposed regulations. Despite low sludge concentrations and high control efficiencies, arsenic and cadmium emissions are expected to exceed limits proposed in recent regulations. Huang et al. reported dibromochlorodibenzodioxins and dibromochlorodibenzofurans characterization in municipal waste incinerator fly ash. Concentrations of these compounds ranged from low to high parts per million depending on the location where the samples were collected.

Brodbelt proposed an interface for on-line process monitoring of gas and liquid samples using a mass spectrometer that was applied to freon, benzene, and pentachloropyridine.

SAMPLING

Reinelt and Grimvall reported that different loading estimation methods can yield significantly different results, even if sampling during peak flows occurs. Theoretical calculations showed that sampling strategies with evenly spaced sampling intervals may systematically over or underestimate the true loading. Characteristics of the collected data, relative influence of point sources, and desired detail of loading estimates influence the choice of the estimation method. Martin et al. compared surface-grab and cross-sectionally integrated sampling methods for determining stream water quality. Surface grab samples underrepresented concentrations of suspended sediments and some sediment-associated constituents.

Foreman et al. encountered analytical interference from mercuric chloride-preserved water samples. This interference occurred in the continuous liquid-liquid extracts that were analyzed by gas chromatography-mass spectrometry. Poor recoveries of spiked samples were observed when using a closed-loop stripping method. Clement described a classroom experiment that illustrates sampling difficulties and analytical errors that can occur in trace environmental determinations. A practical guide for environmental sampling and analysis was presented by Keith.

AUTOMATED ANALYSIS

Sperling et al. published an on-line flow injection preconcentrator coupled to an electrothermal atomic absorption spectrometer for the determination of chromium(VI) and total chromium. Sodium diethylthiocarbamate was used as the