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Waterborne pathogens in urban watersheds

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ABSTRACT

A serious concern for managers of water resources, pathogens in the urban environment easily enter waters through a number of pathways, including discharge of inadequately treated sewage, stormwater runoff, combined sewer overflows and sanitary sewer overflows. Pathogens in US ambient water bodies are regulated under the Clean Water Act (CWA), while pathogens in drinking water supplies are regulated under the Safe Drinking Water Act. Total maximum daily loads (TMDLs) are developed in accordance with CWA regulations for ambient water bodies with bacterial concentrations exceeding the water quality standard, which generally is a measure of a bacterial indicator organism. However, developing a TMDL for a supplementary indicator or pathogen is also required if a use impairment would still exist even after the water body is in compliance with the standard. This occurs because indicator organisms do not reflect the presence of pathogen contamination with complete certainty. The evaluation of pathogen indicators and summary of epidemiological studies presented are resources for those developing TMDLs to achieve water quality standards and restore water bodies to their intended uses.

Key words | bacteria, Clean Water Act, pathogen, protozoa, virus

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INTRODUCTION

Pathogens are disease-causing microorganisms that are a major concern for managers of water resources. Once in a water body, pathogens infect humans through contaminated fish and shellfish, skin contact or ingestion of water. High levels of fecal indicator bacteria are a major cause of impairments of US water bodies (Table 1). The seriousness of pathogen contamination is reflected in the maximum contaminant level goals of zero for microbial pathogens, which were established through Safe Drinking Water Act (SDWA) regulations. These goals conform to the position of the World Health Organization (WHO) (1993) which states: '….there is **no tolerable** lower limit for pathogens, and water intended for consumption, for preparing food and drink, or for personal hygiene, should thus contain no agents pathogenic for humans.'

The WHO estimates that 13 million people die each year from waterborne infections (Warrington 2001*a*). The majority of these deaths occur in developing countries. However, in the US approximately 900,000 cases of illnesses and 900 deaths occur each year as a result of microbial contamination of drinking water (Warrington 2001*a*). The majority of large-scale waterborne disease outbreaks in the past have been attributed to human contamination or inadequacies at water treatment plants. Waterborne outbreaks, upon contact with contaminated recreational water bodies, are attributed to human fecal contamination or sewage (Levy *et al.* 1998; Upton 1999).

The primary pathogens of concern in the US are bacteria, protozoa and viruses. Helminths and fungi are two additional pathogen groups, but not found to be responsible for significant levels of contamination in the US. The sources of most waterborne pathogens are human and animal feces from infected individuals, and from human and animal carriers. These microorganisms are deposited directly into water bodies or transported to water bodies by overland flow and/or subsurface water flow. In urban areas,

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Table 1 | US microbial water quality assessments summary; 1999 and 2000 (USEPA 2002*a*)

Rivers and streams

- 19% of US river and stream miles assessed
- 39% of assessed river and stream miles impaired, totaling 93,000 shoreline miles
- Pathogens (bacteria) are leading cause of impairment
- Agriculture is the primary source of impairment

Ocean shorelines

- 6% of US ocean shoreline miles assessed
- 14% of assessed shoreline miles impaired, totaling 384 shoreline miles
- Pathogens (bacteria) are leading cause of impairment
- Urban runoff/storm sewers are primary source of impairment

Great lakes shorelines

- 92% of US Great Lakes shoreline miles assessed
- 78% of assessed shoreline miles impaired, totaling 102 shoreline miles
- Pathogens (bacteria) are third leading cause of impairment
- Contaminated sediments are the primary source of impairment

Estuaries

- 36% of US estuarine square miles assessed
- 51% of assessed estuaries square miles impaired, totaling 4,764 shoreline miles
- Pathogens (bacteria) are fourth leading cause of impairment
- Municipal point sources are primary source of impairment

Lakes, reservoirs, and ponds

- 43% of US lake, pond and reservoir acres assessed
- 45% of assessed lake acres impaired
- Pathogen (bacteria) are not a leading cause of impairment
- Agriculture is the primary source of impairment

pathogens are transported by stormwater runoff, combined and sanitary sewer overflows, and wastewater treatment plant effluents.

For US water bodies not meeting Clean Water Act (CWA) water quality standards, total maximum daily loads (TMDLs) are developed. For microbial contamination, the EPA has presented the process in depth in *Protocol for Developing Pathogen TMDLs* (USEPA 2001*a*). A microbial water quality standard is generally a measure of a bacterial indicator organism. However, developing a TMDL for a supplementary indicator or pathogen is also required if a use impairment, i.e. waterborne disease outbreak, would still exist even after the water body is in compliance with the standard. This occurs because indicator organisms do not reflect the presence of pathogen contamination with complete certainty.

Presented in this article is information on pathogen sources, health effects of waterborne pathogens, relevant water quality legislation and an evaluation of pathogen indicators. This information should be used in conjunction with the *Protocol for Developing Pathogen TMDLs* (USEPA 2001*a*). In addition to focusing on urban environments, this article addresses complexities associated with the pathogens TMDL process due to the reliance on indicators for estimating pathogen contamination. The waterborne disease outbreaks summaries, summary of epidemiological studies and evaluation of microbial indicators are resources for those developing TMDLs to achieve water quality standards and restore water bodies to intended uses.

PATHOGEN SOURCES IN THE URBAN ENVIRONMENT

Stormwater runoff, generated from pervious and impervious areas during rainfall and snow events, often contains pollutants, including pathogens, which adversely affect receiving water quality. Polluted stormwater runoff is a leading cause of impairment to nearly 40% of the US water bodies that do not meet water quality standards (USEPA 2002*b*). Most stormwater outfalls are considered point sources and require a National Pollutant Discharge Elimination System (NPDES) permit. Urbanization alters the path of stormwater runoff through hydraulic modifications, including catch basins, inlets, curb and gutter, storm sewers, ditches, lined channels, culverts and pavement. Subsequently, flow velocity is increased compared with the original natural conditions (Field & Sullivan 2003). Studies show a linear relationship between runoff volume and watershed imperviousness (Schueler 1987).

Combined sewer systems (CSSs) convey a mixture of sanitary wastewater and stormwater laden with pathogens through a single pipe to a publicly owned treatment facility for treatment prior to discharge to surface waters. The USEPA reports that CSSs are found in 32 states (including the District of Columbia) and concentrated in older communities in the Northeast and Great Lakes regions. Combined sewer overflows (CSOs) occur during moderate or heavy rainfall when capacity is exceeded. The US annual CSO discharge is estimated at 1,269 billion gallons per year (USEPA 2001*b*). CSO receiving waters are 43% rivers, 38% streams, 5% oceans, estuaries and bays, 2% ponds and lakes, and 12% other waters (ditches, canals, unclassified waters). CSOs, a source of impairment for 12% of assessed estuaries (in square miles) and 2% of assessed lakes (in shore miles), discharge water with varying concentrations of sanitary wastewater onto public areas, potentially resulting in a range of adverse health effects (USEPA 2001*b*).

Sanitary sewer overflows (SSOs) are discharges of untreated sewage from municipal sanitary sewer systems. SSOs are associated with wet-weather conditions when sanitary systems receive stormwater in-flow and/or infiltrating groundwater through cracks, broken pipes and equipment failure. SSOs are generally prohibited by the CWA. The USEPA is considering how to better standardize NPDES permit conditions to clarify this prohibition. USEPA (2001c) estimates that 40,000 SSOs occur annually discharging pathogens into US waterways.

HEALTH EFFECTS

Waterborne disease outbreaks

Discharges of stormwater runoff, CSO and SSO to receiving waters create the potential for disease outbreaks. Through climate and epidemiological records, Rose *et al*. (2000) demonstrated a potential correlation between extreme precipitation events (the highest 20% of total intensity over a 20-year period) and waterborne disease outbreaks. The authors found that statistically significant relationships could be identified between these precipitation events and waterborne disease outbreaks owing to contact with water from both surface and groundwater sources. These relationships were much stronger for surface water outbreaks. In addition, illness in swimmers in heavily used recreational waters is due to agents transmitted from swimmer to swimmer (Calderon *et al.* 1991). Exposure pathways of pathogens in recreational waters are dermal contact, ingestion, and inhalation resulting in skin, ear, eye, gastrointestinal and respiratory illnesses. The microorganisms responsible for reported swimming-associated disease outbreaks due to exposure in US waters, excluding man-made hot tubs and pools, between 1986 and 2000 are presented in Table 2.

Few studies other than those related to outbreaks have been conducted to determine the aetiological agents related to swimming-associated illnesses (WHO 1999). One large-scale epidemiological study of swimmers in marine waters receiving stormwater runoff involved interviewing over 15,000 individuals (Haile *et al.* 1999). Researchers reported higher risks of upper respiratory and gastrointestinal infections for swimmers who swam (1) near storm-drain outfalls, (2) in waters with high levels of single bacterial indicators and a low ratio of total to fecal coliforms, and (3) in waters where enteric (intestinal) viruses were detected. These positive associations with adverse health effects indicated a higher risk of illness associated with swimming in ocean water receiving untreated stormwater runoff. More than 1% of the swimmers who swam in front of the outfalls were affected by fevers, chills, ear discharges, vomiting and coughing. Some studies attempting to link health effects to pathogen sources yielded inconclusive results. Perez Guzzi *et al*. (2000) studied 17 haemolytic uraemic syndrome cases to investigate potential contamination from CSOs on California's Mar del Plata beaches. Their investigation detected no pathogenic *Escherichia coli* 0157:H7, although other isolates of *E. coli* were detected in 75% of the samples. None of the 98 strains detected in the outfalls was a strain known to cause human illness.

Exposure pathways for pathogens in drinking water include ingestion, dermal contact and inhalation. Failures in

Table 2 | Outbreaks associated with US recreational waters, 1986–2000 (Levine *et al.* 1990; Herwaldt *et al.* 1992; CDC & EPA 1993; Kramer *et al.* 1996; Levy *et al.* 1998; Barwick *et al.* 2000; Lee *et al.* 2002)

Aetiological agent	No. of cases#	% of total cases	Outbreaks*	% of total outbreaks
Adenovirus 3	595	10.08	1	1.05
AGI**	1,744	29.53	22	23.16
Cryptosporidium parvum	649	10.99	4	4.21
E. coli 0121:H19	11	0.19	1	1.05
E. coli 0157:H7	336	5.69	12	12.63
Giardia lamblia	83	1.41	4	4.21
Leptospira	389	6.59	3	3.16
Naegleria fowleri	16	0.27	16	16.84
Norwalk-like	257	4.35	4	4.21
Schistosoma spp.	203	3.44	7	7.37
Shigella spp.	1,618	27.40	20	21.05
Not reported×	4	0.07	1	1.05
Total	5,905	100	95	100

#A case is defined as a disease occurrence from an aetiological agent.
*An outbreak is defined as: (1) greater than or equal to 2 persons experiencing a similar illness after contact with the recreational water or drinking water; or (2) epidemiologic evidence that implicates the recreational water as the probable source of the illness (i.e. Milwaukee, Wisconsin (1993), 403,000 cases of cryptosporidiosis = one outbreak).
**Acute gastrointestinal illness of unknown aetiology.
×Illness reported was aseptic meningitis.

water treatment systems and the inability of disinfection procedures to inactivate all pathogens allow these microorganisms to remain in the finish water. Of the pathogens identified, *Giardia* and *Cryptosporidium* have been identified to cause the largest number of drinking water-associated illness cases and outbreaks reported to the Centers for Disease Control (CDC) from 1986 to 2000 (Table 3).

Pathogens of concern

Waterborne pathogens of greatest concern have the following characteristics (Rosen 2000):

- They are shed into the environment in high numbers, or they are highly infectious to humans at low doses.
- They have the ability to multiply outside of a host under favourable environmental conditions.
- They can survive and remain infectious in the environment for long periods or are highly resistant to water treatment.

The identified agents for US swimming and drinking water-associated outbreaks are bacteria, protozoa and viruses. Descriptions of these microorganism types and their survival characteristics are provided below. In addition, information on microbial source tracking, a technique used to identify the origin of certain microorganisms, is presented. Although not unique to urban environments, this information pertains to pathogens in these environments.

Bacteria

Bacteria are unicellular microorganisms that exist as either free living organisms or as parasites. Bacteria play a fundamental

Table 3 | Outbreaks associated with drinking water from US surface sources, 1986–2000 (Levine *et al.* 1990; Herwaldt *et al.* 1992; CDC and EPA 1993; Kramer *et al.* 1996; Levy *et al.* 1998; Barwick *et al.* 2000; Lee *et al.* 2002)

Aetiological agent	No. of cases#	% of total cases	Outbreaks *	% of total outbreaks
AGI**	12,169	2.78	15	31.25
Ca. Jejuni	102	0.02	1	2.08
Campylobacter	250	0.06	1	2.08
Cryptosporidium parvum	419,130	95.89	5	10.42
Cyanobacteria-like	21	<0.00	1	2.08
E.coli 0157:H7	38	0.01	3	6.25
Giardia lamblia	3,424	0.78	20	41.67
Shigella sonnei	1,800	0.41	1	2.08
SRSV***	148	0.03	1	2.08
Total	437,082	100	48	100

#A case is defined as a disease occurrence from an aetiological agent.
*An outbreak is defined as: (1) greater than or equal to 2 persons experiencing a similar illness after contact with the recreational water or drinking water; or (2) epidemiologic evidence that implicates the recreational water as the probable source of the illness (i.e. Milwaukee, Wisconsin (1993), 403,000 cases of cryptosporidiosis = one outbreak).
** Acute gastrointestinal illness of unknown aetiology.
*** Small round structured virus.

role in the decomposition and stabilization of organic matter in nature and in biological sewage treatment processes. Bacteria have various shapes: spherical (coccus), rod-shaped (bacillus), comma-shaped (vibrio), spiral (spirillum), or corkscrew-shaped (spirochete), and range from 0.5 to 5.0 micrometres (Rosen 2000). Many types of enteric pathogenic bacteria occur in water supplies and in wastewater. The USEPA (2000, 2002*a*) identified fecal indicator bacteria as one of the leading causes of impairments to surface waters. With increasing demands on water resources, the potential for contamination of surface and groundwater by pathogenic enteric bacteria is expected to rise resulting in an increase in waterborne disease outbreaks. Gastrointestinal illness, e.g. diarrhoea, nausea and cramps, is a common symptom of infections caused by enteric waterborne bacteria. Some pathogens spread through the body from the intestinal mucosa and cause systemic infections known as enteric fevers (WHO 2005). One example of this is typhoid fever. Chlorine disinfection is highly effective for most bacteria (AWWA 1999). Waterborne pathogenic bacteria of concern and their associated diseases are presented in Table 4.

Protozoa

Protozoa are single-cell organisms, varying in size from 2 to 100 micrometres, and undergo significant morphological changes while in their host, progressing through their complex life-cycle (Rosen 2000). Normally they survive in cysts (protective shells) when outside of an organism. Protozoa reproduce rapidly inside a host organism; therefore, ingestion with as few as 30 *Cryptosporidium* oocysts or 10 *Giardia* cysts by a human may result in disease (AWWA 1999). Once in water, protozoa can survive for several weeks, even longer if frozen in ice (Fayer & Nerad 1996). Table 5 lists waterborne pathogenic protozoa of concern and their associated diseases. The waterborne pathogenic protozoans of greatest concern in countries with temperate climates are *Cryptosporidium* and *Giardia*. *Cryptosporidium* oocysts and *Giardia* cysts occur in surface water, where their concentrations are positively associated with the level of fecal pollution (Rosen 2000). Arnone & Walling (2006) identified *Giardia* cysts in 80% of CSO samples and found no *Cryptosporidium* oocysts in these effluents. Further

Table 4 | Waterborne bacteria of concern to human health and their associated diseases (Metcalf & Eddy 1991; AWWA 1999)

Bacteria	Source	Disease	Effects
Campylobacter	Domestic, wild animal feces	Campylobacteriosis	Acute diarrhoea
Escherichia coli 0157:H7 (enteropathogenic)	Cattle feces	Gastroenteritis	Vomiting, diarrhoea
Legionella pneumophilia	Aquatic environments	Legionellosis	Acute respiratory illness
Leptospira interrogans	Urine of dogs, livestock, rodents, wild animals	Leptospirosis (Weil's disease)	Jaundice, fever
Salmonella typhi	Domestic and wild animal feces	Typhoid fever	High fever, diarrhoea, ulceration of small intestine
Salmonella (~1,700 serotypes)	Domestic and wild animal, human feces	Salmonellosis	Diarrhoea
Shigella (4 spp.)	Infected humans	Shigellosis	Bacillary dysentery
Vibrio cholerae	Sediments,shellfish asymptomatic human carriers	Cholera	Extremely heavy diarrhoea
Yersinia entercolitica	Animal feces, pork, unpasteurized milk	Yersinosis	Diarrhoea

investigation by Arnone *et al*. (2005) identified *Giardia* cysts in 60% of stormwater samples and *Cryptosporidium* oocysts in 50% of these effluents. Oocysts and cysts are both very persistent in water. Both are very resistant to chemical disinfectants commonly used in drinking water treatment, with *Crytosporidium* oocysts being more resistant (AWWA 1999). A multi-barrier approach including conventional physical processes of sedimentation, coagulation and filtration can remove 99% or better of most protozoa (AWWA 1999). In industrialized countries, outbreaks of cryptosporidiosis and giardiasis are due to oocysts and cysts entering the drinking water because of treatment failure, contamination of the source water and/or leakage into the distribution system (WHO 1993).

Table 5 | Waterborne protozoans of concern to human health and their associated diseases (Metcalf & Eddy 1991, Personal communication)

Protozoan	Source	Disease	Effects
Cryptosporidium	Human, animal and bird feces	Cryptosporidiosis	Diarrhoea, death in susceptible populations
Cyclospora	Human feces	Cyclosporiasis	Diarrhoea
Entamoeba histolytica	Human feces	Amebiasis (amoebic dysentery)	Prolonged diarrhoea with bleeding, abscesses of the liver and small intestine
Giardia lamblia	Human, animal and bird feces	Giardiasis	Mild to severe diarrhoea, nausea, indigestion
Naegleria fowleri	Bird and aquatic mammal feces	Primary Amoebae meningoencephalitis (PAM)	Inflammation of brain and meninges

Viruses

Viruses are the smallest known agents of disease, ranging in size from 0.02 to 0.30 micrometres. They use host cells for reproduction and are unable to reproduce outside their host. After replication, and subsequent death of the host cell, viral particles are spread to neighbouring cells. This results in infection to the individual (Warrington 2001*b*). Table 6 lists the viruses of concern to human health *via* exposure to water, and their associated diseases. The viruses most significantly affecting water quality and human health are enteric viruses, which are found in the gastrointestinal tract of infected individuals. These viruses are excreted in the feces of infected people and may directly or indirectly contaminate water intended for drinking. Enteric viruses multiply only within living cells, by taking over a living cell and using the cell's reproductive mechanism to replicate. Most waterborne virus disease outbreaks in the US are caused by sewage contamination of untreated or inadequately treated private and semipublic water supplies (Warrington 2001*b*). Conventional physicochemical water treatment processes of coagulation-flocculation and filtration remove up to 99% of most enteric viruses (AWWA 1999). Disinfection of water with free chlorine, chlorine dioxide, ozone and UV light radiation can achieve 99.9% inactivation of most enteric viruses (AWWA 1999). However, Norwalk virus is very resistant to disinfection measures, and more research is needed to determine adenoviruses and caliciviruses disinfection properties (AWWA 1999).

Survival in the environment

Many environmental stressors affect pathogen and microbial indicator survival, most notably sunlight intensity (Chamberlin & Mitchell 1978). Intense ultraviolet sunlight over surface waters enhances bacterial die-off, therefore limiting serious bacterial impacts (Chamberlin & Mitchell 1978). Bacteria in turbid waters and bottom sediments are not as susceptible to sunlight as surface water microorganisms and, therefore, survive longer (Chamberlin & Mitchell 1978). Protozoa and viruses survive UV radiation better than bacteria (Johnson *et al.* 1997). Pathogen survival is also dependent on water temperature. Reduced cell metabolism

Table 6 | Waterborne viruses of concern to human health and their associated diseases (Metcalf & Eddy 1991, personal communication)

Virus	Source	Disease	Effects
Adenovirus (48 serotypes; types 40 and 41 are of primary concern)	Humans	Respiratory disease, gastroenteritis	Acute respiratory disease, pneumonia, conjunctivitis, gastroenteritis
Astroviruses	Humans	Gastroenteritis	Vomiting, diarrhoea
Calicivirus (e.g. Norwalk, Norwalk-like and Sapporo, Sapporo-like viruses)[1]	Humans	Gastroenteritis	Vomiting, diarrhoea
Enterovirus (66 types, e.g. polio, echo, encephalitis, and Coxsackie viruses)	Humans	Gastroenteritis, heart anomalies, meningitis	Respiratory illness, common cold
Hepatitis A	Humans	Infectious hepatitis	Jaundice, fever
Hepatitis E[2]	Humans, pigs	Infectious hepatitis	Jaundice, fever
Reovirus	Humans	Gastroenteritis	Vomiting, diarrhoea
Rotavirus	Humans	Gastroenteritis	Vomiting, diarrhoea

[1]Norovirus and Sapovirus are the new genus names for the Norwalk-like and Sapporo-like viruses.
[2]Hepatitis E is an emerging virus that has caused large outbreaks of infectious hepatitis outside the US.

in cold water enhances bacteria survival (Terzieva & McFeters 1991). Borst & Selvakumar (2004) found that die-off for the five indicator bacteria: total coliform, fecal coliform, fecal streptococci, *E. coli* and enterococci, is approximately twice as fast at 20°C compared with 10°C. Protozoa and virus survival are also increased in cold water (LeChevallier *et al.* 1991; Wait & Sobsey 2000). The inactivation of *Giardia* cysts appears to be positively affected by increased salinity (Johnson *et al.* 1997). However, Borst & Selvakumar (2004) found that salinity concentrations do not affect die-off for the five bacterial indicators listed above. Gantzer *et al.* (1998) found seawater salinity has no effect on virus survival. Competition and predation (Rozen & Belkin 2001), and nutrient supply (Gauthier *et al.* 1989) are additional environmental factors influencing die-off. The USEPA compiled die-off rates of microbial indicators and pathogens in Table 6–1 of *Protocol for Developing Pathogen TMDLs* (USEPA 2001*a*). Struck *et al.* (2005) showed that first order die-off curves for bacteria indicator organisms are applicable for only 24 to 48 hours in wetlands and ponds. Once this time is reached, the organisms appear to reach an irreducible concentration probably because of resuspension, lack of treatment with depth, and particle size association.

Microbial source tracking

Microbial source tracking (MST) is one approach to determining the sources of pathogen contamination in watersheds and has been used in TMDL determinations. The advance of molecular-based methods in recent years has aided source identification by allowing a greater level of discrimination between different microbial strains by relying on universal genetic markers (Simpson *et al.* 2002). MST is also an available tool to identify pathogen sources in evaluations of best management practices (BMP) effectiveness. These methods would provide the ability to distinguish between microbes indigenous to the area from the pathogens entering and leaving the BMP. This would provide for the ability to calculate efficiencies for the organisms identified as relevant for TMDL determination and as posing public health risks through discharge to water bodies (Smith & Perdek 2004).

MST has been used in TMDL determinations, generally identifying sources of microbial indicators. A library-dependent method, pulsed field gel electrophoresis (PFGE) was used to identify coliform sources in Northern Virginia's Four Mile Run Watershed (Simmons *et al.* 2000). The study concluded that nonhuman species (raccoon, dog, deer and Norway rat) were the primary *E. coli* sources in the urban stream. Limitations of MST methods are related to the lack of complete descriptions of the bacteria present in watersheds, how microbial indicators correlate with microbial pathogens, and the effects of watershed microbiota on pathogen survival.

WATER QUALITY LEGISLATION AND REGULATIONS FOR MICROBIAL CONTAMINATION

The CWA, passed by Congress in 1972, authorizes the USEPA to protect surface water quality in the US (USEPA 2002*a*). The statute employs a variety of regulatory and nonregulatory tools to sharply reduce direct pollutant discharges into waterways, finance municipal wastewater treatment facilities and manage polluted runoff. These tools are employed to achieve the broader goal of restoring and maintaining the chemical, physical and biological integrity of the nation's waters as defined by the water quality standards (USEPA 2002*a*). Water quality standards are the benchmark against which monitoring data are compared to assess the health of waters and to list impaired waters in accordance with CWA section 303(d). Achieving the standards allows a water body to support 'the protection and propagation of fish, shellfish, and wildlife and recreation in and on the water'. For microbial contamination, water quality standards generally are based on the presence or concentrations of bacterial indicator organisms and the designated use of the water body (USEPA 2000).

Current US microbial water quality standards are based on criteria developed between 20 and 40 years ago. The USEPA (1976) recommended that states adopt fecal coliform indicator criteria not exceeding a geometric mean of 200 organisms 100 ml^{-1} for recreational waters. The USEPA (1986) revised its recreational water quality criteria based on *Enterococcus* and *E. coli* indicators, and implementation guidance to encourage the use of these indicators is being developed (USEPA 2002*c*). Fresh water criteria geometric means shall not exceed 33–100 mL^{-1} for *Enterococcus* or 126-100 mL^{-1} for *E. coli*. The marine water geometric mean criterion for *Enterococcus* shall not exceed 35–100 mL^{-1}. The Beaches Environmental Assessment, Closure and Health (BEACH) Act of 2000 was established to provide a framework for local governments to develop equally protective and consistent programmes across the US. This Act requires that coastal and Great Lake States adopt EPA published microbial standards (USEPA 2002*c*, 2003) by April 2004. Review of the EPA's 'Water Quality Standards for Coastal and Great Lakes Recreational Waters, Final Rule' (Federal Register 2004) shows that 20 of 33 coastal and Great Lakes states had not adopted BEACH Act required standards in 2004 (Natural Resources Defense Council 2005). Fecal coliform is still widely used by several states because of its use historically.

For US water bodies not meeting state-established water quality standards, CWA regulations require a TMDL to be developed. A TMDL is defined as the maximum amount of a pollutant that a water body can receive and still meet the water quality standard, with an allocation to all pollutant sources (USEPA 2001*a*). The EPA has presented the pathogens TMDL process in depth in *Protocol for Developing Pathogen TMDLs* (USEPA 2001*a*). Inherent in this process are the complexities associated with the use of indicators as estimations of pathogen contamination. For the cases when the numeric water quality standard does not appropriately or sufficiently reflect the use impairment, the use of supplementary indicators might provide additional means for measuring attainment of designated uses. For microbial contamination, this supplementary indicator could be another indicator or a pathogen. Other pertinent legislation, such as the SDWA, must be considered when developing a TMDL.

A significant component of developing a TMDL is estimating source loadings and determining their linkages with receiving-water quality. The complexity of the approach used is based on the nature and complexity of the sources and the level of accuracy desired. The required level of analysis generally becomes more detailed with increases in watershed area, number and type of sources, and diversity of land uses. Simple analyses can be conducted using the Pathogen Loading Estimation Tool, which has been used in over 50 TMDLs (Parker & Lahlou 2001) or similar spreadsheet models. For more intricate analysis, complex computerized models available to estimate loads include Automated QILLUAS (AUTOQI) (USEPA 1997) and SWMM: Storm Water Management Model (USEPA 2005*a*). The EPA's BASINS (USEPA 2002*d*) and the Army Corps of Engineers CEQUAL are publicly available receivingwater models that can be used for the linkage determination for microbial contamination (USEPA 1997). The accuracy of models for pathogens has been questioned because of the variable nature of microbial concentrations in sources and in the environment and the uncertainty of the die-off estimates. Given the current state of understanding with respect to pathogen sources and their fate and effect in water bodies, the incremental benefit gained by using the complex models over basic calculations is uncertain.

The SDWA, originally passed by Congress in 1974, authorizes the USEPA to set national health-based standards for drinking water. The 1986 and 1996 SDWA Amendments require actions to protect drinking water and its various sources such as rivers, lakes, springs and groundwater wells. The SDWA mandates a process for identifying new contaminants which may require regulation in the future, known as the Contaminant Candidate List (CCL). The EPA released the second drinking water CCL in February 2005. This list includes nine microbiological contaminants (Federal Register 2005).

Developed to support SDWA implementation, the Surface Water Treatment Rule (SWTR) (USEPA 1989), Interim Enhanced SWTR (IESWTR) (USEPA 1998), Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR) (USEPA 2002*e*), and Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (USEPA 2005*b*) are designed to prevent the outbreak of waterborne diseases caused by viruses, bacteria and the protozoans *Giardia* and *Cryptosporidium*, which are present in varying concentrations in most surface waters. Built upon the treatment technique requirements of the SWTR, the purposes of the IESWTR are to improve control of microbial pathogens, specifically *Cryptosporidium*, in drinking water, and address risk trade-offs with disinfection by-products. The goal of the LT1ESWTR is improved health protection through enhanced drinking water treatment for the physical removal of microbial contaminants by enhanced filtration and other physical removal processes. The LT2ESWTR goal is to reduce disease incidence associated with *Cryptosporidium* and other pathogenic microorganisms in drinking water. This rule will supplement existing regulations by targeting additional *Cryptosporidium* treatment requirements to higher risk systems.

EVALUATION OF PATHOGEN INDICATORS

Water quality standards are based on the concentrations of bacterial indicator organisms and the designated use of the water body. Measuring microbial indicators is less expensive, easier and more common than measuring pathogens directly. However, indicator organisms used by monitoring programmes are limited in their ability to predict pathogen presence and health risks. Protecting public health may require conducting monitoring and management measures such as watershed evaluations to identify sources of elevated indicator concentrations or any pathogens present. A TMDL is generally developed for the parameter that exceeds water quality standards and possibly for another indicator or pathogen that needs to be addressed to eliminate the use impairment (USEPA 2001*a*).

Total coliforms are a group of closely related, mostly harmless bacteria that live in soil and water as well as the gut of animals. Fecal coliforms, a subset of total coliforms, are the most common microbiological indicator. They are commonly found in the enteric tracts of humans and other warm-blooded animals. If fecal coliforms are detected in sufficiently high concentrations, then there is a high probability of contamination by human fecal matter, which may contain pathogens (USEPA 2001*a*). Fecal coliforms are defined by their ability to grow at an elevated temperature (44.5°C). Most coliforms are not harmful; some strains, including *E. coli* 0157:H7, are pathogenic.

Studies evaluating the use of coliform bacteria as indicators of fecal contamination have shown mixed results (Table 7). Some researchers have reported favourably on coliform testing, often in conjunction with other microorganisms, as an indicator of pathogens. Epidemiological research in the United Kingdom concluded that the European Union's recreational water testing requirements, which include total coliforms, fecal coliforms and other organisms, adequately protect the health of swimmers in coastal waters (Pike 1994). Several other investigators (Seyfried *et al.* 1985; Ferley *et al.* 1989; Corbett *et al.* 1993; Haile *et al.* 1999) have reported positive correlations between fecal coliform concentrations and incidence of general morbidity or total illness. Numerous other studies, however, have found that fecal coliform or total coliform concentrations do not correlate well with illness (Fattal *et al.* 1987; Cheung *et al.* 1990; Calderon *et al.* 1991; Kay *et al.* 1994; Kueh *et al.* 1995; McBride *et al.* 1998). The USEPA epidemiological-microbiological studies conducted in the 1970s concluded that fecal coliform densities showed little or no correlation with gastrointestinal illness among swimmers (Cabelli 1983; Dufour 1984). Although fecal coliforms are primarily associated with the enteric tracts of warm-blooded animals, Dufour (1984) suggests that many bacteria in the environment fit the description of fecal coliforms but do not come from gastrointestinal sources. Thus, these bacteria are of questionable use as fecal indicators.

Several epidemiological studies support the use of *E. coli* and *Enterococcus* as indicators of fecal contamination (Table 7). *Enterococcus*, a subgroup of fecal streptococci, is frequently found in the human digestive tract (USEPA 2001*a*). *Enterococcus* is tolerant of a wide range of environmental conditions and is easy to culture (USEPA 2001*a*). Research in the US has demonstrated high correlations between *Enterococcus* densities and gastrointestinal illness among swimmers in fresh (Dufour 1984) and marine (Cabelli 1983) waters. Dufour (1984) also found a high correlation between *E. coli* and illness in fresh waters. Epidemiological research in Israel and New Zealand demonstrated strong relationships between *Enterococcus* densities and the incidence of illness among swimmers in marine waters receiving raw sewage (Fattal *et al.* 1987) and treated sewage discharges (McBride *et al.* 1998). Other investigators have also reported positive relationships between the incidence of gastrointestinal illness among marine water swimmers and *Enterococcus* densities (Cheung *et al.* 1990; Haile *et al.* 1999).

Table 7 | Key microbial water/discharge epidemiological studies

Author	Country	Water/ discharge types	Indicator best correlated with swimming-associated gastrointestinal illness	Indicator best correlated with other swimming- associated illnesses
Cabelli 1983	US	Marine/sewage	*Enterococcus*; EC to a lesser extent	N/A
Fattal *et al.* 1987	Israel	Marine/raw sewage	*Enterococcus*	N/A
McBride *et al.* 1998	New Zealand	Marine/treated sewage	*Enterococcus*	N/A
Cheung *et al.* 1990	Hong Kong	Marine/sewage and stormwater	EC and *Enterococcus*	N/A
Kueh *et al.* 1995	Hong Kong	Marine/sewage and stormwater	Turbidity, *Clostridium perfringens*, *Aeromonas* spp., *Vibrio cholerae*	None found
Kay *et al.* 1994	United Kingdom	Marine/various	FS	N/A
Corbett *et al.* 1993	Australia	Marine/primary treated	None found	FC for cough, ear symptoms, eye symptoms, fever
Haile *et al.* 1999	US	Marine/ stormwater	FC, *Enterococcus*, EC, viruses, distance from storm drain	FC for skin and respiratory symptoms, enterococcus for skin symptoms, EC for eye, ear and skin symptoms, viruses for fever, chills, eye and respiratory symptoms
Pike 1994	Great Britain	Marine	TC and enteroviruses; FS in cohort study	None found
Dufour 1984	US	Fresh/sewage	EC and *Enterococcus*	N/A
Ferley *et al.* 1989	France	Fresh/untreated sewage	FS	FC for general morbidity; FC, *Aeromonas* and *Pseudomonas aeruginosa* for skin diseases
Seyfried *et al.* 1985	Canada	Fresh/not stated	FS	Total staphylococcus (strongest), FC and FS (weakest) for total illness; total staphylococcus for eye and skin illnesses
Calderon *et al.* 1991	US	Fresh/animal nonpoint source	Staphylococci; bather density	N/A

FC - fecal coliform, FS - fecal streptococcus, EC - *E. coli*, TC - total coliform.

CONCLUSIONS

Regulations developed under the CWA require the development of TMDLs for water bodies not in compliance with established water quality standards. A CWA goal is to remove any use impairments by identifying the amount of pollutants that can be discharged into the water body to maintain the established standards. Because of complexities associated with microbial contamination, this is generally not a straightforward process. Microbial indicator measurement has remained the primary means for assessing microbiological contamination in water because of its low cost and simplicity, as well as its ability to indicate the presence of human fecal material. However, indicator organisms and their associated monitoring programmes are limited in their ability to predict pathogen presence and health risks. Fecal coliform has historically been the microbiological indicator of choice, but its presence does not always correlate well with the incidence of disease. Several epidemiological studies support the use of *E. coli* and the *Enterococcus* group as indicators of fecal contamination. In the US, fecal coliform is being replaced by *Enterococcus* and *E. coli* for fresh waters, and *Enterococcus* for marine waters.

Of the 13 epidemiological research studies from around the world reviewed, *Enterococcus* density appears to be the indicator most strongly correlated with gastrointestinal illness among bathers in recreational waters. *E. coli* is also related to gastrointestinal illness in a number of these studies. Both of these organisms were found to be related to enteric illness more frequently than fecal coliforms. Five of the studies reviewed investigated non-enteric effects in addition to gastrointestinal illness. Fecal coliforms, staphylococcus, *Aeromonas*, *Pseudomonas aeruginosa* and viruses were found to be related to respiratory, eye, and skin symptoms as well as fevers. Climate, water type, survival factors and pollution sources (e.g. sewage or stormwater runoff) are all factors affecting the ability of an indicator to be a predictor of pathogenic pollution and illness.

When approaching the TMDL process, any use impairments have to be addressed to adequately restore water quality and protect human health. For microbial contamination, waterborne disease outbreaks associated with recreation or ingestion can lead to use impairments. Developing a TMDL for an alternate indicator or pathogen, in addition to the indicator identified as the water quality standard, may be required. The US waterborne disease outbreak summaries provide information on the types of bacteria, viruses and protozoa known to have caused outbreaks and serve as a starting point for investigations of outbreaks caused by unknown agents. However, this information is not complete because many waterborne disease outbreaks and cases go unreported, and there are outbreaks where the pathogen responsible is not identified.

DISCLAIMER

Any opinions expressed in this paper are those of the authors and do not, necessarily, reflect the official positions and policies of the USEPA.

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