Microbiological pollution of wastewater

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There have been great improvements in sewage treatment over the past century. Despite this, and our increased knowledge of the etiology of waterborne disease, microbiological pollution of wastewater remains a major threat to public health. In many countries most sewage is discharged without treatment. Because wastewater treatment plants are not designed to remove all pathogens, even treated wastewater may pose health risks. Most microbial wastewater pathogens are enteric in origin, and the majority come from human faeces. Others originate from animals or are part of the indigenous aquatic microflora. Infection may arise directly by drinking or bathing in contaminated water, or – occasionally – from bioaerosols. There are various food routes to illness. When wastewater is discharged to marine environments, filter feeders such as shellfish concentrate any pathogens present. Depuration may not flush them out. Wastewater used to irrigate fruit or vegetables may pose health risks when the product is consumed raw. Three groups of micro-organisms are responsible for microbiological pollution in wastewater: bacteria, viruses and protozoa. The most common species are listed in Table 1. Helminths also pose serious health problems in developing nations. The round worm *Ascaris lumbricoides* is estimated to have infected 1.5 billion humans, causing around 60,000 deaths annually.

The most frequent waterborne illnesses are caused by enteric viruses. Viruses are obligate intracellular parasites, unable to replicate without a living host cell. They are shed in high numbers in faeces and are not removed by filtration during wastewater treatment. Most contain RNA as their genetic material. Two notable exceptions are adenoviruses and polyomaviruses. These double-stranded DNA viruses use host-cell enzymes to repair DNA damage, rendering them highly UV resistant. Although enteric viruses are usually transmitted by the faecal-oral route, infection from polluted water is also significant. Approximately 10-100 infectious particles are needed to cause illness - significantly less than required for most bacterial infections. The highly infectious rotaviruses are responsible world-wide for around 500,000 childhood deaths annually. Although prevalent in the UK, rotaviruses generally cause a mild form of diarrhoea, and are readily inactivated by chlorine. Human caliciviruses, for example noroviruses (responsible for "winter vomiting disease"), are the most common cause of acute gastroenteritis world-wide. Norovirus is highly resistant to chlorine, heat and alcohol. In the UK, noroviruses and hepatitis A are the most frequent cause of illness resulting from consuming contaminated shellfish. Other common wastewater viral pathogens belong to the picornaviruses, which includes poliovirus, coxsackievirus, echovirus and enterovirus. These single-stranded RNA viruses cause various illnesses, including polio. A global vaccination programme has resulted in the incidence of polio decreasing from 350,000 cases annually in 1985 to 1625 in 2008.

Bacteria are the most numerous pathogens in wastewater and a high dose (10^3 to 10^6 bacteria) is usually required to cause illness. The most common include *Salmonella* species, *Vibrio* *cholerae*, *Shigella*, and pathogenic *E. coli* (famously, 0157:H7). These can cause serious disease outbreaks; typhoid, paratyphoid, cholera, dysentery and fatal food poisoning. *Shigella dysenteriae*– responsible for around a million deaths a year - and *E coli* 0157:H7 have very low infectious doses (around 10) and synthesise potent toxins. Wastewater bacterial pathogens may naturally reside in animals and humans, for example *Campylobacter jejuni.* For others, such as *Leptospira*, the main reservoir is animal faeces and urine. Non-enteric pathogens include the agent of Legionnaire's disease and the bacterium *Helicobacter pylori*, the causative agent of stomach ulcers. These show considerable ingenuity in survival. *Legionella* grows as a biofilm, commonly in water tanks; a form of growth that is more resistant to environmental stresses. *H pylori* can enter a viable but non-culturable state, thus evading laboratory detection. Photosynthetic cyanobacteria, which thrive on excess micro-nutrients present in untreated wastewater, comprise an additional type of microbiological wastewater pollution (Figure 1). Their overgrowth causes oxygen depletion resulting in death of aquatic flora and fauna, and cyanobacterial toxins are poisonous to humans and animals.

Protozoa are more complex than bacteria. Most produce cysts or oocysts, a resistant stage in the life cycle than can withstand harsh environments, such as chlorination. Following ingestion by a suitable host, these encysted stages germinate and cause infection. Typically only 10-100 cysts are required to produce illness. The most common causes of illness by this route include *Entamoeba histolytica*, *Giardia intestinalis* and *Cryptosporidium parvum*. Cryptosporidiosis is widespread and thought to cause 250- 500 million infections world-wide annually. *Giardia* and *Entamoeba* are more commonly prevalent in developing countries and annually cause 200 million cases of diarrhoea and 500 million cases of amoebic dysentery, respectively. Other protozoan pathogens are free-living; *Naegleria* and *Acanthamoeba* can enter the body via aerosols or mucous membranes and infection may result merely from swimming in contaminated water. These opportunistic pathogens are ubiquitous in contaminated water and *Acanthamoeba* cysts, unlike those of other protozoa, are highly UV resistant.

The presence of indicator organisms, usually faecal coliforms (e.g. *E. coli*) or *Enterococcus faecalis*, is the most common means of monitoring microbiological wastewater contamination. Enumeration relies on the most probable numbers technique or membrane filtration method. Although simple and cost-effective, this approach has several drawbacks: enteric bacteria are highly susceptible to water treatments and environmental factors, and may go into viable but nonculturable states when released into freshwater, causing an under-estimate of pollution levels. Conversely, non-pathogenic coliforms from animals are not distinguished from human coliforms, resulting in over-estimates. Other, more resistant, indicator organisms may be more suitable, e.g. *Clostridium* spores, faecal bacteriophages (bacterial viruses), or adenoviruses. Traditionally, waterborne bacterial pathogens are identified by culturing on laboratory media. To detect viruses and protozoa, the population is first concentrated. Viruses are identified by electron microscopy, cell culture (if a host is available) or using immunological techniques. Most protozoan pathogens are present in low numbers and not easily cultured. They are commonly identified using immunoflurorescence and infectivity assays. Recently, molecular detection techniques have been successfully employed to identify all types of wastewater pathogens. They include polymerase chain reaction (PCR), DNA array-based techniques and fluorescence *in situ* hybridization. Real-time PCR is quantitative, distinguishes living and non-living cells, and detects RNA viruses, and multiplex PCR allows simultaneous detection of multiple targets.

Although waterborne diseases continue to cause the most devastating effects in poorer nations, disease resulting from wastewater contamination is a problem for all countries. Globalisation of travel and commerce, increased populations, changes in water treatment technologies, and microbial adaptations all contribute to the emergence of new or re-emergence of old pathogens. Molecular biology has improved our ability to identify pathogens; sanitation and vaccination have reduced the incidence of pathogens and disease; and novel technologies can help remove existing pathogens from water supplies. Nonetheless, new wastewater pathogens will continue to emerge providing new challenges for everyone.

References

Brettar I, Höfle MG. (2008) Molecular assessment of bacterial pathogens—a contribution to drinking water safety. *Current Opinion in Biotechnology* **19**, 274-80.

Bitton, G. (2005). Wastewater Microbiology, third edition. John Wiley and Sons, ltd. ISBN 0-471-65071-4

Bouzid M, Steverding D, Tyler KM. (2008) Detection and surveillance of waterborne protozoan parasites. *Current Opinion in Biotechnology* **19**, 302-306.

Carter, M. J. (2005). Enterically infecting viruses: pathogenicity, transmission and significance for food and waterborne infection. *Journal of Applied Microbiology* **98,** 1354-1380.

Hijnen, W. A. M., Beerendonk, E. F. and Medema, G. J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Research* **40**, 3-22.

Nwachcuku, N. and Gerba, C. P. (2004). Emerging waterborne pathogens: can we kill them all? *Current Opinion in Biotechnology* **15**, 175–180.

UNEP-IETC: (2002) International Source Book on Environmentally Sound Technologies for Wastewater and Stormwater Management **[**TP 15**],** United Nations Environment Programme

World Health Organisation (WHO) (2008). Guidelines for drinking-water quality, third edition, incorporating first and second addenda. Volume 1 – Recommendations. Geneva.

Pathogen	Associated illnesses	Major reservoir
Viruses		
Adenoviruses	Gastroenteritis, Respiratory disease, eye infections	Human faeces
Astroviruses	Gastroenteritis	Human faeces
Caliciviruses	Gastroenteritis	Human faeces
Coronavirus (e.g. SARS)	Gastroenteritis, Respiratory disease	Human faeces
Enteroviruses	Gastroenteritis, various	Human faeces
Coxsackie A	Herpangina, aseptic meningitis, respiratory illness	Human faeces
Coxsackie B	Fever; paralysis; respiratory, heart, and kidney disease	Human faeces
Echovirus	Fever, rash, respiratory and heart disease, aseptic meningitis	Human faeces
Noroviruses (e.g. Norwalk)	Gastroenteritis	Human faeces
Poliovirus	Paralysis, aseptic meningitis	Human faeces
Hepatitis A virus	Infectious hepatitis	Human faeces; rarely primate faeces
Hepatitis E virus	hepatitis	Human faeces
Rotaviruses	Gastroenteritis	Human faeces
Bacteria		
Campylobacter jejuni	Gastroenteritis, long term sequelae (*e.g.* arthritis)	Human/animal faeces
Escherichia coli	Gastroenteritis	Human faeces
E. coli 0157:H7	Bloody diarrhoea, hemolytic uremic syndrome	Human faeces
Helicobacter pylori	Abdominal pain, peptic ulcers, gastric cancer	Human faeces
Legionella pneumophila	Legionnaire's disease	Thermally enriched water
Leptospira species	Leptospirosis	Animal faeces and urine
Salmonella (many serotypes)	Salmonellosis, long term sequelae	Human faeces
Salmonella typhi	Typhoid fever	Human faeces
Shigella (several serotypes)	Shigellosis (dysentery), long term sequelae	Human faeces
Vibrio cholerae	Cholera	Human faeces
Yersinia enterocolitica	Yersiniosis, long term sequelae	Human/animal faeces
Opportunistic pathogens	various, mostly gastroenteritis	Natural waters
Protozoa		
Acanthamoeba species	Amoebic meningoencephalitis, eye infection	soil and water
Balantidium coli	Balantidiasis (dysentery)	human faeces
Cryptosporidium parvum	Cryptosporidiosis, diarrhoea, fever	human and animal faeces
Cyclospora cayetanensis	Persistent diarrhoea	faeces, contaminated foods
Entamoeba histolytica	Amoebiasis (amoebic dysentery)	human and animal faeces
Giardia intestinalis	Giardiasis	human and animal faeces
Naegleria fowleri	Amoebic meningoencephalitis	soil and water
Toxoplasma gondii	Toxoplasmosis	animal (mainly cat) faeces

***Table 1** lists common microbial pathogens found in wastewater effluent*