Emerging waterborne pathogens: can we kill them all?

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The rapid emergence of *Cryptosporidium parvum* and *Escherichia coli* 0157:H7 have created a threat to the drinking water industry and there is a growing need to develop a strategy for recognizing potential emerging waterborne pathogens. Globalization of trade, changing population demographics and changes in treatment technology have been driving factors in the emergence of these new pathogens. An understanding of disinfectant action and microbial resistance to treatment processes is needed to better identify those pathogens likely to be of greatest concern. Recent research on microbial resistance to treatment and disinfection demonstrates that the microbial surface structure and composition and the nature of the genome are key to determining the potential for waterborne transmission of emerging pathogens.

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Abbreviations

CCL	Contaminant Candidate List
SARS	severe acute respiratory syndrome
UV	ultraviolet

Introduction

It has been little more than 100 years since the beginning of modern water treatment in the United States. The introduction of filtration followed by disinfection in major cities in the United States led to a dramatic reduction in typhoid and other diseases associated with fecally contaminated water. This ended the age of waterborne epidemics in the United States — or at least so everybody thought. Unexpectedly, in 1993 the largest waterborne outbreak of disease ever documented occurred in Milwaukee, Wisconsin. Over 400 000 persons developed gastroenteritis and perhaps 100 individuals died. Investigations revealed that the protozoan parasite *Cryptosporidium parvum* was responsible [1]. The organism appeared in high concentrations in the city's water source after a period of heavy rains, allowing some of the oocysts of the organism to penetrate the filtration barrier. Chlorine disinfection was found to have little effect on the viability of the oocysts. Surveys indicated that this organism was common in surface waters and was detected in 60% of the treated drinking water supplies in the United States [2,3]. Since that time new rules for protecting surface water supplies and monitoring have been put into place in the United States and the UK [4•].

The United States Environmental Protection Agency (USEPA) is now required under the 1996 amendments of the Safe Drinking Water Act to review and publish a list of unregulated contaminants that are known or expected to occur in public water systems and that might pose a risk in drinking water [4•]. In 1998 the first of these lists was produced, and is referred to as the Drinking Water Contaminant Candidate List, or the CCL [5]. This first CCL contained a list of 10 microorganisms (Box 1); the organisms were selected because of their potential for transmission by drinking water. Over the past few years a good deal of research has been conducted to better understand the potential for these organisms to be removed by drinking water treatment processes. From this list, *Mycobacterium avium*, microsporidia, and adenovirus appear to be the most difficult to control by conventional drinking water treatment, depending on the type of disinfection utilized. It is important that we gain an understanding of the cellular and molecular basis for this resistance and develop molecular methods for their detection. Considering these factors, other emerging groups of potential waterborne agents and our ability to control them are reviewed.

Waterborne pathogens have continued to emerge for a number reasons (listed in Box 2). The application of the polymerase chain reaction (PCR) to pathogen detection in water was a major breakthrough in our ability to demonstrate the responsibility of agents as causes of waterborne disease. This was especially true for agents that are difficult to isolate by cultural methods. PCR also provided a way to demonstrate that the agent in the water was identical to that causing illness in the exposed population. The development of molecular source tracking has led to a new field, which allows the identification of sources of waterborne agents [6]. Application of these tracking methods has demonstrated how globalization of world trade and travel has resulted in the introduction of pathogens largely limited to the developing world into the industrialized nations. Changes in the way we produce food have also led to increased risks from pathogens. For example, the rapid increase in confined feeding

Box 1 Microorganisms on the United States Environmental Protection Agency's Contaminant Candidate List (CCL).

Acanthamoeba (guidance only)
Adenoviruses
Aeromonas hydrophila
Caliciviruses
Coxsackieviruses
Cyanobacteria
Echoviruses
Helicobacter pylori
Microsporidia
Mycobacterium avium intracellulare

Box 2 Reasons why waterborne pathogens continue to emerge.

Increase in sensitive populations
Globalization of commerce and travel
Development of molecular methods for detection and source tracking
Changes in drinking water treatment technology
Changes in food supply production
Evolution (genetic reassortment)

operations for livestock has increased point source surface and groundwater contamination by the wastes generated by these operations. In attempts to improve the quality of drinking water through changes in technology, we can change the relative importance of waterborne pathogens. For example, using ultraviolet (UV) light to disinfect wastewater to eliminate the production of chloramines can make it more difficult to control adenoviruses, which are the most resistant waterborne pathogen to UV light inactivation currently known [7•]. However, adenoviruses are very sensitive to inactivation by chlorine.

Emerging waterborne pathogens

As new waterborne pathogens continue to emerge (Box 3), there is a growing need to develop methodologies for their identification. Below we examine a number of potential waterborne pathogens in relationship to some of their unique properties at the molecular level, which might make them difficult to remove by conventional water treatment.

Microsporidia

Microsporidia is the non-taxonomic name used to describe organisms belonging to the phylum Microspora. Currently, microsporidia are considered protozoa, but appear to be closely related to fungi. They produce an environmentally resistant stage (called a spore) of 1–3 μm in diameter. To date, over 1000 species of microsporidia capable of infecting animals have been described and are usually considered to be opportunistic pathogens in humans. So far, five genera have been associated with infections in humans, with *Enterocytozoon bienusi*, *Encephalitozoon hellem* and *Encephalitozoon intestinalis* causing the majority of infections. *E. intestinalis* has been detected in groundwater and water sources used for drinking water [8,9]. Two recent studies have indicated that drinking water and swimming pools may be routes of transmission of microsporidia infection among AIDS patients [10,11]. Recent research indicates that *E. intestinalis* is more resistant to chlorine than bacteria and viruses, but more sensitive than the cysts of the larger protozoa *Giardia* [12]. Like the waterborne protozoa *Giardia* and *Cryptosporidium*, it is very easily inactivated by UV light [13]. The effectiveness with which *E. intestinalis* can be removed by physical methods, conventional coagulation, sedimentation and mixed media filtration, is similar to that observed for *E. coli* (removal of ~99%) [7•].

Box 3 Emerging potential waterborne pathogens.

Microsporidia
Mycobacterium avium intracellulare
Adenoviruses
Parvoviruses
Coronaviruses (SARS)
Picobirnaviruses
Circoviruses
Polyoma virus

Mycobacteria

Members of *Mycobacterium avium* complex (e.g. *M. avium intracellulare*) are acid-fast, rod-shaped bacteria the cell walls of which contain high levels of lipid (waxy) material. They are opportunistic pathogens that can infect the lungs, producing cough, fatigue and low-grade fever. The organisms are found in natural waters and drinking water distribution systems throughout the United States at concentrations ranging from 0.8 to 45 000/100 ml [3]. An outbreak in a hospital among immunocompromised individuals was traced to the chlorinated drinking water supply [14]. Recent research has demonstrated this organism to be the most resistant non-spore-forming bacteria to all disinfectants commonly used to treat drinking water [7•]. As other mycobacteria show a similar resistance, the ability of this bacterium to resist inactivation probably results from the waxy material in its cell wall.

Adenoviruses

Adenoviruses (49 different human types) are double-stranded DNA viruses, about 70 nm in diameter. They primarily infect children causing respiratory disease, pneumonia, eye infections and gastroenteritis. Several studies have suggested that they might be the most common enteric viruses in domestic sewage [15]. Along with hepatitis A virus, they may also be the longest surviving enteric viruses in water [16]. Although sensitive to inactivation by oxidizing disinfectants, they are known to be the most resistant waterborne pathogen to inactivation by UV light [7•,17]. This is because of the double-stranded DNA genome, which allows adenoviruses to use the host-cell repair enzymes during replication to repair damage in the DNA caused by the UV light [18].

Parvoviruses

Parvoviruses are single-stranded human enteric pathogenic viruses, which have been associated with gastroenteritis [19]. They are also the smallest known enteric viruses (18–25 nm) and have the lowest isoelectric point [20]. They are the most resistant of the enteric viruses to inactivation by heat [21].

Coronaviruses

Severe acute respiratory syndrome (SARS), which resulted in thousands of deaths in 2003, is a coronavirus. Its source is believed to be live animals sold in food markets of southern China [22]. Although the virus is excreted in respiratory secretions, large numbers are also excreted in the feces; as many as 1.31×10^7 viruses are excreted per gram of feces. Almost 40% of SARS patients have diarrhea during the course of the illness and the virus can be detected in the stool for more than 10 weeks after the infection [23]. The virus does not appear to be spread by aerosols, but by close contact with infected individuals or fomites. Limited research indicates that SARS is fairly stable in the environment and can survive for at least 96 h in feces and on surfaces at room temperature [24].

Polyomaviruses

JC virus (JCV) is a polyoma virus etiologically associated with a fatal demyelinating disease known as progressive multifocal leukoencephalopathy (PML). An association with colon cancer has also been suggested [25]. JCV produces persistent infections in the kidney and is excreted in the urine of healthy individuals and in PML patients [26]. The virus has been detected in sewage worldwide and is stable in the environment [26]. Transmission by the fecal oral route has been suggested, but not proven [27]. Polyoma viruses are small (38–43 nm) non-enveloped and contain a supercoiled double-stranded DNA that is very heat stable. The simian polyoma virus SV40 was found to be more sensitive to chlorine inactivation than the enteroviruses [28].

Picobirnaviruses

Picobirnaviruses are small non-enveloped (30–40 nm) double-stranded RNA viruses associated with gastroenteritis in AIDS patients, as well as the elderly and children [29].

Circoviruses

TT virus (TTV) and TTV-like mini virus (TLMV) were the first human circoviruses to be described [30•]. They both contain circular single-stranded DNA. TTV is 30–32 nm in diameter, whereas TLMV is less than 30 nm in diameter. Both are present in feces, saliva, skin and hair and TTV appears to be enterically transmitted [31]; infection is common throughout the world. TTV was detected in sewage with the same frequency as hepatitis E virus in an endemic area [32]. TTV was originally isolated from patients with hepatitis of unknown etiology; however, the role of TTV and TLMV in human disease is still uncertain. TTV and other circoviruses appear to be very resistant to inactivation by heat [30•,33].

Resistance of waterborne pathogens to treatment removal

Conventional drinking water treatment consists of a series of barriers to remove contaminants from water. The stages of treatment include coagulation (usually using aluminum sulfate and polymers), followed by sedimentation, filtration and disinfection. At a minimum, all drinking water from surface supplies in the United States must receive at least filtration and disinfection [4•]. Although coagulation can reduce the concentration of pathogenic microorganisms, filtration and disinfection are the primary barriers. Filtration is the main barrier for the removal of waterborne protozoan parasites and enteric pathogenic bacteria. Virus removal is enhanced by coagulation, but filtration cannot be totally relied upon because of the small size of viruses. Thus, disinfection becomes the main barrier for viruses. Use of membranes (e.g. ultrafiltration, nanofiltration and reverse osmosis) in the water treatment processes can cause large reductions in all classes of pathogens, but are not absolute barriers for pathogen removal. Herath *et al.* [34] observed that coliphage removal by microfiltration was related to the isoelectric point, with greatest removal near the isoelectric point of the virus.

In summary, the main mechanisms of pathogen removal by drinking water treatment depend upon size exclusion (filtration), chemically enhanced coagulation (bridging between like charged organisms by a chemical), surface adsorption (to the flocs formed during coagulation or filter media), and loss of viability (disinfection). The ability of microorganisms to penetrate any or all of these barriers then depends upon several intrinsic factors (see Box 4). In addition, non-intrinsic water quality factors will also influence removal, such as pH, the presence of soluble and particulate matter, soluble chemical species, and temperature.

Most of the emerging pathogens discussed in this review are viruses, because they have the ability to penetrate

Box 4 Factors that make microorganisms resistant to water treatment.

- Cell walls containing waxy material
- Thick protective resistant stage (e.g. cyst, oocyst, spore)
- Viruses with double-stranded DNA
- Small genome
- Low isoelectic point
- Low hydrophobicity
- Small size
- Clumping factor (genetically controlled surface structures of the specific microbe)
- Ability to associate with organic particulate matter

most filtration systems. The parvoviruses and the newly discovered TLMV appear to be the smallest known waterborne pathogens, and potentially could be removed less effectively than other groups of enteric viruses. Parvoviruses also have the lowest known isoelectric point of enteric viruses, which would be expected to reduce their potential to be removed by filtration. Although a 99.6% reduction of *M. avium* and *E. intestinalis* can be expected by mixed media filtration, they are fairly resistant to disinfectant processes. In the case of *M. avium* any organisms reaching the distribution system have the ability to grow, because of their resistance to disinfectants [35].

The molecular basis of pathogen resistance to disinfection and physical treatment processes

Adsorption is involved in the removal of microorganisms by both flocculation and filtration, and most viruses removal probably occurs by this mechanism. The effectiveness of these processes depends upon the nature of the surface chemistry of the adsorbent and the microorganism. The degree of interaction of these two types of particulates is governed by both electrostatic and hydrophobic interactions. The surfaces of most filtration media and microorganisms are characterized by a net negative charge, which explains the poor removal of most viruses by filter media: enteric viruses can vary greatly in both their isoelectric point and hydrophobicity [36•]. Dowd *et al.* [9] demonstrated that virus transport through soils was dependent upon both the size and isoelectric point of viruses: the smaller the virus and lower the isoelectric point the less virus retention. This was also proven to be the case in field studies in a sand and gravel aquifer [37]. The hydrophobicity of the virus might also have a role, depending upon ionic conditions [36•,38].

Surface properties of microorganisms are also likely to influence the resistance of viruses to disinfectants. The ease with which disinfectants penetrate the outer structures of the organism and the ability of the organism to clump are important factors in the action of many disinfectants. The cell wall of mycobacteria is highly hydrophobic, resulting in a reduction in the permeability of hydrophilic disinfectants [39•]. The clumping of viruses might be related to their isoelectric point, with some viruses clumping in water at a pH near their isoelectric point [40]. Conformational changes affected by the isoelectric point can also affect resistance to disinfection [41].

The mode and site of disinfectant action also affect the resistance of different viral groups. The disinfecting ability of UV light is dependent upon thymine dimerization. In general, viruses with high molecular weight and double-stranded DNA or RNA are easier to inactivate than those with low molecular weight and single-stranded genomes. Likewise, viruses with single-stranded nucleic acids of high molecular weight are easier to inactivate than those with single-stranded nucleic acids of low molecular weight; this is presumably because the target density is higher in larger genomes. Viruses with double-stranded genomes are less susceptible than those with single-stranded genomes, because naturally occurring enzymes within the host cell are able to repair damaged sections of the double-stranded genome, using the non-damaged strand as a template [42]. Thus, it is not surprising that recent research in both the laboratory and field have shown adenoviruses to be the most UV light resistant organism transmitted by the ingestion route [17,43,44,45]. Small genome target organisms like MS2 coliphage are also very resistant to inactivation by UV light [42].

Oxidizing disinfectants may inactivate a virus by interaction with the lipid membrane, capsid and/or the nucleic acid. Generally, enveloped viruses are more sensitive to inactivation by these types of disinfectants [39•]. Damage to the capsid may prevent attachment to the receptor sites on the host cell or release of the genome into the environment [46]. The ease with which disinfectants can penetrate the capsid of various viruses is unknown and probably varies dramatically between viruses and disinfectants. In general, the smaller enteric viruses appear to be more resistant to chemical disinfectants.

Conclusions

The rapid emergence of new, potentially waterborne pathogens has created a greater need to understand the intrinsic factors responsible for microbial resistance to water treatment processes and disinfectants [47–51, 52•,53,54]. Increasing demands are being placed on the water treatment industry to reduce the risks of illness from both chemicals and microorganisms. To address these needs we require a better understanding of microbial resistance at the molecular level and need to develop more rapid ways to assess the effectiveness of treatment.

Recent research on microbial resistance to treatment and disinfection demonstrates that the outer surfaces and the nature of the genome are critical to our understanding of resistance to disinfectants and removal by physical methods.

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