Review Paper

Disinfection resistance of waterborne pathogens on the United States Environmental Protection Agency's Contaminant Candidate List (CCL)

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ABSTRACT

In 1999, the United States Environmental Protection Agency developed a list of emerging waterborne microbial pathogens that may pose a risk in drinking water. This review deals with the disinfection resistance of microorganisms on the Contaminate Candidate List or CCL. Current disinfection practices in the United States appear to be capable of dealing with most of the microorganisms on the CCL, with the exception of *Mycobacterium avium* and adenoviruses. *Mycobacterium avium* is more resistant to most disinfectants than other waterborne bacteria and adenoviruses are the most resistant waterborne microorganisms to inactivation by ultraviolet disinfection. The microsporidium, *Encephalitozoon intestinalis*, shows significant resistance to inactivation by chemical disinfectants and further research on additional species of microsporidia appears to be warranted.

Key words | adenovirus, calicivirus, Contaminant Candidate List, disinfection, drinking water, *Encephalitozoon intestinalis*, *Mycobacterium*

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INTRODUCTION

The United States Environmental Protection Agency (EPA) is required under the Safe Drinking Water Act (as amended in 1996) to publish a list of unregulated contaminants that are known or expected to occur in public water systems that may pose a risk in drinking water (NRC 1999). In 1998, the first of these lists was produced and is referred to as the Drinking Water Contaminant Candidate List, or CCL (EPA 1998). The CCL includes 50 chemical and 10 microbiological contaminants. Table 1 lists the microorganisms of the Contaminant Candidate List (CCL). The list contains four bacteria, four viruses, and two protozoa. The organisms were selected for their potential to be transmitted by water. All of the bacteria and the *Acanthamoeba* occur naturally in water and are considered water-based pathogens. The remaining microorganisms on the list are transmitted by the faecal-oral route. The purpose of this review is to summarize existing information on disinfectant resistance of the CCL microorganisms and to identify significant data gaps.

CHARACTERISTICS OF CCL MICROORGANISMS

Aeromonas hydrophila is a gram-negative rod and occurs in surface waters at concentrations from 10^3–10^4/ml, and has been found at concentrations of 10^2–10^3/ml in some finished and distribution system waters (Moyer 1999).

Table 1 | Microorganisms on the Contaminant Candidate List (CCL)

Bacteria	Viruses	Protozoa
Aeromonas hydrophila	Adenovirus	*Acanthamoeba*
Helicobacter pylori	Calicivirus	*Microsporidia*
Mycobacterium avium intracellulare (*MAC*)	Coxsackievirus	
Cyanobacteria	Echovirus	

Aeromonas hydrophila has been associated with food (Krovacek *et al.* 1995) and waterborne outbreaks of gastroenteritis (Holmberg *et al.* 1986). Counts of aeromonads in contaminated well water associated with cases of gastroenteritis range from 0.7 to 460/ml. *Helicobacter pylori* is a gram-negative curve-shaped bacteria and is the major cause of peptic ulcers in humans (Taylor & Blaser 1991). The organism is associated with the development of stomach cancer (Eurogast Study Group 1993). Epidemiological studies have shown that the consumption of untreated drinking water can cause infection with the organism (Klein et al. 1991). Members of the *Mycobacterium avium* complex (i.e. *M. avium intracellulare*) are acid-fast, rod-shaped bacteria whose cell walls contain high levels of lipid (waxy) material. They are opportunistic pathogens which can infect the lungs, producing cough, fatigue and low grade fever. Opportunistic infections are those that are caused in individuals whose immune system has been compromised in some manner. Infections in the immunocompromised individual constitute a relatively new and severe problem magnified by the current AIDS epidemic and by escalation in organ and tissue transplantations. *Mycobacterium* spp are agents that take advantage of the impaired or destroyed immune system to set up persistent and generalized infections in the immunocompromised host. Such infections are difficult to treat and tend to be long term. From the lungs the organism can be disseminated throughout the body. 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 (LeChevallier 1999).

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 recreational outbreaks have been associated with eye infections, sore throats, and fever (Hunter 1997). Human caliciviruses are single-stranded RNA viruses, 27 to 40 nm in size, that cause gastroenteritis usually lasting two to three days where vomiting is a common symptom. The human caliciviruses are now divided into two groups based on their nucleic acid composition, i.e. Norwalk-like and Sapparo-like (Atmar & Estes 2001). They are believed to be the most common cause of foodborne illness in the United States and have been associated with numerous waterborne disease outbreaks (Hunter 1997). The human caliciviruses have never been grown in the laboratory. Coxsackie and echoviruses are single-stranded RNA viruses, 25 to 30 nm in diameter, and belong to the enterovirus group. They can cause a wide range of diseases including aseptic meningitis, paralysis, rash, fever, heart disease, and have been associated with certain types of diabetes in children. They are excreted in the faeces and are common in sewage polluted waters. Two aseptic meningitis outbreaks caused by coxsackieviruses have been associated with recreational waters (Hawley *et al.* 1973; Denis *et al.* 1994). Echovirus 30 was associated with an outbreak of gastroenteritis among bathers in a swimming pool (Kee *et al.* 1994).

By definition, disinfection does not necessarily destroy all microorganisms; rather disinfection reduces the number of disease-causing microorganisms to an acceptable level. In the United States, disinfection is currently the most widely used method of controlling disease caused by pathogenic microorganisms in water systems. Disinfection is an essential barrier for protection from waterborne disease and includes such practices as chlorination, ozonation, and ultraviolet irradiation (Bitton 1999). Chlorination is the most common method of disinfection in the United States (Craun 1993). This is mostly due to its proven efficacy and low cost. A disadvantage of using chlorine as a disinfectant is the potential for the production of disinfection by-products such as trihalomethanes and haloacetic acids (Craun 1993). These disinfection by-products form when organic carbon concentrations are high in water. For this reason, other disinfectants such as ozone and ultraviolet irradiation are being considered as alternatives to chlorination. The efficacy of disinfection is dependent upon variables such as water pH, temperature, turbidity, and the microorganism (Hoff 1986; Gerba & Rose 1990; Roessler & Severin 1996; Bitton 1999). Additionally, each type of microorganism, and even species within each genus, may have different resistance to each disinfectant (Sobsey 1989; Roessler & Severin 1996).

Laboratory experiments are used to assess the relative resistance of microorganisms to disinfectants. These types of studies typically generate one of three types of

inactivation curves: straight, shouldered, or tailed. First-order kinetics is shown by a straight line or curve. First-order kinetics as seen with a straight line is also considered 'mixed second order'. Because of the relationship of disinfectant concentration and the microorganisms involved, these organisms are considered to be of a sensitive homogeneous population. The shouldered curve represents the response of multi-target kinetics. Shouldered curves are probably due to clumping in microbial suspensions (Rubin *et al.* 1983). The third commonly found disinfection curve is the tailed curve. Tailed curves represent varying populations and/or resistance within the exposed microorganisms. This curve also indicates that interfering factors may be present in the experimental suspension (Roessler & Severin 1996; Bitton 1999). In terms of resistance to chemical disinfectants (e.g. chlorine) it is generally accepted that protozoan cysts are the most resistant followed in order by bacterial spores and acid fast bacteria, viruses and vegetative bacteria (Sobsey 1989; Bitton 1999). However, for each method of disinfection, microorganisms respond differently and inactivation curves may vary for each type of microorganism (Hoff 1986).

An assessment of *Ct* (concentration × time) values for the CCL microorganisms are difficult due to limited data, the variable manner in which the experiments were conducted, how the organism was prepared, measurement of residual disinfectant or how the data were reported. Unfortunately in many studies, residual disinfectant concentration was not determined. The *Ct* values in the tables are estimated from the available data reported by the author when possible. It is important to remember that these *Ct* values for many of the studies are crude estimates. For this reason caution should be exercised when drawing conclusions from the data presented.

DISINFECTANT RESISTANCE OF CCL MICROORGANISMS

A literature search was conducted and information collected on the disinfectant resistance of microorganisms on the CCL. Information on type of suspending media, free available disinfectant concentration, temperature, pH, and time of exposure are included in the tables if the authors provided the information. From this information *Ct* (concentration × time) values were estimated if they were not provided by the authors (EPA 1990).

Chlorine

Table 2 lists the available data for free chlorine inactivation of CCL bacteria. There are very little data available on the inactivation of *Helicobacter pylori* and *A. hydrophila* by free chlorine. These data suggest that *H. pylori* and *A. hydrophila* appear to be very susceptible to chlorine, similar to those of other enteric bacteria (Sobsey 1989). Various strains of *Mycobacterium avium* and *intracellulare* were found to have a *Ct* ranging from 51 to 204 at 23°C (Table 2). This is probably due to the presence of waxy material in its cell wall and ability of some strains to clump more than others. This resistance and its ability to grow in biofilms probably explain the common occurrence of this organism in distribution systems (Van Reyn *et al.* 1993, 1994).

Tables 3 through 6 summarize the available data for free chlorine inactivation of CCL viruses and protozoa. Most studies have shown that coxsackievirus B5 is more resistant to chlorine than poliovirus type 1 (Liu *et al.* 1971; Payment *et al.* 1985; Harakeh 1987). Data suggest that it could be at least ten times more resistant than poliovirus type 1. A study by Liu *et al.* (1971) indicates that poliovirus 1 and coxsackievirus B5 are the most resistant to chlorine, followed by adenovirus 5 and several types of echovirus. Echovirus is less resistant to inactivation by free chlorine than coxsackievirus (Table 3). The enteroviruses are significantly more resistant to chlorine inactivation at pH 10.0 than 6.0 (Tables 3 & 4). Payment *et al.* (1985) suggested that isolates from chlorinated tapwater were significantly more resistant to free chlorine than strains maintained in the laboratory. The non-enteric adenoviruses appear to have similar sensitivity to chlorine, while enteric adenovirus 40 appears very sensitive. This may have been due to differences in the tendency of the different strains to clump. Human caliciviruses cannot be grown in cell culture so the feline calicivirus has been used

Table 2 | Inactivation of CCL bacteria by chlorine

Bacteria (strain)	Water	Free Cl_2 residual (mg/l)	Temp (°C)	pH	Time (min)	Reduction (%)	Est. Ct_{99} mg-min/l	Reference
Aeromonas hydrophila (TW11)	CDF	0.2	21	6	7*	99	1.4*	Massa *et al.*, 1999
A. hydrophila (TW27)	CDF	0.2	21	6	1*	99	0.2*	Massa *et al.*, 1999
A. hydrophila (10693H)	SDW	0.14 ± 0.026	10	ND	5	99.999	ND	Chamorey & Drancourt, 1999
A. hydrophila (10693H)	SDW	0.55 ± 0.39	20	ND	5	99.999	ND	Chamorey & Drancourt, 1999
A. hydrophila (10693H)	SDW	0.60 ± 0.28	30	ND	5	99.999	ND	Chamorey & Drancourt, 1999
A. hydrophila (10693H)	SDW	0.52 ± 0.14	37	ND	5	99.999	ND	Chamorey & Drancourt, 1999
A. hydrophila (clinical)	Tap	0.05	5	7.8	20	90	1.0*	Sisti *et al.*, 1998
A. hydrophila (clinical)	Tap	0.3	5	7.8	20	90	6.0*	Sisti *et al.*, 1998
Helicobacter pylori	CDF	0.5	5	6	1.33	99	0.12	Johnson *et al.*, 1997
Mycobacterium intracellulare	BDF	0.15	ND	7.0	60	70	⩾480	Pelletier & Du Moulin, 1987
M. avium strain A5	BDF	0.15	23	7.0	55	99.9	106	Taylor *et al.*, 2000
M. avium strain 1060	BDF	0.15	23	7.0	55	99.9	204	Taylor *et al.*, 2000
M. avium strain 1508	BDF	0.15	23	7.0	55	99.9	164	Taylor *et al.*, 2000
M. avium strain 5002	BDF	0.15	23	7.0	55	99.9	126	Taylor *et al.*, 2000
M. avium strain 5502	BDF	0.15	23	7.0	40	99.9	51	Taylor *et al.*, 2000

CDF—chlorine demand free; BDF—buffer demand free; SDW—sterile distilled water; Tap—treated drinking water; ND—not done or no data; *—estimated from data, N_0/N, or calculated from data in reference.

as a model. Feline calicivirus appears to have a similar sensitivity to inactivation by chlorine as the other human enteric viruses (Bitton 1999).

De Jonckheere & Van De Voorde (1976) found *Acanthamoeba cubertsoni* cysts to be very resistant to chlorine, but did not define their suspending media. Cursons *et al.* (1980) found *A. cubertsoni* and *A. castellantii* to be somewhat more resistant than enteric viruses under similar conditions. The spores of microsporidium *Encephalitozoon intestinalis* are slightly less resistant to chlorine than *Giardia* cysts, and significantly more resistant than bacteria and enteric viruses (AWWARF 2001).

Chlorine dioxide

Table 7 lists the available data for chlorine dioxide (ClO_2) on the CCL microorganisms. All of the CCL organisms for which data could be found appear fairly sensitive to chlorine dioxide, except *Acanthamoeba*. No studies for *Helicobacter pylori*, *Mycobacterium avium*, calicivirus or

Table 3 | Inactivation of CCL viruses by chlorine

Virus (strain)	Water	Free Cl_2 residual (mg/l)	Temp (°C)	pH	Time (min)	Reduction (%)	Est. Ct_{99} mg-min/l	Reference
Coxsackie B5	BDF	0.5	5	6.0	6.5	99	3.25*	Sobsey *et al.*, 1988
Coxsackie B5	BDF	0.5	5	8.0	19	99	9.5*	Sobsey *et al.*, 1988
Coxsackie B5	Tap	11.8	25	8.1	5*	99	59*	Grabow *et al.*, 1984
Coxsackie B5	HTE	27.0	25	8.5	4*	99	108*	Grabow *et al.*, 1984
Coxsackie B5	CDF	0.51–0.52	5 ± 0.2	6.0–6.06	3.4	99	1.73*	Engelbrecht *et al.*, 1980
Coxsackie B5	CDF	0.48–0.50	5 ± 0.2	7.81–7.82	4.5	99	2.16*	Engelbrecht *et al.*, 1980
Coxsackie B5	CDF	0.50–0.51	5 ± 0.2	9.93–10.05	66.0	99	33.0*	Engelbrecht *et al.*, 1980
Coxsackie A9	CDF	0.46–0.49	5 ± 0.2	6.0	0.3	99	0.14*	Engelbrecht *et al.*, 1980
Coxsackie A9	CDF	0.48–0.50	5 ± 0.2	10.0–10.01	1.5	99	0.72*	Engelbrecht *et al.*, 1980
Echo 1	CDF	0.48–0.49	5 ± 0.2	6.0	0.5	99	0.24*	Engelbrecht *et al.*, 1980
Echo 1	CDF	0.47–0.49	5 ± 0.2	7.81–7.82	1.2	99	0.56*	Engelbrecht *et al.*, 1980
Echo 1	CDF	0.49–0.51	5 ± 0.2	10.0–10.4	96.0	99	47.0*	Engelbrecht *et al.*, 1980

Tap—treated drinking water; CDF—chlorine demand free; BDF—buffer demand free; HTE—humus tank effluent; *—estimated from data, N_o/N, or calculated from data in cited reference.

microsporidia inactivation by chlorine dioxide were found.

Ozone

The resistance of the CCL bacteria and protozoa to ozone disinfection is shown in Table 8. Table 9 lists the available data for ozone (O_3) for viruses. The cysts of *A. castellantii* were also more resistant than the other protozoa and viruses. As a group, the viruses were the most sensitive to ozone of the CCL organisms.

Ultraviolet light

The resistance of CCL bacteria and protozoa to UV light inactivation is shown in Table 10. Table 11 lists the available data for ultraviolet irradiation of CCL enteric viruses. To obtain accurate information on ultraviolet inactivation, it is necessary to use a collimated beam apparatus. The collimator provides a radiation field that can be quantified accurately by using a radiometer equipped with an appropriate detector. The collimator also provides reproducibility in the experimental protocol because the ultraviolet irradiation is directed to the microbial suspension at a uniform intensity. Few studies utilized the collimated beam or similar apparatus and it was difficult to determine the intensity and/or dose necessary for inactivation. Double-stranded RNA rotavirus is known to be more resistant than the single-stranded RNA enteroviruses, although only a few enteroviruses have been studied. Adenoviruses appear to be the most resistant waterborne organisms to inactivation by UV light, probably because of the double-stranded DNA in their genome (Day *et al.* 1975).

Table 4 | Inactivation of CCL viruses by chlorine

Virus (strain)	Water	Free Cl_2 residual (mg/l)	Temp (°C)	pH	Time (min)	Reduction (%)	Est. Ct_{99} mg-min/l	Reference
Echo 5	CDF	0.5	5	6.0	1.3	99	0.65*	Engelbrecht *et al.*, 1980
Echo 5	CDF	0.5	5	10.0	27	99	13.5*	Engelbrecht *et al.*, 1980
Coxsackie B5#	$CaCl_2$	0.4	5	7.0	1,000	99.92*	400*	Payment *et al.*, 1985
Coxsackie B5‡	$CaCl_2$	0.4	5	7.0	1,000	99.95*	400*	Payment *et al.*, 1985
Coxsackie B5‡	$CaCl_2$	0.4	5	7.0	100	99.8*	40*	Payment *et al.*, 1985
Coxsackie B5 (Lab Strain)	$CaCl_2$	0.4	5	7.0	100	98.8*	40*	Payment *et al.*, 1985
Coxsackie B4##	$CaCl_2$	0.4	5	7.0	100	99.3*	40*	Payment *et al.*, 1985
Coxsackie B4‡	$CaCl_2$	0.4	5	7.0	10	99.7*	4*	Payment *et al.*, 1985
Coxsackie B4‡	$CaCl_2$	0.4	5	7.0	10	99.5*	4*	Payment *et al.*, 1985
Coxsackie B4‡	$CaCl_2$	0.4	5	7.0	10	99.7*	4*	Payment *et al.*, 1985
Coxsackie B4 (Lab Strain)	$CaCl_2$	0.4	5	7.0	10	99.2*	4*	Payment *et al.*, 1985
Echo 1	PEW	0.5	2	7.8	26.1	99.99	13.1*	Liu *et al.*, 1971
Echo 7	PEW	0.5	2	7.8	7.1	99.99	3.6*	Liu *et al.*, 1971
Echo 9	PEW	0.5	2	7.8	12.4	99.99	6.2*	Liu *et al.*, 1971

CDF—chlorine demand free; PEW—Potomac estuarine water; $CaCl_2$—calcium chloride; *—estimated from data, N_o/N, or calculated from data in cited reference; ‡—isolated from chlorinated water; #—isolated from raw sewage; ##—isolated from treated sewage.

Data available for *Acanthamoeba*, *M. avium intracellulare*, and *E. intestinalis* were not obtained with a collimated beam apparatus. One study with *A. hydrophila* suggested similar resistance among enteric bacteria.

DISCUSSION

The treatment of surface waters in the United States requires that enteric viruses be reduced by at least 99.99% (4 logs) and *Giardia* by 99.9% (3 logs) by a combination of physical removal (flocculation and filtration) and disinfection (EPA 1990). The physical processes of conventional treatment are expected to remove 2.5 logs of *Giardia* cysts and 2 logs of viruses. Disinfection is expected to achieve the remaining requirements for removal. The United States Environmental Protection Agency provides guidance for different water disinfectants by providing *Ct* requirements under various water quality parameters (i.e. pH and temperature) for achieving these requirements. These requirements were based upon studies using either poliovirus type 1 or hepatitis A as model viruses and *Giardia* cysts as the model protozoan. To assess if current treatment requirements are adequate to control the waterborne disease transmission of emerging pathogens it is essential to know their resistance to disinfectants.

Table 5 | Inactivation of CCL viruses by chlorine

Virus (strain)	Water	Free Cl_2 residual (mg/l)	Temp (°C)	pH	Time (min)	Reduction (%)	Est. Ct_{99} mg-min/l	Reference
Echo 11	PEW	0.5	2	7.8	13.4	99.99	6.7*	Liu *et al.*, 1971
Echo 12	PEW	0.5	2	7.8	14.5	99.99	7.25*	Liu *et al.*, 1971
Echo 29	PEW	0.5	2	7.8	20.0	99.99	10*	Liu *et al.*, 1971
Coxsackie A5	PEW	0.5	2	7.8	33.5	99.99	16.8*	Liu *et al.*, 1971
Coxsackie A9	PEW	0.5	2	7.8	6.8	99.99	3.4*	Liu *et al.*, 1971
Coxsackie B1	PEW	0.5	2	7.8	8.5	99.99	4.3*	Liu *et al.*, 1971
Coxsackie B3	PEW	0.5	2	7.8	16.2	99.99	8.1*	Liu *et al.*, 1971
Coxsackie B5	PEW	0.5	2	7.8	39.5	99.99	19.8*	Liu *et al.*, 1971
Adeno 3	PEW	0.5	2	7.8	4.8	99.99	2.4*	Liu *et al.*, 1971
Adeno 7A	PEW	0.5	2	7.8	12.5	99.99	6.25*	Liu *et al.*, 1971
Adeno 12	PEW	0.5	2	7.8	13.5	99.99	6.75*	Liu *et al.*, 1971
Adeno 5	Tap	0.2	25	7.5	30	99	6.0*	Abad *et al.*, 1994
Adeno 40	CDF	Yes	5	7.0	ND	99	0.03†	AWWARF, 2001
Adeno 40	CDF	Yes	5	8.0	ND	99	0.11†	AWWARF, 2001
Feline calicivirus	CDF	Yes	5	7.0	ND	99	0.0016†	AWWARF, 2001
Feline calicivirus	CDF	Yes	5	8.0	ND	99	0.83†	AWWARF, 2001
Feline calicivirus aggregated	CDF	Yes	5	7.0	ND	99	1.25†	AWWARF, 2001
Coxsackie B5	ASE	7.8	15	7.2	ND	99	ND	Harakeh, 1987

Tap—treated drinking water; ASE—activated sludge effluent; PEW—Potomac estuarine water; ND—Not done or no data; *—estimated from data, N_o/N, or calculated from data in cited reference; CDF—chlorine demand free; †—predicted by Hom Power Model.

Ultraviolet light

The EPA Guidance Manual (EPA 1990) gives values for 1 and 3 log reductions based on studies done by Battigelli *et al.* (1993) for hepatitis A virus (HAV). These data were used because HAV had been established as an important waterborne virus for some time (EPA 1990). The recommended doses for UV light were derived by applying a safety factor of three to the HAV inactivation data, i.e. three times the observed value for hepatitis A virus. For a one log reduction, a dose of 21 was used and 36 mWs/cm^2 for a three log reduction. It was felt that these doses would be more than enough to inactivate any waterborne bacteria.

Like *G. lamblia* (Belosevic *et al.* 2001) and *Cryptosporidium parvum* (Clancy *et al.* 2000), *E.*

Table 6 | Inactivation of CCL protozoa by chlorine

Protozoa (strain)	Water	Free Cl_2 residual (mg/l)	Temp (°C)	pH	Time (min)	Reduction (%)	Est. Ct_{99} mg-min/l	Reference
Acanthamoeba sp. 4A	ND	4–8	25	7.3–7.4	24 h	99.99	960–7200	De Jonckheere & Van de Voorde, 1976
A. cubertsoni (A1)								
A. castellantii (1501)	BDF	0.80	25	7	30	99.9	5.25*	Cursons *et al.*, 1980
A. culbersoni (A1)	BDF	0.95	25	7	30	99.9	6.0*	Cursons *et al.*, 1980
Encephalitozoon intestinalis	CDF	2.0	ND	ND	16	>99.9	ND	Wolk *et al.*, 2000
E. intestinalis	CDF	Yes	5	6	120	99	36–42 (18)†	AWWARF, 2001
E. intestinalis	CDF	Yes	5	7	120	99	24–114 (39)†	AWWARF, 2001
E. intestinalis	CDF	Yes	5	8	120	99	42–73 (59)†	AWWARF, 2001

BDF—buffer demand free; CDF—chlorine demand free; ND—not done or no data; *—estimated from data, N_0/N, or calculated from data in cited reference; †—predicted by Hom Power Law Model.

Table 7 | Inactivation of CCL microorganisms by chlorine dioxide

Organism (strain)	Water	ClO_2 conc. (mg/l) initial (residual)	Temp (°C)	pH	Time (min)	Reduction (%)	Est. Ct_{99} mg-min/l	Reference
Aeromonas hydrophila (M800[##])	BDF	0.2	2 ± 2	8	ND	ND	0.11–0.14	Medema *et al.*, 1991
A. hydrophila (M800[#])	BDF	0.2	2 ± 2	8	ND	ND	0.04–0.14	Medema *et al.*, 1991
Mycobacterium avium strain 1060	BDF	0.1–1.2	23	7.0	ND	99.9	8	Taylor *et al.*, 2000
M. avium strain 5002	BDF	0.1–1.2	23	7.0	ND	99.9	11	Taylor *et al.*, 2000
M. avium strain 5502	BDF	0.1–1.2	23	7.0	ND	99.9	2	Taylor *et al.*, 2000
Acanthamoeba castellantii (1501)	BDF	2.9 (0.65)	25	7.0	30	99.995	19.5*	Cursons *et al.*, 1980
A. cubertsoni (A1)	BDF	2.5 (0.6)	25	7.0	30	99.995	18.0*	Cursons *et al.*, 1980
Feline calicivirus	BDF	1.0 (yes)	5	7.0	5	99	3–15 (0.99)†	AWWARF, 2001
Adenovirus 40	BDF	0.5 (yes)	5	7.0	ND	99	0.28	AWWARF, 2001

BDF—buffer demand free; ND—not done or no data; *—estimated from data, N_0/N, or calculated from data in cited reference; †—predicted by Hom Power Law Model; [#]—culture grown in drinking water; [##]—culture grown in TSB.

Table 8 | Inactivation of CCL bacteria and protozoa by ozone

Organism (strain)	Water	O_3 Conc. (mg/l) initial (residual)	Temp (°C)	pH	Time (min)	Reduction (%)	Est. Ct_{99} mg-min/l	Reference
Mycobacterium avium strain 1060	ODF	ND	23	ND	ND	99.9	0.17	Taylor *et al.*, 2000
M. avium strain 5002	ODF	ND	23	7.0	ND	99.9	0.12	Taylor *et al.*, 2000
M. avium strain 5502	ODF	ND	23	7.0	ND	99.9	0.10	Taylor *et al.*, 2000
Acanthamoeba (3 spp)	DW	0.4	25	7.0	4	>98.9	1.6*	Langlais & Perrine, 1986
A. castellantii (1501)	ODF	6.75 (0.078)	25	7.0	30	99.9*	2.34*	Cursons *et al.*, 1980
A. cubertsoni (A1)	ODF	6.75 (0.08)	25	7.0	30	99.9997	2.4*	Cursons *et al.*, 1980
Encephalitozoon intestinalis	SDW	0.5	Room	ND	2	Some growth	ND	Naumovitz *et al.*, 1998
E. intestinalis	SDW	1.0	Room	ND	2	Some growth	ND	Naumovitz *et al.*, 1998
E. intestinalis	ODF	(0.15)	5	7.0	ND	99	0.3-0.4	AWWARF, 2001

ND—not done or no data; ODF—ozone demand free; SDW—sterile demand free; *—estimated from data, N_0/N, or calculated from data in cited reference; DW—drinking water.

Table 9 | Inactivation of CCL viruses by ozone

Virus	Water	O_3 Conc. (mg/l) initial (residual)	Temp (°C)	pH	Time (min)	Reduction (%)	Est. Ct_{99} mg-min/l	Reference
Coxsackie A9#	ODF	0.032 (0.027)	ND	ND	0.16	99	0.0043*	Emerson *et al.*, 1982
Coxsackie A9#	ODF	0.012 (0.010)	ND	ND	0.33	99.2	0.0033*	Emerson *et al.*, 1982
Coxsackie A9‡	ODF/1 NTU	3.59 (2.66)	ND	ND	0.16	99.4	0.43*	Emerson *et al.*, 1982
Coxsackie A9‡	ODF/5 NTU	3.69 (2.93)	ND	ND	0.16	99.0	0.47*	Emerson *et al.*, 1982
Coxsackie B5	ASE	0.25	20	7.2	5.5	99	1.3*	Harakeh & Butler, 1985
Coxsackie B5	ASE	0.32	20	7.2	2.0	99	0.64*	Harakeh & Butler, 1985
Echo 1	ASE	0.26	20	7.2	10	99	2.6*	Harakeh & Butler, 1985
Adeno 40	ODF	(≤0.1)	5–7	7.0	ND	99	<0.1†	AWWARF, 2001
Feline calicivirus	ODF	(0.02)	5	7.0	ND	99	0.04†	AWWARF, 2001

ND—not done or no data; †predicted by Hom Power Law Model; ODF/1 NTU—ozone demand free samples adjusted to a turbidity of 1 NTU; ODF/5 NTU—ozone demand free samples adjusted to a turbidity of 5 NTU; ASE—activated sludge effluent; *—estimated from data, N_0/N, or calculated from data in cited reference; ‡—associated; #—unassociated.

Table 10 | Inactivation of CCL bacteria and protozoa by UV irradiation

Organism	Water	UV dose mWs/cm^2	Reduction (%)	Reference
Aeromonas hydrophila‡	PBW	~2.5*	99*	Wilson *et al.*, 1992
Aeromonas hydrophila‡	ND	3	90	EPA, 2002
Aeromonas hydrophila‡	ND	8	99	EPA, 2002
Mycobacterium avium intracellulare	GM	0.14	99*	David, 1973
Mycobacterium fortuitum‡	PBW	66	90	EPA, 2002
Mycobacterium fortuitum‡	PBW	103	99	EPA, 2002
Mycobacterium intracellulare‡	PBW	7	90	EPA, 2002
Mycobacterium intracellulare‡	PBW	25	99	EPA, 2002
Acanthamoeba castellantii	ND	~35	90	Wolfe, 1990
Acanthamoeba castellantii	SBW	~70	99*	Chang *et al.*, 1985
Anacystis nidulans	GM	~91	99*	Amla, 1979
Encephalitozoon intestinalis	SDW	50	No growth	Naumovitz *et al.*, 1998
Encephalitozoon intestinalis	SDW	100	No growth	Naumovitz *et al.*, 1998
Encephalitozoon intestinalis	SDW	200	No growth	Naumovitz *et al.*, 1998
Encephalitozoon intestinalis‡	PBW	2.2	90	EPA, 2002
Encephalitozoon intestinalis‡	PBW	8.4	99.9	EPA, 2002

PBW—phosphate buffer water; SDW—sterile distilled water; SBW—sterile buffered water; GM—growth medium; ND—not done or no data; *—estimated from data, N_o/N, or calculated from data in cited reference; ‡—collimated beam apparatus used.

intestinalis appears to be very sensitive to inactivation by UV light. Adenoviruses are the most resistant waterborne organisms to UV light disinfection. The greater resistance of adenoviruses compared to other waterborne microorganisms is because of its double-stranded DNA genome which allows it to use the host cell enzymes during replication to repair damages in the DNA caused by the UV irradiation (Day 1993). The presence and availability of these enzymes in the host cells vary in different cells and it is possible that different cell lines or cells prepared in different ways may give different results. The availability of repair enzymes results in a greater number of infectious viruses that would be detected after UV light exposure (Piperakis & McLennan 1984).

Chlorine

The *Ct* values found for *E. intestinalis* for a 99% reduction are close to those found for *G. lamblia*, but far below those of *Cryptosporidium parvum* (Bitton 1999). Thus, *E. intestinalis* appears to be somewhat more susceptible to chlorine than *Giardia* cysts, but much more resistant than

Table 11 | Inactivation of CCL enteric viruses by UV irradiation

Organism	Water	UV dose mWs/cm^2	Reduction (%)	Reference
Adeno 40‡	SDW	30	90	Meng & Gerba, 1996
Adeno 41‡	SDW	23.6	90	Meng & Gerba, 1996
Adeno 40‡	SDW	124	99.99	Meng & Gerba, 1996
Adeno 41‡	SDW	111.8	99.99	Meng & Gerba, 1996
Adeno 40‡	PBW	58	90	AWWARF, 2001
Adeno 40‡	PBW	170	99.9	AWWARF, 2001
Adeno 2‡	PBW	78	90	EPA, 2002
Adeno 2‡	PBW	119	99.9	EPA, 2002
Coxsackie B5‡	PBW	15	99*	Battigelli *et al.*, 1993
Coxsackie B5‡	PBW	18	90	EPA, 2002
Coxsackie B5‡	PBW	27	99.9	EPA, 2002
Coxsackie B3‡	PBW	16	90	EPA, 2002
Coxsackie B3‡	PBW	24.5	99.9	EPA, 2002
Coxsackie A9‡	SEW	1.66*	99*	Hill *et al.*, 1970
Feline calici‡	PBW	16.8	90	EPA, 2002
Feline calici‡	PBW	25.2	99.9	EPA, 2002
Echo 1‡	SEW	1.25*	99*	Hill *et al.*, 1970
Echo 11‡	SEW	1.41*	99*	Hill *et al.*, 1970

PBW—phosphate buffer water; SDW—sterile distilled water; SEW—sterile estuarine water (salinity 21.8 ppt); ND—not done or no data; *—estimated from data, N_0/N, or calculated from data in cited reference; ‡—collimated beam apparatus used.

enteric bacteria and viruses. *Mycobacterium avium* strains show a great deal of variability in their resistance to free chlorine (Taylor *et al.* 2000) and appear to be the most resistant bacterial species to chlorine. This resistance probably accounts for their common isolation in finished tap water (Van Reyn *et al.* 1993, 1994). The *Ct* for inactivation of 2 log of poliovirus type 1 at pH 7.0 and 5°C is 2.0 in the Guidance Manual (EPA 1990). This appears to be adequate to control most of the enteric viruses (Tables 4 & 5), except for coxsackievirus B5. The work of Payment *et al.* (1985) suggests that isolates from disinfected tap water may be more resistant. Payment *et al.* (1985) suggested that this could be due to a greater tendency of these isolates to clump.

Chlorine dioxide

Acanthamoeba cysts were the most resistant organisms on the CCL for which data could be located. None of the organisms appeared unusually resistant to chlorine dioxide, although the available data were more limited for this disinfectant compared to the other disinfectants.

Ozone

The Guidance Manual (EPA 1990) recommends a *Ct* for *Giardia lamblia* of 1.3 based on studies using *in vitro* excystation to assess viability (Wickramanayake *et al.* 1985). The highest *Ct* value for 99% inactivation observed at 5°C and pH 7.0 was 0.64. Assuming first order kinetics and applying a safety factor of two, a value of 1.3 was recommended. Studies of poliovirus type 1 as a model apply a safety factor of two. For a 2 log reduction of viruses, 0.6 was recommended at 5°C. Application of these *Ct* values would appear adequate to control both the protozoa and viruses on the CCL list.

SUMMARY AND DATA GAPS

Overall it appears that adenoviruses and *M. avium* are the most difficult to disinfect applying current guidance recommended by the United States Environmental Protection Agency for treatment of surface waters (EPA 1990). Adenoviruses are clearly the most resistant of all waterborne microorganisms to ultraviolet disinfection and current guidance is inadequate for their control. Adenoviruses are the most common viruses usually detected in sewage (Hurst *et al.* 1988), and a screening of surface water samples collected during the Information Collection Rule in the United States found 48.3% contained adenovirus 40, 41, and 11 by molecular methods (Chapron *et al.* 2000). Thus, it is important that additional studies be conducted to assess how preparation of adenovirus in the laboratory and assay conditions (i.e. assessment of different cell lines) affects the estimated resistance of this virus to UV light. *Mycobacterium* spp resistance to chlorine indicates that it will be difficult to control in distribution systems even with a residual. Since *Mycobacterium* spp usually only cause health problems in compromised individuals (i.e. individuals whose immune systems are impaired) it may only be of concern to sensitive populations (Van Reyn *et al.* 1994). Given the significant resistance of *E. intestinalis* to chlorine and ozone, additional studies should be conducted on other species of microsporidia that infect humans.

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