Chlorine Inactivation of *Escherichia coli* O157:H7

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We analyzed isolates of *Escherichia coli* O157:H7 (which has recently caused waterborne outbreaks) and wild-type *E. coli* to determine their sensitivity to chlorination. Both pathogenic and nonpathogenic strains were significantly reduced within 1 minute of exposure to free chlorine. Results indicate that chlorine levels typically maintained in water systems are sufficient to inactivate these organisms.

Escherichia coli O157:H7 is becoming increasingly recognized as a waterborne pathogen. Two recent outbreaks during summer 1998, one involving a drinking water supply in Wyoming (1) and another involving recreational water exposure at a water park in Georgia (2), have underscored the role of water in transmission. Contaminated drinking water (3,4) and recreational water have been associated with outbreaks of hemorrhagic colitis caused by *E. coli* O157:H7 (5-7). Chlorination of water is one of the primary public health measures used to ensure that both potable water and water used in recreational settings are free of microbial pathogens. Our study was undertaken to determine the chlorine resistance of *E. coli* O157:H7 and compare this resistance with that of wild-type *E. coli.*

Seven strains of *E. coli* O157:H7, isolated from cattle from geographically distinct areas (Florida, Idaho, Illinois, Missouri, Texas, Washington, and Wisconsin), were obtained from the U.S. Department of Agriculture (D. Miller, Ames, IA). The isolates exhibited the characteristic phenotypic traits: sorbitol-negative, ß-glucuronidase–negative, lactose-positive, indole-positive, and positive for glutamate decarboxylase (8). All enterohemorrhagic isolates were active toxin producers, as determined by in vitro enzyme immunoassay (Meridian Diagnostics, Inc., Cincinnati, OH). These cattle isolates were chosen as representative strains that might contaminate water supplies after surface run-off from pastures and fields. Four wild-type *E. coli* isolates from cattle manure from a local dairy farm (Ohio) were characterized by biochemical test kits (bioMerieux Vitek, Hazelwood, MO). All bacterial cultures used in the disinfection experiments were grown for 18 to 20 hours at 35ºC in brain heart infusion broth, concentrated by centrifugation, and washed three times in phosphate buffer (9) before testing.

The results of the disinfection experiments, including the rates of inactivation, are shown in the Table. Initial levels for all isolates were 5.52 to 5.79 $\log_{10}$ CFU/ml. The mean chlorine levels at each exposure time were 1.1 mg/L free chlorine and 1.2 mg/L total chlorine. For both the pathogenic and the wild-type strains, exposure to these levels of chlorine for 1 minute reduced the viable populations by approximately four orders of magnitude. The inactivation rates and corresponding correlation coefficient (r^2) values are listed in the Table. Little difference was observed in the rates of inactivation for the pathogenic and wild-type organisms.

These results indicate that the *E. coli* O157:H7 isolates used in this study were sensitive to chlorination and were similar in resistance to that of wild-type *E. coli* isolates. The biocidal activity of chlorine decreases with decreasing temperature (not done in this study). The 5ºC temperature we used represents a worst-case condition for both ground water or winter surface-water temperature. A survey of disinfection practices in the United States found that water utilities maintain a median chlorine residual of 1.1 mg/L and a median exposure time of 45 minutes before the point of first use in the distribution system (10). At this level of

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Table. Chlorine inactivation of *Escherichia coli* O157:H7 and wild-type *E. coli* [a]

	Log_{10}CFU/ml					
	Initial	After exposure time of			Inactivation	
Isolate	inoculum	30 sec	60 sec	120 sec	rate (sec^{-1})	r^2
E. coli O157:H7						
N009-6-1	5.63	2.60	1.88	0.82	-2.96	0.82
N6001-8-10	5.78	2.52	1.44	0.72	-3.06	0.68
N6021-5-1	5.78	2.54	1.52	0.66	-3.06	0.54
N60049-26-1	5.68	2.35	1.40	0.54	-3.00	0.86
N6059-7-2	5.72	2.42	1.74	0.86	-3.02	0.72
N6104-5-9	5.62	2.40	1.69	0.72	-2.96	0.89
N6114-7-2	5.63	2.52	1.66	0.89	-2.96	0.82
Mean	5.69	2.48	1.62	0.74	-2.93	0.82
E. coli (wild type)						
A	5.53	2.66	1.80	1.52	-2.51	0.61
B	5.79	2.60	1.48	0.81	-2.68	0.60
C	5.68	2.48	0.92	0.84	-2.61	0.61
D	5.52	2.34	0.95	0.39	-2.50	0.61
Mean	5.63	2.52	1.28	0.89	-2.93	0.71

[a]In chlorine demand-free chlorinated (CDF) buffer, 5°C, pH 7.0, 1.1 mg/L free chlorine, 1.2 mg/L total chlorine. Duplicate chlorine inactivation experiments were conducted in CDF buffer at pH 7.0. All experiments were conducted at 5°C in a recirculating, refrigerated water bath. The chlorinated buffer was prepared by the addition of reagent-grade sodium hypochlorite (Fisher Scientific, Fair Lawn, NJ). Reaction vessels were continuously mixed (250 rpm) by using an overhead stirring apparatus equipped with sterile stainless steel paddles. Chlorine concentrations were determined by the N,N-dimethyl-*p*-phenylenediamine colorimetric method (9). Samples were removed from the reaction vessels at the desired exposure times, and the chlorine was immediately neutralized by the addition of 0.5 ml of 10% (wt/vol) sodium thiosulphate. Vessels containing CDF buffer without chlorine served as controls for determining unexposed concentrations of the bacteria. Initial levels and the number of survivors after chlorine exposure were determined by the membrane filtration procedure using mT7 agar incubated for 22 to 24 hours at 35°C. This medium was chosen because of its ability to recover oxidant-stressed organisms (9). Levels of bacteria were determined by duplicate filtrations of appropriate dilutions for each exposure time.The log_{10}-transformed data were used to determine the levels of inactivation for each isolate. The means for the inactivation data for the *E. coli* O157:H7 isolates and for the wild-type *E. coli* isolates at each exposure time were used to compare the inactivation rates between the pathogenic and the wild-type organisms. The following first order model was used to describe the inactivation rate: $y = y_{10}10^{-at}$, where t = time in seconds, y = CFU/ml at any time t, y_{10} = CFU/ml at time zero, and a = the inactivation rate in sec^{-1}. The log transformation of this equation was used to calculate the inactivation rate. A regression analysis using least squares was conducted for experiments with each individual isolate and for the mean values for each of the two types of isolates (serotype O157 and wild-type) to determine the inactivation rates ("a" values).

chlorination, *E. coli* O157:H7 is unlikely to survive conventional water treatment practices in the United States. *E. coli* O157:H7 survives at a similar rate to that of wild-type *E. coli* in nondisinfected drinking water (11). Survival patterns and sensitivity to chlorination previously observed for the strains used in this study suggest that wild-type *E. coli* could serve as an adequate indicator organism for fecal contamination of water. Using wild-type *E. coli* to indicate *E. coli* O157:H7 would be useful because most analytical procedures for detecting *E. coli* in drinking water (e.g., assays for lactose fermentation at 44°C to 45°C or production of the enzyme ß-glucuronidase) cannot detect pathogenic *E. coli* O157:H7 strains (8).

Although chlorination appears to adequately control this pathogen, not all municipal water supplies use chlorine disinfection. In addition, chlorine residual can dissipate under adverse conditions, and exposure to sunlight or organic chlorine-demand substances can greatly diminish chlorine levels. Protection of organisms associated with particulate matter, such as fecal material, can also readily decrease the biocidal activity of chlorine. These considerations are particularly important in determining the efficacy of chlorination in a recreational water setting. The results of this study indicate that the isolates studied were sensitive to chlorination. Evaluation of other isolates under differing environmental conditions would be worthy of further consideration.

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