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Effects of Disinfectants on *Campylobacter jejuni*

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Because of the increasing recognition that *Campylobacter jejuni* is an important enteric pathogen of humans, we studied the effects of widely used disinfectants on the viability of this organism. At an inoculum size of 10^3 to 10^4 CFU/ml, 1.25 mg of hypochlorite per liter killed three strains within 1 min. At an inoculum size of 10^6 to 10^7 CFU/ml, 5 mg of hypochlorite per liter killed three strains within 15 min. Killing of similar concentrations of *C. jejuni* and *Escherichia coli* by hypochlorite was approximately the same. At the high inoculum, 0.15% phenolic compound, 10 mg of iodophor per liter, 1:50,000 quaternary ammonium compound, 70% ethyl alcohol, and 0.125% glutaraldehyde killed all three strains within 1 min. These studies demonstrate that, under the conditions we tested (pH 7.0; 24 to 26°C), the recommended standard concentrations of disinfecting agents are adequate to destroy *C. jejuni*.

Campylobacter jejuni is a common cause of endemic and epidemic enteritis in humans. Contaminated food or water and direct contact with infected animals have been incriminated in transmission of infection caused by this bacterium (3, 4, 9, 11, 12). Therefore, knowledge of the effects of common disinfectants against *C. jejuni* is needed. Because chlorine is such a widely used agent (disinfection of water, sewage, and other potential vehicles and decontamination of kitchens, restaurants, and hospitals), we tested its effects against *C. jejuni* under laboratory conditions. Other disinfectants commonly used in hospitals and laboratories, including iodophors, alcohol, Formalin, glutaraldehyde, and phenolic compounds, were also tested. As an example of a quaternary ammonium compound, we also tested susceptibility to benzalkonium chloride, not because it is the most effective in its class, but because it is widely used.

MATERIALS AND METHODS

Test organisms. Three strains of *C. jejuni* (DVAMC 80-134, 80-135, and 80-137) isolated from humans with acute enteritis in Denver, Colo., were used. Organisms were stored at −70°C after isolation and reconstituted before testing. The isolates used were passaged a total of not more than three to five times on artificial media before testing. We used brucella broth and agar (BBL Microbiology Systems, Cockeysville, Md.) for reconstituting and subculturing the organisms. Overnight broth cultures, usually yielding 5×10^8 CFU/ml, were used for all tests.

Disinfectants. In these studies, we tested disinfectants from commercial sources manufactured for domestic, institutional, and hospital use. Table 1 lists the sources and their brand and common names, the active ingredients, and the range of concentrations used for testing. Sterile triple-distilled water (or Sorenson buffer) at pH 7.0 was used as the diluent. The concentrations of disinfectants used in this study were based on the concentration of the active ingredients listed in the formulation. For the hypochlorite experiments, concentrations of chlorine diluted by chlorine demand-free water (pH 7.0) were determined by the amperometric method (5) before testing.

Test methods. An overnight culture of *C. jejuni* was centrifuged twice for 20 min at 1,500 × *g*, and the sediment was resuspended in 2.0 ml of Sorenson buffer (pH 7.0) for use. The bacterial concentration of the suspension was assayed by the method of Miles and Misra (6) to ensure the accuracy of the number of organisms used for testing. In the disinfectant assays, 0.2 ml of the bacterial suspension was added to 9.8 ml of the test agent to achieve the desired bacterial and disinfectant concentrations. Sorenson buffer without disinfectant was used as a test control. For the hypochlorite experiments, two inocula of *C. jejuni*, high (10^6 to 10^7 CFU/ml) and low (10^3 to 10^4 CFU/ml), were used for testing; for the other disinfectant experiments, only the high inoculum was used. For all studies, the period of contact ranged from 1 to 240 min, and all tests were carried out at 24 to 26°C.

The commonly used neutralizer of halogens, sodium thiosulfate (0.1%), was found to be inhibitory to at least one of the three test strains in duplicate experiments. Further testing showed that brucella broth neutralized 10 mg of hypochlorite per liter in 1 min. Therefore, we used brucella broth as a neutralizer as well as a growth medium. Determination of the survival of organisms in hypochlorite was done by inoculating in duplicate 1.0 ml of the assay suspension into 9.0 ml of brucella broth. For the other disinfectants, such

TABLE 1. Type, source, and concentration of disinfectants tested

Disinfectant and source	Active ingredients	Range of concn tested
Chlorox (Chlorox Co. Oakland, Calif.)	Sodium hypochlorite, 5.25%	0.312–5 mg/liter
Amphyl (National Laboratories, Toledo, Ohio)	*o*-Phenylphenol, 15.0%; *p*-test-amylphenol, 6.3%	0.02–0.15%
Betadine (Purdue Fredrick, Norwalk, Conn.)	Iodine-polyvinylpyrrolidine; available iodine, 1%	2.5–10 mg/liter
Zephiran (Winthrop Laboratories, New York, N.Y.)	Alkylbenzyl dimethylammonium chloride, 17%	1:400,000–1:50,000
Cidex[a] (Arbrook Co., Summerville, N.J.)	Glutaraldehyde, 2%	0.0005–0.125%
Formalin (J.C. Baker Diagnostic, Bethlehem, Pa.)	Formaldehyde solution, buffered (pH 6.92), 10%	0.078–5%
Ethyl alcohol	Ethyl alcohol, 70%	70%

[a] Currently distributed by Surgikos, Arlington, Tex.

as the phenolic and quaternary ammonium compounds, Tween 80 and azolectin, in recommended concentrations for neutralization (1), were also found to be inhibitory or to delay the growth of at least one test strain of the organism. To minimize the effects of residual disinfectant activity, organisms in test agents were diluted 1:100 in Sorenson buffer before inoculation into brucella broth. Subcultures of bacterial suspensions in the quaternary ammonium and phenolic compounds in the dilutions in which they were used showed no growth, whereas subcultures of these solutions diluted 1:100 showed full growth identical to that in buffer controls.

All broths were incubated for 48 h at 42°C in a jar with an atmosphere of 5 to 6% O_2, 8% CO_2, and the remainder N_2 (2). The broths were subcultured onto sheep blood agar and incubated in the same microaerobic atmosphere for 48 h for growth of *C. jejuni*. The endpoint was defined as the concentration of disinfectant at which none of the three strains survived. All experiments were performed in duplicate.

For comparison, experiments were also performed on the effect of hypochlorite on high and low inocula of *Escherichia coli* (ATCC 25922). One experiment using 10^2 CFU/ml (low inoculum) and two experiments using 10^6 to 10^7 CFU/ml (high inoculum) of *E. coli* were done in parallel with the *Campylobacter* studies.

RESULTS

The effects of hypochlorite on the strains of *C. jejuni* tested are shown in Table 2. For the high inoculum, 5 mg/liter was required to kill all three strains in 15 min; lower concentrations took longer to kill. No killing was detected at 4 h in the presence of 0.312 mg/liter. In contrast, all organisms in the control solutions showed growth. For the low inoculum, 1.25 mg/liter killed the organisms in 1 min, whereas 0.156 mg/liter killed the organisms in 4 h.

Table 3 shows the results of a comparison of the effects of hypochlorite on *C. jejuni* and *E. coli*. For the high inoculum, the effect of hypochlorite on those two organisms was similar. For the low inoculum, although only 10^2 CFU/ml of *E. coli* was tested, compared with 10^3 to 10^4 CFU/ml of *C. jejuni*, the times until killing occurred were similar for each of the concentrations tested.

Table 4 shows the effects of the other disinfectants on the three strains of *C. jejuni*. For the high inoculum (10^6 to 10^7 CFU/ml) for each of the three strains, 0.15% phenolic compound, free iodine in iodophor (10 mg/liter), 1:50,000 quaternary ammonium compound, 70% ethyl alcohol, and 0.125% glutaraldehyde killed the organisms within 1 min. Lower concentrations of these agents took longer to kill. Fifteen minutes was required for 2.5% Formalin to kill the inoculum.

DISCUSSION

A limitation of these studies is that we used *C. jejuni* strains that had been passaged on artificial

TABLE 2. Killing of three strains of *C. jejuni* by hypochlorite

No. of organisms (per ml) tested (range)	Concn (ppm)	Growth after the following times (min)[a]:				
		1	15	30	60	240
10^6–10^7	5	+/−	−	−	−	−
	2.5	+/−	+/−	−	−	−
	1.25	+	+	+	+/−	−
	0.625	+	+	+	+	−
	0.312	+	+	+	+	+
	Control	+	+	+	+	+
10^3–10^4	1.25	−	−	−	−	−
	0.625	+	+	−	−	−
	0.312	+	+	+/−	−	−
	0.156	+	+	+	+/−	−
	0.078	+[b]	+	+	+	+/−
	Control	+	+	+	+	+

[a] +, Growth of all strains tested; +/−, no growth of at least one strain; −, no growth of any strain.

[b] Two strains were tested at this dilution.

TABLE 3. Comparison of killing of *C. jejuni* and *E. coli* by hypochlorite[a]

Hypochlorite concn (mg/liter)	Time (min) until no growth[b]			
	High inoculum		Low inoculum	
	C. jejuni	*E. coli*	*C. jejuni*	*E. coli*
5	15	15	<1	<1
2.5	30	15	<1	<1
1.25	240	240	<1	<1
0.62	240	240	30	60

[a] Three strains of *C. jejuni* (see text) and one strain of *E. coli* (ATCC 25922) were tested.
[b] High inoculum, 10^6 to 10^7 CFU/ml; low inoculum, 10^3 to 10^4 CFU/ml (*C. jejuni*) and 10^2 CFU/ml (*E. coli*).

media for several generations and that the studies were performed in the laboratory, not in natural settings. Nevertheless, for these preliminary studies, we attempted to establish laboratory conditions that would approximate those in the field. In the hypochlorite studies, the killing of *E. coli* we observed was somewhat higher than that reported previously (8), perhaps due to differences in experimental technique. Further studies under field conditions are needed to

TABLE 4. Killing of three strains of *C. jejuni* (10^6 to 10^7 CFU/ml) by common disinfectants and antiseptics

Disinfectant	Concn tested	Growth after the following times (min)[a]:				
		1	15	30	60	240
Phenolic	0.15%	−	−	−	−	−
	0.078%	+/−	−	−	−	−
	0.04%	+	+/−	−	−	−
	0.02%	+	+	+	+	+
	Control	+	+	+	+	+
Iodophor	10 ppm	−	−	−	−	−
	5 ppm	+/−	+/−	+/−	+/−	+/−
	2.5 ppm	+	+	+	+	+
	Control	+	+	+	+	+
Quaternary ammonium compound	1:50,000	−	−	−	−	−
	1:100,000	+	−	−	−	−
	1:200,000	+	+	+/−	−	−
	1:400,000	+	+	+	+	+
	Control	+	+	+	+	+
Ethyl alcohol	70%	−	−	−	−	−
	Control	+	+	+	+	+
Formalin	2.5%	+	−	−	−	−
	1.25%	+	+	−	−	−
	0.625%	+	+	+	−	−
	0.312%	+	+	+	+	−
	0.156%	+	+	+	+	+/−
	0.078%	+	+	+	+	+
	Control	+	+	+	+	+
Glutaraldehyde	0.125%	−	−	−	−	−
	0.0625%	+/−	−	−	−	−
	0.0312%	+/−	−	−	−	−
	0.0156%	+	−	−	−	−
	0.008%	+	+/−	−	−	−
	0.004%	+	+	+/−	−	−
	0.002%	+	+	+	+	−
	0.001%	+	+	+	+	+/−
	0.0005%	+	+	+	+	+
	Control	+	+	+	+	+

[a] +, Growth of all strains tested; +/−, no growth of at least one strain; −, no growth of any strain.

definitively answer questions on susceptibilities.

Because some of the standard neutralizing agents were found to be inhibitory to some *C. jejuni* strains, we used alternative methods of neutralization. The protein-rich brucella broth was found to neutralize chlorine, and dilution of the phenolic and quaternary ammonium compounds was found to eliminate residual disinfectant activity.

The effectiveness of chlorine is known to be influenced by temperature, pH, and concentration of organic compounds (5). As pH decreases, the effectiveness of chlorine for killing microorganisms increases. In most municipal systems, tap water has a pH of 7.5 or above to minimize corrosion of pipes. Although our tests were performed at pH 7.0, because of the low concentrations needed to kill *C. jejuni*, our results suggest that present water treatment chlorination standards are probably effective for clearing *C. jejuni*. Nevertheless, further studies are needed to document the effectiveness of chlorine and chloramines for killing *C. jejuni* under those conditions actually encountered in water treatment operations.

The experiments utilizing high inocula were designed to determine the effects of hypochlorite on contamination with *C. jejuni* in such environments as laboratories, hospitals, restaurants, and slaughterhouses, in which levels of contamination are high. Our results show that the concentration needed to kill the high inoculum of *C. jejuni* are much lower than those commonly recommended (2,000 mg/liter) for general disinfection (10). Although in solutions in which high concentrations of organic materials are present the effectiveness of chlorine disinfection will be reduced, our results indicate a wide margin of safety.

Ethyl alcohol and iodophor compounds are commonly used in hospitals to disinfect skin and thermometers. Our results indicate that in the recommended concentration, ethyl alcohol is effective against *C. jejuni*. The iodophor tested was effective at a concentration of free iodine three logarithms below that recommended.

Although nosocomial infections due to the misuse of quaternary ammonium compounds have been reported (7), these compounds are widely employed for disinfection in hospitals and laboratories. The newly increased recommended concentration, 1:500, has improved the safety of this compound and is 100 times greater than the concentration necessary to kill *C. jejuni* in 1 min. Compared with quaternary ammonium compounds developed later, benzalkonium chloride is one of the least effective. However, the low concentration of this agent needed to kill *C. jejuni* suggests that the other compounds would also be effective.

Glutaraldehyde has been recommended for use in disinfecting delicate instruments at a concentration of 2% at pH 8. Although the final pH achieved in our test solutions was only 7.4, at the low concentration we used, complete killing of *C. jejuni* occurred in 1 min at a level many times lower than that recommended.

Formalin (10%) is commonly used to fix and disinfect tissues. Our results showed that 2.5% killed the organisms in 15 min. In our laboratory, tissues are preserved in 10% Formalin for at least 8 h before further handling; thus, killing of *C. jejuni* should be complete. Because our experimental studies were done in buffer rather than in tissue, further corroboration of the efficacy of Formalin for killing *C. jejuni* is needed.

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