

Antimicrobial effect of electrolyzed water for inactivating *Campylobacter jejuni* during poultry washing

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Abstract

The effectiveness of electrolyzed (EO) water for killing *Campylobacter jejuni* on poultry was evaluated. Complete inactivation of *C. jejuni* in pure culture occurred within 10 s after exposure to EO or chlorinated water, both of which contained 50 mg/l of residual chlorine. A strong bactericidal activity was also observed on the diluted EO water (containing 25 mg/l of residual chlorine) and the mean population of *C. jejuni* was reduced to less than 10 CFU/ml (detected only by enrichment for 48 h) after 10-s treatment. The diluted chlorine water (25 mg/l residual chlorine) was less effective than the diluted EO water for inactivation of *C. jejuni*. EO water was further evaluated for its effectiveness in reducing *C. jejuni* on chicken during washing. EO water treatment was equally effective as chlorinated water and both achieved reduction of *C. jejuni* by about 3 log₁₀ CFU/g on chicken, whereas deionized water (control) treatment resulted in only 1 log₁₀ CFU/g reduction. No viable cells of *C. jejuni* were recovered in EO and chlorinated water after washing treatment, whereas high populations of *C. jejuni* (4 log₁₀ CFU/ml) were recovered in the wash solution after the control treatment. Our study demonstrated that EO water was very effective not only in reducing the populations of *C. jejuni* on chicken, but also could prevent cross-contamination of processing environments. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Campylobacter jejuni infection has been recognized as the most common bacterial cause of human diarrhea (Altekruse et al., 1999). Outbreaks of *Cam-*

pylobacter enteritis have been associated with the consumption of contaminated meat, particularly poultry and dairy products (Tauxe, 1992). Human infection is largely confined to the alimentary track, resulting in severe diarrhea, abdominal pain, stomach cramps, nausea, vomiting, fever, and flaccid paralysis. The bacterium colonizes the intestinal tracks of many animals used for foods. Because of the relatively high frequency of contamination of poultry with *C. jejuni*, raw poultry products have been perceived to be responsible for a significant amount of human illness

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(White et al., 1997). *C. jejuni* has been detected on chicken skin and exposed surfaces during slaughter operation, whereas internal tissues (meat) are intrinsically sterile (Hood et al., 1988; Humphrey et al., 1993). Therefore, the development of an effective process to reduce or eliminate *C. jejuni* on the surface of poultry is crucial.

While chlorine rinses are generally used during processing of poultry for pathogen reduction (James et al., 1992), various other processes have been proposed as alternatives to eliminate or substantially decrease bacterial population on poultry carcasses. These include treatment with trisodium phosphate (Vareltzis et al., 1997; Xiong et al., 1998), cetylpyridinium chloride (Xiong et al., 1998), hydrogen peroxide (Lillard and Thomson, 1983), gamma irradiation (Katta et al., 1991), microwave (Göksoy et al., 2000), and chilling (Vivien et al., 2000). However, most of these processes have not been completely acceptable due to the chemical residues, discoloration of chicken carcasses, high cost or limited effectiveness.

Electrolyzed (EO) water is generated by electrolysis of a dilute salt (NaCl) solution in an electrolysis chamber where anode and cathode electrodes are separated by a membrane. On the anode side, acidic EO water is generated and has strong bactericidal effect on most known pathogenic bacteria, due to its low pH, high oxidation–reduction potential (ORP) (about 1100 mV) and the presence of hypochlorous acid (Kim et al., 2000; Len et al., 2000). Researches conducted in our laboratories have shown that EO water is very effective for inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* (Venkitanarayanan et al., 1999a; Kim et al., 2000). Venkitanarayanan et al. (1999b) also demonstrated that EO water treatment of kitchen cutting boards inoculated with *L. monocytogenes* and *E. coli* O157:H7 reduced populations of the pathogens to undetectable levels. Therefore, we expect that EO water will be an effective process not only in reducing the population of microorganisms on chicken, but also in preventing cross-contamination during poultry washing.

This study was designed to evaluate the effectiveness of EO water for killing *C. jejuni* and its potential application in reducing *C. jejuni* on chicken during washing. Comparative inactivation studies were also performed with chlorinated water.

2. Materials and methods

2.1. Bacterial culture

A six-strain mixture of *C. jejuni* (obtained from the Poultry Microbiology Safety Research Unit, USDA, Athens, GA) was used for this study: *C. jejuni* EPI 4 (chicken carcass isolate, biotype I, serotype R), EPI 7 (chicken carcass isolate, biotype II, serotype 2), EPI 19 (chicken carcass isolate, biotype II, serotype UT), EPI 21 (human feces isolate, biotype II, serotype 2), EPI 47 (chicken feces isolate, biotype II, serotype 9), and EPI 66 (human feces isolate, biotype III, serotype 27). Each strain was grown on *Campylobacter* selective agar (Difco Laboratory, Detroit, MI) that contained 10% lysated horse blood and *Campylobacter* selective supplement (Oxoid No. SR098E) at 42 °C for 48 h under a microaerobic condition (5% O₂, 10% CO₂, 85% N₂). Each strain grown separately on the selective plates was suspended in 0.1 M of phosphate-buffered saline (PBS, pH 7.4) and a six-strain cocktail was then prepared by combining approximately equal portions of each strain. Cells in the cocktail were harvested, washed twice with PBS by centrifugation at 5000 × g for 10 min, and diluted in the same buffer to obtain final populations of about 10⁷–10⁸ CFU/ml.

2.2. Preparation of treatment solutions

EO water was generated using an EO water generator (ROX 20 TA, Hoshizaki Electric, Japan) by electrolysis of a dilute salt solution (about 0.1% sodium chloride, Sigma, St. Louis, MO) at a setting of 14 amperage. After the machine stabilized at a reading of 14 amperage (about 1 min after turning on the machine), EO water was collected from the anode side of the EO water generator. Chlorinated water used in this study was prepared to have similar residual chlorine (50 mg/l) to the EO water by diluting chlorine water (Fisher Scientific Fair Lawn, NJ) with deionized water. The diluted EO or chlorinated water were prepared by diluting the EO or chlorinated water (containing 50 mg/l of residual chlorine) in deionized water to obtain a final residual chlorine concentration of approximately 25 mg/l. Freshly prepared EO and chlorinated water were used for the study and deionized water was used as

the control. The pH and ORP of EO and chlorinated water were measured immediately before and after treatment using a dual-scale pH meter (Acumet model 15, Fisher Scientific Fair Lawn) with pH and ORP electrodes. The residual chlorine was determined by an iodometric method using a chlorine test kit (Hach Ames, IA).

2.3. Treatment of pure culture

A volume of 9 ml of sterile deionized (control), EO, or chlorinated water was transferred to sterile screw-cap tubes. One milliliter of the six-strain mixture of *C. jejuni* was added to each tube at either room temperature (23 ± 2 °C) or 4 °C for 10 or 30 s. Following treatment, 1 ml of each sample was serially diluted (1:10) in sterile 0.1% peptone water and the surviving population of *C. jejuni* was determined by plating 0.1 ml of each dilution in duplicate on *Campylobacter* selective plates. Plates were incubated at 42 °C for 48 h under a microaerobic condition. To detect the presence of lower numbers of survivors that would not be detected by direct plating, a volume of 2 ml of each sample solution was transferred into 25 cm² cell culture flasks (Corning, Corning, NY) containing 10 ml of Bolton's broth (Med-Ox Diagnostics, Ogdensburg, NY) that contained 5% lysated horse blood and *Campylobacter* enrichment supplement (MED-Ox Diagnostics No. BMX131), and incubated at 42 °C for 48 h. Following enrichment in Bolton's broth, the culture was streaked on to *Campylobacter* selective agar, and the plates were incubated at 42 °C for 48 h under a microaerobic condition. The experiment was replicated four times.

2.4. Preparation and inoculation of chicken sample

Middle sections of chicken wings (approximately 50 ± 5 g per sample) were purchased from a local grocery store and stored at 4 °C for no more than 3 h before testing. For inoculation, the skin of each chicken wing was checked to ensure complete coverage of each wing. Each sample was then inoculated with a six-strain mixture of *C. jejuni* by gently spreading 0.1 ml of inoculum onto the skin surface and air-dried under a biosafety hood for 10 min at room temperature (23 ± 2 °C) to allow for bacterial attachment.

2.5. Treatment and bacteriological analysis of chicken samples

Inoculated chicken samples (50 ± 5 g) were placed individually in sterile stomacher bags (Model 400 Bags 6041, Seward, London, UK) containing 500 ml of sterile deionized water (control), EO water, or chlorinated water. Each time, two bags were placed in a Nalgene polypropylene basket ($15.6 \times 16.8 \times 17.8$ cm) and shaken gently (100 rpm) using a platform shaker (New Brunswick Scientific, Edison, NJ) at a temperature of 4 °C or room temperature (23 ± 2 °C) for 10 or 30 min. At the end of the treatment, each sample was placed immediately into 100 ml of sterile neutralizing buffer solution (Difco) and rubbed gently with hands from the outside of the stomacher bag for 2 min. The viable cells in washed treatment solutions and neutralizing buffer solution were assayed through serially diluting in 9 ml of sterile 0.1% peptone water, and then directly plating 0.1 ml of each dilution in duplicate on *Campylobacter* selective plates and incubated at 42 °C for 48 h before counting. Enrichment was performed to detect the presence of lower numbers of survivors that would not be detected by direct plating as described above. The experiment was replicated three times.

2.6. Statistical analysis

For each treatment, the data from the independent replicate trials were pooled and the mean value and standard deviation were determined (Steel and Torrie, 1980).

3. Results and discussion

The pH, ORP, and residual chlorine concentration of each treatment solutions are presented in Table 1. The initial pH and ORP of sterile deionized water were 6.49 ± 0.12 and 418 ± 7 mV, respectively. No residual chlorine was detected in deionized water. Room-temperature EO water generated at a setting of 14 amperage had initial pH, ORP, and residual chlorine of 2.57, 1082 mV, and 51.6 mg/l, respectively. Room temperature chlorinated water prepared by diluting concentrated chlorine water with deionized water had initial pH, ORP, and residual chlorine

Table 1
Properties of EO and chlorinated water^a

Temperature (°C)	Solutions	pH	ORP (mV)	Residual chlorine (mg/l)
23	Deionized water	6.49 ± 0.12	418 ± 7	0
	EO water (50 mg/l) ^b	2.57 ± 0.03	1082 ± 8	51.6 ± 2.7
	EO water (25 mg/l) ^c	2.84 ± 0.02	1050 ± 10	25.6 ± 1.1
	Chlorinated water (50 mg/l) ^d	2.97 ± 0.07	1020 ± 10	52.8 ± 2.1
	Chlorinated water (25 mg/l) ^e	3.03 ± 0.04	990 ± 7	25.3 ± 1.7
4	Deionized water	6.56 ± 0.07	425 ± 3	0
	EO water (50 mg/l)	2.67 ± 0.05	1092 ± 4	53.3 ± 1.5
	EO water (25 mg/l)	2.95 ± 0.03	1072 ± 3	25.7 ± 1.3
	Chlorinated water (50 mg/l)	3.07 ± 0.08	1046 ± 9	53.9 ± 2.1
	Chlorinated water (25 mg/l)	3.22 ± 0.06	1026 ± 5	26.1 ± 1.3

^a Values are the means of four replicated measurements ± SD.

^b EO water containing 50 mg/l of residual chlorine.

^c EO water containing 25 mg/l of residual chlorine.

^d Chlorinated water containing 50 mg/l of residual chlorine.

^e Chlorinated water containing 25 mg/l of residual chlorine.

of 2.97, 1020 mV, and 52.8 mg/l, respectively. The EO and chlorinated water were also further diluted in deionized water to have a final residual chlorine concentration of approximately 25 mg/l, and their water properties are shown in Table 1.

The initial population of *C. jejuni* used for this study was approximately 7.5 log₁₀ CFU/ml. For a 10-s treatment, the *C. jejuni* count in the samples treated with EO or chlorinated water containing 50 mg/l residual chlorine was reduced to undetectable levels (as determined by both direct plating and enrichment

procedures) at both temperatures (Table 2). However, no reduction in bacterial counts was achieved in the control samples. The EO and chlorinated water containing approximately 25 mg/l residual chlorine also had strong bactericidal activity. The mean population of *C. jejuni* treated with EO water was reduced to less than 1.0 log₁₀ CFU/ml (detected only by enrichment for 48 h) regardless of treatment time and temperature. The diluted chlorine water (25 mg/l residual chlorine) was less effective than the EO water at the same residual chlorine concentration for inactivation of *C.*

Table 2
Inactivation of *C. jejuni* by EO and chlorinated water

Temperature (°C)	Time (s)	Surviving population (log ₁₀ CFU/ml) ^a					
		Initial population	Control ^b	EO water ^c (50 mg/l)	EO water ^d (25 mg/l)	Chlorinated water ^e (50 mg/l)	Chlorinated water ^f (25 mg/l)
23	10	7.47 ± 0.13	7.48 ± 0.36	ND ^g	<1 ^h	ND	2.16 ± 0.35
	30	7.47 ± 0.13	7.33 ± 0.43	ND	<1	ND	2.23 ± 0.39
4	10	7.42 ± 0.26	7.41 ± 0.34	ND	<1	ND	2.71 ± 0.54
	30	7.42 ± 0.26	7.51 ± 0.21	ND	<1	ND	2.72 ± 0.69

^a Values are the means of four replicated measurements ± SD.

^b Deionized water.

^c EO water containing 50 mg/l of residual chlorine.

^d EO water containing 25 mg/l of residual chlorine.

^e Chlorinated water containing 50 mg/l of residual chlorine.

^f Chlorinated water containing 25 mg/l of residual chlorine.

^g Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

^h Positive by an enrichment procedure and no detectable survivors by a direct plating procedure.

jejuni (Table 2). After a 10-s exposure to the chlorinated water, the surviving population of *C. jejuni* was more than $2 \log_{10}$ CFU/ml, and treatment time and temperature had no significant effect ($p > 0.05$) on the ability of chlorinated water to inactivate *C. jejuni*.

The mechanism of inactivation of microbial cells by EO water is not clear, but is believed to involve the presence of hypochlorous acid and the high ORP. Previous studies suggested that hypochlorous acid inactivates bacterial cells by (1) oxidation of cell surface sulfhydryl compounds (Leyer and Johnson, 1997), (2) inactivation of enzymes involved in respiration (Albrich et al., 1986; Hurst et al., 1991), (3) inhibition of ATP generation (Barrette et al., 1989), and (4) retardation of active transport (Albrich et al., 1986; Hurst et al., 1991).

Since diluted EO and chlorinated water both contain similar amount of residual chlorine (25 mg/l), the greater antimicrobial effect observed in EO water (Table 2) can possibly be explained by its lower pH and/or high ORP value than those of chlorinated water (Table 1). Previous reports (Carson, 1991; Kim et al., 2000) suggested that ORP of a treatment solution might be the primary factor affecting microbial inactivation. For the current study, higher ORP in EO water appears to be responsible for greater antimicrobial effect than the chlorinated water. This high ORP may be due to the presence of other oxidants. Little is known about the action of extracellular ORP on microbial metabolism. The high ORP in the EO water could cause the modification of metabolic fluxes and ATP production, probably due to the change in the electron

flow in the cells. Further investigation is required to define the characteristics of components in EO water responsible for its inhibitory effects on pathogens.

Table 3 shows survival characteristics of *C. jejuni* on chicken wings treated with EO or chlorinated water. No detectable *C. jejuni* was found on the chicken samples purchased from the local grocery store. The initial population of *C. jejuni* inoculated on each sample was about $5 \log_{10}$ CFU/g. For EO water treatment, the populations of *C. jejuni* were reduced by $3 \log_{10}$ CFU/g, whereas the control resulted in only $1 \log_{10}$ CFU/g reduction. Although greater antimicrobial effect was observed on the EO water than the chlorinated water for the pure culture studies (Table 2), chlorinated water was equally effective as EO water in killing or removing *C. jejuni* on chicken skins. This can be explained by the nature of chicken skin and limited penetration effects of disinfectants. Mandrell and Wachtel (1999) reported that pathogens were attached to a water–skin interface, and further entrapped in folds, crevices, and pores (follicles). Chicken skin also has been shown to provide a microenvironment for the survival of *C. jejuni* in folds and follicles where there is protein supply, and possibly fatty acids and oils, which help cells persist from freezing or cold environments (Lee et al., 1998). For the current study, no significant difference ($P > 0.05$) on survival of *C. jejuni* was observed between 10- and 30-min treatment time. This result suggests that disinfectants in washing solutions have an efficacy to rapidly kill cells located at the water–skin interface, where their penetration seems to be easier, but disinfectants could not pene-

Table 3
Inactivation of *C. jejuni* on chicken by EO and chlorinated water^a

Temperature (°C)	Time (min)	Initial population on chicken (\log_{10} CFU/g)	Surviving population on chicken (\log_{10} CFU/g)			Surviving population in washed solution (\log_{10} CFU/ml)		
			Control ^b	EO water ^c	Chlorinated water ^d	Control	EO water	Chlorinated water
23	10	5.05 ± 0.14	3.91 ± 0.13	2.09 ± 0.09	2.27 ± 0.17	3.83 ± 0.13	ND ^e	ND
	30	5.05 ± 0.14	3.84 ± 0.14	1.83 ± 0.09	2.08 ± 0.07	3.89 ± 0.12	ND	ND
4	10	5.03 ± 0.08	3.89 ± 0.12	2.23 ± 0.13	2.42 ± 0.01	3.97 ± 0.09	ND	ND
	30	5.03 ± 0.08	3.82 ± 0.10	2.08 ± 0.08	2.22 ± 0.20	3.98 ± 0.14	ND	ND

^a Values are the means of three replicated measurements \pm SD.

^b Deionized water.

^c EO water containing 50 mg/l of residual chlorine.

^d Chlorinated water containing 50 mg/l of residual chlorine.

^e Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

Table 4
Properties of EO and chlorinated water before and after treatment^a

Solutions	Temperature (°C)	Time (min)	Before treatment			After treatment		
			pH	ORP (mV)	Residual chlorine (mg/l)	pH	ORP (mV)	Residual chlorine (mg/l)
EO water	23	10	2.55 ± 0.02	1083 ± 4	52.3 ± 1.6	2.67 ± 0.05	681 ± 5	22.5 ± 1.2
		30	2.55 ± 0.02	1083 ± 4	52.3 ± 1.6	2.79 ± 0.03	669 ± 5	18.3 ± 1.0
	4	10	2.54 ± 0.03	1082 ± 6	50.2 ± 1.5	2.65 ± 0.04	648 ± 8	24.7 ± 1.5
		30	2.54 ± 0.03	1082 ± 6	50.2 ± 1.5	2.76 ± 0.05	675 ± 9	19.8 ± 2.0
Chlorinated water	23	10	2.99 ± 0.02	1026 ± 7	53.3 ± 1.4	3.15 ± 0.04	641 ± 3	22.8 ± 0.8
		30	2.99 ± 0.02	1026 ± 7	53.3 ± 1.4	3.37 ± 0.05	628 ± 7	20.1 ± 1.7
	4	10	2.98 ± 0.03	1022 ± 5	51.8 ± 1.7	3.20 ± 0.06	646 ± 6	23.3 ± 1.1
		30	2.98 ± 0.03	1022 ± 5	51.8 ± 1.7	3.42 ± 0.06	634 ± 7	19.2 ± 2.3

^a Values are the means of three replicated measurements ± SD.

trate into the crevices or pores that appear to protect cells.

C. jejuni populations in wash solutions immediately after treatments are also shown in Table 3. No viable cells of *C. jejuni* were observed in either the EO or chlorinated water after treatment. However, an average count of 4.0 log₁₀ CFU/ml was recovered in the wash solution after control treatment. This indicated that EO and chlorinated water reduced the population of *C. jejuni* on chicken and cross contamination to processing environment or other chicken were also prevented due to no survival of *C. jejuni* in the wash solution after treatment. After the washing treatment, the ORP of EO water decreased from initial 1080 to 670 mV, whereas ORP of chlorinated water decreased from 1020 to 630 mV (Table 4). Residual chlorine concentration decreased from 50 mg/l for both EO and chlorinated water to 20 mg/l, probably due to vaporization of molecular chlorine at lower pH during stirring and reactions of chlorine with organic materials in chicken carcasses or with *C. jejuni* cells.

Our study demonstrated that EO water was very effective not only in reducing the population of *C. jejuni* on chicken, but also could prevent cross-contamination of processing environments due to no survival of *C. jejuni* in the EO water after washing treatment. The advantages of using EO water in the poultry processing are: (1) it is a nonthermal treatment for microbial inactivation; (2) no other chemicals except very dilute NaCl solution are required to generate the EO water; (3) it has strong antimicrobial effect to prevent cross-contamination of processing

environments; (4) it is produced on site and on demand with the concentration for direct usage and no dilution from concentrated chemicals is needed; (5) it is a less potential health hazard to the worker due to the lack of need to handle concentrated chemicals for microbial inactivation.

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