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Research note

Influence of nalidixic acid adaptation on sensitivity of various Shiga toxin-producing *Escherichia coli* to EO water treatment

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ABSTRACT

This study was designed to validate the use of nalidixic acid-adapted strains of various STEC for electrolyzed oxidizing (EO) water efficacy testing. The resistance of total 48, parent (NalS) and adapted (NalA) strains of *Escherichia coli* O157:H7 and six major serotypes of non-O157, STEC were tested against EO water using minimum inhibitory concentration (MIC) and inoculated beef trims. MIC was conducted for 15 s testing period with free chlorine concentrations of 3.00, 2.50, 2.00, 1.50, 1.00, 0.50 and 0.25 mg/L. While, beef trims (5 cm cubes) were inoculated with a cocktail of the same serogroup strains and treated with EO water (50 mg/L available chlorine) for 1 min. The MIC values of individual strains ranged from 0.41 to 1.66 mg/L free chlorine of EO water. When treated on beef trim, pathogen load reductions ranging from 0.44 to 1.54 log CFU/cm^2 were observed. No significant differences in sensitivity towards EO water treatment were observed between NalS and their NalA derivatives in either study which validates the use of NalA strains in EO water efficacy study. In addition, the EO water treatment that reduced *E. coli* O157:H7 was equally or more effective in reducing non-O157 STEC on beef trim.

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) in general and seven major STEC serogrops; O26, O45, O103, O111, O121, O145 and O157 have been recognized as major foodborne pathogen (Hussein & Bollinger, 2005). STEC infections can lead to serious health issues such as hemolytic uremic syndrome and kidney failure (Blanco et al., 2003). Because of the severity of the disease caused by STEC the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) announced that as of June 2012, raw nonintact beef products contaminated with STEC O26, O45, O103, O111, O121 and O145 would also be considered adulterated same as *E. coli* O157:H7 (USDA-FSIS 2011). In this scenario, it is important for the beef industry to examine the effectiveness of various current and new pathogen intervention steps in beef processing to control non-O157 STEC.

Often antimicrobial efficacy determination studies become intricate because of high levels of background flora present on food matrices. One of the common approaches is to use antibiotic resistant pathogens such as nalidixic acid adapted strains as markers for inoculation studies. For successful application of this approach, antibiotic adapted pathogens must be validated to have similar resistance to interventions as their antibiotic sensitive parents (Niemira, 2005). In a number of previous studies, nalidixic acid adapted pathogens were validated for use in various interventions (Blackburn & Davies, 1994; Taormina & Beuchat, 1999). In contrast, some studies also reported that nalidixic acid resistance increases sensitivity of several *E. coli* O157:H7 and *Salmonella* isolates to ionizing radiation (Niemira, 2005; Niemira & Lonczynske, 2006). Based on previous studies it is clear that suitability of nalidixic acid adapted strains as marker organisms should be determined for all new interventions.

Electrolyzed oxidizing (EO) water is one of the emerging environment friendly antimicrobial treatments (Huang, Hung, Hsu, Huang & Hwang, 2007). EO water is produced by electrolysis of dilute salt solution (<0.1%NaCl) in electrolysis chamber containing an anode and a cathode separated by a diaphragm membrane. As a result of electrolysis of dilute salt solution, the anode side of the chamber produces water containing chlorine gas, hypochlorous acid and hydrochloric acid. Therefore, this type of EO water is also known as acidic EO water. It has pH <2.7, oxidation reduction potential (ORP) >1050 mV and high free chlorine concentrations (Jadeja, Hung & Bosilevac, 2013). These properties make acidic EO water effective as an antimicrobial agent (Hsu, 2003). EO water has been successfully used to control pathogens from fresh produce (Hung, Tilly & Kim, 2010; Pangloli, Hung, Beuchat, King & Zhao,

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2009), fish and seafoods (Huang, Shaiu, Hung & Hwang, 2006). Bosilevac, Shackelford, Brichta & Koohmaraie (2005) used EO water to decontaminate cattle hide and reported 3.5 log CFU/100 cm^2 and 4.3 log CFU/100 cm^2 reductions for aerobic and *Enterobacteriaceae* counts respectively. In the same study Bosilevac et al., (2005) reported that after treating hides with EO water prevalence of *E. coli* O157 was reduced from 82 to 35%. Kalchayanand et al., (2008) demonstrated that EO water could reduce *E. coli* O157:H7 on beef heads by 0.5 logs.

This study was designed to validate the use of nalidixic acid adapted strains of seven major STEC, in EO water efficacy determination experiments, using pure cultures as well as on spiked beef trims.

2. Materials and methods

2.1. Bacterial strains

A total of 24 nalidixic acid sensitive (NalS) parent strains of 'top 7' STEC, *E. coli* O26, O45, O103, O121, O111, O145 and O157 were used in this study (Table 1). Subcultures of all 24 strains were adapted to nalidixic acid (50 mg/L) (Sigma chemicals Co., Mo.) using the method described by Taormina & Beuchat (1999). Briefly, each of the NalS bacterial strains were cultured separately in 10 ml tryptic soy broth (TSB, Difco, Becton Dickinson, MD) for 18 h at 37 °C. These overnight grown strains were transferred to TSB supplemented with an increasing amount of nalidixic acid (5, 10, 20, 30, 40 and 50 mg/L) for 24 h. At the end of each 24 h, 200 μl bacterial culture was taken from lower concentration solution and transferred to next elevated nalidixic acid concentration containing TSB solution. All cultures were grown at 37 °C with 150 RPM agitation.

Table .1
Minimum inhibitory concentrations of NalS and NalA STEC strains for EO water treatment.

Bacterial strains	Origin	Source[a]	Free chlorine mg/L NalS	Free chlorine mg/L NalA
E .coli O157:H7				
932	Human	CFS	A 1.66 ± 0.28ab	A 1.66 ± 0.28ab
1	Beef	USDA	A 1.50 ± 0.00abc	A 1.50 ± 0.50abc
4	Human	USDA	A 1.50 ± 0.00abc	A 1.33 ± 0.28abc
5	Human	USDA	A 1.66 ± 0.28ab	A 1.50 ± 0.50abc
E009	Beef	CFS	A 1.83 ± 0.28a	A 1.66 ± 0.28ab
E. coli O26:H11				
DEC9E	Cattle	CFS	A 1.33 ± 0.28abc	A 1.16 ± 0.28abc
DEC10B	Cattle	CFS	A 1.33 ± 0.28abc	A 1.00 ± 0.00abc
3079-97	Human	CFS	A 1.16 ± 0.28abc	A 1.00 ± 0.50abc
1	Human	USDA	A 1.33 ± 0.28abc	A 1.33 ± 0.28abc
2	Beef	USDA	A 1.50 ± 0.00abc	A 1.16 ± 0.28abc
E. coli O111				
NM-3208-95	Human	CFS	A 1.16 ± 0.50abc	A 0.83 ± 0.57abc
NM-:0944-95	Cattle	CFS	A 0.66 ± 0.28bc	A 0.66 ± 0.28bc
NM-3287-97	Human	CFS	A 0.50 ± 0.00c	A 0.41 ± 0.14c
NM-4543-95	Cattle	CFS	A 1.00 ± 0.28abc	A 0.83 ± 0.00abc
H:8-1	Human	USDA	A 0.75 ± 0.28abc	A 0.66 ± 0.66bc
E. coli O103:H2				
O103-1	Human	USDA	A 1.83 ± 0.28a	A 1.66 ± 0.28ab
O103-2	Cattle	USDA	A 1.33 ± 0.50abc	A1.00 ± 0.28abc
E. coli O145				
NM-O145-1	Human	USDA	A 0.91 ± 0.91abc	A 0.66 ± 0.28bc
H28-O145-2	Beef	USDA	A 0.83 ± 0.38abc	A 0.66 ± 0.28bc
E. coli O45:H2				
O45-1	Human	USDA	A 0.58 ± 0.38bc	A 0.50 ± 0.00c
O45-2	Beef	USDA	A 1.00 ± 0.00abc	A 1.16 ± 0.28abc
E. coli O121				
O121-1	Human	USDA	A 0.58 ± 0.38bc	A 0.50 ± 0.00c
H19-O121-2	Beef	USDA	A 0.50 ± 0.00c	A 0.41 ± 0.14c

Means bearing no common capital case letter in the same row are significantly different ($P \leq 0.05$).
a–c means bearing no common lower case letter in the same column are significantly different ($P \leq 0.05$).
[a] USDA – USDA-ARS Meat and Animal Research Center, CFS – Center for Food Safety, University of Georgia.

2.2. Bacterial culture preparation for minimum inhibitory concentration (MIC) determination

Bacterial strains were cultured separately in 10 ml TSB at 37 °C for 24 h. Bacterial population of each culture was determined by plating 0.1 ml appropriate dilution of bacterial suspension on tryptic soy agar (TSA, Difco, Becton Dickinson, MD). Culture were then sedimented thrice by centrifugation (4000 × g for 15 min) and pellets were resuspended in phosphate buffered saline (PBS, pH-7) and appropriate dilutions were made to achieve the final concentration of approximately 10^7 CFU/ml. Bacterial population of each culture was determined by plating appropriate dilutions of bacterial suspensions in 0.1 ml volume on tryptic soy agar plates. Plates were incubated at 37 °C for 24 h before enumeration.

2.3. EO water preparation

EO water was produced by electrolyzing 0.1% NaCl solution in ROX 20 TA EO water generator (Hoshizaki Electric Company Ltd., Japan) with electric current set at 15 A. The pH and oxidation reduction potential (ORP) of EO water were measured using an ACCUMET pH meter (AR50, Fisher Scientific, Pittsburgh, PA). The initial free chlorine concentrations of samples were determined by a DPD- FEAS method (Hach Co., Loveland, CO). Based on initial free chlorine concentration appropriate dilutions of EO water sample were prepared with the help of deionizined water to achieve final free chlorine concentrations of 50 mg/L or a series of 3.00, 2.50, 2.00, 1.50, 1.00, 0.50, 0.25 mg/L.

2.4. MIC

EO water samples with free chlorine concentrations of 3.00, 2.50, 2.00, 1.50, 1.00, 0.50, 0.25 mg/L were prepared as described above. The average pH and ORP values of samples were found as 3.62, 3.79, 3.93, 4.23, 4.49, 4.71, 4.86 and 930, 873, 816, 800, 756, 678, 633 mV respectively.

Each freshly prepared EO water sample (9 ml) was placed in sterilized glass tube and inoculated with 1 ml of bacterial suspension. Tubes were then vortexed (5 s) and allowed to stand for 15 s. At the end of 15 s, an aliquot of 100 μl was taken out from treatment tube and mixed with 100 μl 2× neutralizing buffer (Becton, Dickinson and company, Sparks, MD) by vortexing and the sample was then added to 9 ml TSB and followed by incubation at 37 °C for 48 h. At the end of the incubation period tubes were observed for growth and the lowest concentration of EO water that inhibit the visible growth of bacteria was considered as MIC for that particular strain.

2.5. Survival of NalS vs NalA STEC on meat surface

Seven different STEC cocktails were prepared by mixing 5 ml of each individually grown bacterial strain of that respective serotype and bacterial counts were adjusted to achieve approximately 10^7 CFU/ml. Beef trims (5 × 5 cm in size and 5 cm in thickness with 25–35% fat) were first exposed to ultraviolet light for 30 min (15 min each side) in a biological safety cabinet (Class II Type A/B3, NuAire, Inc., MN) with a 30 W UV light source (Osram Sylvania lighting Inc., MA), and then spot inoculated by applying ten drops of 10 μl bacterial suspension on to the one side of beef trim. The uniform distribution of inoculums was ensured by spreading bacterial suspension on beef trim using a sterile hokey stick. After inoculation, bacteria were allowed to attach for 1 h at 4 °C.