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Validation of Various Antimicrobial Interventions for Use in a Bone Dust Cabinet in a Commercial Beef Harvest Facility

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Objectives

The objective of this study was to evaluate the efficacy of 3 antimicrobial spray interventions (peroxyacetic acid, PAA; lactic acid, LA; lactic/citric acid blend, LCA) in reducing inoculated populations of Shiga toxin-producing *Escherichia coli* (STEC) on pre-rigor beef tissue. A secondary objective was to validate *E. coli* biotype I to serve as surrogates for STEC.

Materials and Methods

The efficacy of each intervention was assessed using a 14-strain mixture of rifampicin-resistant STEC, comprised of 2 strains of *E. coli* O157:H7 and 2 strains each of *E. coli* serogroups O26, O45, O103, O111, O121, and O145. In addition, this study served to validate the utility for a non-pathogenic 5-strain mixture of rifampicin-resistant *E. coli* biotype I to serve as surrogates for the aforementioned STEC mixture. For 3 sampling days, 90 tissue samples from pre-rigor plate subprimals were obtained from beef carcasses immediately following slaughter. The tissue samples were evenly split into 2 inoculation groups (n = 45 samples/group, 15/group/d): i) STEC, or ii) surrogate. Within each inoculation group, tissue samples were randomly assigned to 1 of 9 treatments: i) 200 ppm PAA; ii) 1% LCA; iii) 1.5% LCA; iv) 2.5% LCA; v) 5% LA; vi) 8% LA; vii) 10% LA; viii) potable water; or ix) untreated control. The external fat surface of pre-rigor tissues samples was spot inoculated (5 to 6 log CFU/cm^2) with 100 µL of the STEC or surrogate inoculum and was spread over a 50 cm^2 area using a sterile plastic spreader. Within each inoculation group, treatments were applied using a custom-built, laboratory-scale spray cabinet (0.53 lpm, 137.9 kPA over 8 floodjet spray nozzles). Tissue surfaces were sampled approximately 10 min after spray-treatment application, using sponges hydrated with D/E neutralizing broth, and analyzed for surviving STEC and surrogate populations on tryptic soy agar supplemented with rifampicin (100 µg/ml). This experiment was conducted as a randomized complete block design. Data were evaluated using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). To compare surviving populations of the surrogates and STEC, data were analyzed using the MIXED Procedure in SAS with microbial population of the untreated control samples used as a covariate to adjust least-squares means to a common pre-treatment inocluated plate count.

Results

When applied as a spray treatment to pre-rigor beef tissue, LA applied as a 10% solution was more ($P < 0.05$) effective at reducing STEC and surrogate populations than water, PAA, 1, 1.5 or 2.5% LCA, and 5 and 8% LA (Table 1). Additionally, the 5, 8, and 10% LA treatments were more ($P < 0.05$) effective at reducing both inoculum types than water, PAA, or 1, 1.5 or 2.5% LCA. No differences ($P \geq 0.05$) in surviving STEC populations were observed for tissue samples treated with PAA, 1.5% LCA or 2.5% LCA. Pairwise comparisons indicated surviving STEC and surrogate populations did not differ ($P \geq 0.05$).

Conclusion

When all treatments were compared, LA, at 10%, was found to have the greatest effect against STEC populations. As evidenced by similar surviving populations of STEC and surrogate populations, the 5-strain, non-pathogenic *E. coli* would effectively serve as a surrogate inoculum for the 14-strain STEC cocktail used in this study.

Table 1. Surviving populations (log CFU/cm^2) of Shiga toxin-producing *Escherichia coli*[1] (STEC) and *E. coli* surrogates[2] on hot beef tissue following spray-treatment with various interventions[1] in a custom-built laboratory-scale spray cabinet.

Treatment	Microorganism	
	STEC[2]	Surrogate[3]
Untreated Control	5.51^{bc}	5.67^{a}
Water	5.70^{a}	5.66^{a}
200 ppm PAA	5.39^{c}	5.45^{b}
1% LCA	5.55^{ab}	5.38^{b}
1.5% LCA	5.51^{bc}	5.40^{b}
2.5% LCA	5.46^{bc}	5.33^{b}
5% LA	4.93^{d}	4.96^{c}
8% LA	4.85^{d}	4.73^{d}
10% LA	4.59^{e}	4.22^{e}
SEM[4]	0.13	0.13

[a-e] Within each inoculum type (STEC and Surrogate), LSmeans with different superscripts are different ($P < 0.05$).

[1] Except for untreated control samples, all samples were subject to spray treatment application (0.14 gpm at 20 psi over 8 nozzles) using a custom-built laboratory-scale spray cabinet.

[2] STEC cocktail: two strains of *E. coli* O157:H7 and two strains each of the "Big Six" non-O157 STEC serogroups (i.e., O26, O45, O103, O111, O121, and O145)

[3] Surrogate cocktail: Five strain mixture of *E. coli* biotype I strains (ATCC BAA-1427, ATCC BAA-1428, ATCC BAA-1429, ATCC BAA-1430, and ATCC BAA-1431)

[4] Pooled standard error of the mean.