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Method of Inoculum Preparation Impacts Efficiency of E. Coli O157:H7 and Salmonella Surrogate Organism Inoculation onto Intact Beef During Antimicrobial Intervention Validation

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Objectives

Meat processors must validate the efficacy of antimicrobial interventions for food safety verification purposes. The methods by which pathogen surrogates are prepared for in-plant challenge trials may impact their attachment onto meat surfaces, potentially impacting challenge trial results. This study compared 2 procedures for preparing an inoculum of non-pathogenic *Escherichia coli* for completion of antimicrobial intervention validation.

Materials and Methods

Chilled beef bottom rounds were purchased from a local purveyor; each was halved into 2 portions and assigned to 1 of 2 inoculum preparation methods. Five non-pathogenic *E. coli* Biotype I strains were activated from culture stocks by loop transfer of each strain from a tryptic soy agar (TSA) slant to 10 mL sterile tryptic soy broth and incubating aerobically at 35°C (18 to 24 h). This process was completed in identical fashion twice sequentially. Upon activation completion, tubes from each culture were combined together into a 50 mL vol. mixture (Preparation Method 1). For Preparation Method 2, cells produced in identical fashion to those for Method 1 were at this point collected by centrifugation. The supernatant was decanted and pellet resuspended in 50 mL 0.1% (w/v) peptone diluent. Washing and resuspension procedures were repeated thrice identically. Final pellets were stored in insulated coolers containing coolant packs for 48 h prior to resuspending in 50 mL 0.1% diluent. Inocula were sprayed onto beef (2 mL delivered) using a hand-held sprayer. After application, *E. coli* cells were allowed to attach to meat surfaces for 30 min (25°C). Following attachment, 3.5% lactic acid (15 mL, 25°C) or Citrilow (15 mL; pH 1.05, 25°C) were sprayed onto inoculated beef. Prior to, and following intervention application, surface tissue excisions (5 × 10 cm^2 each; 2 mm depth) were collected via sterile implements. Excisions were composited, and stomached in 100 mL sterile diluent. Decimal dilutions in 0.1% peptone diluent were prepared and spread on TSA surfaces, then overlaid with 12 mL MacConkey Agar. Plates were incubated 48 h (35°C) prior to colony inspection and enumeration. Data were collected from 2 identically completed replications (n = 12). Mean *E. coli* attaching to beef, and *E. coli* mean reductions as a function of inoculum preparation and antimicrobial treatment, were then compared.

Results

Method of cocktail preparation impacted numbers of *E. coli* cells recovered from inoculated beef samples following post-attachment (P = 0.003). Mean numbers of *E. coli* on beef samples via inoculation methods 1 and 2 were 6.5 and 6.1 $\log_{10}$ CFU/cm^2, respectively. However, no differences in *E. coli* reductions as a function of the interaction of main effects (inoculation method*antimicrobial treatment) were detected (P = 0.407).

Conclusion

Analysis of data indicates that method of pathogen surrogate preparation for in-plant food safety intervention validation will impact the numbers of cells successfully inoculated onto experimental product surfaces. However, method of inoculum preparation did not impact observed surrogate reductions following treatment. Researchers and industry specialists engaged in food safety intervention validation should carefully consider all aspects of challenge trial development, execution, and the potential impacts on resulting data.

