FINAL REPORT

EVALUATION OF CHEMICAL DECONTAMINATION TREATMENTS FOR BEEF TRIMMINGS AGAINST *ESCHERICHIA COLI* O157:H7, NON-O157 SHIGA TOXIN-PRODUCING *E. COLI* AND ANTIBIOTIC RESISTANT AND SUSCEPTIBLE *SALMONELLA* TYPHIMURIUM AND *SALMONELLA* NEWPORT

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Project Title: Evaluation of chemical decontamination treatments for beef trimmings against *Escherichia coli* O157:H7, non-O157 Shiga toxin-producing *E. coli* and antibiotic resistant and susceptible *Salmonella* Typhimurium and *Salmonella* Newport

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Objective: The overall goal of the project was to determine whether interventions known for reducing *Escherichia coli* O157:H7 contamination on beef trimmings are also effective in reducing non-O157 Shiga toxin-producing *E. coli* (STEC), and multiple drug resistant (MDR) and susceptible *Salmonella* Newport and *Salmonella* Typhimurium.

Conclusions: Studies were conducted to evaluate the antimicrobial effects of chemical decontamination treatments against *E. coli* O157:H7, six non-O157 STEC serotypes (O26, O45, O103, O111, O121, and O145), and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) phenotypes of *S*. Newport and *S*. Typhimurium inoculated on beef trimmings; individual strains or mixtures were evaluated. The antimicrobial treatments evaluated were lactic acid (5%, at 25 or 55°C), acidified sodium chlorite (0.1%), peroxyacetic acid (0.02%), sodium metasilicate (4%), Bromitize® Plus (225 ppm active bromine), SYNTRx 3300 (pH 1.0), and AFTEC 3000 (pH 1.2). Findings indicated that the antimicrobial effects of these decontamination treatments against the non-O157 STEC serotypes and *S*. Newport/Typhimurium antibiotic resistance phenotypes were generally the same as those against *E. coli* O157:H7. Thus, decontamination interventions applied on beef trimmings against *E. coli* O157:H7 should be at least equally effective against strains of the top six CDC non-O157 STEC serotypes and susceptible as well as multidrug resistant *S*. Newport and *S*. Typhimurium.

Deliverable: The results of this project are submitted to the American Meat Institute Foundation as this final report. Additional deliverables will include technical scientific presentations (an overview of data was presented by John Sofos during his presentation on "The Science and Purpose of Laboratory Challenge Studies" at the symposium on "Validation of Enteric Pathogen Interventions: Scientific, Regulatory and Applied Approaches for Beef Slaughter and Further Processors" presented at the 98th Annual Meeting of the International Association for Food Protection, held in Milwaukee, WI (July 31-August 3, 2011), and peer-reviewed scientific papers as well as trade magazine articles. These deliverables will be available to industry and regulators as they develop interventions and policies for control of STEC other than *E. coli* O157:H7 and multidrug resistant *Salmonella* in beef trimmings.

II. TECHNICAL ABSTRACT

Published studies have evaluated the antimicrobial effects of various chemical decontamination treatments for beef trimmings; however, in most of these studies, *Escherichia coli* O157:H7 was used as the target pathogen. Data are, thus, needed to determine whether these antimicrobial interventions are also effective against other pathogens of recent concern in fresh beef. The objective of this project was to determine whether interventions known for reducing *E. coli* O157:H7 contamination on beef trimmings are also effective in reducing non-O157 Shiga toxin-producing *E. coli* (STEC) serotypes, and multiple drug resistant and susceptible *Salmonella* Newport and *Salmonella* Typhimurium. Prior to initiation of studies with beef trimmings, individual strains of wild-type and rifampicin-resistant (100 μg/ml) variants of six non-O157 STEC serotypes (the CDC top six: O26, O45, O103, O111, O121, and O145) and two or three antibiotic resistance phenotypes (susceptible, MDR and/or MDR-AmpC) of *S*. Newport and *S*. Typhimurium were screened for their tolerance to 5% lactic acid in a sterile beef homogenate. The acid tolerance of the individual strains was compared with that of a 5-strain mixture of wild-type or rifampicin-resistant *E. coli* O157:H7. Results of the acid challenge showed that in most cases, individual strains of non-O157 STEC (wild-type and rifampicin-resistant), *S*. Newport and *S*. Typhimurium were less ($P<0.05$) acid tolerant than the *E. coli* O157:H7 5-strain mixture (wild-type and rifampicin-resistant). Findings of this acid challenge study were used to select strains for inclusion in inoculum mixtures (four strains per *E. coli* serotype or *S*. Newport/Typhimurium antibiotic resistance profile) for the studies with beef trimmings. Where possible, the more acid tolerant strains of human, food, or food animal origin were preferentially selected. In all cases, rifampicin-resistant cultures of *E. coli* O157:H7 and non-O157 STEC were used in subsequent studies. To evaluate the antimicrobial effects of chemical decontamination treatments for trimmings, studies with the non-O157 STEC serotypes were conducted separately from those with the *S.* Newport/Typhimurium antibiotic resistance profiles. Furthermore, each antimicrobial was evaluated independently; therefore, no comparisons are being made between the chemical treatments. Beef trimmings (10 cm length × 5 cm width × 1 cm thick) were separately inoculated (approximately 3-4 log CFU/cm^2) with 4-strain mixtures of rifampicin-resistant *E. coli* O157:H7, rifampicin-resistant non-O157 STEC (serotypes O26, O45, O103, O111, O121, O145), *S.* Newport (antibiotic susceptible, MDR-AmpC), and *S.* Typhimurium (antibiotic susceptible, MDR, MDR-AmpC). Inoculated trimmings were then immersed, for 30 s (or 5 s for SYNTRx), in solutions of lactic acid (5%, pH 2.2, 25 or 55°C), acidified sodium chlorite (0.1%, pH 2.5±0.1, 25°C), peroxyacetic acid (0.02%, pH 3.8±0.1, 25°C), sodium metasilicate (4%, pH 12.5±0.1, 25°C), Bromitize® Plus (225 ppm active bromine, pH 6.6±0.1, 25°C), SYNTRx 3300 (pH 1.0, 25°C), and AFTEC 3000 (pH 1.2, 25°C). Counts of non-O157 STEC serotypes and *S.* Newport/Typhimurium antibiotic resistance phenotypes on treated samples were statistically compared with counts of the reference pathogen, *E. coli* O157:H7. In general, results of the statistical analysis indicated that the chemical decontamination treatments tested against *E. coli* O157:H7 were equally ($P\geq0.05$) or more ($P<0.05$) effective against the non-O157 STEC and *Salmonella* inocula. Additional studies were conducted to evaluate (i) decontamination of beef trimmings inoculated with individual strains (i.e., instead of 4-strain mixtures) of non-O157 STEC and *S.* Newport; (ii) microbial populations on decontaminated beef trimmings and in subsequently ground product; (iii) decontamination of beef trimmings comprised of lean muscle or fatty tissue; and, (iv) decontamination of beef trimmings by immersion or spraying. Major findings from these studies were (i) counts of individual strains

(four strains per pathogen serotype/antibiotic resistance phenotype) of *E. coli* O26, *E. coli* O103, antibiotic susceptible *S*. Newport and MDR-AmpC *S*. Newport on trimmings treated with lactic acid (5%, 55°C) were similar ($P \geq 0.05$) or lower ($P < 0.05$) than counts of a 4-strain *E. coli* O157:H7 mixture; (ii) counts of treated beef trimmings were the same ($P \geq 0.05$) as those of subsequently ground samples; (iii) surviving pathogen (*E. coli* O157:H7 or non-O157 STEC) counts on fatty tissue samples were 0.6-1.2 log CFU/cm^2 lower ($P < 0.05$) than those on lean muscle tissue samples after treatment with lactic acid (5%, 55°C); and, (iv) no differences ($P \geq 0.05$) in surviving *E. coli* O157:H7 counts were obtained for trimmings decontaminated with lactic acid (5%, 25°C) by immersion (30 s) or spraying (2.76 bar; flow rate, 5.68 liters/min; conveyor belt speed, 5 cm/s); however, for samples treated with sodium metasilicate (4%), surviving pathogen counts on samples immersed in the solution were 0.5 log CFU/cm^2 lower ($P < 0.05$) than those on sprayed samples. Overall, the findings presented in this report on the effectiveness of chemical decontamination treatments for beef trimmings against *E. coli* O157:H7, non-O157 STEC serotypes, and antibiotic susceptible and resistant *S*. Newport and *S*. Typhimurium should be useful to regulatory authorities and the meat industry as they consider these pathogens in beef trimmings.

III. INTRODUCTION

Escherichia coli O157:H7 is the most well recognized Shiga toxin-producing *E. coli* (STEC) in the United States. Its association with outbreaks of human illness, some of which were highly publicized, linked to the consumption of undercooked ground beef and other non-intact beef products has led to extensive research and regulatory activities related to its control in/on these products. Based on data published by the research community, various physical and chemical decontamination interventions have been adopted by the beef industry to reduce pathogen contamination on the hide, carcasses, and trimmings during processing. Examples of such interventions include knife trimming, steam vacuuming, spraying or washing carcasses with hot or cold water and/or chemical solutions, steam pasteurization, and carcass chilling (Sofos and Smith, 1998). Recently, there has been increased interest in the application of antimicrobial treatments to beef trimmings prior to grinding for the reduction of microbial contamination in ground beef. Antimicrobial agents that have been evaluated and some applied for decontamination of beef trimmings include lactic acid, acidified sodium chlorite, peroxyacetic acid, sodium metasilicate, potassium lactate, chlorine dioxide, and cetylpyridinium chloride (Bosilevac et al., 2004; Ellebracht et al., 1999, 2005; Harris et al., 2006; Pohlman et al., 2009; Ransom et al., 2003; Stivarius et al., 2002a,b).

Aside from *E. coli* O157:H7, there are more than 100 other STEC serotypes that have also been implicated or have the potential to cause human illness (Brooks et al., 2005; Mathusa et al., 2010). According to recent estimates, non-O157 STEC is responsible for 112,752 cases of foodborne illness in the United States annually (Scallen et al., 2011). The Centers for Disease Control and Prevention Foodborne Diseases Active Surveillance Network (FoodNet) has identified O26, O45, O103, O111, O121, and O145 as the six most common non-O157 STEC serotypes in clinical cases (http://www.cdc.gov/foodnet/factsandfigures.htm). The main reservoir of non-O157 STEC, as is the case for *E. coli* O157:H7, is the intestinal tract of ruminants (Hussein and Bollinger, 2005). As such, it is not surprising that non-O157 STEC have been isolated from beef cattle, beef carcasses and ground beef (Barkocy-Gallagher et al., 2003;

Bosilevac and Koohmaraie, 2011; Hussein, 2007; Hussein and Bollinger, 2005). However, there has only been one reported non-O157 STEC outbreak, and associated recall, linked to beef in the United States. The *E. coli* serotype implicated in this 2010 outbreak was O26 (http://www.fsis.usda.gov/News_&_Events/Recall_050_2010_Release/index.asp). Other foods that have been involved in non-O157 STEC outbreaks include iceberg lettuce, milk, punch, and apple cider (Grant et al., 2011; Mathusa et al., 2010).

Antibiotic resistant pathogens are of concern because of the challenges they present in treating the clinical disease (Arthur et al., 2008). According to epidemiological and outbreak investigations (Greene, 2008; Schneider et al., 2011; Varma et al., 2006), undercooked ground beef has been implicated as a vehicle for infection with multiple drug resistant (MDR) or MDR-AmpC *Salmonella*. The MDR phenotype is defined as resistance to at least ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (ACSSuT), and the MDR-AmpC phenotype is defined as resistance to at least ACSSuT, amoxicillin-clavulanic acid and ceftiofur, and a decreased susceptibility to ceftriaxone (MIC $\geq$2 μg/ml) (CDC, 2009; Greene, 2008). A concern associated with these antibiotic resistant *Salmonella* strains is whether their antibiotic resistance properties also make them less susceptible to chemical decontamination interventions applied during beef processing (Arthur et al., 2008).

As stated previously, numerous chemical interventions have been evaluated, and some adopted by the meat industry, for decontamination of beef trimmings prior to grinding. Although these chemical decontamination treatments are targeted at controlling pathogen contamination, in general, associated with beef trimmings, research studies evaluating the effectiveness of these treatments have mostly been conducted with *E. coli* O157:H7 as the target pathogen. Data are, thus, needed on the efficacy of these interventions against other pathogens of recent concern in fresh beef, including non-O157 STEC and multidrug resistant *Salmonella*.

IV. OVERALL PROJECT GOAL

The overall project goal was to determine whether interventions known for reducing *E. coli* O157:H7 contamination on beef trimmings are also effective in reducing non-O157 STEC, and multiple drug resistant and susceptible *S*. Newport and *S*. Typhimurium.

The report describes six studies that were conducted:

1. *Acid tolerance of non-O157 STEC,* S. *Newport and* S. *Typhimurium strains* (this work was in addition to what was included in the proposal)
    - Individual strains of the CDC (Centers for Disease Control and Prevention) top six non-O157 STEC serotypes, and antibiotic susceptible, MDR and/or MDR-AmpC *S*. Newport and *S*. Typhimurium were screened for their acid tolerance, specifically to 5% lactic acid, to aid in the selection of strains for inclusion in inocula for subsequent studies. The acid tolerance of these individual strains was compared to the acid tolerance of a 5-strain mixture of *E. coli* O157:H7.

2. *Lactic acid decontamination of beef trimmings inoculated with individual strains of* E. coli *O26,* E. coli *O103, and antibiotic susceptible and MDR-AmpC* S. *Newport*

- The purpose of this study was to determine whether it would be meaningful to evaluate the chemical interventions for beef trimmings against individual strains of non-O157 STEC and *Salmonella*, instead of as mixtures of strains within each *E. coli* serotype or *S.* Newport/Typhimurium antibiotic resistance profile.

3. *Comparison of microbial populations on decontaminated beef trimmings and in subsequently ground product* (this work was in addition to what was included in the proposal)
   - The methodology provided in the project proposal indicated that untreated and treated beef trimmings would be ground before analysis of samples for microbial counts. The objective of this study was to determine whether the microbial counts obtained for treated beef trimmings reflected the counts of subsequently ground product. If counts were similar, subsequent studies would analyze beef trimmings, instead of ground samples, for microbial survivors.

4. *Comparison of decontamination of beef trimmings comprised of lean muscle or fatty tissue* (this work was in addition to what was included in the proposal)
   - The objective of this study was to evaluate decontamination of beef trimmings that were comprised of lean muscle or fatty tissue.

5. *Comparison of chemical decontamination of beef trimmings by immersion or spraying* (this work was in addition to what was included in the proposal)
   - The objective of this study was to compare two treatment application methods (i.e., immersion and spraying) for decontamination of beef trimmings.

6. *Evaluation of chemical decontamination treatments for beef trimmings against* E. coli *O157:H7, non-O157 STEC, and antibiotic resistant and susceptible* S. *Newport and* S. *Typhimurium*
   - The work conducted under this study specifically addressed the overall goal of the project.
   - Chemical interventions for beef trimmings were evaluated against the six non-O157 STEC serotypes (i.e., O26, O45, O103, O111, O121, and O145), two or three antibiotic resistance phenotypes (susceptible, MDR, and/or MDR-AmpC) of *S.* Newport and *S.* Typhimurium, and *E. coli* O157:H7. Studies were conducted with mixtures of strains within each *E. coli* serotype or *S.* Newport/Typhimurium antibiotic resistance profile (based on findings from Study 2).
   - The antimicrobial treatments evaluated were lactic acid (5%, at 25 or 55°C), acidified sodium chlorite (0.1%), peroxyacetic acid (0.02%), sodium metasilicate (4%), and Bromitize® Plus (225 ppm active bromine), as listed in the proposal; two additional antimicrobials, SYNTRx 3300 (pH 1.0) and AFTEC 3000 (pH 1.2), were also evaluated.

V. MATERIALS AND METHODS

Study 1: Acid tolerance of non-O157 STEC, *S.* Newport and *S.* Typhimurium strains

The objective of this study was to screen individual strains of non-O157 STEC (serotypes O26, O45, O103, O111, O121, and O145), and individual strains of two or three antibiotic resistance

phenotypes (susceptible, MDR, and/or MDR-AmpC) of *S*. Newport and *S*. Typhimurium, for their tolerance to 5% lactic acid in a sterile beef homogenate, to aid in the selection of strains for inclusion in inocula for subsequent studies with beef trimmings. The acid tolerance of these individual strains was compared to the acid tolerance of a 5-strain mixture of *E. coli* O157:H7.

Bacterial strains. By working with different groups we were able to acquire four to seven strains each of the six non-O157 STEC serotypes (i.e., O26, O45, O103, O111, O121, and O145), and four to 13 strains each of MDR and/or MDR-AmpC and susceptible *S*. Newport and *S*. Typhimurium (Tables 1 and 2). The non-O157 STEC strains were kindly provided by Dr. Chitrita DebRoy (*E. coli* Reference Center, The Pennsylvania State University, University Park, PA), Dr. Pina Fratamico (Eastern Regional Research Center, USDA-ARS-NAA, Wyndmoor, PA), and Dr. Tommy Wheeler (U.S. Meat Animal Research Center, USDA-ARS-NPA, Clay Center, NE). Dr. Martin Wiedmann (Department of Food Science, Cornell University, Ithaca, NY) and Dr. Shaohua Zhao (Center for Veterinary Medicine, U.S. FDA, Laurel, MD) kindly provided the *Salmonella* strains. The *E. coli* O157:H7 strains needed for this study were already available in our laboratory, and included ATCC 43888, ATCC 43895, C1-057, C1-072, and C1-109 (C1 strains were of bovine fecal origin; Carlson et al., 2009).

To facilitate selective enumeration of the non-O157 STEC inocula from the natural meat microflora in studies with beef trimmings, rifampicin-resistant (100 μg/ml) variants of the non-O157 STEC strains were selected as described by Kaspar and Tamplin (1993). Use of strains with a selective marker, like antibiotic resistance, also allows recovery of cells injured by exposure to chemical decontamination treatments, by plating the cells on a non-selective medium (tryptic soy agar, in our studies) supplemented with the antibiotic (100 μg/ml rifampicin, in our studies). Rifampicin-resistant cultures of the *E. coli* O157:H7 strains used in this and subsequent studies were already available in our laboratory and have been used by our group in all our recent work with fresh beef. The lactic acid challenge was conducted on both, wild-type (parental) and rifampicin-resistant strains of non-O157 STEC and *E. coli* O157:H7.

The antibiotic resistance profiles of the *Salmonella* isolates was confirmed using the Sensititre® antimicrobial susceptibility system (Trek Diagnostic Systems, Cleveland, OH), specifically panel CMV2AGNF which was designed for the National Antimicrobial Resistance Monitoring System (NARMS). With this panel, minimum inhibitory concentrations (MIC) were determined, in accordance with the manufacturer's instructions, for ampicillin, amoxicillin/clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. Results were interpreted based on available breakpoints (http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm237109.htm) (Table 2). It should be noted that azithromycin was also included on the panel but no breakpoints were found for this antimicrobial. *Salmonella* strains with a MDR phenotype (Table 2) were resistant to at least ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (ACSSuT), and strains with a MDR-AmpC phenotype were resistant to at least ACSSuT, amoxicillin-clavulanic acid and ceftiofur, and had a decreased susceptibility to ceftriaxone (MIC ≥2 μg/ml) (CDC, 2009; Greene, 2008).

Preparation of inocula. Strains were individually cultured and subcultured at 35°C for 20-24 h in 10 ml tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) (for *Salmonella*, and wild-type *E. coli* O157:H7 and non-O157 STEC strains) or TSB supplemented with rifampicin (100 µg/ml, Sigma-Aldrich, St. Louis, MO; TSB+rif) (for rifampicin-resistant *E. coli* O157:H7 and non-O157 STEC strains). Broth cultures were then streak-plated onto tryptic soy agar (TSA; Acumedia, Lansing, MI) (for wild-type *E. coli* O157:H7 and non-O157 STEC strains), TSA supplemented with 100 µg/ml rifampicin (TSA+rif) (for rifampicin-resistant *E. coli* O157:H7 and non-O157 STEC strains), or xylose lysine deoxycholate (XLD) agar (Acumedia) (for *Salmonella* strains); plates were incubated at 35°C for 24 h. Suspensions of each strain were prepared by suspending single colonies from the culture plates into 5 ml phosphate-buffered saline (PBS, pH 7.4; 0.2 g/liter KH_2PO_4, 1.5 g/liter $Na_2HPO_4 \cdot 7H_2O$, 8.0 g/liter NaCl, and 0.2 g/liter KCl). The bacterial suspensions were standardized to a 0.5 McFarland standard (cell concentration of approximately 1.5×10^8 CFU/ml) using a spectrophotometer (600 nm) and nephelometer (Sensititre, Trek Diagnostics). For the inoculum comprised of a composite of five *E. coli* O157:H7 strains, bacterial suspensions to a 0.5 McFarland standard were initially prepared for each of the five strains separately, before combining the strains. All bacterial suspensions were diluted tenfold in PBS before use.

Lactic acid challenge. The acid challenge was performed in a beef homogenate acidified with 5% lactic acid. The homogenate was prepared by pummeling (2 min; Masticator, IUL Instruments, Barcelona, Spain) fresh beef with distilled water to yield a 10% (w/w) suspension. The suspension was passed through cheesecloth, and the liquid portion was sterilized by autoclaving. For each challenge, 28 ml of the sterile homogenate was pipetted into a sterile 100 ml Erlenmeyer flask containing a magnetic stir bar. The flask was placed onto a magnetic stirrer, and while stirring, 0.3 ml of the diluted bacterial suspension was added to the homogenate. The target inoculation level was approximately 6 log CFU/ml. Prior to addition of lactic acid, an aliquot of the inoculated homogenate was removed for microbiological analysis to determine the inoculation level (control). A 1.7 ml volume of lactic acid (88%, Purac America, Lincolnshire, IL) was then added to the stirring inoculated homogenate, giving a final concentration of 5% lactic acid. Immediately after addition of the lactic acid (time-0 min), and at intervals of 2, 4, 6, and 8 min, aliquots were removed for microbial analysis. The pH of the beef homogenate ranged from 5.78±0.31 to 5.91±0.30 before addition of lactic acid, and 2.32±0.18 to 2.64±0.70 after addition of lactic acid.

Microbiological analysis. Aliquots (1 ml) removed from the challenge medium at each time interval were neutralized in 9 ml D/E neutralizing broth (Difco), and subsequently serially diluted in 0.1% buffered peptone water (Difco). Appropriate dilutions were surface-plated on TSA (for wild-type *E. coli* O157:H7, wild-type non-O157 STEC, and *Salmonella* strains), TSA+rif (for rifampicin-resistant *E. coli* O157:H7 and rifampicin-resistant non-O157 STEC strains) and XLD agar (for *Salmonella* strains). Colonies were counted after incubation of plates at 35°C for 24-48 h. The detection limit of the analysis was 1.0 log CFU/ml.

Statistical analysis. The study was conducted twice, with three acid challenges per strain or strain mixture (for *E. coli* O157:H7) performed within each repetition. Each repetition was considered as a blocking factor in a randomized block design. Mean microbial counts (log CFU/ml) of individual strains (or strain mixture) within each time interval (control, and 0, 2, 4,

6, 8 min) were compared statistically with ANOVA-based procedures followed by Dunnett-adjusted multiple comparison methods for further mean separation using the PROC MIXED command of SAS (v9.2; SAS Institute, Inc., Cary, NC). Using this procedure, surviving microbial counts of wild-type non-O157 STEC or *Salmonella* strains, within each time interval, were compared with surviving counts of the wild-type 5-strain *E. coli* O157:H7 mixture. Similarly, surviving counts of rifampicin-resistant non-O157 STEC or *Salmonella* strains (wild-type), within each time interval, were compared with surviving counts of the rifampicin-resistant 5-strain *E. coli* O157:H7 mixture. In addition to this analysis, a repeated measures analysis, using the PROC GLM command of SAS, was used to analyze the effect of lactic acid exposure (up to 8 min) on surviving counts within each strain. For both analyses, *P* values less than 0.05 ($P<0.05$) were considered statistically significant.

Study 2: Lactic acid decontamination of beef trimmings inoculated with individual strains of *E. coli* O26, *E. coli* O103, and antibiotic susceptible and MDR-AmpC *S.* Newport

This work was conducted to determine whether it would be meaningful to test strains individually, instead of as mixtures of strains within each *E. coli* serotype or *S.* Newport/Typhimurium antibiotic resistance profile. Thus, the objective of this study was to evaluate lactic acid decontamination of beef trimmings against individual strains of *E. coli* O26, *E. coli* O103, and antibiotic susceptible and MDR-AmpC *S.* Newport. The antimicrobial effect of lactic acid decontamination against the individual strains was compared with that of a 4-strain mixture of *E. coli* O157:H7.

Bacterial strains and preparation of inocula. In this (Study 2) and in all subsequent studies (Studies 3-6), rifampicin-resistant, instead of wild-type, cultures of non-O157 STEC and/or *E. coli* O157:H7 were used for inoculation of beef samples. Based on results of the lactic acid challenge (Study 1), four strains each of rifampicin-resistant *E. coli* O26 (O26:H11 hSTEC_03, O26:H2 93.0494, O26 0.1302, and O26:H11 5.2217), rifampicin-resistant *E. coli* O103 (O103 MDR0089, O103:H2 87.1368, O103:H2 90.1764, and O103:H2 92.0084), antibiotic susceptible *S.* Newport (FSL S5-639, CVM N4505, CVM N18445, and CVM N1509) and MDR-AmpC *S.* Newport (FSL S5-436, FSL S5-920, CVM 22698, and CVM N19852) were selected for evaluation in this study. Criteria used for selection of strains were acid tolerance (Study 1) and/or source of the strain; strains of human, food, or food animal origin were preferentially selected (see Tables 1 and 2 for sources of strains). The rifampicin-resistant 4-strain mixture of *E. coli* O157:H7, used as the control in this study, was comprised of strains ATCC 43895, C1-057, C1-072, and C1-109.

Strains were individually cultured (35°C, 20-24 h) in 10 ml TSB (for *Salmonella* strains) or TSB+rif (for rifampicin-resistant strains of *E. coli* O157:H7 and non-O157 STEC) and then subcultured (35°C, 20-24 h) by transferring 0.1 ml of the activated culture into fresh 10 ml TSB or TSB+rif. Broth cultures of the four *E. coli* O157:H7 strains were combined in a centrifuge tube before harvesting of cells, while the individual *E. coli* O26, *E. coli* O103, and *S.* Newport strains were harvested separately. Cells were harvested by centrifugation (4,629×g, 15 min, 4°C; Eppendorf model 5810 R, Hamburg, Germany). Cells were washed with 10 ml PBS, centrifuged again, and resulting cell pellets were resuspended in PBS (40 ml for *E. coli* O157:H7, and 10 ml

for each *E. coli* O26, *E. coli* O103, and *S*. Newport strain). Each inoculum was then serially diluted in 9 ml PBS to a final concentration of approximately 6 log CFU/ml.

Inoculation of beef trimmings. Purchased fresh (approximately 48 h post-slaughter) beef chuck rolls were collected from the production line (prior to the application of any chemical intervention) of a slaughter facility in Northern Colorado. The meat was vacuum-packaged, transported to the Department of Animal Sciences at Colorado State University (within 1 h of collection), and then either used immediately or stored at 4°C for up to 48 h. Beef chuck rolls were cut into 10 × 5 × 1 cm (length × width × thickness) trim samples with a weight of approximately 100 g. The beef trimmings were spot-inoculated by depositing 0.1 ml of the specific inoculum over the surface of one side of the meat sample, followed by a 10 min cell attachment period at 4°C. The same procedure was followed for inoculation of the second side. The target inoculation level of the beef trimmings was approximately 3 log CFU/cm^2.

Lactic acid decontamination of beef trimmings. Inoculated beef trimmings were either left untreated (control) or treated with 5% lactic acid (55°C, pH 2.1-2.2) by completely immersing individual beef trimming samples, for 30 s, in 150 ml of the solution in a Whirl-Pak bag (19 × 30 cm, Nasco, Modesto, CA). Fresh solutions were used for treatment of each sample. Following treatment, samples were removed from the bag with a pair of forceps and placed in a strainer for draining for 60 s (30 s per side). After draining, samples were transferred to a Whirl-Pak filter bag (19 × 30 cm, Nasco) and held at 4°C for 1 h before microbial analysis. The 1 h period before microbial analysis of samples simulated the potential time lapse between collection of treated beef trimming samples from the production floor in a grinding facility and their subsequent analysis for microbial contamination.

Microbiological analyses. Immediately after the 1 h period at 4°C, 100 ml of D/E neutralizing broth was added to the beef sample in the Whirl-Pak bag followed by pummeling (Masticator) for 2 min. Samples were serially tenfold diluted in 0.1% buffered peptone water and appropriate dilutions were surface-plated on TSA (for total bacteria counts) and the following selective medium/media depending on the inoculum and experiment (for inoculated pathogen counts):

- For samples inoculated with rifampicin-resistant *E. coli* O157:H7 in experiments with rifampicin-resistant *E. coli* O26 and *E. coli* O103 strains
  → TSA+rif
- For samples inoculated with rifampicin-resistant *E. coli* O157:H7 in experiments with *S*. Newport strains
  → TSA+rif
  → Modified sorbitol MacConkey agar (mSMAC; MacConkey sorbitol agar [Difco] supplemented with 2.5 mg/liter potassium tellurite [Sigma-Aldrich] and 20 mg/liter novobiocin [Sigma-Aldrich])
- For samples inoculated with rifampicin-resistant *E. coli* O26 and *E. coli* O103 strains
  → TSA+rif
- For samples inoculated with *S*. Newport strains
  → XLD agar

TSA plates were incubated at 25±2°C for 72 h, and TSA+rif, mSMAC, and XLD agar plates at 35°C for 24 h before counting of colonies. Uninoculated beef samples were also microbially

analyzed for any background rifampicin-resistant bacterial populations on TSA+rif, sorbitol-negative populations on mSMAC, and hydrogen sulfide-producing populations on XLD agar. The detection limit of the microbial analysis was 0.0 log CFU/cm^2.

Physicochemical analyses. The moisture pickup of treated beef trimmings was determined by weighing the meat samples before and after application of the treatment (i.e., after the 60 s draining period). The percent moisture pickup was calculated as follows:

$$\frac{\text{treated weight - green weight}}{\text{green weight}} \times 100$$

For pH measurements, uninoculated untreated and treated (samples were held at 4°C for 1 h following treatment before measurement of pH) trimmings were homogenized (Masticator, 2 min) with distilled water (1:1 ratio), and the pH of the homogenate was measured with a Denver Instruments (Arvada, CO) pH meter fitted with a glass electrode.

Statistical analysis. Studies with the individual strains of the two non-O157 STEC serotypes were conducted separately to those with the individual strains of the antibiotic susceptible and MDR-AmpC *S*. Newport. All experiments were conducted twice, with three samples analyzed per repetition. Each repetition was considered as a blocking factor in a randomized block design. Microbial counts, transformed into log CFU/cm^2, were statistically compared with ANOVA-based procedures followed by Dunnett-adjusted multiple comparison methods for further mean separation using the PROC MIXED command of SAS (v9.2). Using this procedure, counts (before or after lactic acid treatment) of each *E. coli* O26 and *E. coli* O103 strain were directly compared with counts (before or after antimicrobial treatment) of the 4-strain composite of *E. coli* O157:H7. Similarly, counts of each antibiotic susceptible and MDR-AmpC *S*. Newport strain were directly compared with counts of the 4-strain *E. coli* O157:H7 mixture. The pH of samples, and the antimicrobial effect of lactic acid within each strain or the *E. coli* O157:H7 composite, were statistically analyzed with a student-based *t*-test using the PROC GLM command of SAS. *P* values less than 0.05 ($P<0.05$) were considered statistically significant.

<u>Study 3.</u> Comparison of microbial populations on decontaminated beef trimmings and in subsequently ground product

The objective of this study was to compare microbial populations on decontaminated beef trimmings and in subsequently ground product. The findings of the study were used to determine which sample type (i.e., trimmings or ground product) should be analyzed for microbial survivors in studies evaluating chemical interventions against pathogen populations on beef trimmings.

Bacterial strains, preparation of inocula, and inoculation of beef trimmings. Two inoculum composites were used in this study; one comprised of six rifampicin-resistant *E. coli* O157:H7 strains, and the second of one rifampicin-resistant strain each of the six non-O157 STEC serotypes. The rifampicin-resistant *E. coli* O157:H7 mixture consisted of strains ATCC 43888, ATCC 43895, ATCC 51658, C1-057, C1-072, and C1-109, and the rifampicin-resistant non-O157 STEC mixture consisted of strains O26:H11 hSTEC_03, O45:H2 05-6545, O103 MDR0089, O111 4.0522, O121:NM 03-4064, and O145:NM 03-4699 (see Table 1 for strain information). The strains were individually cultured and subcultured as before (Study 2), and

then combined to form the two inoculum mixtures as indicated above. Cells of the two inocula were harvested, washed, resuspended in 60 ml PBS, and further diluted to a concentration of 6 log CFU/ml, as previously described (Study 2).

Beef chuck rolls were fabricated into 10 cm length × 5 cm width × 1 cm thick trimmings (approximately 100 g) and spot-inoculated (Study 2) with either the rifampicin-resistant *E. coli* O157:H7 or rifampicin-resistant non-O157 STEC inoculum to a target concentration of approximately 3-4 log CFU/g.

Decontamination of beef trimmings and grinding. The decontamination treatments evaluated were applied by immersion (as outlined in Study 2) and included:

1. No treatment (control)
2. Lactic acid (5%, pH 1.9±0.1, 55°C) for 30 s
3. Lactic acid (5%, 55°C) for 30 s, followed by rinsing in sterile distilled water (25°C) for 30 s

For the sequential treatment (i.e., lactic acid followed by water), samples were immersed in lactic acid for 30 s, allowed to drain (30 s per side), and then immersed in sterile distilled water for 30 s, and then drained again (30 s per side). After draining, trimmings were either held at 4°C for 1 h before microbial analysis, or were ground using an electric meat grinder with a 0.95-cm diameter plate (The Sausage Maker Inc., Buffalo, NY). Ground samples were also held at 4°C (1 h) before microbiological analysis. Thus, the treatments for this study can be summarized as follows:

1. Untreated control + no grinding
2. Untreated control + grinding
3. Lactic acid + no grinding
4. Lactic acid + grinding
5. Lactic acid + water rinsing + no grinding
6. Lactic acid + water rinsing + grinding

Microbiological and physicochemical analyses. D/E neutralizing broth was added to the beef samples (approximately 100 g for non-ground samples, and 50 g for ground samples) at a ratio of 1:1 (w/w) and analyzed for microbial populations (total bacteria, rifampicin-resistant *E. coli* O157:H7, and rifampicin-resistant non-O157 STEC), as described in Study 2. The detection limit of the microbial analysis was 0.3 log CFU/g. Also, moisture pickup determinations were conducted, and sample pH measurements were taken. Additional sets of uninoculated untreated and treated samples were stored at 4°C in Whirl-Pak bags and pH measurements were taken after 24, 48, and 72 h; this was done to determine whether the initial pH (i.e., 1 h after treatment) of treated samples would change during storage.

Statistical analysis. The study was conducted twice with three samples analyzed per repetition. Microbial counts were converted to log CFU/g before statistical analysis. Each repetition was considered as a blocking factor in a randomized block design. Mean microbial counts and pH values were compared statistically with ANOVA-based procedures followed by Tukey-adjusted multiple comparison methods for further mean separation using the PROC GLM command of SAS (v.9.2). In addition, a student-based *t*-test was used to compare counts of *E. coli* O157:H7 and non-O157 STEC within each treatment (i.e., antimicrobial treatment, and grinding/no

grinding of samples). In all cases, *P* values less than 0.05 ($P<0.05$) were considered statistically significant.

<u>Study 4:</u> Comparison of decontamination of beef trimmings comprised of lean muscle or fatty tissue

The objective of this study was to evaluate lactic acid decontamination of beef trimmings that were comprised of lean muscle or fatty tissue.

Bacterial strains and preparation of inocula. Two inocula were used in this study; the 6-strain mixture of rifampicin-resistant *E. coli* O157:H7 (strains ATCC 43888, ATCC 43895, ATCC 51658, C1-057, C1-072, and C1-109) and the 6-strain mixture of rifampicin-resistant non-O157 STEC (strains O26:H11 hSTEC_03, O45:H2 05-6545, O103 MDR0089, O111 4.0522, O121:NM 03-4064, and O145:NM 03-4699) previously used in Study 3. The strains were cultured as before (Study 2), combined to form the two inoculum mixtures, and then harvested, washed, resuspended in PBS, and further diluted to a concentration of 6 log CFU/ml (Studies 2 and 3).

Inoculation and treatment of samples. Purchased fresh (approximately 48 h post-slaughter) beef inside rounds, collected from the slaughter facility in Northern Colorado prior to the application of a chemical intervention, were fabricated into 10 × 5 × 1 cm (length × width × thickness) pieces comprised of (i) lean muscle tissue (from the bottom surface of the inside round); and, (ii) fatty tissue (from the top surface of the inside round). The pieces were spot-inoculated only on one of the two surfaces (i.e., the surface that was originally on the outside of the beef inside round) with 0.1 ml of either the rifampicin-resistant *E. coli* O157:H7 or rifampicin-resistant non-O157 STEC inoculum. Samples were held at 4°C for 20 min for bacterial cell attachment. The target inoculation level was approximately 3 log CFU/cm^2.

The treatments applied to the inoculated samples were:
1. No treatment (control)
2. Lactic acid (5%, pH 2.1±0.1, 55°C) for 30 s
3. Lactic acid (5%, 55°C) for 30 s, followed by rinsing in sterile distilled water (25°C) for 30 s

The method used for treatment of the lean muscle and fatty tissue samples was the same as the one described previously (Study 2). Samples were allowed to drain for 60 s (30 s per side) following each treatment. After draining, samples were placed into a Whirl-Pak filter bag and held at 4°C for 1 h before microbial analysis.

Microbiological and physicochemical analyses. Lean muscle and fatty tissue samples were analyzed for surviving microbial populations on TSA (total bacteria counts) and TSA+rif (pathogen counts). Sample pH measurements (after 1 and 24 h at 4°C), and moisture pickup determinations were also conducted (as described in Studies 2 and 3).

Statistical analysis. The study was conducted twice with three samples analyzed per repetition. Bacterial counts were converted to log CFU/cm^2 and means and standard deviations were calculated. Data (bacterial counts and pH values) were analyzed with ANOVA-based procedures followed by Tukey-adjusted multiple comparison methods for further mean separation using the

PROC GLM command of SAS (v.9.2) with independent variables including serotype (*E. coli* O157:H7 or non-O157 STEC), antimicrobial treatment, tissue type, and their interactions. Means were considered significantly different when *P* values were less than 0.05 ($P<0.05$).

<u>Study 5:</u> Comparison of chemical decontamination of beef trimmings by immersion or spraying

The objective of this study was to compare two treatment application methods (i.e., immersion and spraying) for decontamination of beef trimmings with lactic acid or sodium metasilicate.

Bacterial strains, inoculum preparation, and inoculation of beef trimmings. One inoculum was used in this study, specifically, the rifampicin-resistant *E. coli* O157:H7 4-strain mixture used in Study 2 (i.e., strains ATCC 43895, C1-057, C1-072, and C1-109). The strains were cultured, harvested, washed, and diluted in PBS to a concentration of 6 log CFU/ml, as previously described (Study 2). Beef chuck rolls were fabricated into 10 cm length × 5 cm width × 1 cm thick trimmings and inoculated (approximately 3 log CFU/cm^2) on both sides (Study 2) with the rifampicin-resistant *E. coli* O157:H7 mixture.

Decontamination of beef trimmings. The decontamination treatments and treatment application methods evaluated were:

1. No treatment (control)
2. Lactic acid (5%, pH 2.1±0.1, 25°C); applied by immersion
3. Lactic acid; applied by spraying
4. Lactic acid followed by rinsing with sterile distilled water (25°C); applied by spraying
5. Sodium metasilicate (4%, pH 12.3±0.2, 25°C; AvGard® XP, Danisco, New Century, KS); applied by immersion
6. Sodium metasilicate; applied by spraying
7. Sodium metasilicate followed by rinsing with sterile distilled water (25°C); applied by spraying

For treatment of the inoculated beef trimmings by immersion, samples were placed on a wire mesh (40.6 cm length × 26.7 cm width) and submerged, for 30 s, in 2 liters of the antimicrobial solution in a polypropylene container (54 cm length × 44 cm width × 13 cm height).

The spraying treatments were applied by using a custom-built spray cabinet (Chad Co., Olathe, KS) that was equipped with a conveyor belt of adjustable speeds and seven spraying nozzles (four above, and three below, the conveyor belt, H1/8VV-110015; Spraying Systems Co., Wheaton, IL) (Figure 1). Before and after application of the antimicrobial treatments (without or with rinsing with water), the spray cabinet was rinsed with a chlorine solution and then with water three times. The spray treatments were applied at 2.76 bar to obtain a flow rate of 5.68 liters/min, and the conveyor belt speed was set at 5 cm/s. The same spraying parameters were used for samples receiving a water rinsing treatment after the application of lactic acid or sodium metasilicate. The spray cabinet was rinsed with water once between application of the antimicrobial solution and the water rinsing treatment (the time lapse was approximately 5 min between application of the antimicrobial and water rinsing treatment). Samples treated by immersion or spraying were drained for 30 s per side, transferred to a Whirl-Pak bag and held at 4°C for 1 h before microbial analysis.

Microbiological and physicochemical analyses. Beef trimmings were analyzed for surviving microbial populations on TSA and TSA+rif. The pH of uninoculated untreated and treated beef trimmings was measured after holding the samples at 4°C for 1, 24, 48 and 72 h. Moisture pickup determinations of trimmings treated by immersion or spraying were also conducted. Procedures followed for these analyses are outlined in Study 2.

Statistical analysis. The study was conducted twice with three samples analyzed per repetition. Bacterial counts were converted to log CFU/cm^2 before statistical analysis. Microbial counts (before and after treatment using the different application methods) and pH data were analyzed with ANOVA-based procedures followed by Tukey-adjusted multiple comparison methods for further mean separation using the PROC GLM command of SAS (v9.2). Pairwise *t*-tests were used to compare the antimicrobial effects of the two chemicals (lactic acid, sodium metasilicate), within each treatment application method. Means were considered significantly different when *P* values were less than 0.05 ($P<0.05$).

Study 6: Evaluation of chemical decontamination treatments for beef trimmings against *E. coli* O157:H7, non-O157 STEC, and antibiotic resistant and susceptible *S.* Newport and *S.* Typhimurium

Work conducted under this study specifically addressed the overall project goal, which was to determine whether chemical interventions known for reducing *E. coli* O157:H7 contamination on beef trimmings are also effective in reducing the CDC top six non-O157 STEC serotypes (i.e., O26, O45, O103, O111, O121, and O145), and two or three antibiotic resistance phenotypes (susceptible, MDR, and/or MDR-AmpC) of *S.* Newport and *S.* Typhimurium.

Bacterial strains and preparation of inocula. The results of the lactic acid challenge (Study 1) were used to select four strains each (i.e., if more than four strains were available) of the six non-O157 STEC serotypes (rifampicin-resistant cultures were used) and up to three antibiotic resistance phenotypes (susceptible, MDR, and/or MDR-AmpC) of *S.* Newport and *S.* Typhimurium for use in this study (Table 3). Criteria used for selection of strains were acid tolerance (Study 1) and/or source of the strain; strains of human, food, or food animal origin were preferentially selected. Furthermore, based on findings of Study 2, which indicated that in most cases counts of individual strains (four strains per pathogen serotype/antibiotic resistance phenotype) of *E. coli* O26, *E. coli* O103, antibiotic susceptible *S.* Newport and MDR-AmpC *S.* Newport on trimmings treated with lactic acid (5%, 55°C) were similar ($P\geq0.05$) to those of the 4-strain *E. coli* O157:H7 mixture, it was decided to use mixtures of strains within each *E. coli* serotype or *S.* Newport/Typhimurium antibiotic resistance profile. The rifampicin-resistant *E. coli* O157:H7 4-strain mixture, used as the reference pathogen in these studies, was comprised of strains ATCC 43895, C1-057, C1-072, and C1-109.

Strains were individually cultured and subcultured in TSB (for *Salmonella*) or TSB+rif (for rifampicin-resistant strains of *E. coli* O157:H7 and non-O157 STEC) per the procedure given in Study 2. Broth cultures of the four strains belonging to the same *E. coli* serotype or *S.* Newport/Typhimurium antibiotic resistance phenotype (as shown in Table 3) were combined in a centrifuge tube, and cells were harvested and washed with PBS (Study 2). Resulting cell pellets

from the 4-strain mixture were resuspended in 40 ml PBS, and serially diluted in 9 ml PBS to a final concentration of approximately 6 log CFU/ml.

Inoculation of beef trimmings. Beef trimmings (10 cm length × 5 cm width × 1 cm thickness) were inoculated to a target level of approximately 3-4 log CFU/cm^2 as described in Study 2.

Application of chemical decontamination treatments. Antimicrobial treatments were applied at approved concentrations (USDA-FSIS, 2011) or at a pH recommended by the manufacturer, and were prepared according to the manufacturers' instructions. The treatments evaluated were:

1. No treatment (control)
2. Lactic acid (5%, pH 2.2±0.1, 25°C) (Purac America)
3. Lactic acid (5%, pH 2.2±0.0, 55°C)
4. Acidified sodium chlorite (0.1%, pH 2.5±0.1, 25°C) (Sanova®, Ecolab, St. Paul, MN)
5. Peroxyacetic acid (0.02%, pH 3.8±0.1, 25°C) (Inspexx™ 200, Ecolab)
6. Sodium metasilicate (4%, pH 12.5±0.1, 25°C) (AvGard® XP)
7. Bromitize® Plus (225 ppm active bromine, pH 6.6±0.1, 25°C) (Advanced Food Technologies, LLC, Shreveport, LA)
8. SYNTRx 3300 (pH 1.0, 25°C) (Synergy Technologies, Inc., Shreveport, LA)
9. AFTEC 3000 (pH 1.2, 25°C) (Advanced Food Technologies)

The decontamination treatments were applied per the procedure given in Study 2. Briefly, individual beef trimming samples were completely immersed, for 30 s (except SYNTRx), in 150 ml of the solution in a Whirl-Pak bag. SYNTRx was applied for 5 s per the manufacturer's recommendations. Fresh solutions were used for treatment of each sample. Following treatment, samples were drained for 60 s (30 s per side), then transferred to a new Whirl-Pak bag and held at 4°C for 1 h before microbial analysis.

Microbiological and physicochemical analyses. Untreated and treated trimmings were analyzed for total bacteria counts and inoculated pathogen counts as described in Study 2. The pH of uninoculated untreated and treated beef trimmings was measured after holding the samples at 4°C for 1 and 24 h (Studies 2 and 3). Moisture pickup determinations of treated trimmings were also conducted (Study 2).

Statistical analysis. Each antimicrobial was evaluated independently; therefore, separate statistical analyses were conducted for each antimicrobial. Furthermore, studies with the non-O157 STEC serotypes were conducted separately from those with the *S*. Newport/Typhimurium antibiotic resistance profiles. All studies were conducted twice (lactic acid decontamination of trimmings inoculated with non-O157 STEC was conducted three times), with three samples analyzed per repetition. Each repetition was considered as a blocking factor in a randomized block design. Microbial counts, transformed into log CFU/cm^2, were statistically compared with ANOVA-based procedures followed by Dunnett-adjusted multiple comparison methods for further mean separation using the PROC MIXED command of SAS (v9.2). Using this procedure, counts (before or after antimicrobial treatment) of each non-O157 STEC serotype were directly compared with counts (before or after antimicrobial treatment) of the reference pathogen, *E. coli* O157:H7. Similarly, counts of each *S*. Newport or *S*. Typhimurium antibiotic resistance phenotype (susceptible, MDR and/or MDR-AmpC) were directly compared with counts of *E. coli* O157:H7. In addition to this analysis, a student-based *t*-test, using the PROC GLM

command of SAS, was used to compare counts of samples before and after antimicrobial treatment within each *E. coli* serotype or *S*. Newport/Typhimurium antibiotic resistance phenotype. The pH values were also statistically analyzed with a student-based *t*-test. In all cases, *P* values less than 0.05 ($P<0.05$) were considered statistically significant.

VI. RESULTS

Study 1: Acid tolerance of non-O157 STEC, *S.* Newport and *S.* Typhimurium strains

- Overall, results of the acid challenge, conducted in a beef homogenate acidified with 5% lactic acid, showed that in most cases, individual strains of non-O157 STEC (wild-type and rifampicin-resistant), *S*. Newport and *S*. Typhimurium were less ($P<0.05$) acid tolerant than the *E. coli* O157:H7 5-strain mixture (wild-type and rifampicin-resistant) (Figures 2-12).
- Inoculated levels (6.1±0.1 log CFU/ml) of the wild-type *E. coli* O157:H7 5-strain mixture were reduced ($P<0.05$) to 1.1±0.2 log CFU/ml by the end of the challenge (i.e., 8 min of exposure).
- For the wild-type non-O157 STEC strains, irrespective of serotype, 85.7% (30 out of 35 strains) reached the detection limit (<1.0 log CFU/ml) within 0 min (i.e., immediately following the addition of lactic acid to the inoculated beef homogenate) to 6 min of exposure (Figures 2-7).
- For the *Salmonella* isolates, 87.9% (29 out of 33 isolates) reached the detection limit within 0 to 4 min (based on TSA counts) or 0 to 2 min (based on XLD agar counts) of exposure, irrespective of *Salmonell*a serotype or antibiotic resistance phenotype (Figures 8-12). The difference in results obtained with TSA and XLD agar indicate that sublethally injured cells were unable to recover on the selective medium.
- With respect to the rifampicin-resistant *E. coli* O157:H7 5-strain mixture, inoculated levels (5.9±0.2 log CFU/ml) reached the detection limit after 6 min of exposure. Most (82.9%; 29 out of 35) of the rifampicin-resistant variants of the non-O157 STEC strains reached the detection limit within 0 to 6 min of exposure (Figures 2-7).
- As indicated, results of the lactic acid challenge were used to select strains for inclusion in inoculum mixtures for the studies with beef trimmings. Where possible, the more acid tolerant strains of human, food, or food animal origin were selected.

Study 2: Lactic acid decontamination of beef trimmings inoculated with individual strains of *E. coli* O26, *E. coli* O103, and antibiotic susceptible and MDR-AmpC *S.* Newport

a) *E. coli* O26 and *E. coli* O103 strains

- Total bacteria counts of uninoculated beef trimmings were 3.3±0.3 log CFU/cm^2. Also, counts of <0.5±0.6 log CFU/cm^2 were obtained for rifampicin-resistant populations (on TSA+rif) in uninoculated samples.
- The 4-strain *E. coli* O157:H7 mixture was reduced ($P<0.05$) from 3.2 log CFU/cm^2 on untreated samples, to 2.1 log CFU/cm^2 on samples treated with warm (55°C) 5% lactic acid (reduction of 1.1 log CFU/cm^2) (Figure 13).
- Similarly, counts of the individual strains of *E. coli* O26 and *E. coli* O103 were reduced ($P<0.05$) from 3.1-3.3 log CFU/cm^2 on untreated trimmings, to 1.7-2.3 log CFU/cm^2 on treated samples, with total reductions of 1.0-1.5 log CFU/cm^2 (Figure 13).

- Overall, counts of the individual strains on treated trimmings were similar ($P \geq 0.05$) or lower ($P < 0.05$; by 0.4 log CFU/cm^2) than counts of the 4-strain *E. coli* O157:H7 mixture.
- Total bacteria counts of inoculated samples were reduced ($P < 0.05$) from 3.4-3.7 log CFU/cm^2 on untreated trimmings, to 2.0-2.5 log CFU/cm^2 on treated samples (Figure 14).
- The pH values of untreated and lactic-acid treated samples were 5.67±0.10 and 4.18±0.20, respectively.
- The moisture pickup of treated trimmings ranged from 5.56-8.60%.

b) Antibiotic susceptible/MDR-AmpC *S.* Newport strains

- Total bacteria counts of uninoculated beef trimmings were 2.6±0.3 log CFU/cm^2. Rifampicin-resistant (TSA+rif), sorbitol-negative (mSMAC), and hydrogen sulfide-producing (XLD agar) populations were not detected (<0.0 log CFU/cm^2) in uninoculated samples.
- The initial level of the 4-strain mixture of *E. coli* O157:H7 on inoculated beef samples was 3.1 (TSA+rif) and 2.9 (mSMAC) log CFU/cm^2, while inoculated *S*. Newport populations ranged from 3.0 to 3.2 log CFU/cm^2 (Figures 15 and 16).
- Lactic acid (55°C) decontamination of trimmings reduced ($P < 0.05$) *E. coli* O157:H7 counts by 1.0 and 1.1 log CFU/cm^2 based on counts recovered from TSA+rif and mSMAC, respectively (Figures 15 and 16).
- *Salmonella* counts, irrespective of the strain, were ($P < 0.05$) reduced by 1.5-1.9 log CFU/cm^2 after immersion of trimmings in lactic acid (Figures 15 and 16).
- *Salmonella* counts of the individual strains, irrespective of antibiotic resistance profile, were similar ($P \geq 0.05$) or lower ($P < 0.05$; by 0.3-0.7 log CFU/cm^2) than mSMAC counts of the 4-strain *E. coli* O157:H7 mixture on decontaminated samples.
- Initial levels of total bacterial populations (3.2-3.4 log CFU/cm^2) on inoculated samples were reduced ($P < 0.05$) to 1.9-2.3 log CFU/cm^2 after lactic acid treatment (Figure 17).
- Warm lactic acid treatment of beef trimmings had a significant effect ($P < 0.05$) on the pH of samples; pH values of control (untreated) and treated samples were 5.74±0.10 and 4.26±0.18, respectively.
- The moisture pickup of the samples after treatment with warm lactic acid was 4.41-7.23%.

Based on the results of this study, it was decided to use mixtures of strains within each serotype and/or antibiotic resistance phenotype for the work conducted under Study 6.

<u>Study 3.</u> Comparison of microbial populations on decontaminated beef trimmings and in subsequently ground product

- The total bacteria count of uninoculated chuck roll samples was 4.2±0.5 log CFU/g, and rifampicin-resistant populations were not detected (<0.3 log CFU/g).
- Results indicated that pathogen (*E. coli* O157:H7 or non-O157 STEC) and total bacteria counts of trimmings (non-ground) and ground samples were not ($P \geq 0.05$) different, regardless of antimicrobial treatment (untreated or treated with lactic acid, or lactic acid followed by a water rinse) (Figures 18 and 19). Therefore, for the main study (Study 6) and all other studies with trimmings (Studies 2, 4, and 5), trimmings samples, instead of ground samples were analyzed for microbial counts.

- Within each lactic acid (55°C) treatment (i.e., without or with a water [25°C] rinse), no differences ($P \geq 0.05$) were obtained between counts of *E. coli* O157:H7 and non-O157 STEC (Figure 18).
- Pathogen (*E. coli* O157:H7 or non-O157 STEC) counts of non-ground lactic acid-treated samples (without or with water rinsing) were 0.9-1.1 log CFU/g lower ($P<0.05$) than untreated non-ground samples. Similarly, pathogen counts of ground lactic acid-treated samples (without or with water rinsing) were 0.7-0.9 log CFU/g lower ($P<0.05$) than untreated ground samples (Figure 18).
- Initial pH values of uninoculated, untreated beef trimmings and ground beef were 5.72 and 5.57 units, respectively. Corresponding pH values of samples treated with lactic acid (without water rinsing) were 4.11 and 4.68, respectively.
- Samples treated with lactic acid followed by a water rinse had pH values that were 0.35 (non-ground samples) and 0.30 (ground samples) units higher ($P<0.05$) than those of samples only treated with lactic acid.
- The pH of non-ground and ground samples, generally, did not ($P \geq 0.05$) change during 72 h of storage at 4°C.
- Samples treated with lactic acid had a moisture pickup of 6.23-10.13%, while samples treated with lactic acid followed by water rinsing had a moisture pickup of 9.24-10.87%.

<u>Study 4:</u> Comparison of decontamination of beef trimmings comprised of lean muscle or fatty tissue

- Total bacteria counts of uninoculated lean muscle and fatty tissue samples were 3.3±0.2 and 3.4±0.3 log CFU/cm^2, respectively. Rifampicin-resistant bacteria were not detected (<0.0 log CFU/cm^2) in uninoculated samples.
- Pathogen counts (*E. coli* O157:H7 or non-O157 STEC) of inoculated, untreated samples were 3.2 and 3.1 log CFU/cm^2 for lean muscle and fatty tissue samples, respectively (Figure 20).
- Overall, *E. coli* O157:H7 and non-O157 STEC counts of lean muscle tissue treated with lactic acid (without or with subsequent water rinsing) were higher ($P<0.05$) than those of corresponding fatty tissue samples (Figure 20).
- Lactic acid (55°C) treatment reduced ($P<0.05$) initial pathogen populations of lean muscle tissue samples to 2.0 (*E. coli* O157:H7; reduction of 1.2 log units) or 2.1 (non-O157 STEC; reduction of 1.1 log units) log CFU/cm^2 (Figure 20).
- Lactic acid treatment of fatty tissue samples reduced ($P<0.05$) initial pathogen populations to 0.8 (*E. coli* O157:H7; reduction of 2.3 log units) or 1.5 (non-O157 STEC; reduction of 1.6 log units) log CFU/cm^2 (Figure 20).
- Pathogen counts (*E. coli* O157:H7 or non-O157 STEC) of lean muscle or fatty tissue samples decontaminated with lactic acid were not ($P \geq 0.05$) different than those of samples decontaminated with lactic acid followed by rinsing with distilled water (Figure 20).
- In general, trends of total bacterial populations, recovered on TSA, for untreated and treated lean muscle and fatty tissue samples were similar to those of pathogen (*E. coli* O157:H7 and non-O157 STEC) populations (recovered on TSA+rif) (Figure 21).
- Samples treated with lactic acid or lactic acid followed by water rinsing had lower ($P<0.05$) pH values (pH of 4.27 and 4.47, respectively, for lean muscle tissue; 3.59 and 3.90,

respectively, for fatty tissue) than untreated lean muscle tissue (pH 5.57) and fatty tissue (pH 5.52) samples.
- The pH values of treated (lactic acid or lactic acid followed by water rinsing) lean muscle tissue samples were higher (P<0.05) than those of treated fatty tissue samples.
- Overall, the pH of all treated samples stored at 4°C for 24 h was higher than that of corresponding samples held at 4°C for 1 h after treatment.
- The moisture pickup of lean muscle and fatty tissue samples treated only with lactic acid ranged from 5.70±2.22 to 7.83±3.18%. Samples treated with lactic acid and then rinsed with water had moisture pickups of 8.77±2.76 to 14.24±4.86%.

<u>Study 5:</u> Comparison of chemical decontamination of beef trimmings by immersion or spraying

- The background, natural contamination level of the beef trimmings was 2.6±0.1 log CFU/cm^2 (recovered on TSA), and no rifampicin-resistant populations were detected (<0.0 log CFU/cm^2) in uninoculated samples.
- The *E. coli* O157:H7 inoculation level of beef samples was 3.2±0.0 log CFU/cm^2 (Figure 22).
- Irrespective of treatment application method (immersion, spraying, spraying followed by rinsing with water), sodium metasilicate was more (P<0.05) effective than lactic acid (applied at 25°C) in reducing pathogen pathogens on trimmings. Overall, decontamination of trimmings with sodium metasilicate or lactic acid reduced (P<0.05) pathogen counts by 0.9-1.4 and 0.5-0.6 log CFU/cm^2, respectively (Figure 22).
- Pathogen counts of samples treated with lactic acid were not (P≥0.05) different among the treatment application methods (immersion, spraying, spraying followed by rinsing with water) (Figure 22).
- For sodium metasilicate, lower (by 0.5 log CFU/cm^2; P<0.05) pathogen counts were obtained for samples treated by immersion than by spraying (without water rinsing) (Figure 22).
- Rinsing of samples with water after spray application of lactic acid or sodium metasilicate did not (P≥0.05) have an effect on surviving pathogen counts as compared to those that did not receive the water rinse treatment (Figure 22).
- In general, microbial populations enumerated on TSA were similar to those on TSA+rif, regardless of the antimicrobial or the treatment application method (Figures 22 and 23).
- The pH of untreated beef trimmings was 5.73±0.09. Overall, irrespective of treatment application method, samples treated with lactic acid had a lower (P<0.05) pH (pH of 4.58-4.94), and samples treated with sodium metasilicate had a higher (P<0.05) pH (pH of 6.52-8.37), than untreated samples on the day of the experiment (i.e., 1 h after treatment).
- Application of lactic acid by immersion or spraying resulted in a similar drop (P<0.05) in the pH of samples (pH values of 4.62 and 4.58, respectively). The pH values of samples sprayed with lactic acid and then rinsed with water were 0.32 and 0.36 pH units higher (P<0.05) than the pH values of samples immersed or sprayed with the antimicrobial without water rinsing.
- The pH of samples after immersion in sodium metasilicate was 8.37, while the pH of samples after spraying with this antimicrobial (without or with rinsing with water) was significantly lower (P<0.05; pH of 6.52-6.65).
- Storage of uninoculated samples at 4°C for up to 72 h did not (P≥0.05) affect the pH of the untreated beef trimmings. In contrast, compared to initial pH values, samples treated with

lactic acid, by immersion or spraying, had higher (P<0.05) pH values after storage for 24 h at 4°C (pH values of 5.12 and 5.10, respectively).
- Samples immersed in sodium metasilicate and stored at 4°C for 24 h had lower (by 1.39 units; P<0.05) pH values than the pH of samples analyzed 1 h after treatment.
- In general, pH values of treated samples stored at 4°C for 48 or 72 h were not (P≥0.05) different than those of samples stored for 24 h, regardless of the antimicrobial or treatment application method.
- The average moisture pickup of beef trimmings after immersion or spraying was 2.98±0.51% and 2.47±0.48%, respectively, irrespective of the antimicrobial. For samples sprayed with lactic acid or sodium metasilicate and then rinsed with water, the average moisture pickup was 4.91±1.08%.

<u>Study 6:</u> Evaluation of chemical decontamination treatments for beef trimmings against *E. coli* O157:H7, non-O157 STEC, and antibiotic resistant and susceptible *S.* Newport and *S.* Typhimurium

As stated in the statistical analysis section of the Materials and Methods, each antimicrobial was evaluated independently; as such, no comparisons are being made between the chemical treatments; it should be noted, that no such comparison was included in the overall goal of the proposal. Additionally, studies with the non-O157 STEC serotypes were conducted separately from the studies with the *S*. Newport and *S*. Typhimurium antibiotic resistance profiles. Thus, results are presented separately for each antimicrobial and pathogen group (i.e., non-O157 STEC and *Salmonella*). Furthermore, as previously described, microbial counts (before or after antimicrobial treatment) of each non-O157 STEC serotype were statistically compared with counts (before or after antimicrobial treatment) of the reference pathogen, *E. coli* O157:H7. Similarly, counts of each *S*. Newport or *S*. Typhimurium antibiotic resistance phenotype (susceptible, MDR and/or MDR-AmpC) were statistically compared with counts of *E. coli* O157:H7. No statistical comparisons were made between the six non-O157 STEC serotypes or between the five *Salmonella* inocula, as this was also considered unnecessary and it was not part of the project goal.

For all the experiments conducted under Study 6, mean total bacteria counts of uninoculated beef trimmings ranged from 2.2 to 3.9 log CFU/cm^2. Background rifampicin-resistant (on TSA+rif), sorbitol-negative (on mSMAC), and hydrogen sulfide-producing (on XLD agar) microbial populations were not detected (<0.0 log CFU/cm^2) in uninoculated samples.

a) Lactic acid (25 and 55°C) treatment of beef trimmings inoculated with *E. coli* O157:H7 or the non-O157 STEC serotypes
- Initial levels of inoculated *E. coli* O157:H7 and the six non-O157 STEC serotypes on beef trimmings ranged from 3.1 to 3.3 log CFU/cm^2 (Figure 24).
- Overall, irrespective of lactic acid treatment (25 or 55°C), surviving counts of all six non-O157 STEC serotypes on treated samples were not (P≥0.05) different than surviving counts of *E. coli* O157:H7.
- Treatment of samples with lactic acid applied at 25 or 55°C reduced (P<0.05) *E. coli* O157:H7 counts by 0.7 and 1.4 log CFU/cm^2, respectively. Corresponding reductions of

the six non-O157 STEC serotypes were 0.4-0.9 and 1.0-1.3 log CFU/cm^2, respectively (Figure 24).

- Overall, initial counts of total bacterial populations were 3.5-3.6 log CFU/cm^2 on all inoculated trimmings. Total bacteria counts were reduced (P<0.05) by 0.7-1.1 and 1.0-1.4 log CFU/cm^2 following treatment with lactic acid at 25 or 55°C, respectively (Figure 25).
- The pH of untreated beef trimmings was 5.41±0.31, and was reduced (P<0.05) to 4.12±0.32 and 4.03±0.24 after treatment with 25 or 55°C lactic acid solutions, respectively. Following storage of samples at 4°C for 24 h, pH values of 5.44±0.33, 4.36±0.31, and 4.23±0.22 were obtained for untreated trimmings, and trimmings treated with 25 or 55°C lactic acid, respectively.
- The moisture pickup of samples decontaminated with lactic acid ranged from 4.73 to 6.71% for the 25°C solution, and 5.39 to 6.48% for the 55°C solution.

b) Lactic acid (25 and 55°C) treatment of beef trimmings inoculated with *E. coli* O157:H7 or the *S.* Newport/Typhimurium antibiotic resistance phenotypes

- Counts of inoculated populations of *E. coli* O157:H7 and *Salmonella* on untreated beef trimmings were 3.0-3.3 log CFU/cm^2 (Figures 26 and 27).
- *E. coli* O157:H7 counts were reduced (P<0.05) by 0.5 (TSA+rif) and 0.8 (mSMAC) log CFU/cm^2 after treatment of samples with 25°C lactic acid, and 1.2 (TSA+rif) and 1.5 (mSMAC) log CFU/cm^2 after treatment with 55°C lactic acid (Figures 26 and 27).
- Overall, decontamination of trimmings with 25 or 55°C lactic acid solutions reduced *Salmonella* counts by 1.2-1.5 and 1.5-1.9 log CFU/cm^2, respectively (Figures 26 and 27).
- *Salmonella* counts, irrespective of serotype or antibiotic resistance profile, were similar (P≥0.05) or lower (P<0.05; by 0.7-1.1 and 0.4-0.6 log CFU/cm^2 compared with TSA+rif and mSMAC counts of *E. coli* O157:H7, respectively,) than counts of *E. coli* O157:H7 following decontamination of samples with 25 or 55°C lactic acid solutions.
- Initial total bacteria counts of 3.9-4.2 log CFU/cm^2 on inoculated samples were reduced (P<0.05) to 2.9-3.2 (25°C solution) and 2.3-3.0 (55°C solution) log CFU/cm^2 following lactic acid treatment (Figure 28).
- Lactic acid decontamination of trimmings reduced (P<0.05) the pH of samples from 4.90±0.23 (untreated control) to 4.22±0.21 (25°C solution) and 4.23±0.11 (55°C solution). The pH values of treated samples were 4.53±0.21 (25°C solution) and 4.60±0.11 (55°C solution) after 24 h at 4°C.
- The moisture pickup of samples treated with 25 or 55°C solutions of lactic acid was 4.58-6.60% and 4.65-7.95%, respectively.

c) Acidified sodium chlorite treatment of beef trimmings inoculated with *E. coli* O157:H7 or the non-O157 STEC serotypes

- Populations of *E. coli* O157:H7 and non-O157 STEC inocula were 3.7 and 3.7-3.9 log CFU/cm^2, respectively, on untreated beef trimmings, and were reduced (P<0.05) to 2.8 (*E. coli* O157:H7; reduction of 0.9 log units) and 2.8-3.1 (reductions of 0.7-1.0 log units) log CFU/cm^2 after treatment with acidified sodium chlorite for 30 s (Figure 29).
- Statistical analysis of the data indicated that counts of *E. coli* O103 and *E. coli* O111 on treated trimmings were higher (P<0.05) than those of *E. coli* O157:H7; however, the difference in counts between these three STEC serotypes (O157, O103, and O111) was only 0.3 log CFU/cm^2, which microbiologically is not considered a significant difference

(NACMCF, 2010). Counts of the remaining tested non-O157 STEC serotypes on treated samples were not ($P \geq 0.05$) different than those of *E. coli* O157:H7.

- Total bacteria counts of inoculated untreated samples were reduced ($P < 0.05$) by 0.7-1.1 log CFU/cm^2 after treatment with acidified sodium chlorite (Figure 30).
- The pH values of untreated and treated trimmings were 5.74±0.20 and 5.69±0.11, respectively. Corresponding values after 24 h at 4°C were 5.67±0.17 and 5.63±0.19, respectively.
- The moisture pickup of trimmings treated with acidified sodium chlorite ranged from 2.21 to 3.19%.

d) Acidified sodium chlorite treatment of beef trimmings inoculated with *E. coli* O157:H7 or the *S.* Newport/Typhimurium antibiotic resistance phenotypes

- Initial levels of inoculated *E. coli* O157:H7 populations on beef samples were 3.1 (TSA+rif) and 2.7 (mSMAC) log CFU/cm^2, while inoculated *Salmonella* populations ranged from 2.9 to 3.1 log CFU/cm^2 (Figures 31 and 32).
- Acidified sodium chlorite decontamination of trimmings reduced ($P < 0.05$) *E. coli* O157:H7 counts by 0.5 (TSA+rif/mSMAC) and *Salmonella* counts by 0.4-0.6 log CFU/cm^2 (Figures 31 and 32).
- *Salmonella* counts, irrespective of serotype or antibiotic resistance profile, were similar ($P \geq 0.05$) or lower ($P < 0.05$; by 0.3 log CFU/cm^2) than TSA+rif counts of *E. coli* O157:H7 on decontaminated samples. However, based on mSMAC counts of *E. coli* O157:H7, surviving populations of treated samples for four out of the five tested *Salmonella* inocula were statistically higher ($P < 0.05$) than those of *E. coli* O157:H7, but, microbiologically, counts of these *Salmonella* inocula were only 0.3-0.5 log CFU/cm^2 higher than the mSMAC counts of *E. coli* O157:H7.
- Total bacteria counts of inoculated samples before and after treatment were 4.1-4.2 and 3.8-4.2 log CFU/cm^2, respectively (Figure 33).
- The pH values of untreated and treated samples were initially (i.e., after 1 h at 4°C) 5.79±0.11 and 5.75±0.16, respectively, and 6.23±0.11 and 5.63±0.14, respectively, after 24 h at 4°C.
- The weight gain of samples decontaminated with acidified sodium chlorite ranged from 2.25 to 4.37%.

e) Peroxyacetic acid treatment of beef trimmings inoculated with *E. coli* O157:H7 or the non-O157 STEC serotypes

- Counts of inoculated populations of *E. coli* O157:H7 and the non-O157 STEC serotypes on untreated meat samples were 3.7 and 3.7-3.9 log CFU/cm^2, respectively (Figure 34).
- After decontamination with peroxyacetic acid, counts of 3.1 and 2.8-3.1 log CFU/cm^2 were obtained for samples inoculated with *E. coli* O157:H7 (reduction of 0.6 log units) or the non-O157 STEC serotypes (reductions of 0.7-1.0 log units), respectively (Figure 34).
- Counts of the non-O157 STEC serotypes on treated samples were similar ($P \geq 0.05$) or lower ($P < 0.05$; by 0.3 log CFU/cm^2) than counts of *E. coli* O157:H7.
- Total bacteria counts of inoculated trimmings before and after decontamination were 3.8-3.9 and 3.1-3.2 log CFU/cm^2, respectively (Figure 35).

- The pH values of untreated and treated trimmings were 5.90±0.17 and 5.72±0.08, respectively. Corresponding values for samples held at 4°C for 24 h were 5.77±0.10 and 5.82±0.07, respectively.
- The moisture pickup of trimmings decontaminated with peroxyacetic acid was 2.20-3.35%.

f) Peroxyacetic acid treatment of beef trimmings inoculated with *E. coli* O157:H7 or the *S.* Newport/Typhimurium antibiotic resistance phenotypes

- Initial levels of inoculated *E. coli* O157:H7 populations on trimmings were 3.1 (TSA+rif) and 2.8 (mSMAC) log CFU/cm^2, and were reduced (P<0.05) to 2.4 (TSA+rif; reduction of 0.7 log units) and 2.3 (mSMAC; reduction of 0.5 log units) log CFU/cm^2 after treatment with peroxyacetic acid (Figures 36 and 37).
- *Salmonella* inocula were reduced (P<0.05) from 3.0-3.3 log CFU/cm^2 on untreated samples, to 2.4-2.6 log CFU/cm^2 on treated samples, with total reductions of 0.5-0.7 log CFU/cm^2 (Figures 36 and 37).
- *Salmonella* counts, irrespective of serotype or antibiotic resistance profile, were similar (P≥0.05) or higher (P<0.05; by 0.2-0.3 log CFU/cm^2) than counts of *E. coli* O157:H7 on treated samples. As previously stated, a 0.2-0.3 log unit difference is not considered microbiologically meaningful.
- Initial counts of total bacterial populations were 3.3-3.6 log CFU/cm^2 on inoculated trimmings. These counts were reduced (P<0.05) to 2.8-3.2 log CFU/cm^2 after decontamination of samples with peroxyacetic acid (Figure 38).
- The pH values of untreated and treated samples were initially (i.e., after 1 h at 4°C) 6.04±0.13 and 5.98±0.14, respectively, and 5.44±0.11 and 5.42±0.08, respectively, after 24 h at 4°C.
- The weight gain of trimmings after immersion in peroxyacetic acid was 1.81-3.99%.

g) Sodium metasilicate treatment of beef trimmings inoculated with *E. coli* O157:H7 or the non-O157 STEC serotypes

- Populations of *E. coli* O157:H7 and non-O157 STEC inocula were 3.5 and 3.4-3.6 log CFU/cm^2, respectively, on untreated beef trimmings. These initial counts were reduced (P<0.05) to 2.0 (*E. coli* O157:H7; reduction of 1.5 log units) and 1.9-2.3 (non-O157 STEC serotypes; reductions of 1.3-1.5 log units) log CFU/cm^2 after decontamination of samples with sodium metasilicate (Figure 39).
- Counts of all six non-O157 STEC serotypes on treated samples were not (P≥0.05) different from counts of *E. coli* O157:H7.
- Total bacteria counts of 3.5-3.7 log CFU/cm^2 on untreated inoculated trimmings were reduced (P<0.05) to 2.0-2.5 log CFU/cm^2 after treatment with sodium metasilicate (Figure 40).
- The initial (1 h after treatment) pH of untreated and treated trimmings was 5.77±0.16 and 8.23±0.98, respectively, and 5.61±0.13 and 7.25±0.76, respectively, after 24 h storage at 4°C.
- The moisture pickup of trimmings immersed in sodium metasilicate was 3.18±1.24 to 4.95±2.01%.

h) Sodium metasilicate treatment of beef trimmings inoculated with *E. coli* O157:H7 or the *S.* Newport/Typhimurium antibiotic resistance phenotypes

- Pathogen counts of inoculated, untreated beef samples were 3.1 (TSA+rif) and 2.8 (mSMAC) log CFU/cm^2 for *E. coli* O157:H7, and 3.0-3.3 log CFU/cm^2 for the five *Salmonella* inocula (Figures 41 and 42).
- Sodium metasilicate decontamination of trimmings reduced (P<0.05) *E. coli* O157:H7 counts by 1.3 (TSA+rif) and 1.4 (mSMAC) log CFU/cm^2, and *Salmonella* counts by 1.3-1.5 log CFU/cm^2 (Figures 41 and 42).
- Overall, counts of *Salmonella* on treated samples were not (P≥0.05) different than those of *E. coli* O157:H7, with one exception. Specifically, counts of MDR *S.* Typhimurium on decontaminated trimmings were 0.6 log CFU/cm^2 higher (P<0.05) than mSMAC counts of *E. coli* O157:H7. No significant differences (P≥0.05) were obtained when comparing the counts of the five tested *Salmonella* inocula with TSA+rif counts of *E. coli* O157:H7.
- Total bacteria counts of inoculated samples before and after treatment were 3.3-3.6 and 2.1-2.6 log CFU/cm^2, respectively (Figure 43).
- Beef samples had a pH of 6.04±0.13 before treatment, and a pH of 8.66±0.55 after treatment with sodium metasilicate. Samples held for 24 h (4°C) had pH values of 5.44±0.11 (untreated control) and 6.52±0.16 (treated).
- The moisture pickup of decontaminated trimmings was 4.67-6.12%.

i) Bromitize Plus treatment of beef trimmings inoculated with *E. coli* O157:H7 or the non-O157 STEC serotypes

- Counts of inoculated populations of *E. coli* O157:H7 and the non-O157 STEC serotypes on untreated trimmings were 3.1 and 3.1-3.2 log CFU/cm^2, respectively (Figure 44). Initial counts of total bacterial populations on untreated samples were 3.2-3.4 log CFU/cm^2, irrespective of pathogen inoculum treatment (Figure 45).
- Application of Bromitize Plus by immersion for 30 s resulted in 0.3, 0.2-0.3, and 0.1-0.3 log CFU/cm^2 reductions of *E. coli* O157:H7, the non-O157 STEC serotypes, and total bacteria counts, respectively (Figures 44 and 45).
- Counts of the non-O157 STEC serotypes on treated samples were similar (P≥0.05) to those of *E. coli* O157:H7, except for *E. coli* O103. Although the 0.2 log CFU/cm^2 difference in counts between *E. coli* O157:H7 and *E. coli* O103 was statistically significant (P<0.05), microbiologically, it was not meaningful.
- The pH of beef samples was 5.71±0.08 before treatment and 5.72±0.16 after treatment with the antimicrobial. These sample pH values remained unchanged (5.73±0.03 and 5.72±0.06, respectively; P≥0.05) after storage (4°C) for 24 h.
- The average moisture pickup of treated samples was 2.85±0.01%.

j) Bromotize Plus treatment of beef trimmings inoculated with *E. coli* O157:H7 or the *S.* Newport/Typhimurium antibiotic resistance phenotypes

- Initial levels of inoculated *E. coli* O157:H7 populations on trimmings were 3.1 (TSA+rif) and 2.9 (mSMAC) log CFU/cm^2, and were reduced to 2.9 (TSA+rif) and 2.7 (mSMAC) log CFU/cm^2 after treatment with Bromotize Plus. Thus, a reduction of 0.2 log CFU/cm^2 was obtained irrespective of plating medium (Figures 46 and 47).

- *Salmonella* inocula were reduced ($P<0.05$) from 2.8-3.1 log CFU/cm^2 on untreated samples to 2.3-2.7 log CFU/cm^2 on treated samples, and reductions ranged from 0.2-0.5 log CFU/cm^2 (Figures 46 and 47).
- *Salmonella* counts, irrespective of serotype or antibiotic resistance profile, were similar ($P\geq0.05$) or lower ($P<0.05$; by 0.2-0.6 log CFU/cm^2) than counts of *E. coli* O157:H7 following decontamination of samples with this antimicrobial.
- Total bacteria counts of untreated and treated trimmings were 3.3-3.8 and 3.1-3.3 log CFU/cm^2, respectively (Figure 48).
- Initial pH values of untreated and treated samples (5.61±0.09 and 5.68±0.22, respectively) were not ($P\geq0.05$) different than those of samples held at 4°C for 24 h (5.76±0.10 and 5.73±0.07, respectively).
- The moisture pickup of trimmings decontaminated with Bromitize Plus was 2.75-4.25%.

k) SYNTRx 3300 treatment of beef trimmings inoculated with *E. coli* O157:H7 or the non-O157 STEC serotypes

- Following treatment of trimmings with SYNTRx (pH 1.0) for 5 s, counts of *E. coli* O157:H7 and the non-O157 STEC serotypes were 0.2 and 0.1-0.3 log CFU/cm^2, respectively, lower than initial levels (3.1-3.2 log CFU/cm^2) (Figure 49).
- On treated samples, counts of the six non-O157 STEC serotypes were statistically similar ($P\geq0.05$) or lower ($P<0.05$; by 0.1 log CFU/cm^2) than those of *E. coli* O157:H7.
- Total bacteria counts of trimmings before and after decontamination were 3.2-3.3 and 2.9-3.1 log CFU/cm^2, respectively (Figure 50).
- Beef samples treated with SYNTRx had a lower ($P<0.05$) pH (pH 5.13±0.24) than untreated samples (pH 5.92±0.18). After storage of untreated and treated trimmings at 4°C (24 h), pH values were 5.79±0.11 and 5.41±0.22, respectively.
- The average moisture pickup of decontaminated samples was 3.91±1.38%.

l) SYNTRx 3300 treatment of beef trimmings inoculated with *E. coli* O157:H7 or the *S.* Newport/Typhimurium antibiotic resistance phenotypes

- Inoculated levels of the pathogen inocula were 3.1 (TSA+rif) and 3.0 (mSMAC) log CFU/cm^2 for *E. coli* O157:H7, and 2.9-3.2 log CFU/cm^2 for the non-O157 STEC serotypes (Figures 51 and 52).
- SYNTRx decontamination of trimmings reduced ($P<0.05$) *E. coli* O157:H7 counts by 0.3 (TSA+rif) and 0.5 (mSMAC) log CFU/cm^2, and *Salmonella* counts by 0.4-0.5 log CFU/cm^2 (Figures 51 and 52).
- Statistical analysis of *Salmonella* counts with those of *E. coli* O157:H7 on treated samples showed some statistical differences ($P<0.05$); however, in these cases, *Salmonella* counts were either 0.2-0.3 log CFU/cm^2 lower, or 0.3 log CFU/cm^2 higher than counts of *E. coli* O157:H7.
- Total bacteria counts of trimmings were 3.9-4.1 log CFU/cm^2 before treatment with SYNTRx and 3.4-3.8 log CFU/cm^2 after treatment (Figure 53).
- The pH of untreated beef trimmings was 5.47±0.09 and was reduced ($P<0.05$) to 4.77±0.24 after treatment with SYNTRx. Following storage of samples at 4°C for 24 h, pH values of 5.71±0.07 and 5.23±0.02 were obtained for untreated and treated beef samples, respectively.

- The moisture pickup of trimmings following immersion in SYNTRx for 5 s was 3.73-4.27%.

m) AFTEC 3000 treatment of beef trimmings inoculated with *E. coli* O157:H7 or the non-O157 STEC serotypes

- *E. coli* O157:H7 counts were 3.1 and 2.7 log CFU/cm^2 on untreated and treated (AFTEC 3000, pH 1.2, 30 s) beef trimmings, respectively (i.e., reduction of 0.4 log units) (Figure 54).
- Counts of non-O157 STEC serotypes were 3.1-3.2 log CFU/cm^2 on untreated trimmings and 2.7-2.8 log CFU/cm^2 on treated samples (i.e., reductions of 0.3-0.4 log units) (Figure 54).
- Surviving counts on treated samples for all six non-O157 STEC serotypes were not ($P\geq0.05$) different than surviving counts of *E. coli* O157:H7.
- Total bacteria counts of treated samples were 0.3-0.5 log CFU/cm^2 lower than those of untreated trimmings (Figure 55).
- The pH of samples was 5.98±0.10 prior to, and 4.92±0.16 after treatment with AFTEC, while the corresponding values after storage at 4°C (24 h) were 5.90±0.14 and 5.34±0.11, respectively.
- Treated samples had an average moisture pickup of 3.98±1.83%.

n) AFTEC 3000 treatment of beef trimmings inoculated with *E. coli* O157:H7 or the *S.* Newport/Typhimurium antibiotic resistance phenotypes

- Inoculated *E. coli* O157:H7 populations on trimmings were 3.1 (TSA+rif) and 3.0 (mSMAC) log CFU/cm^2, and were reduced ($P<0.05$) to 2.8 (TSA+rif) and 2.6 (mSMAC) log CFU/cm^2 after decontamination with AFTEC (reductions of 0.3 and 0.4 log CFU/cm^2, respectively) (Figures 56 and 57).
- *Salmonella* inocula were reduced ($P<0.05$) from 2.9-3.2 log CFU/cm^2 on untreated samples to 2.3-2.6 log CFU/cm^2 on treated samples, and reductions ranged from 0.5-0.7 log CFU/cm^2 (Figures 56 and 57).
- *Salmonella* counts, irrespective of serotype or antibiotic resistance profile, were similar ($P\geq0.05$) or lower ($P<0.05$; by 0.2-0.5 log CFU/cm^2) than TSA+rif/mSMAC counts of *E. coli* O157:H7 on decontaminated samples.
- Total bacteria counts of untreated and treated trimmings were 3.9-4.1 and 3.3-3.6 log CFU/cm^2, respectively (Figure 58).
- The pH of AFTEC-treated trimmings (pH 4.68±0.23) on the day of the experiment was lower ($P<0.05$) than that of untreated samples (pH 5.47±0.09). Similarly, the pH of treated samples (pH 5.04±0.08) held at 4°C (24 h) was lower ($P<0.05$) than that of untreated samples (pH 5.71±0.07).
- The moisture pickup of trimmings decontaminated with AFTEC ranged from 3.44±0.64 to 4.78±1.32%.

VII. CONCLUSIONS

Under the conditions of the above studies:

- Results of the acid challenge, conducted in a sterile beef homogenate acidified with 5% lactic acid, showed that in most cases, individual strains of non-O157 STEC (wild-type and

rifampicin-resistant), *S*. Newport and *S*. Typhimurium were less ($P<0.05$) acid tolerant than the *E. coli* O157:H7 5-strain mixture (wild-type and rifampicin-resistant) (Study 1).

- Individual strains (four strains per pathogen serotype/antibiotic resistance phenotype) of *E. coli* O26, *E. coli* O103, antibiotic susceptible *S*. Newport and MDR-AmpC *S*. Newport inoculated on beef trimmings were similarly ($P\geq0.05$) or more ($P<0.05$) sensitive to decontamination with lactic acid (5%, 55°C) than those of the *E. coli* O157:H7 4-strain mixture (Study 2). Based on these results, it was decided to use mixtures of strains within each serotype and/or antibiotic resistance phenotype for the work conducted under Study 6.
- *E. coli* O157:H7 and non-O157 STEC counts of trimmings and ground samples were not ($P\geq0.05$) different, irrespective of antimicrobial treatment (i.e., untreated or treated with lactic acid [5%, 55°C]) (Study 3). Based on these findings, trimmings, instead of ground samples, were analyzed for microbial counts in all other studies with trimmings (Studies 2, 4, 5, and 6).
- Higher reductions (by 0.6-1.2 log CFU/cm^2) of *E. coli* O157:H7 or non-O157 STEC were obtained on fatty tissue samples than on lean muscle tissue samples after treatment with lactic acid (5%, 55°C) (Study 4).
- Lactic acid decontamination (5%, 25°C) of trimmings by immersion (30 s) or spraying (2.76 bar; flow rate, 5.68 liters/min; conveyor belt speed, 5 cm/s) resulted in similar ($P\geq0.05$) surviving counts of *E. coli* O157:H7. When trimmings were decontaminated with sodium metasilicate (4%), pathogen counts of samples sprayed with the antimicrobial were 0.5 log CFU/cm^2 higher ($P<0.05$) than those immersed in the solution (Study 5).
- Chemical decontamination treatments for beef trimmings tested against *E. coli* O157:H7, including lactic acid (5%, pH 2.2, 25 or 55°C), acidified sodium chlorite (0.1%, pH 2.5±0.1, 25°C), peroxyacetic acid (0.02%, pH 3.8±0.1, 25°C), sodium metasilicate (4%, pH 12.5±0.1, 25°C), Bromitize Plus (225 ppm active bromine, pH 6.6±0.1, 25°C), SYNTRx 3300 (pH 1.0, 25°C), and AFTEC 3000 (pH 1.2; 25°C), were generally equally ($P\geq0.05$) or more ($P<0.05$) effective against non-O157 STEC (serotypes O26, O45, O103, O111, O121, and O145) and *S*. Newport/Typhimurium (antibiotic susceptible, MDR, and/or MDR-AmpC) inocula. In a few cases, surviving counts of *E. coli* O157:H7 on treated trimmings were statistically lower ($P<0.05$) than surviving counts of some non-O157 STEC and *Salmonella* inocula; however, biologically, the difference in counts between *E. coli* O157:H7 and these non-O157 STEC and *Salmonella* inocula was 0.2-0.6 log CFU/cm^2, which microbiologically is not considered a significant difference (Study 6).
- Overall, it can be concluded that chemical antimicrobial interventions used against *E. coli* O157:H7 on beef trimmings will be at least equally effective against non-O157 STEC and multidrug resistant *Salmonella*.

VIII. RECOMMENDATIONS FOR FUTURE RESEARCH

- Evaluate the chemical interventions in other fresh meat products and under other conditions that may be employed by some processors.
- Evaluate other antimicrobials as they are developed, proposed or approved.

IX. PRESENTATIONS AND PUBLICATIONS

Abstracts will be prepared for presentation at upcoming scientific meetings. Also, all data will be prepared for publication in scientific journals and trade magazines. An overview of data was presented by John Sofos during his presentation on "The Science and Purpose of Laboratory Challenge Studies" at the symposium on "Validation of Enteric Pathogen Interventions: Scientific, Regulatory and Applied Approaches for Beef Slaughter and Further Processors" presented at the 98th Annual Meeting of the International Association for Food Protection, held in Milwaukee, WI (July 31-August 3, 2011).

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Table 1. Sources of non-O157 STEC strains

E. coli serotype	Strain (alternate ID given by providing institution)	Source	Provided by
O26:H11	hSTEC_03 (O26-1)	human	Dr. Wheeler
O26:H11	imp_113.1 (O26-2)	beef	Dr. Wheeler
O26:H11	81.0211 (A1-006)	antelope	Dr. DebRoy
O26	85.1150 (A1-007)	pig	Dr. DebRoy
O26:H2	93.0494 (A1-008)	human	Dr. DebRoy
O26	0.1302 (A1-009)	cow	Dr. DebRoy
O26:H11	5.2217 (A1-010)	human	Dr. DebRoy
O45	99E_2750 (O45-1)	human	Dr. Wheeler
O45	O45-2	human	Dr. Wheeler
O45:H2	05-6545	human	Dr. Fratamico
O45:H2	96-3285	human	Dr. Fratamico
O103:H2	hSTEC_05 (O103-1)	human	Dr. Wheeler
O103	MDR0089 (O103-2)	beef	Dr. Wheeler
O103:H2	3.2607 (A1-011)	horse	Dr. DebRoy
O103:H11	86.0765 (A1-012)	mouse	Dr. DebRoy
O103:H2	87.1368 (A1-013)	goat	Dr. DebRoy
O103:H2	90.1764 (A1-014)	cow	Dr. DebRoy
O103:H2	92.0084 (A1-015)	human	Dr. DebRoy
O111:H8	hSTEC_08 (O111-1)	human	Dr. Wheeler
O111:NM	18DA (O111-2)	human	Dr. Wheeler
O111	93.0523 (A1-001)	human	Dr. DebRoy
O111	4.0005 (A1-002)	cow	Dr. DebRoy
O111	4.0522 (A1-003)	cow	Dr. DebRoy
O111	85.1154 (A1-004)	pig	Dr. DebRoy
O111	93.0522 (A1-005)	human	Dr. DebRoy
O121	10896 (O121-1)	human	Dr. Wheeler
O121	imp_450 (O121-2)	beef	Dr. Wheeler
O121:H19	97-3068	human	Dr. Fratamico
O121:NM	03-4064	human	Dr. Fratamico
O121:H19	08023	human	Dr. Fratamico
O145:NM	hSTEC_22 (O145-1)	human	Dr. Wheeler
O145	MAY109 (O145-2)	beef	Dr. Wheeler
O145:NM	03-4699	human	Dr. Fratamico
O145:NM	83-75	human	Dr. Fratamico
O145:H28	07865	cow	Dr. Fratamico

Table 2. Sources and antibiotic resistance profiles of *S*. Newport and *S*. Typhimurium strains

Salmonella serotype	Strain	Source	Antibiotic resistance[a]	Phenotype: Susceptible/MDR/ MDR-AmpC	Provided by
Newport	FSL S5-639	human	S	Susceptible	Dr. Wiedmann
	CVM N4505	ground turkey	S	Susceptible	Dr. Zhao
	CVM N18445	ground beef	S	Susceptible	Dr. Zhao
	CVM N1509	ground turkey	S	Susceptible	Dr. Zhao
	FSL R6-531	human	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Wiedmann
	FSL R8-0104	human	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Wiedmann
	FSL S5-413	human	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Wiedmann
	FSL S5-436	bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
	FSL S5-577	bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Wiedmann
	FSL S5-920	bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
	FSL R8-2926	human	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
	FSL R8-2350	bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Wiedmann
	CVM N635	ground beef	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, SXT	MDR-AmpC	Dr. Zhao
	CVM 22698	pork chop	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, SXT	MDR-AmpC	Dr. Zhao
	CVM 29461	ground beef	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Zhao
	CVM 22707	ground beef	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Zhao
	CVM N19852	ground beef	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Zhao

Salmonella serotype	Strain	Source	Antibiotic resistance[a]	Phenotype: Susceptible/MDR/ MDR-AmpC	Provided by
Typhimurium	FSL S5-536	human	S	Susceptible	Dr. Wiedmann
	CVM N7300	chicken breast	S	Susceptible	Dr. Zhao
var. O 5- (Copenhagen)	CVM N15788	ground beef	S	Susceptible	Dr. Zhao
var. O 5- (Copenhagen)	CVM N18534	chicken breast	S	Susceptible	Dr. Zhao
	FSL R6-215	human	AMP, CHL, STR, FIS, TET	MDR	Dr. Wiedmann
	FSL S9-165	bovine	AMP, CHL, STR, FIS, TET	MDR	Dr. Wiedmann
	FSL R8-2540	human	AMP, CHL, STR, FIS, TET	MDR	Dr. Wiedmann
	CVM N6431	chicken breast	AMP, CHL, STR, FIS, TET	MDR	Dr. Zhao
var. O 5- (Copenhagen)	CVM 30662	chicken breast	AMP, CHL, STR, FIS, TET	MDR	Dr. Zhao
	CVM N497	pork chop	AMP, CHL, STR, FIS, TET	MDR	Dr. Zhao
var. Copenhagen	FSL S5-786	bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
	FSL S5-916	bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
var. Copenhagen	FSL S5-385	bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
var. O 5- (Copenhagen)	CVM N176	chicken breast	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Zhao
	CVM 33831	cattle	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, SXT, KAN	MDR-AmpC	Dr. Zhao
var. O 5- (Copenhagen)	CVM 30034	ground turkey	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, NAL, KAN	MDR-AmpC	Dr. Zhao

[a] Per results of the Sensititre® antimicrobial susceptibility system CMV2AGNF panel (Trek Diagnostic Systems). Antibiotics included on the panel include ampicillin (AMP), amoxicillin/clavulanic acid (AUG2), cefoxitin (FOX), ceftiofur (XNL), ceftriaxone (AXO), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfisoxazole (FIS), tetracycline (TET), trimethoprim/sulfamethoxazole (SXT)

MDR: resistant to at least ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (ACSSuT)

MDR-AmpC: resistant to at least ACSSuT, amoxicillin-clavulanic acid and ceftiofur, and a decreased susceptibility to ceftriaxone (MIC $\geq$2 μg/ml)

S: sensitive to all tested antibiotics

Table 3. Rifampicin-resistant *E. coli* O157:H7, rifampicin-resistant non-O157 STEC, and antibiotic susceptible, MDR and/or MDR-AmpC *S*. Newport and *S*. Typhimurium strains included in inocula (four strains per *E. coli* serotype or *S*. Newport/Typhimurium antibiotic resistance profile) for studies with beef trimmings (Study 6)

E. coli serotype	Strain	Source	*Salmonella* serotype	Antibiotic resistance phenotype	Strain	Source
O157:H7	ATCC 43895	raw hamburger	Newport	Susceptible	FSL S5-639	human
O157:H7	C1-057	bovine feces	Newport	Susceptible	CVM N4505	ground turkey
O157:H7	C1-072	bovine feces	Newport	Susceptible	CVM N18445	ground beef
O157:H7	C1-109	bovine feces	Newport	Susceptible	CVM N1509	ground turkey
O26:H11	hSTEC_03	human	Newport	MDR-AmpC	FSL S5-436	bovine
O26:H2	93.0494	human	Newport	MDR-AmpC	FSL S5-920	bovine
O26	0.1302	cow	Newport	MDR-AmpC	CVM 22698	pork chop
O26:H11	5.2217	human	Newport	MDR-AmpC	CVM N19852	ground beef
O45	99E_2750	human	Typhimurium	Susceptible	FSL S5-536	human
O45	O45-2	human	Typhimurium	Susceptible	CVM N7300	chicken breast
O45:H2	05-6545	human	Typhimurium var. O 5- (Copenhagen)	Susceptible	CVM N15788	ground beef
O45:H2	96-3285	human	Typhimurium var. O 5- (Copenhagen)	Susceptible	CVM N18534	chicken breast
O103	MDR0089	beef	Typhimurium	MDR	FSL R6-215	human
O103:H2	87.1368	goat	Typhimurium	MDR	FSL R8-2540	human
O103:H2	90.1764	cow	Typhimurium	MDR	CVM N6431	chicken breast
O103:H2	92.0084	human	Typhimurium var. O 5- (Copenhagen)	MDR	CVM 30662	chicken breast
O111:H8	hSTEC_08	human	Typhimurium var. Copenhagen	MDR-AmpC	FSL S5-786	bovine
O111	93.0523	human	Typhimurium var. O 5- (Copenhagen)	MDR-AmpC	CVM N176	chicken breast
O111	4.0005	cow	Typhimurium	MDR-AmpC	CVM 33831	cattle
O111	4.0522	cow	Typhimurium var. O 5- (Copenhagen)	MDR-AmpC	CVM 30034	ground turkey
O121	10896	human				
O121	imp_450	beef				
O121:H19	97-3068	human				
O121:NM	03-4064	human				
O145:NM	hSTEC_22	human				
O145	MAY109	beef				
O145:NM	03-4699	human				
O145:H28	07865	cow				

Figure 1. Custom-built spray cabinet used to apply treatments (Study 5)

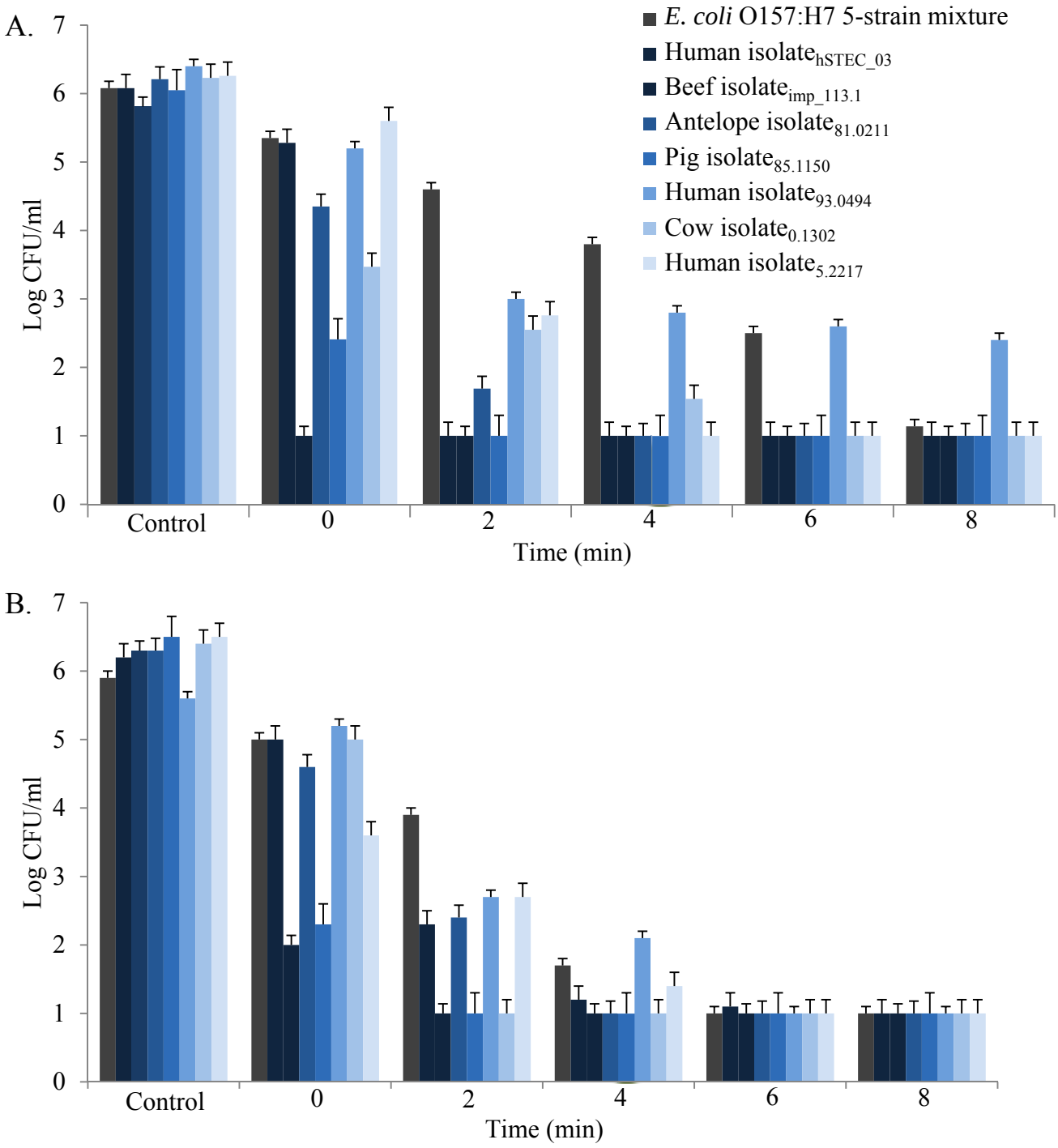

Figure 2. Counts (log CFU/ml) of wild-type (A; recovered on tryptic soy agar, TSA) and rifampicin-resistant (B; recovered on TSA + rifampicin) *E. coli* O26 strains and a 5-strain mixture of *E. coli* O157:H7 during exposure to an acidified (5% lactic acid) beef homogenate (error bars indicate standard error)

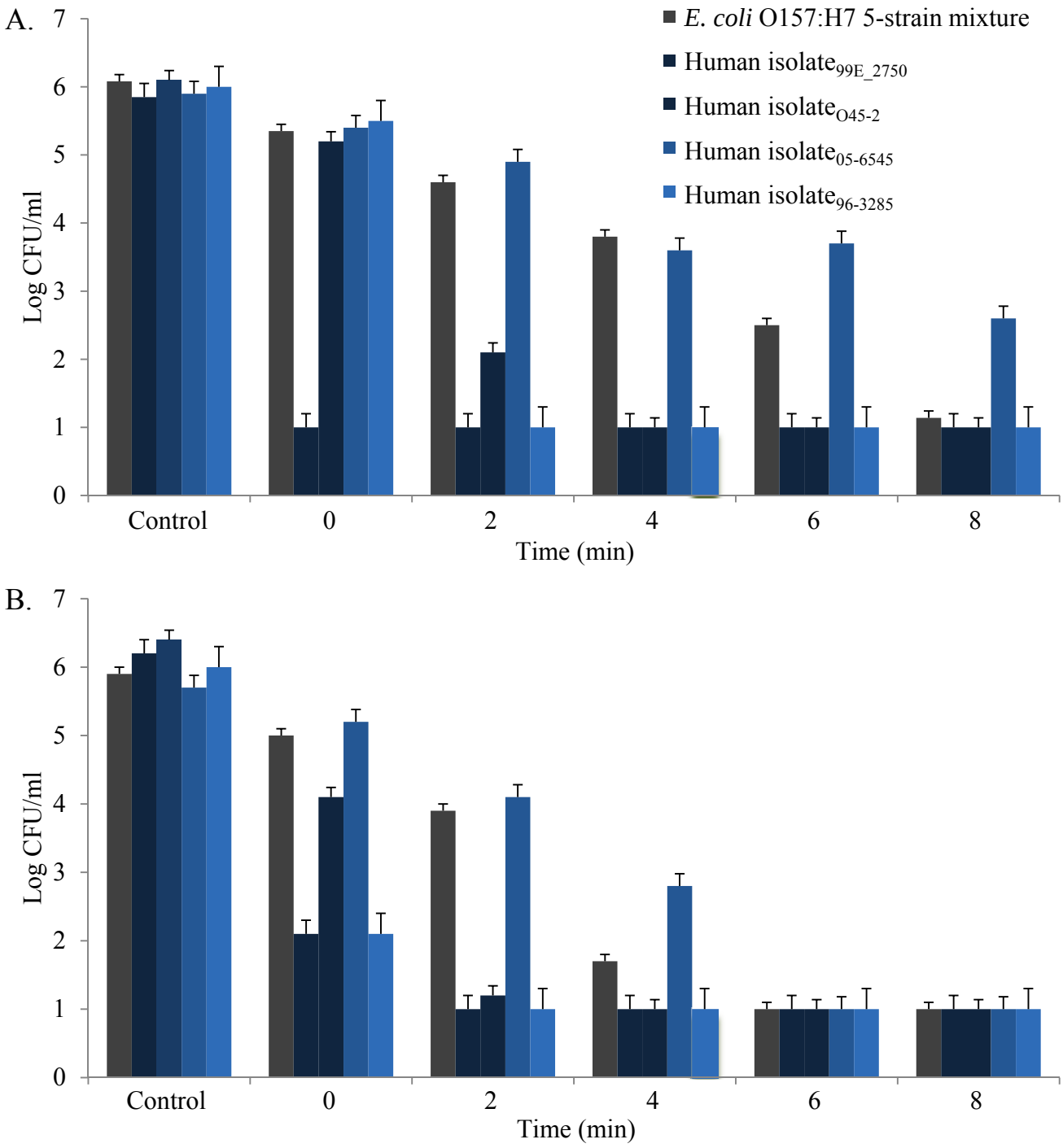

Figure 3. Counts (log CFU/ml) of wild-type (A; recovered on tryptic soy agar, TSA) and rifampicin-resistant (B; recovered on TSA + rifampicin) *E. coli* O45 strains and a 5-strain mixture of *E. coli* O157:H7 during exposure to an acidified (5% lactic acid) beef homogenate (error bars indicate standard error)

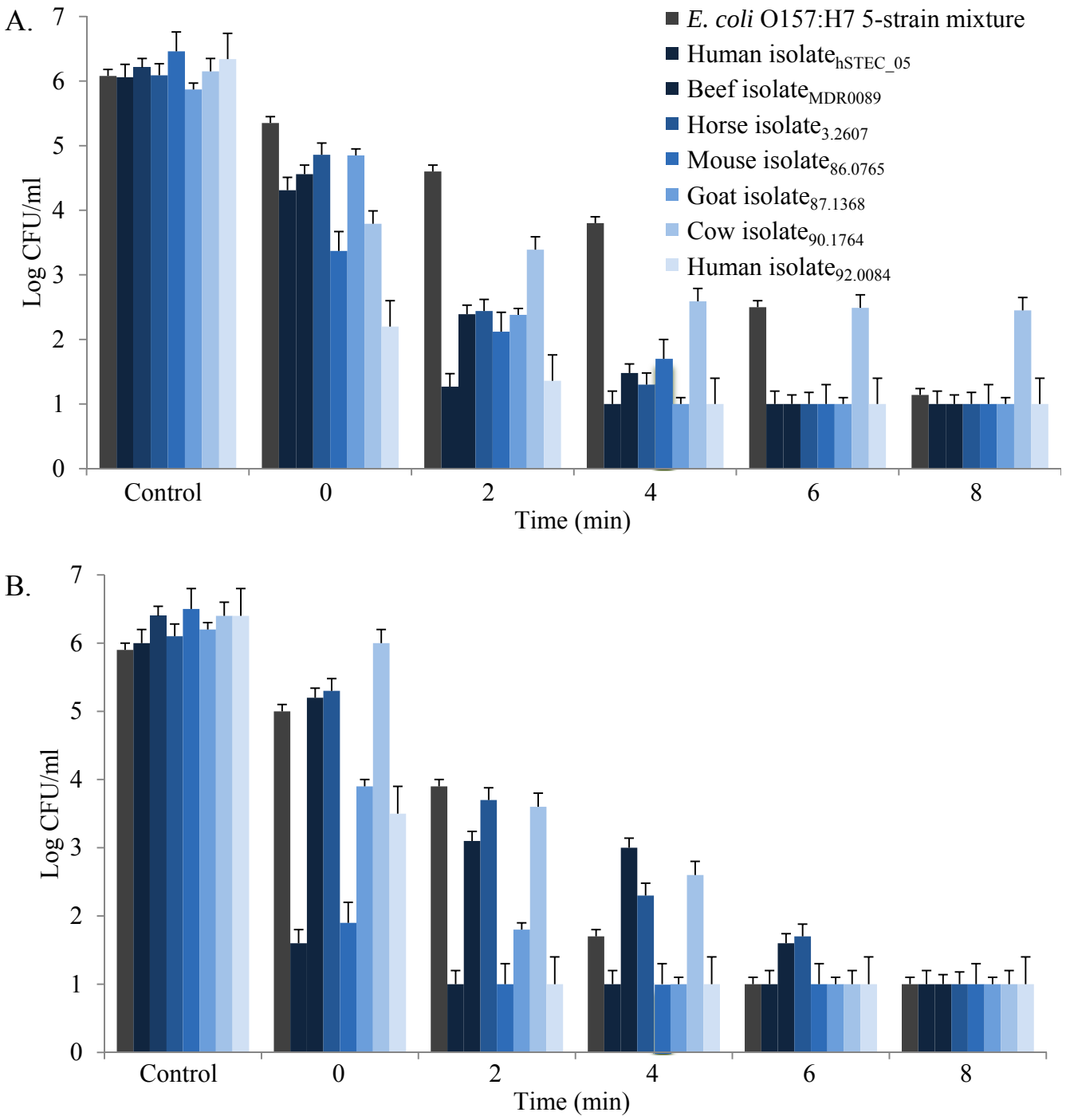

Figure 4. Counts (log CFU/ml) of wild-type (A; recovered on tryptic soy agar, TSA) and rifampicin-resistant (B; recovered on TSA + rifampicin) *E. coli* O103 strains and a 5-strain mixture of *E. coli* O157:H7 during exposure to an acidified (5% lactic acid) beef homogenate (error bars indicate standard error)

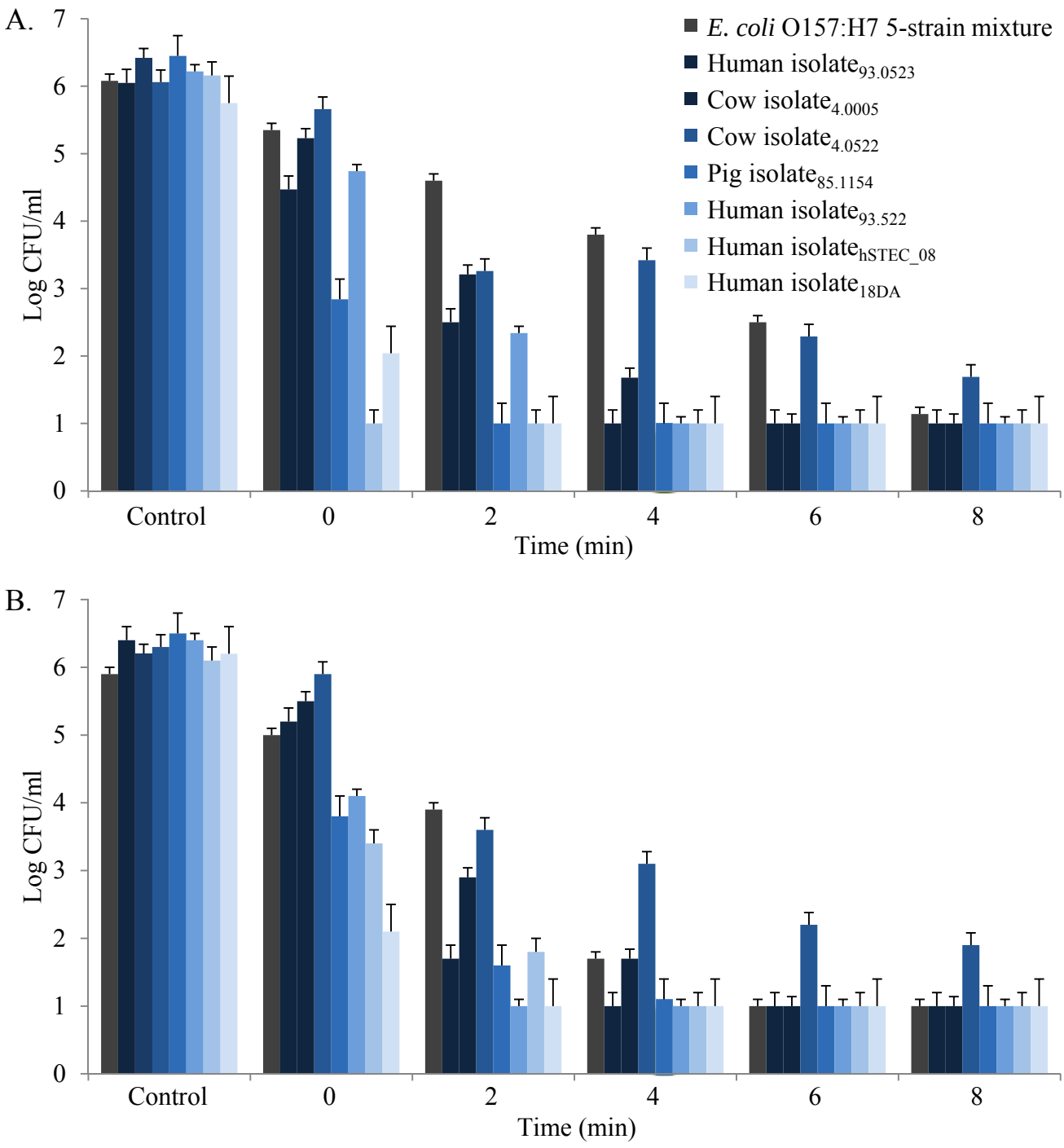

Figure 5. Counts (log CFU/ml) of wild-type (A; recovered on tryptic soy agar, TSA) and rifampicin-resistant (B; recovered on TSA + rifampicin) *E. coli* O111 strains and a 5-strain mixture of *E. coli* O157:H7 during exposure to an acidified (5% lactic acid) beef homogenate (error bars indicate standard error)

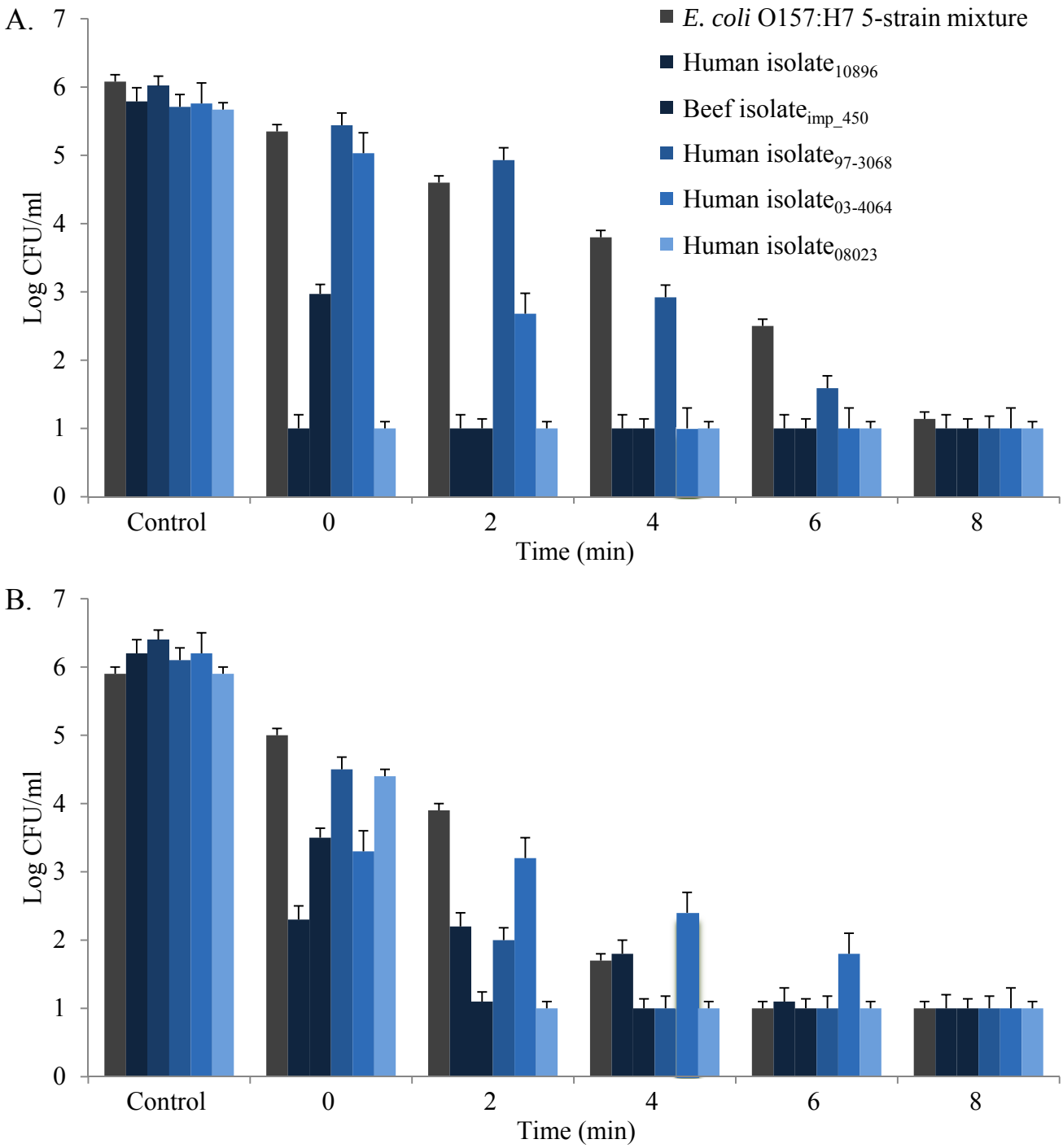

Figure 6. Counts (log CFU/ml) of wild-type (A; recovered on tryptic soy agar, TSA) and rifampicin-resistant (B; recovered on TSA + rifampicin) *E. coli* O121 strains and a 5-strain mixture of *E. coli* O157:H7 during exposure to an acidified (5% lactic acid) beef homogenate (error bars indicate standard error)

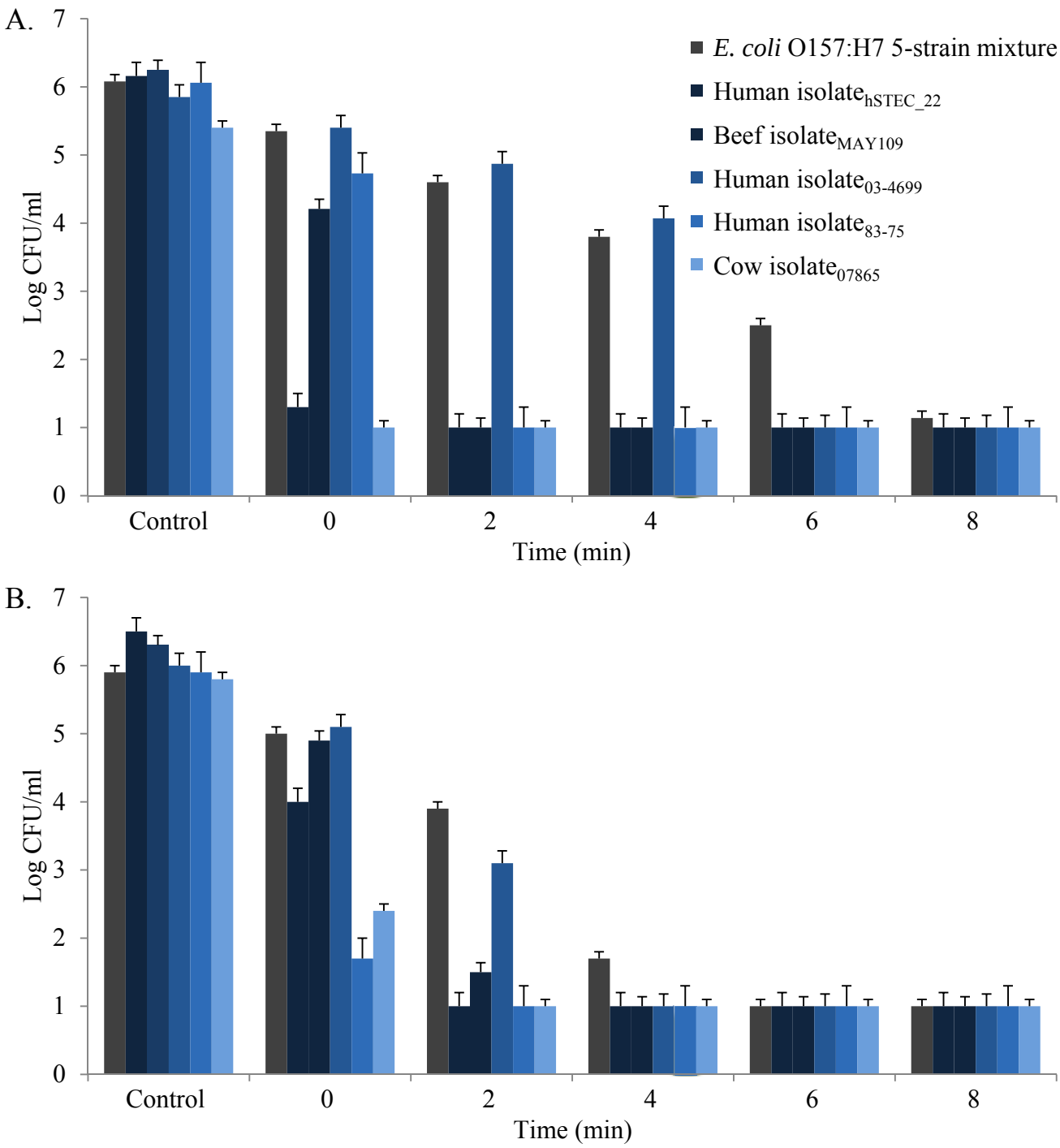

Figure 7. Counts (log CFU/ml) of wild-type (A; recovered on tryptic soy agar, TSA) and rifampicin-resistant (B; recovered on TSA + rifampicin) *E. coli* O145 strains and a 5-strain mixture of *E. coli* O157:H7 during exposure to an acidified (5% lactic acid) beef homogenate (error bars indicate standard error)

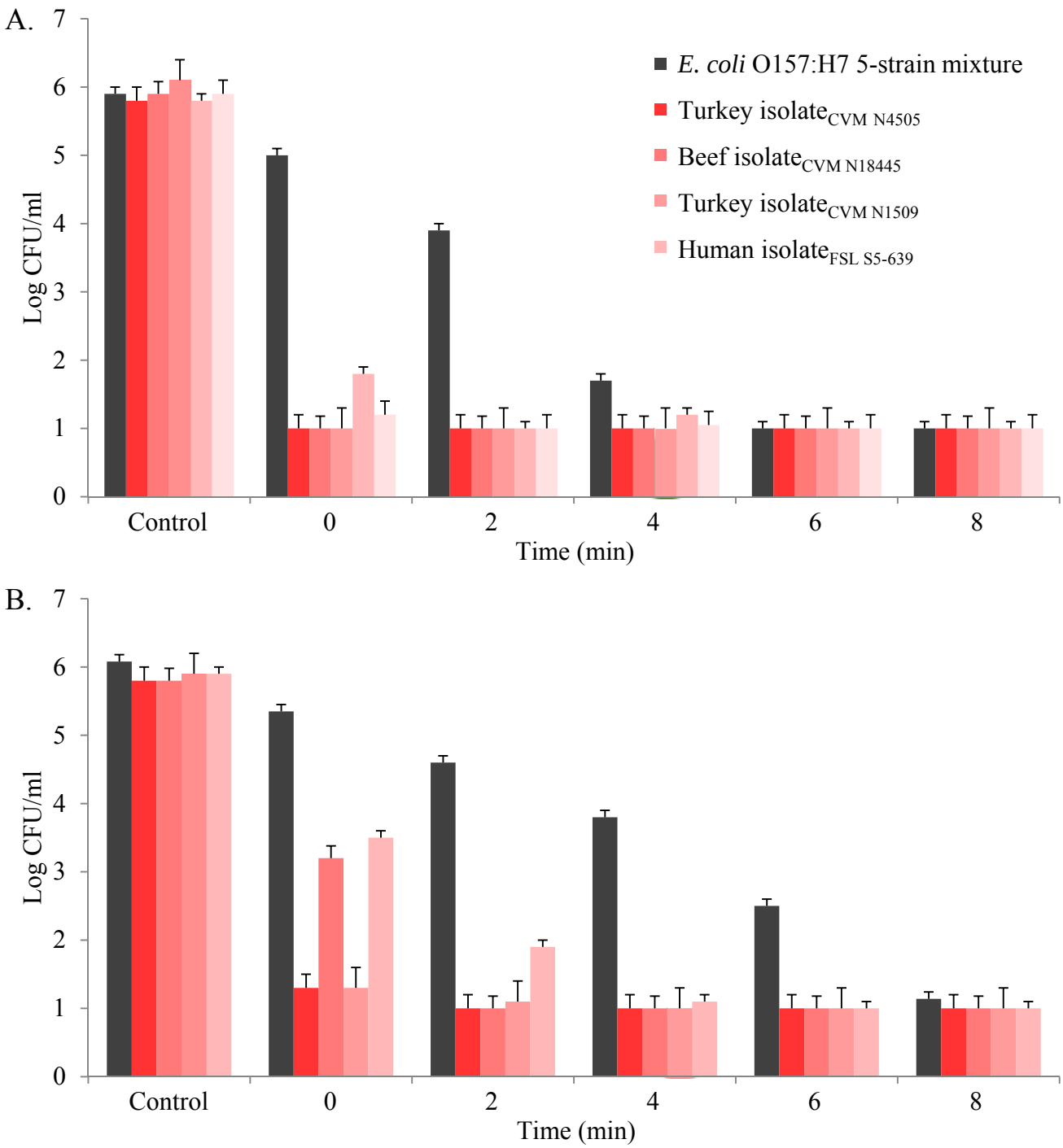

Figure 8. Counts (log CFU/ml) of antibiotic susceptible *S*. Newport strains, recovered on XLD agar (A) and tryptic soy agar (B; TSA), and a 5-strain rifampicin-resistant (A; recovered on TSA + rifampicin) or wild-type (B; recovered on TSA) mixture of *E. coli* O157:H7, during exposure to an acidified (5% lactic acid) beef homogenate (error bars indicate standard error)

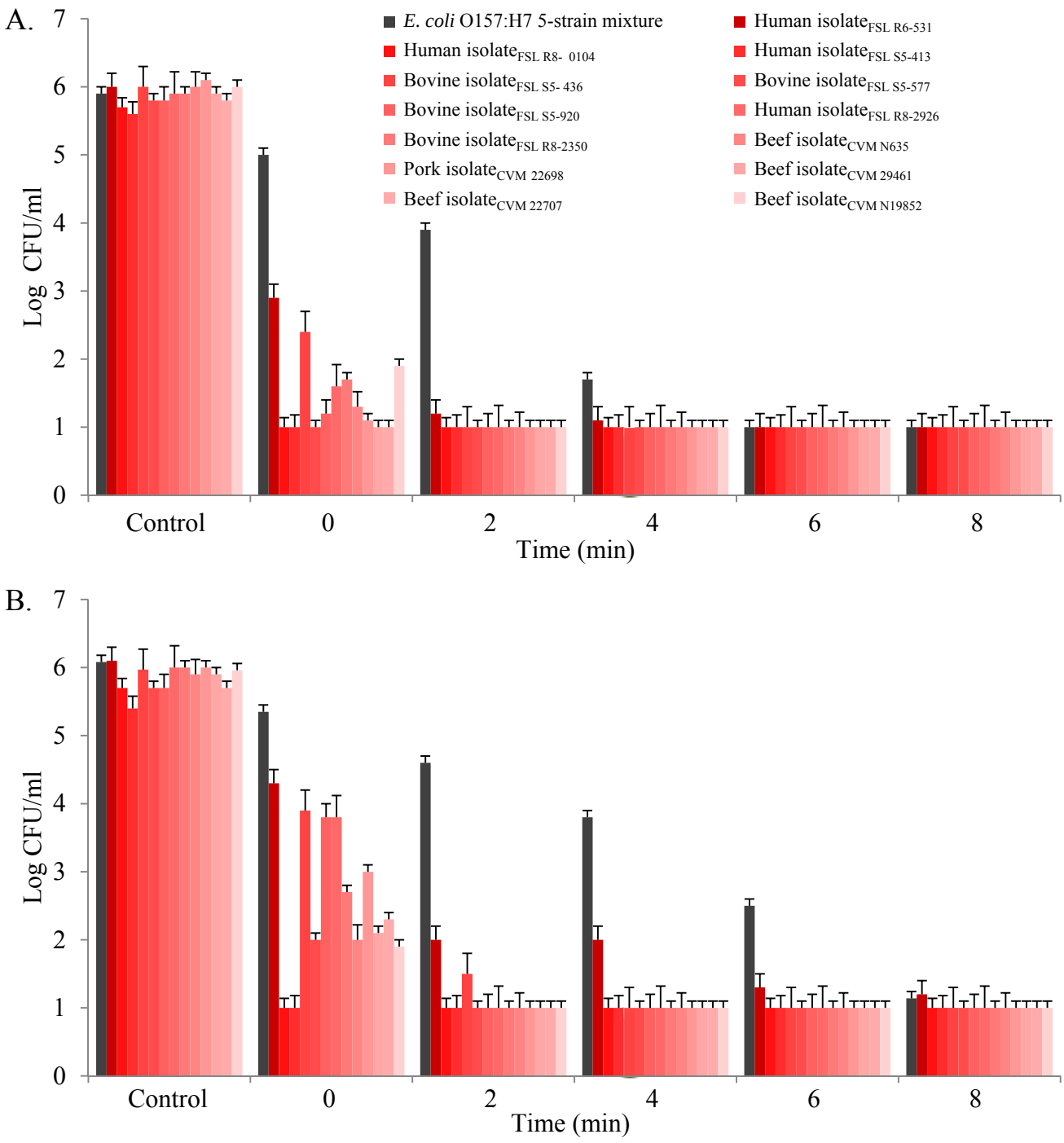

Figure 9. Counts (log CFU/ml) of MDR-AmpC *S.* Newport strains, recovered on XLD agar (A) and tryptic soy agar (B; TSA), and a 5-strain rifampicin-resistant (A; recovered on TSA + rifampicin) or wild-type (B; recovered on TSA) mixture of *E. coli* O157:H7, during exposure to an acidified (5% lactic acid) beef homogenate (error bars indicate standard error)

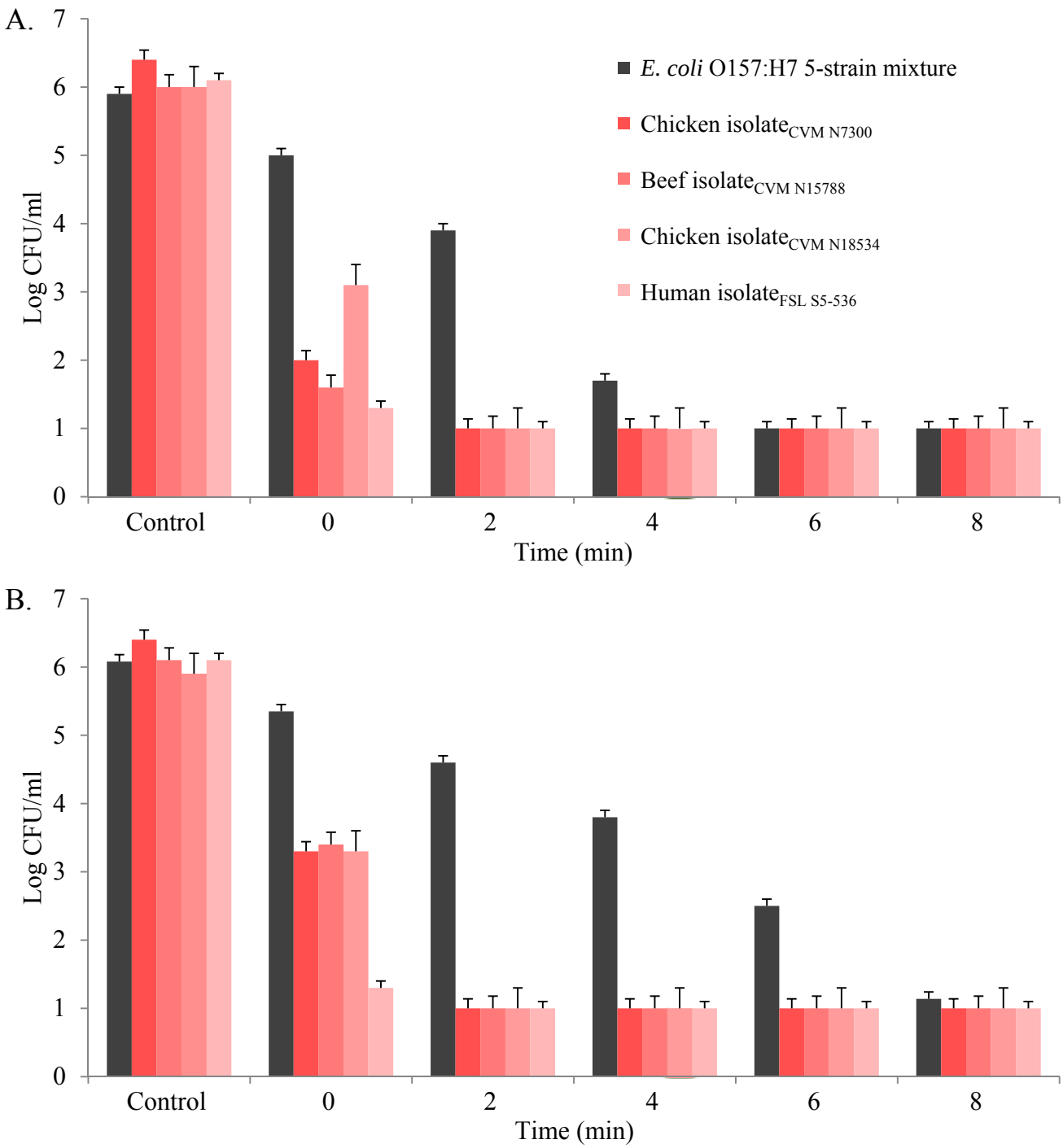

Figure 10. Counts (log CFU/ml) of antibiotic susceptible *S.* Typhimurium strains, recovered on XLD agar (A) and tryptic soy agar (B; TSA), and a 5-strain rifampicin-resistant (A; recovered on TSA + rifampicin) or wild-type (B; recovered on TSA) mixture of *E. coli* O157:H7, during exposure to an acidified (5% lactic acid) beef homogenate (error bars indicate standard error)

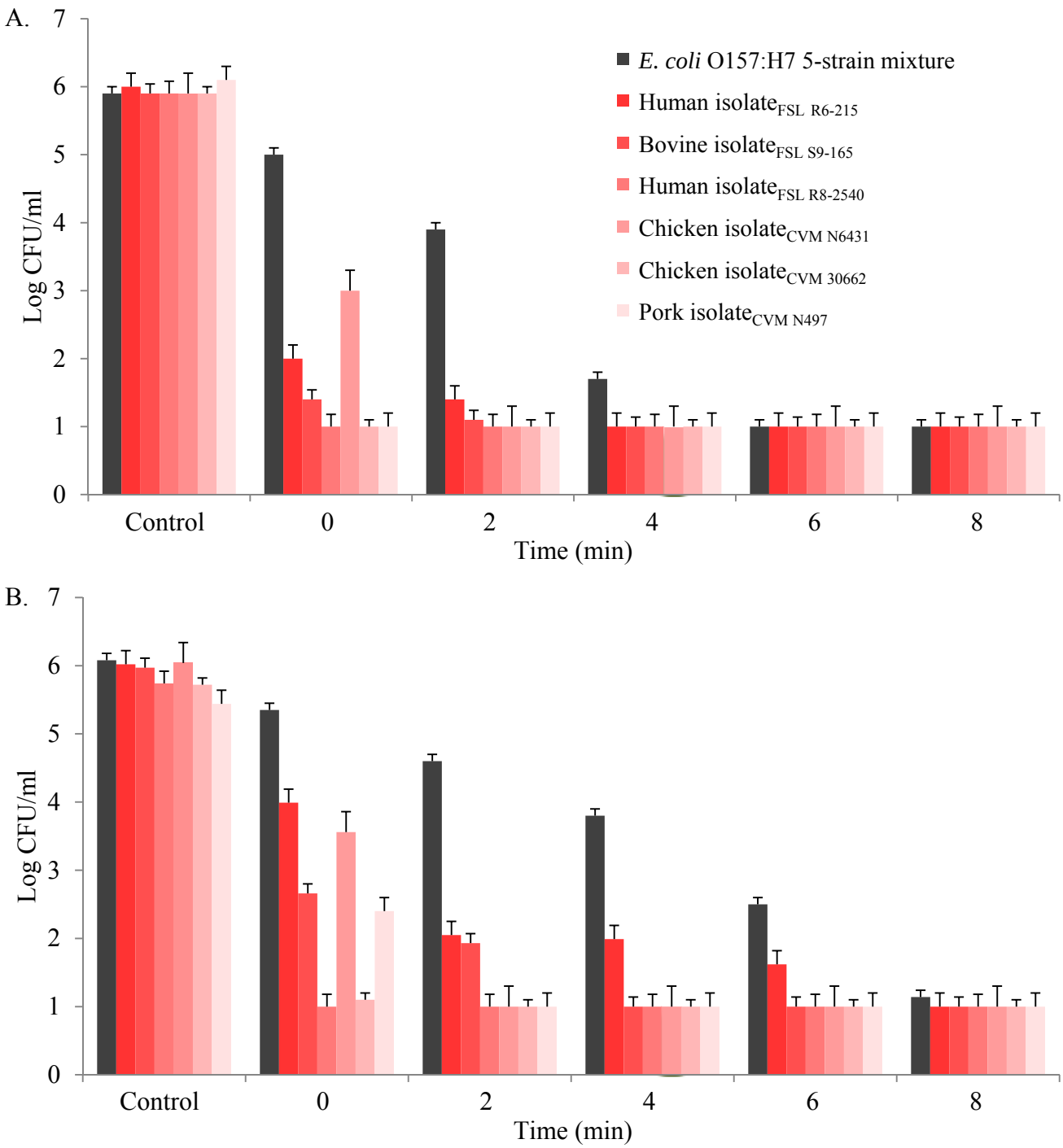

Figure 11. Counts (log CFU/ml) of MDR *S.* Typhimurium strains, recovered on XLD agar (A) and tryptic soy agar (B; TSA), and a 5-strain rifampicin-resistant (A; recovered on TSA + rifampicin) or wild-type (B; recovered on TSA) mixture of *E. coli* O157:H7, during exposure to an acidified (5% lactic acid) beef homogenate (error bars indicate standard error)

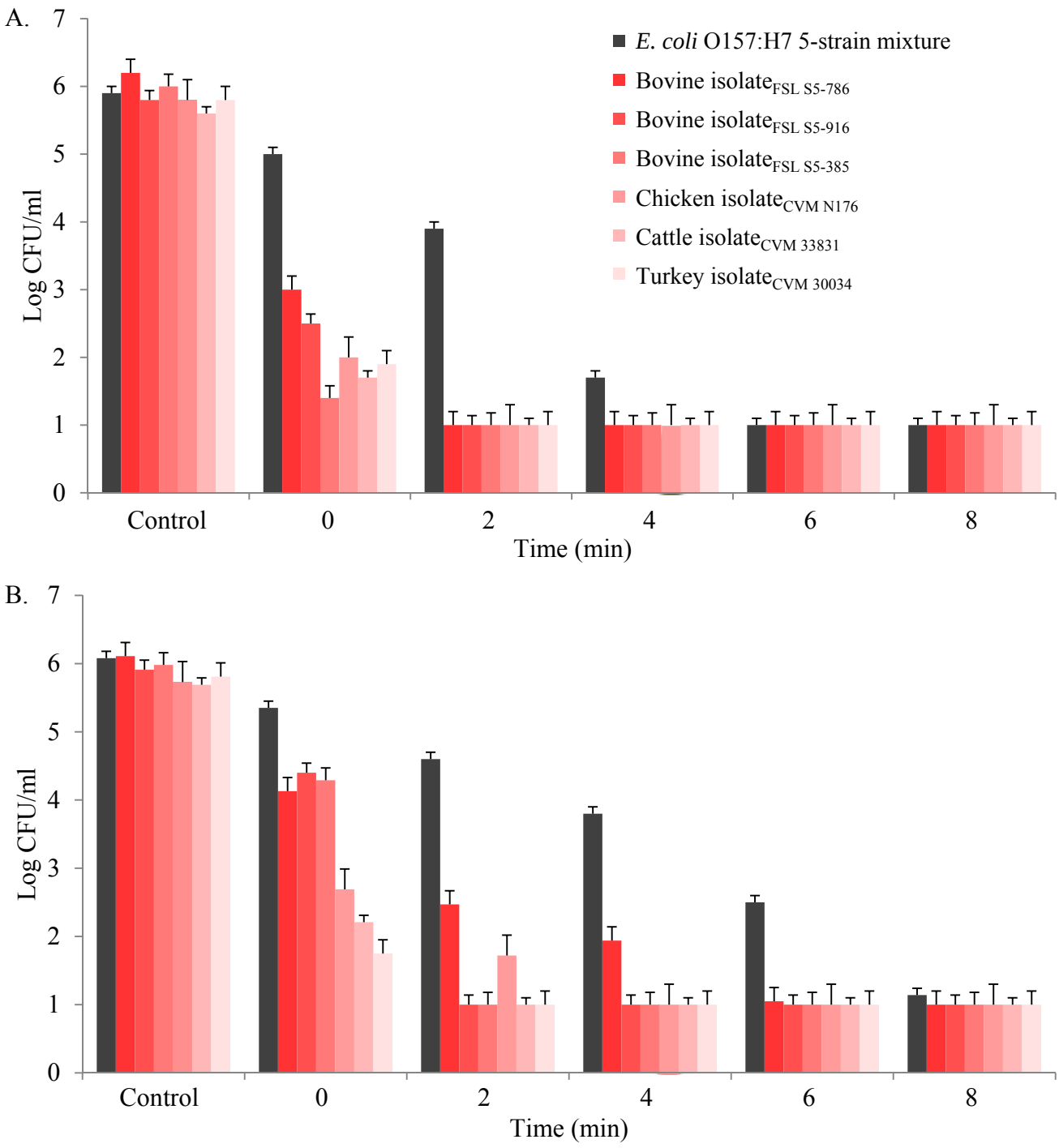

Figure 12. Counts (log CFU/ml) of MDR-AmpC *S.* Typhimurium strains, recovered on XLD agar (A) and tryptic soy agar (B; TSA), and a 5-strain rifampicin-resistant (A; recovered on TSA + rifampicin) or wild-type (B; recovered on TSA) mixture of *E. coli* O157:H7, during exposure to an acidified (5% lactic acid) beef homogenate (error bars indicate standard error)

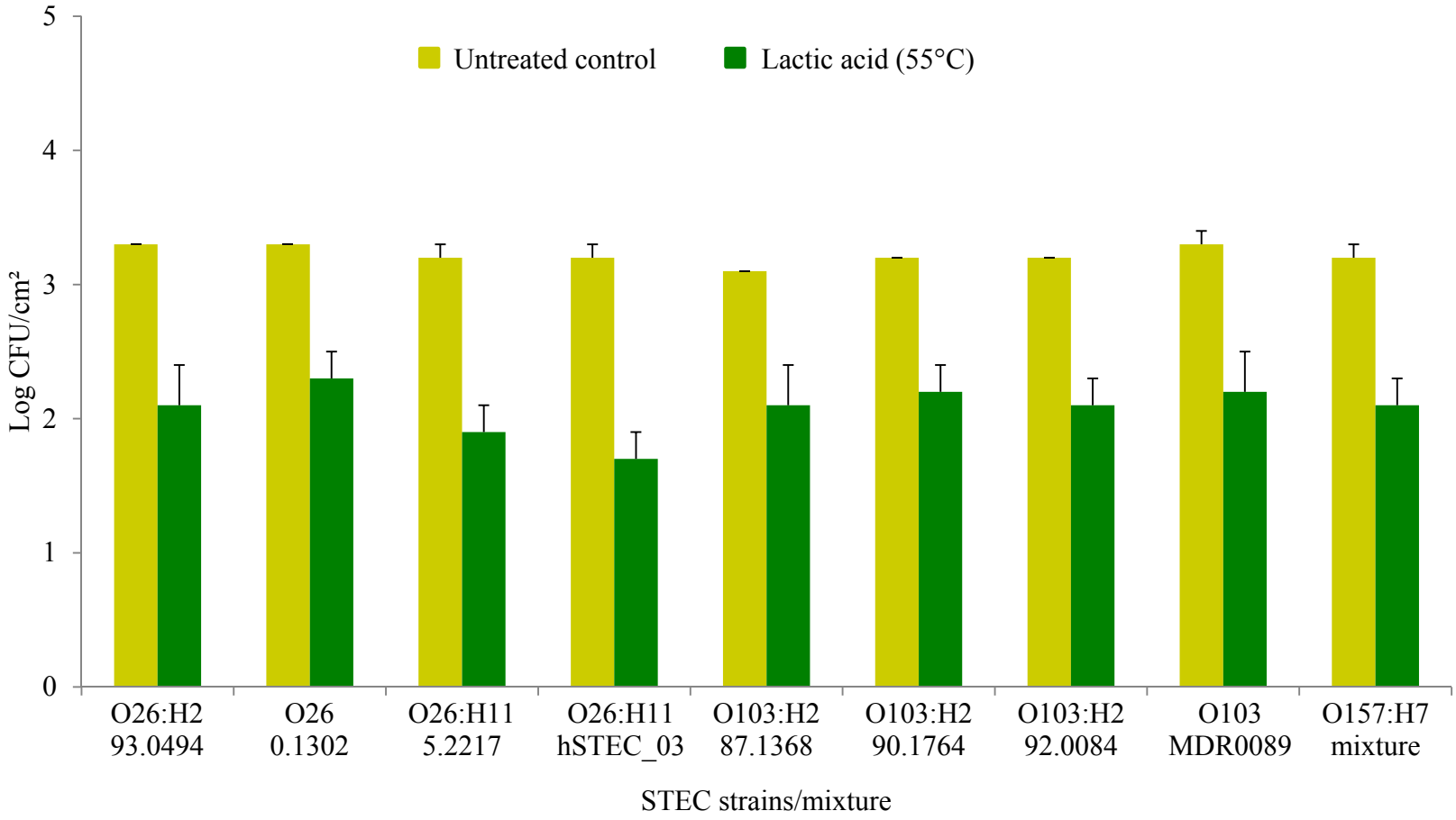

Figure 13. Populations (log CFU/cm^2) of individual rifampicin-resistant *E. coli* O26 and *E. coli* O103 strains, and a 4-strain mixture of *E. coli* O157:H7 (recovered on tryptic soy agar + rifampicin) on beef trimmings before and after decontamination with lactic acid (5%, 55°C)

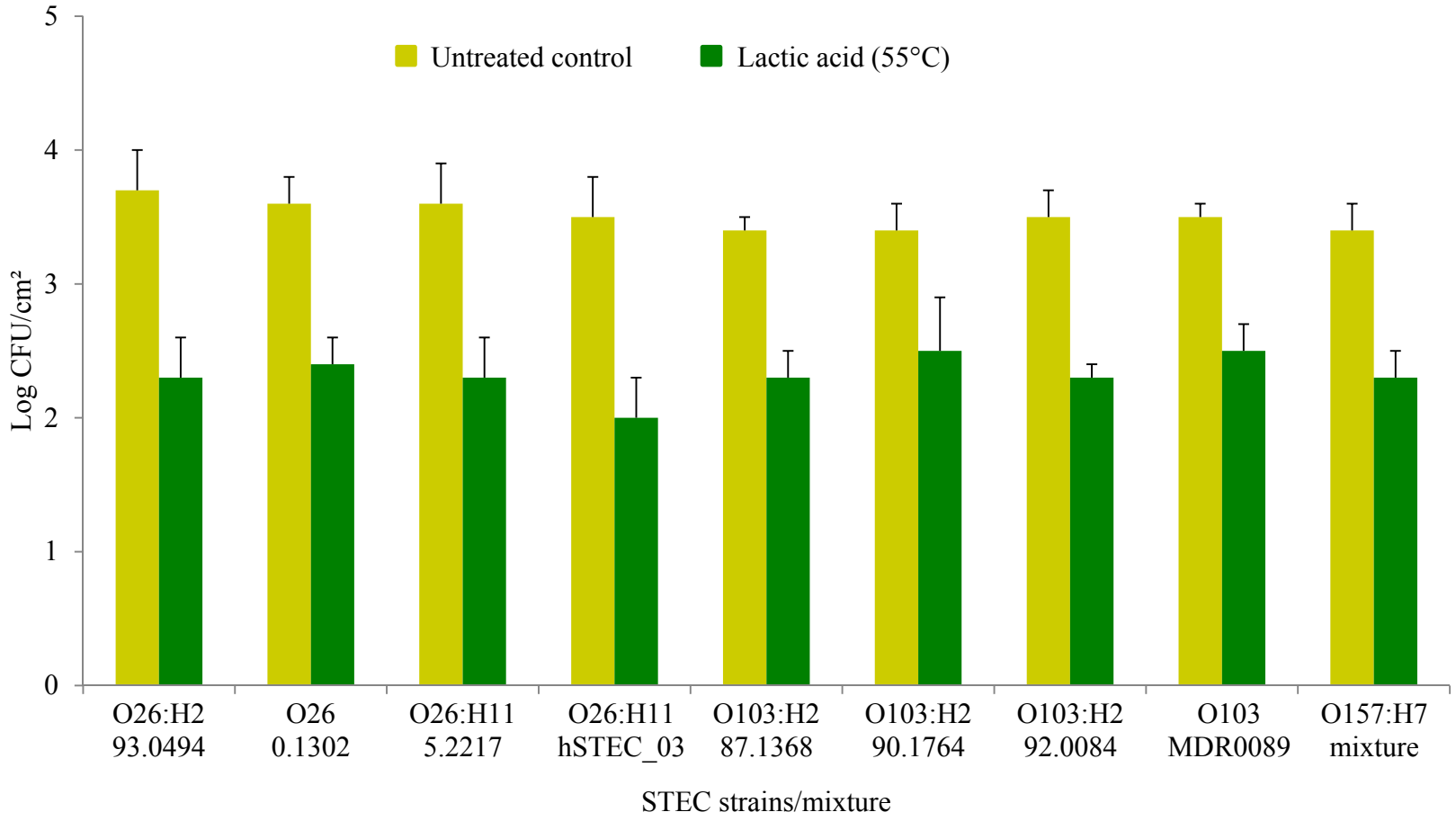

Figure 14. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with individual rifampicin-resistant *E. coli* O26 and *E. coli* O103 strains, or a 4-strain mixture of *E. coli* O157:H7) before and after decontamination with lactic acid (5%, 55°C)

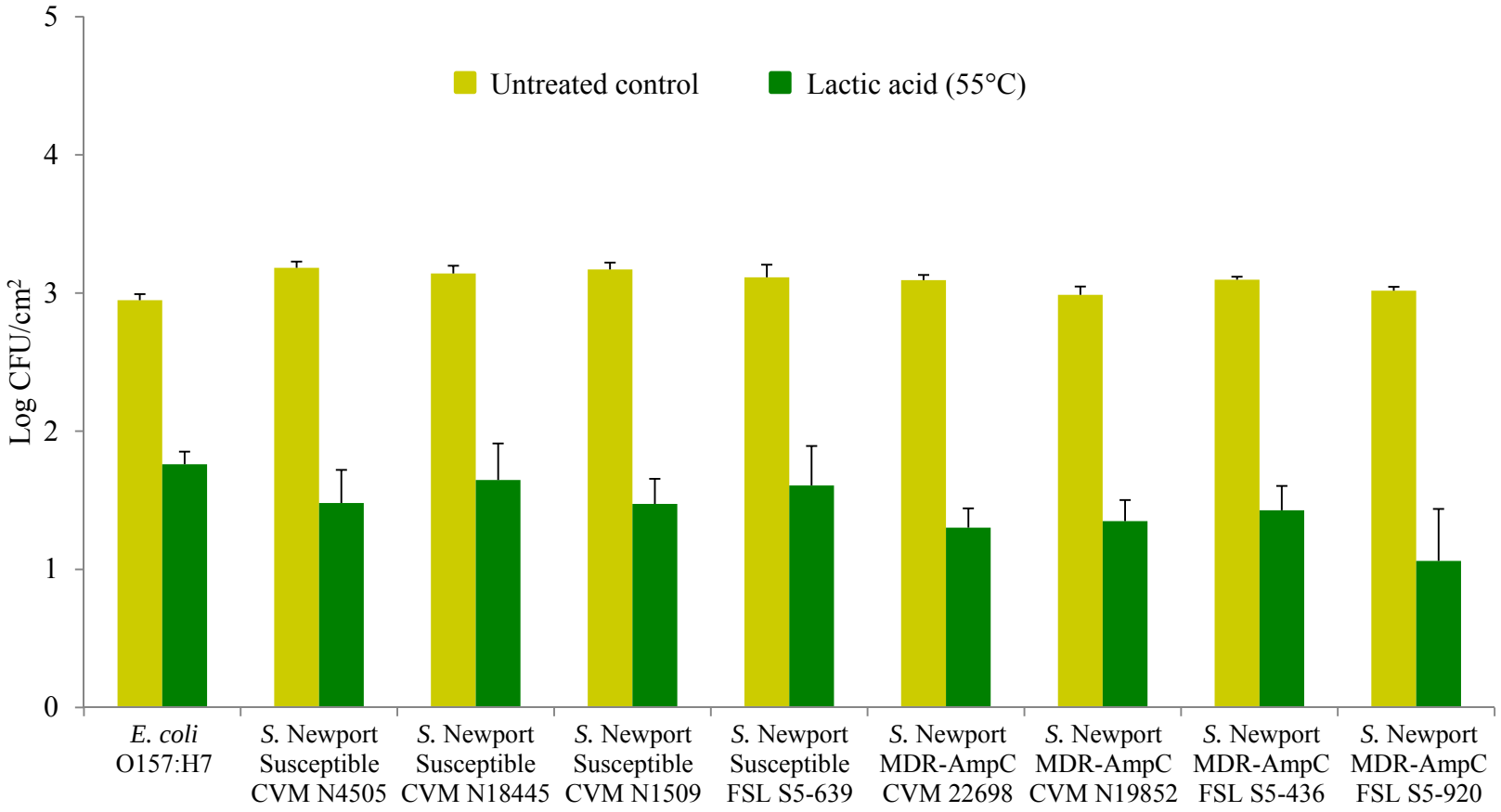

Figure 15. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (4-strain mixture; recovered on modified sorbitol MacConkey agar) and individual strains of antibiotic susceptible or MDR-AmpC *S.* Newport (recovered on XLD agar) on beef Trimmings before and after decontamination with lactic acid (5%, 55°C)

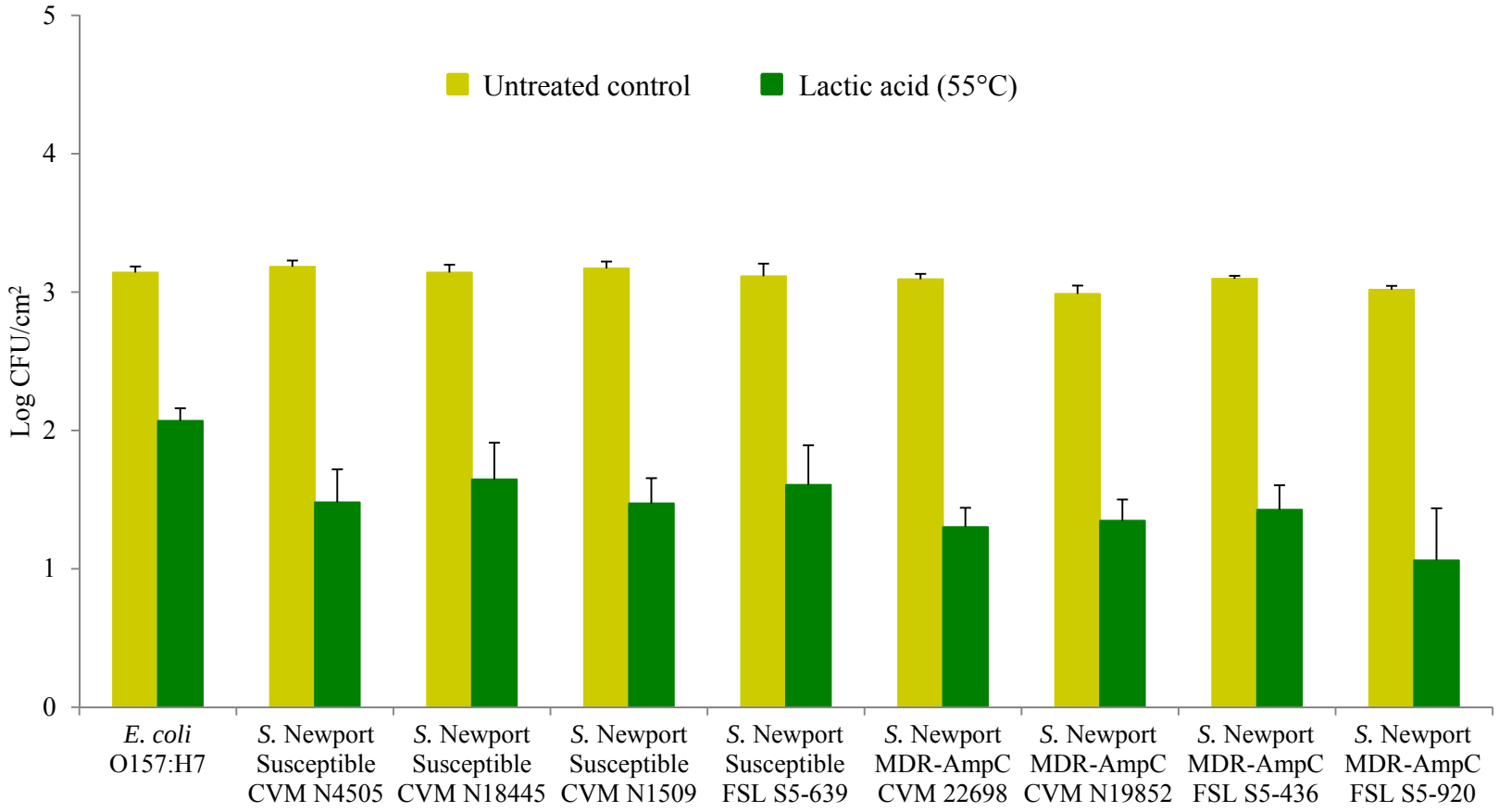

Figure 16. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (4-strain mixture; recovered on tryptic soy agar + rifampicin) and individual strains of antibiotic susceptible or MDR-AmpC *S.* Newport (recovered on XLD agar) on beef trimmings before and after decontamination with lactic acid (5%, 55°C)

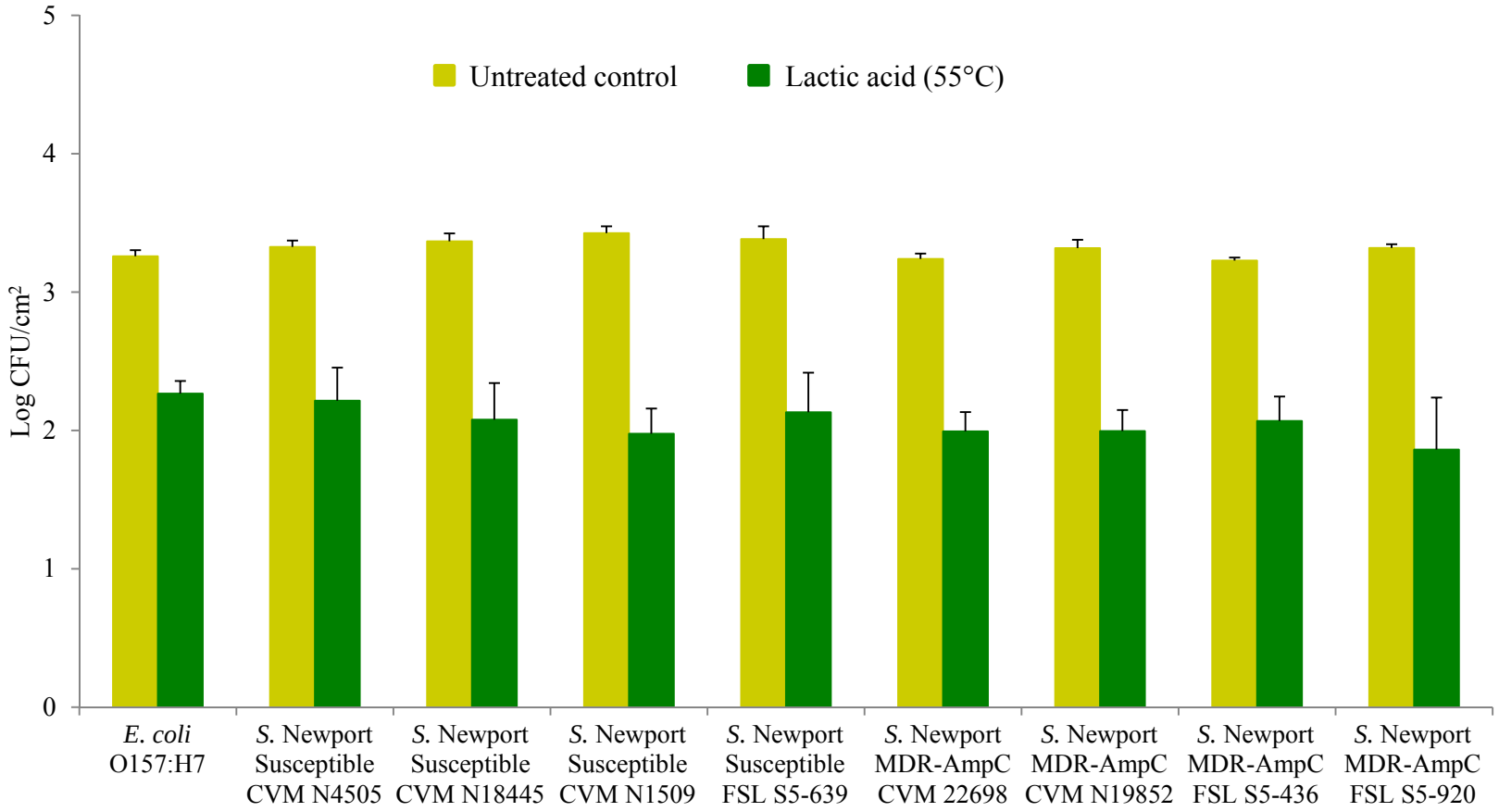

Figure 17. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with a 4-strain mixture of rifampicin-resistant *E. coli* O157:H7 or individual strains of antibiotic susceptible or MDR-AmpC *S.* Newport) before and after decontamination with lactic acid (5%, 55°C)

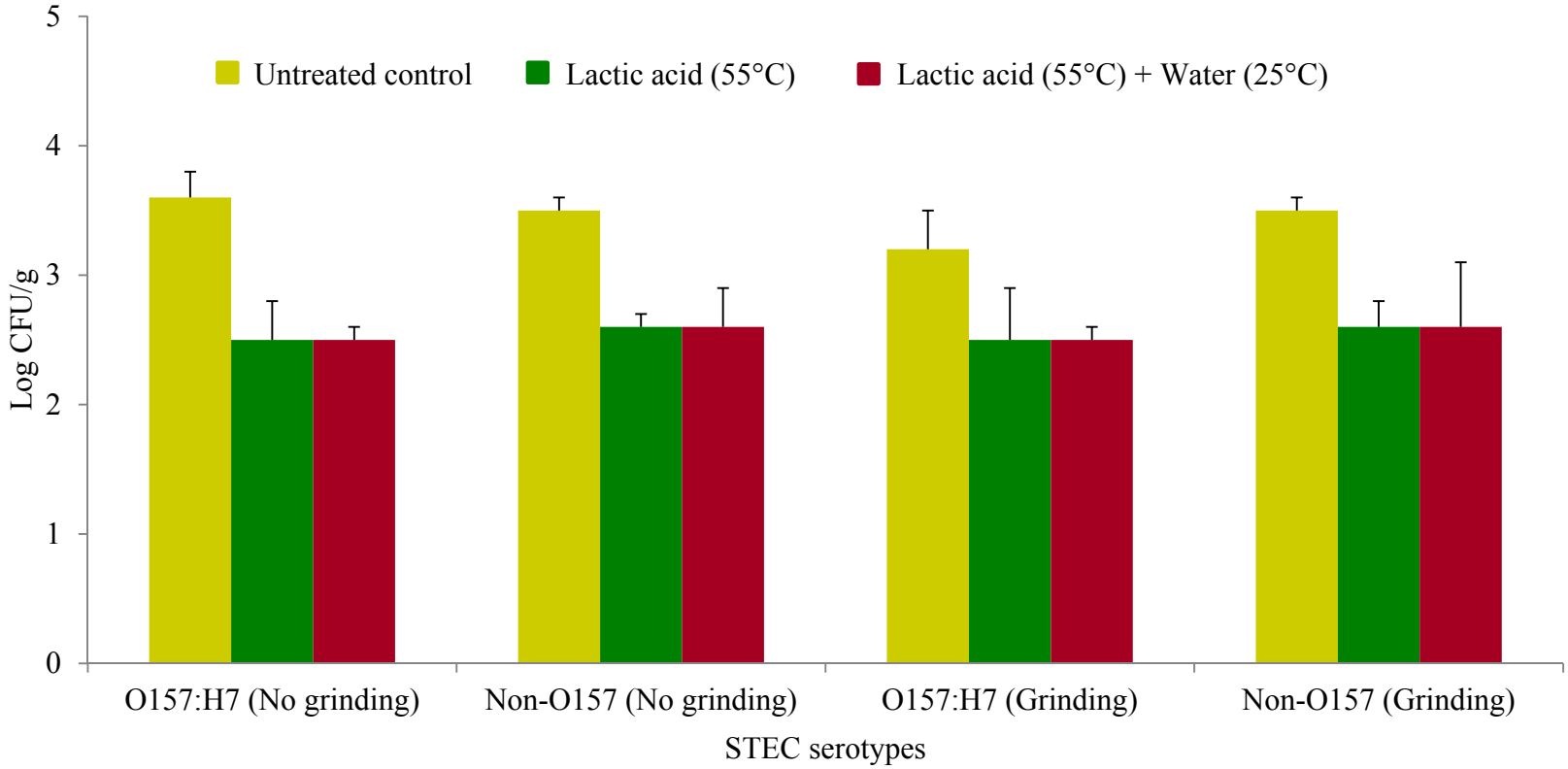

Figure 18. Populations (log CFU/g) of rifampicin-resistant *E. coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* (STEC) inocula (recovered on tryptic soy agar + rifampicin) on beef trimmings ("no grinding") before and after decontamination with lactic acid (5%, 55°C) without or with a water (25°C) rinse, and in subsequently ground ("grinding") product

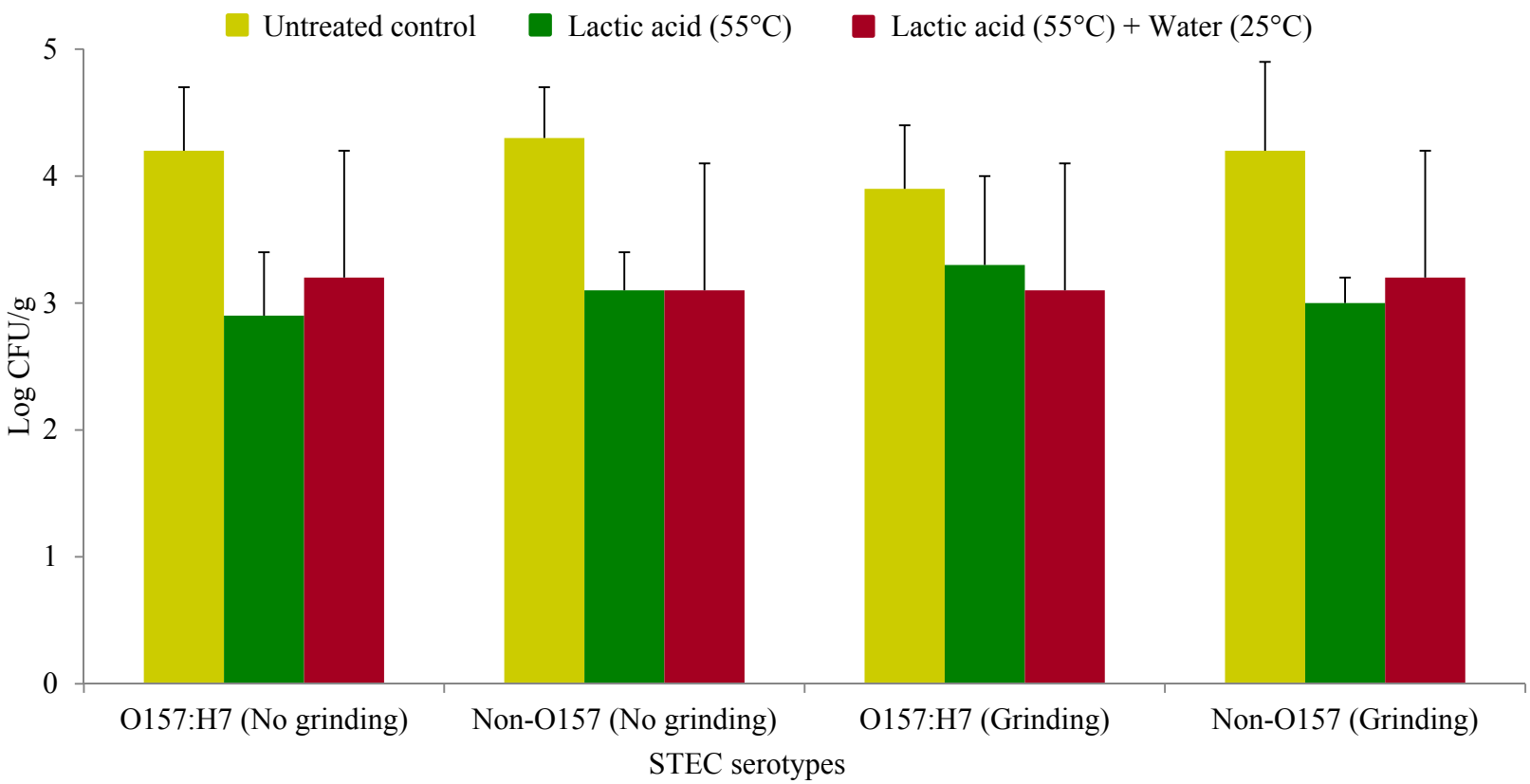

Figure 19: Total bacterial populations (log CFU/g), recovered on tryptic soy agar, of beef trimmings ("no grinding") and in subsequently ground product ("grinding") (inoculated with rifampicin-resistant *E. coli* O157:H7 or non-O157 Shiga toxin-producing *E. coli* [STEC]); the trimmings were either left untreated or were decontaminated with lactic acid (5%, 55°C) without or with a water (25°C) rinse

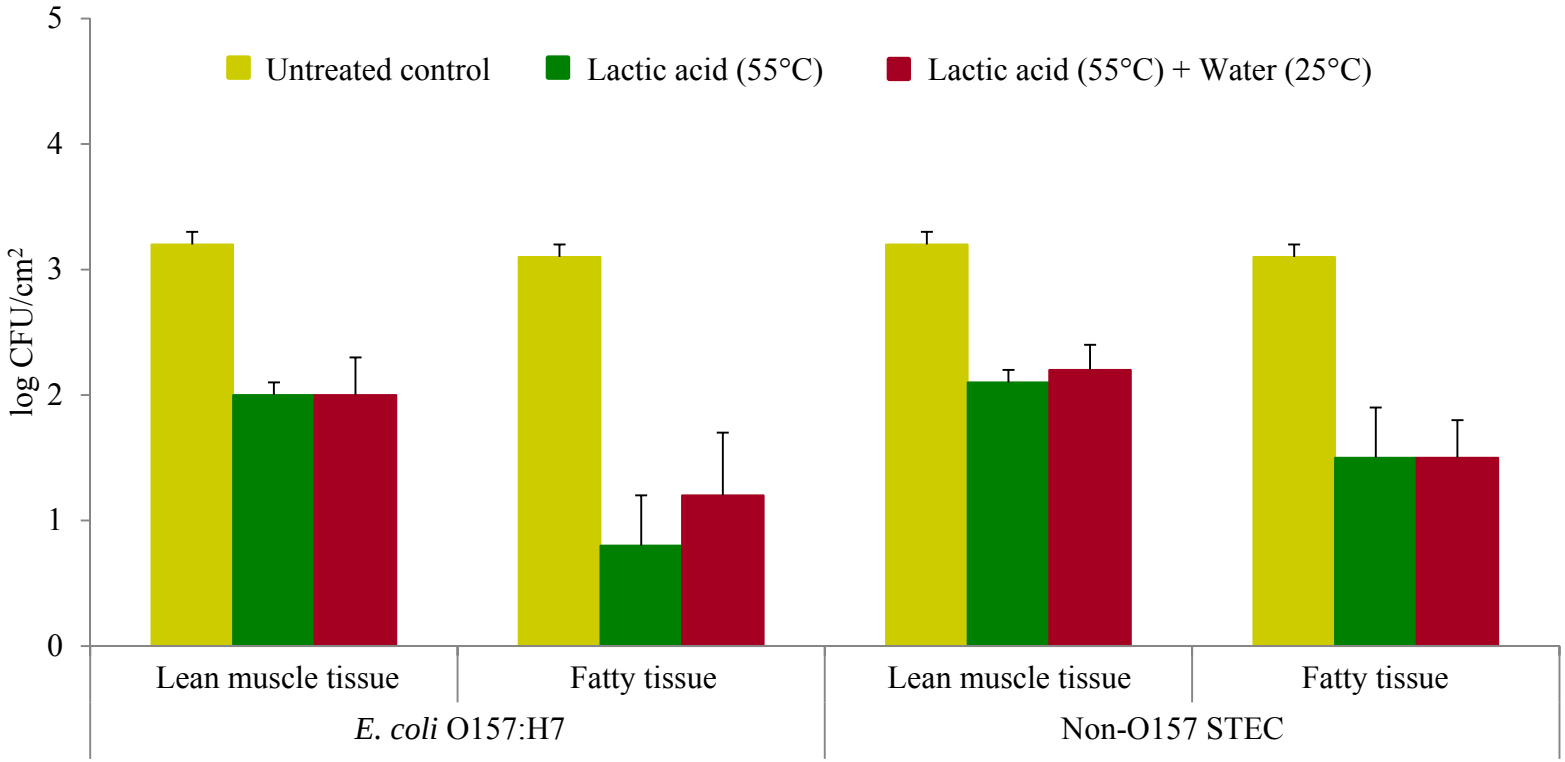

Figure 20. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* (STEC) inocula (recovered on tryptic soy agar + rifampicin) on beef trimmings comprised of lean muscle or fatty tissue before and after decontamination with lactic acid (5%, 55°C) without or with a water (25°C) rinse

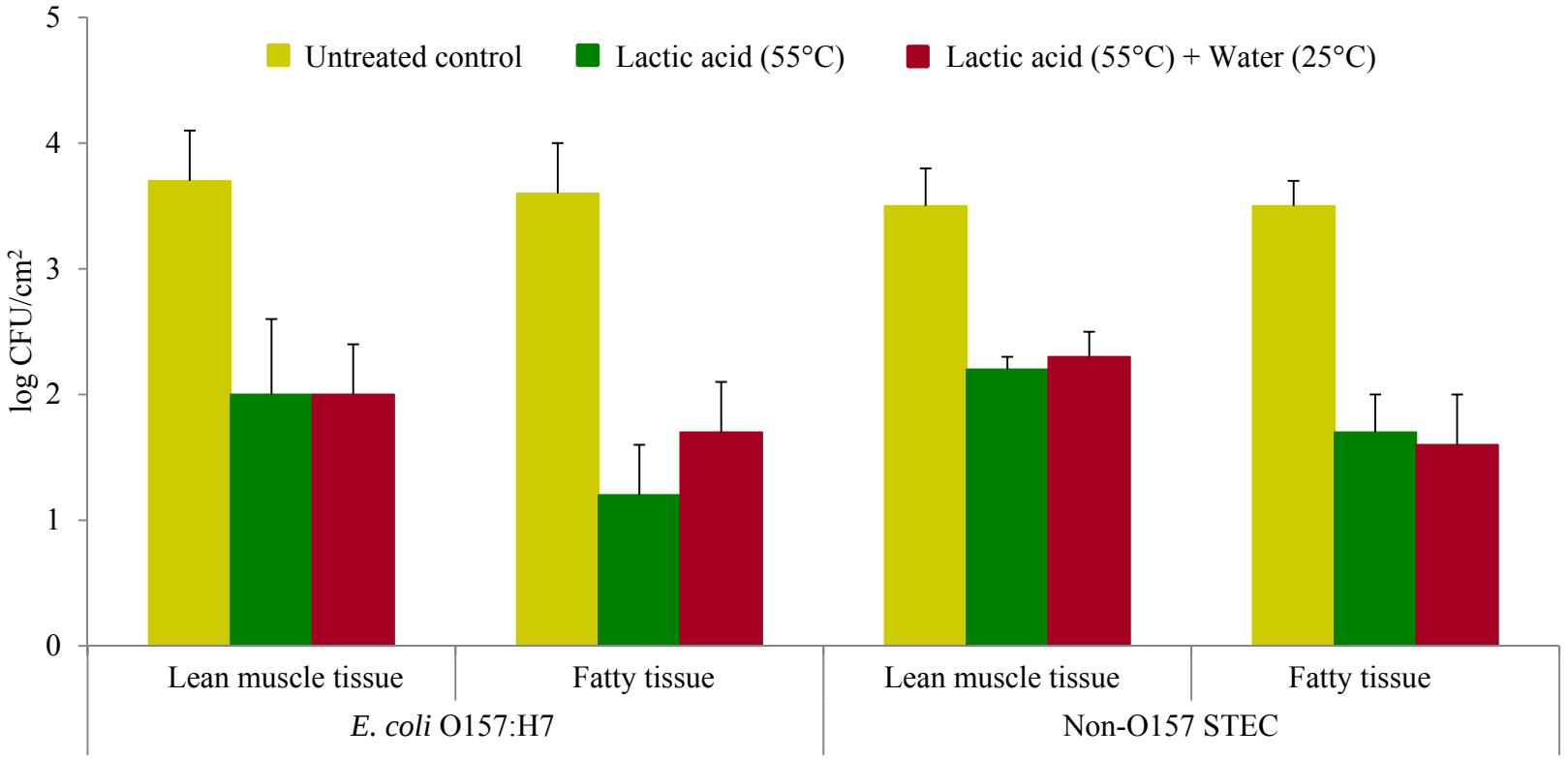

Figure 21. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings comprised of lean muscle or fatty tissue (inoculated with rifampicin-resistant *E. coli* O157:H7 or non-O157 Shiga toxin-producing *E. coli* [STEC]) before and after decontamination with lactic acid (5%, 55°C) without or with a water (25°C) rinse

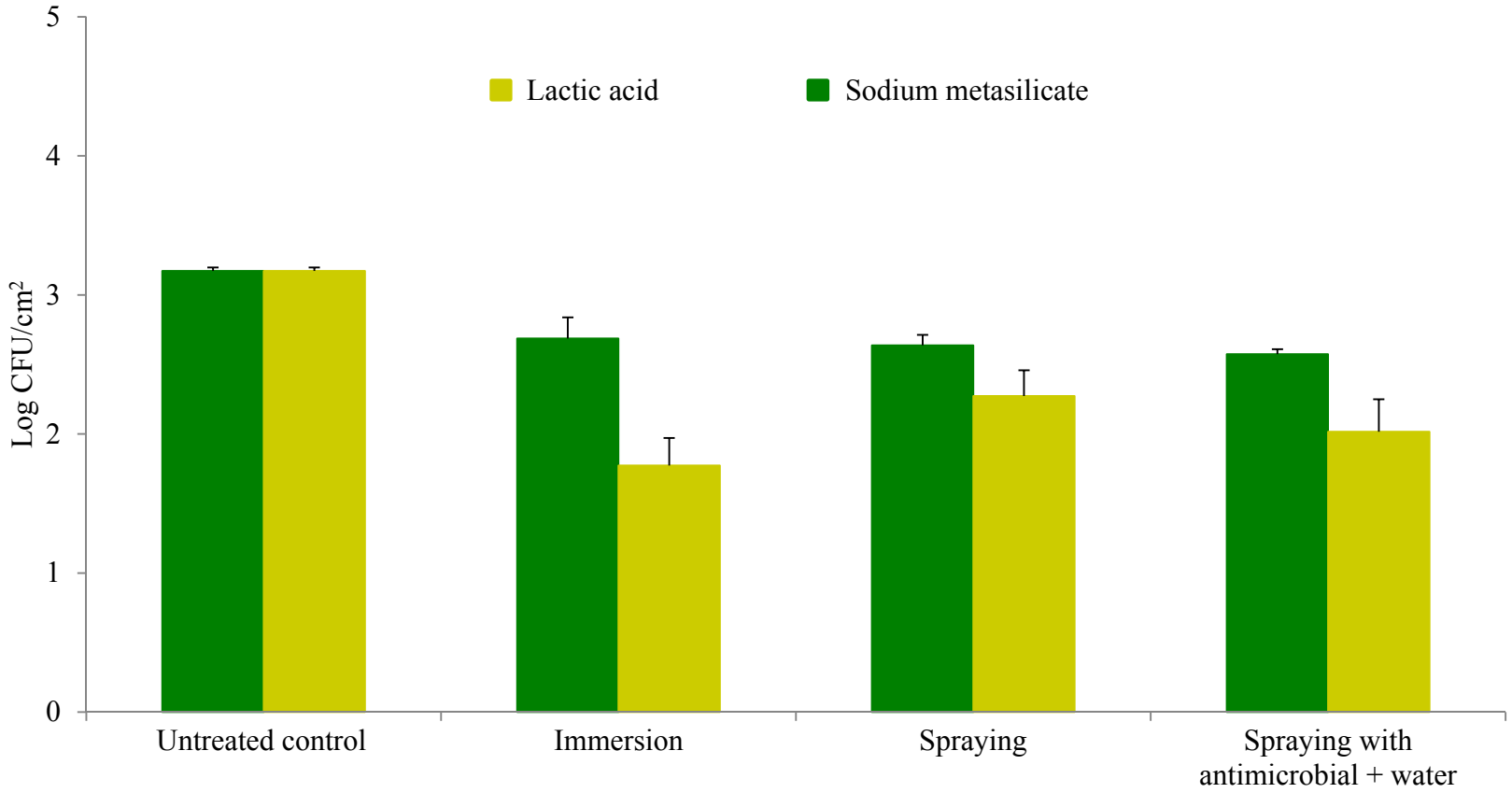

Figure 22. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on tryptic soy agar + rifampicin) on beef trimmings left untreated or treated with lactic acid (5%, 25°C) or sodium metasilicate (4%) by immersion, spraying, or spraying with the antimicrobial followed by a water (25°C) rinse

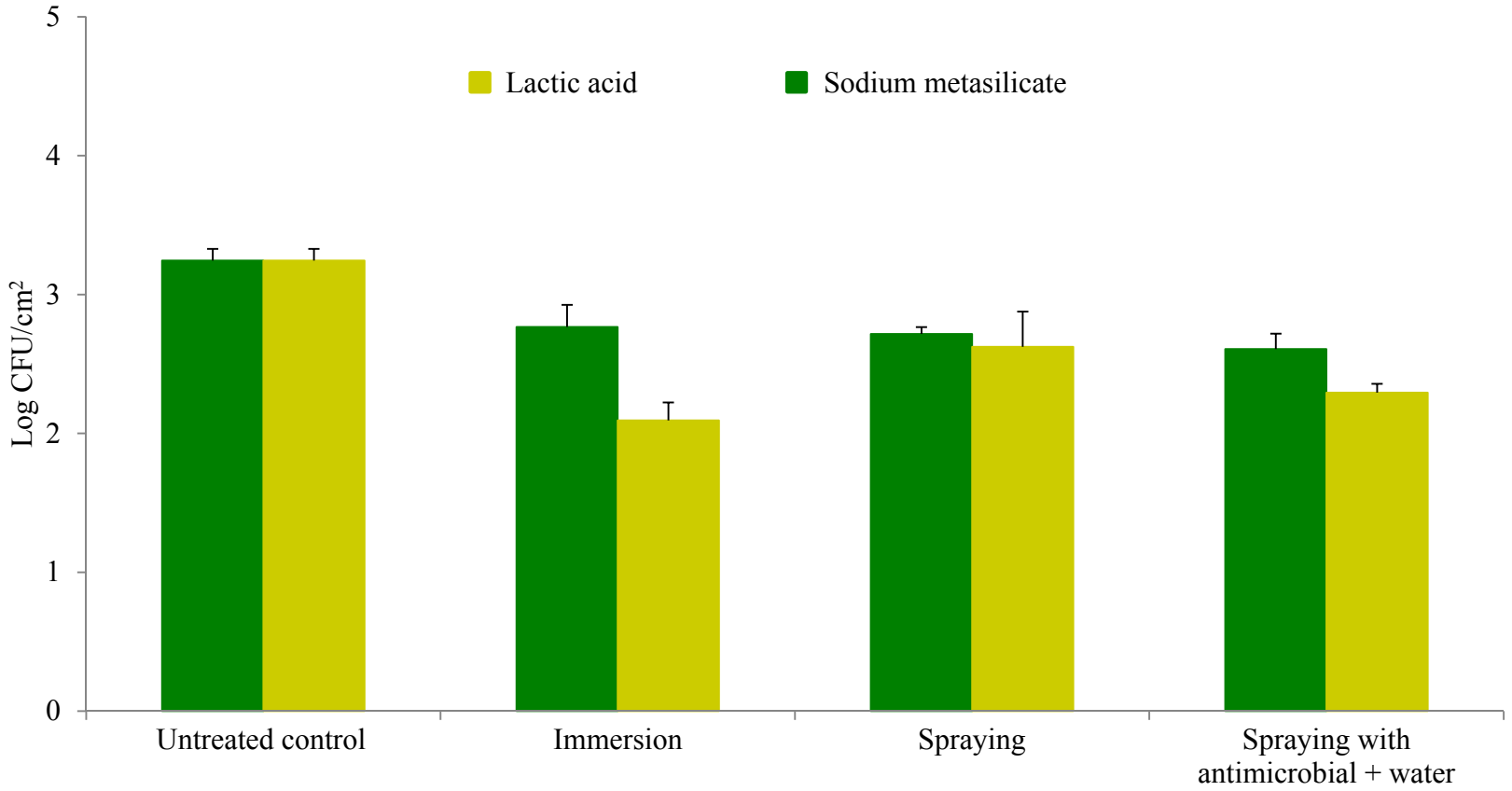

Figure 23. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7) left untreated or treated with lactic acid (5%, 25°C) or sodium metasilicate (4%) by immersion, spraying, or spraying with the antimicrobial followed by a water (25°C) rinse

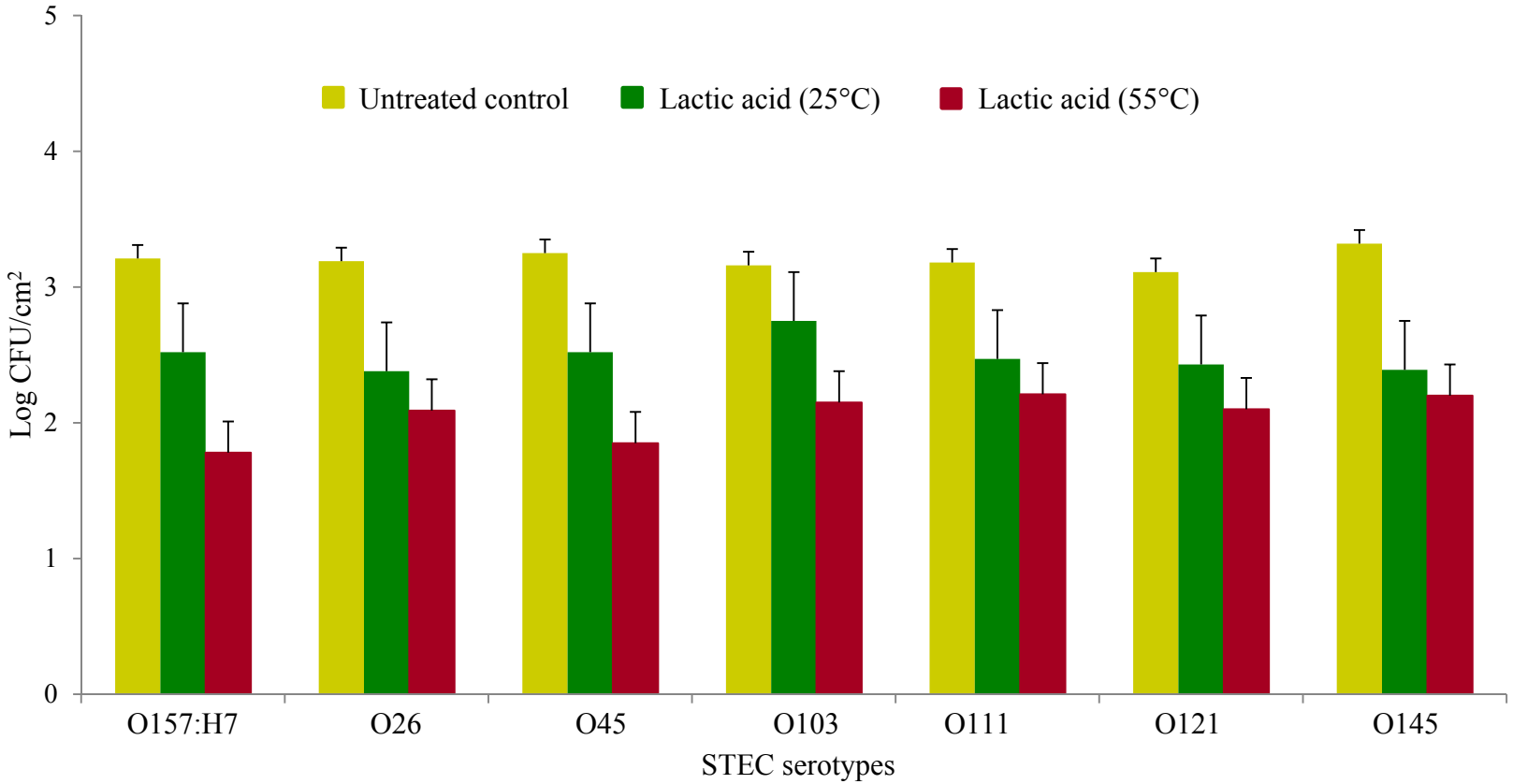

Figure 24. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 and six non-O157 STEC serotypes (recovered on tryptic soy agar + rifampicin) on beef trimmings before and after decontamination with lactic acid (5%, 25 or 55°C)

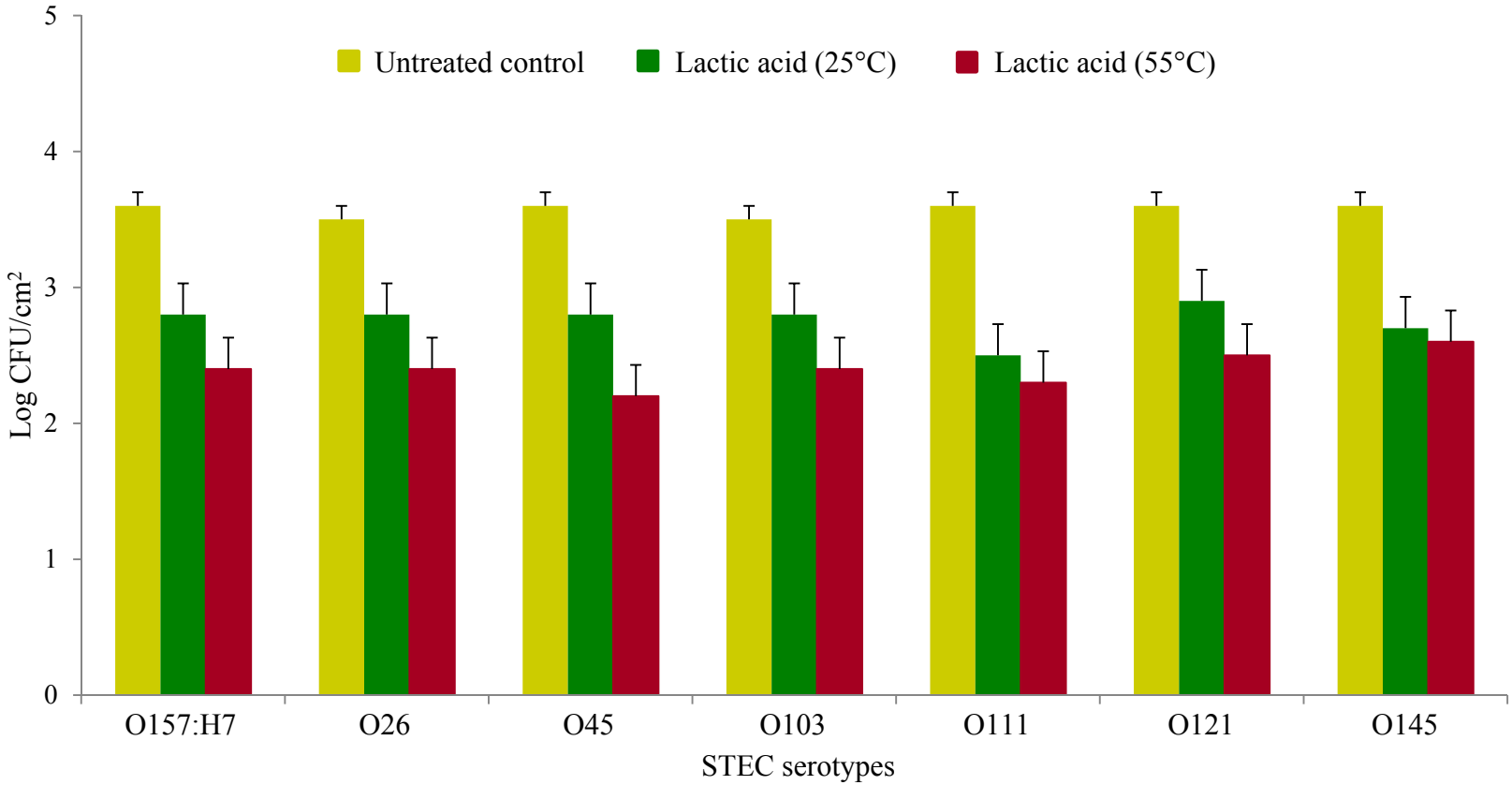

Figure 25. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or six non-O157 STEC serotypes) before and after decontamination with lactic acid (5%, 25 or 55°C)

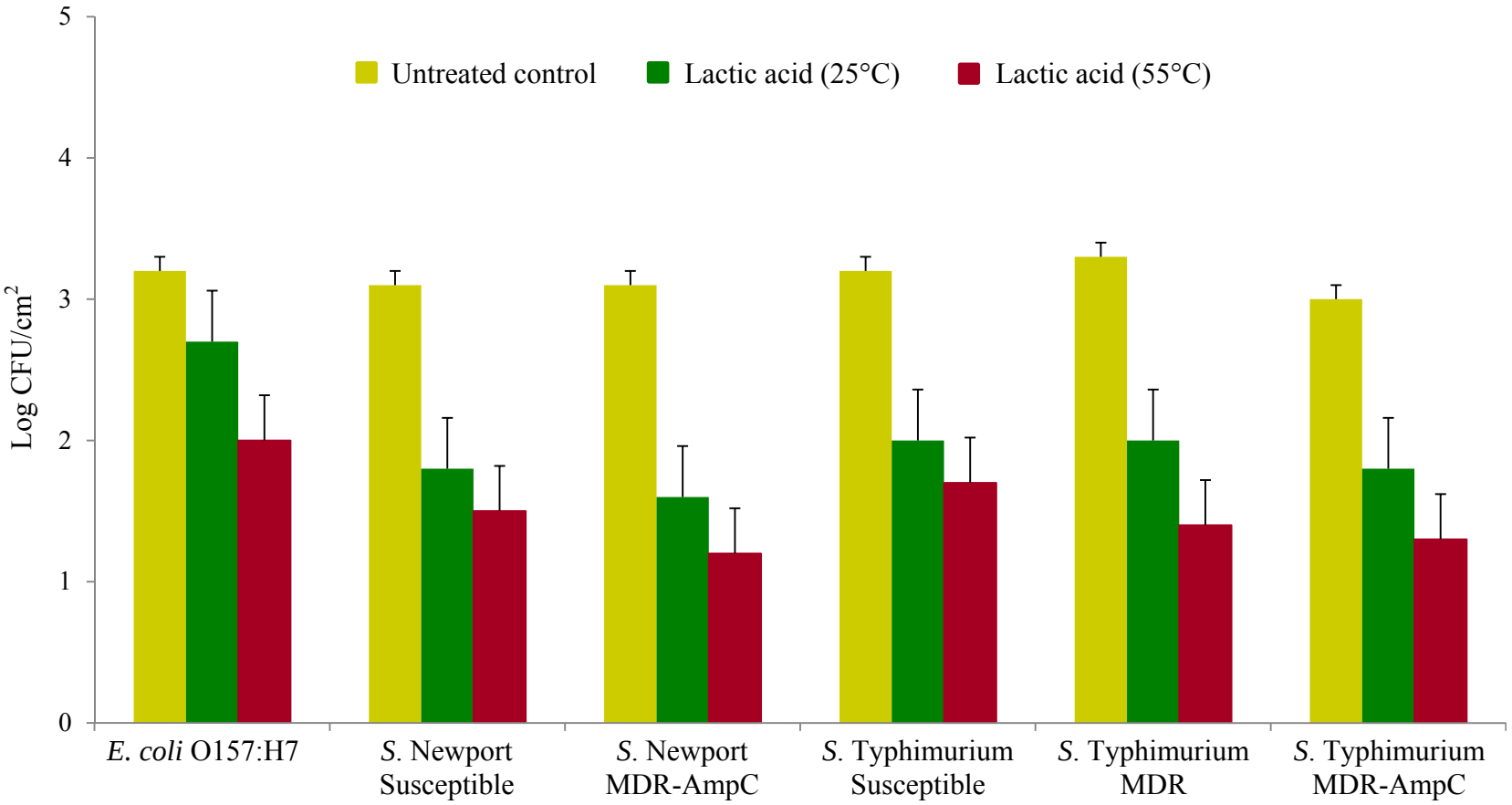

Figure 26. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on tryptic soy agar + rifampicin) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with lactic acid (5%, 25 or 55°C)

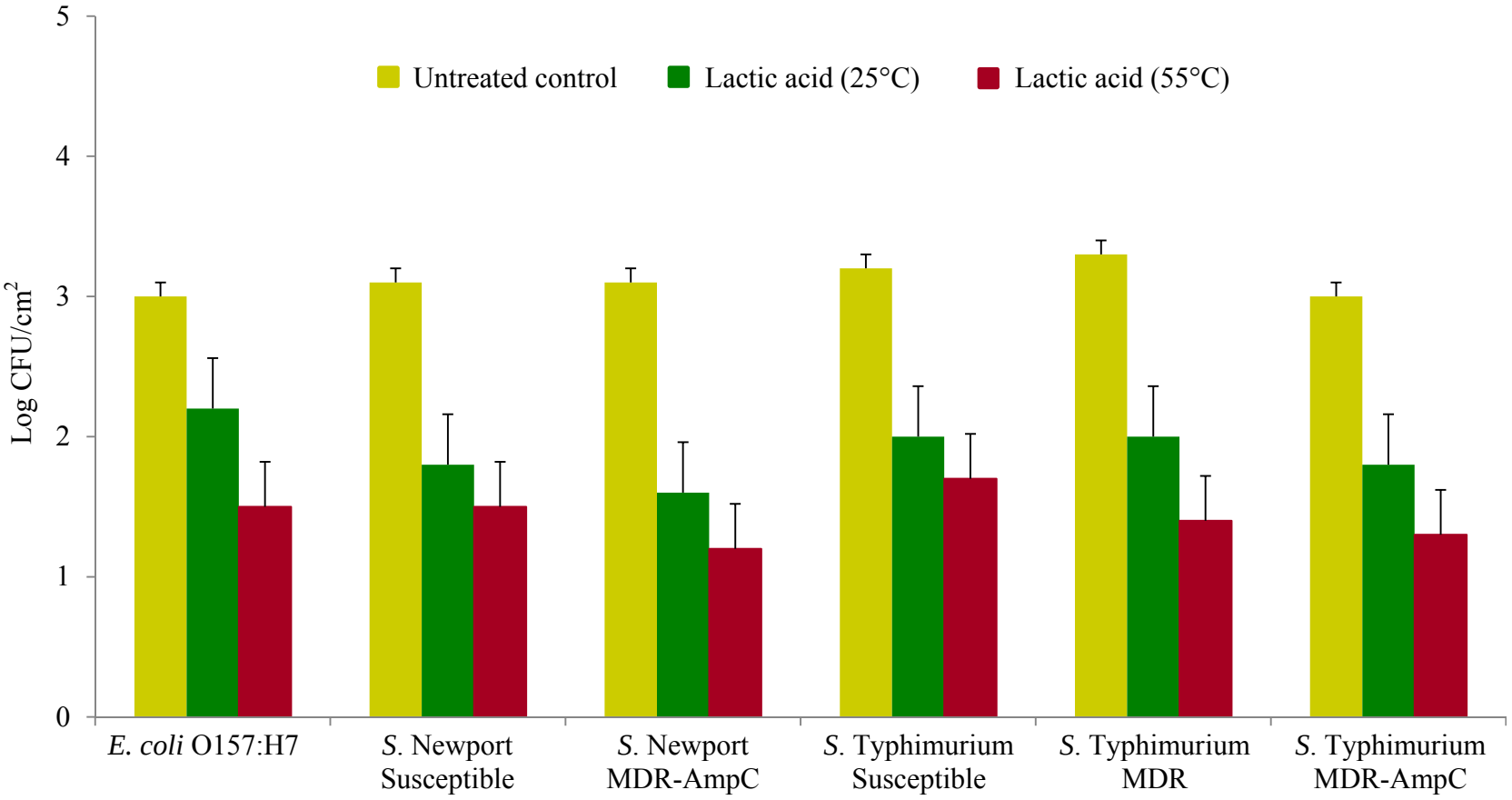

Figure 27. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on modified sorbitol MacConkey agar) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with lactic acid (5%, 25 or 55°C)

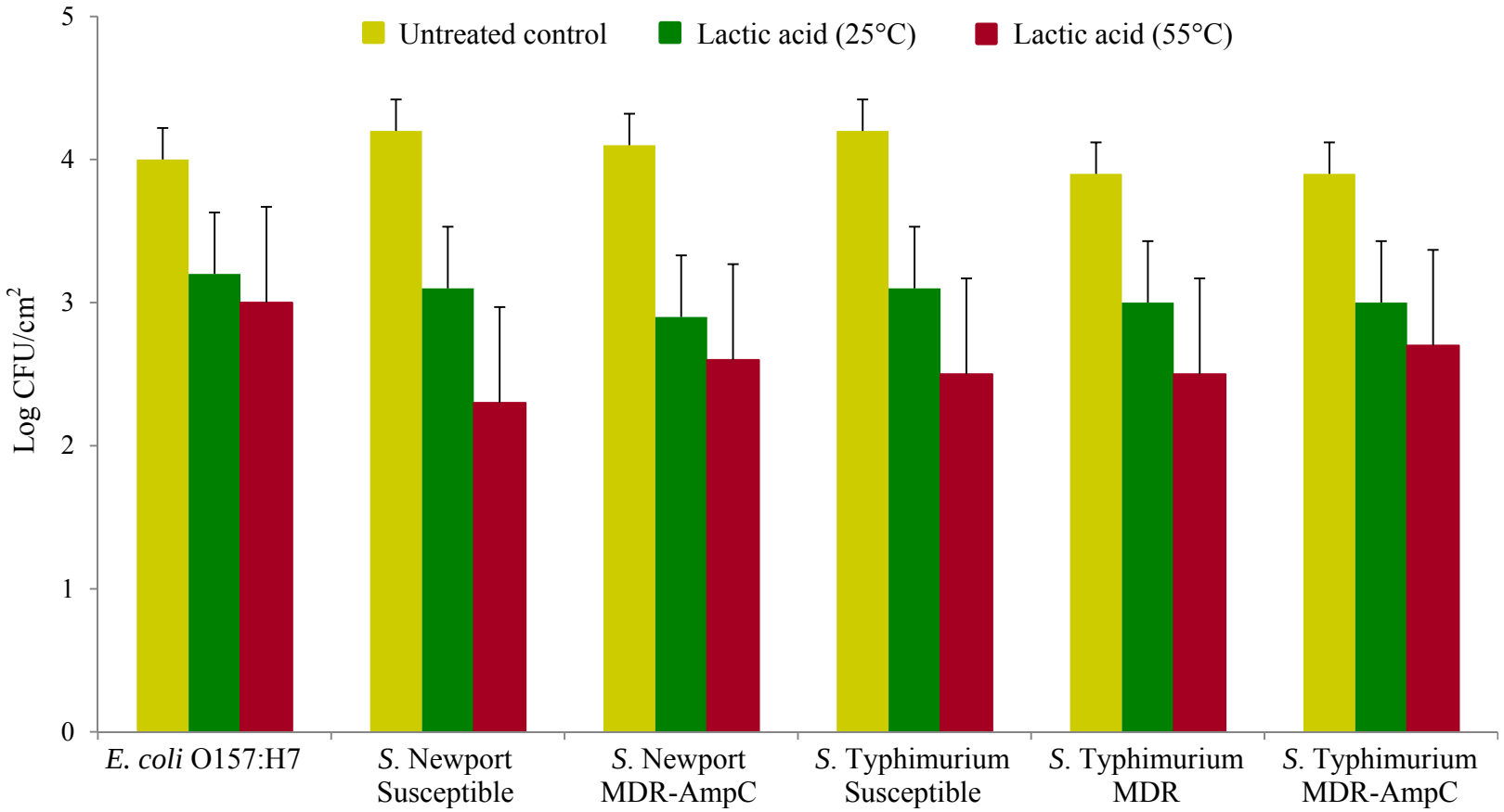

Figure 28. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or antibiotic susceptible or resistant [MDR and/or MDR-AmpC] *S.* Newport and *S.* Typhimurium) before and after decontamination with lactic acid (5%, 25 or 55°C)

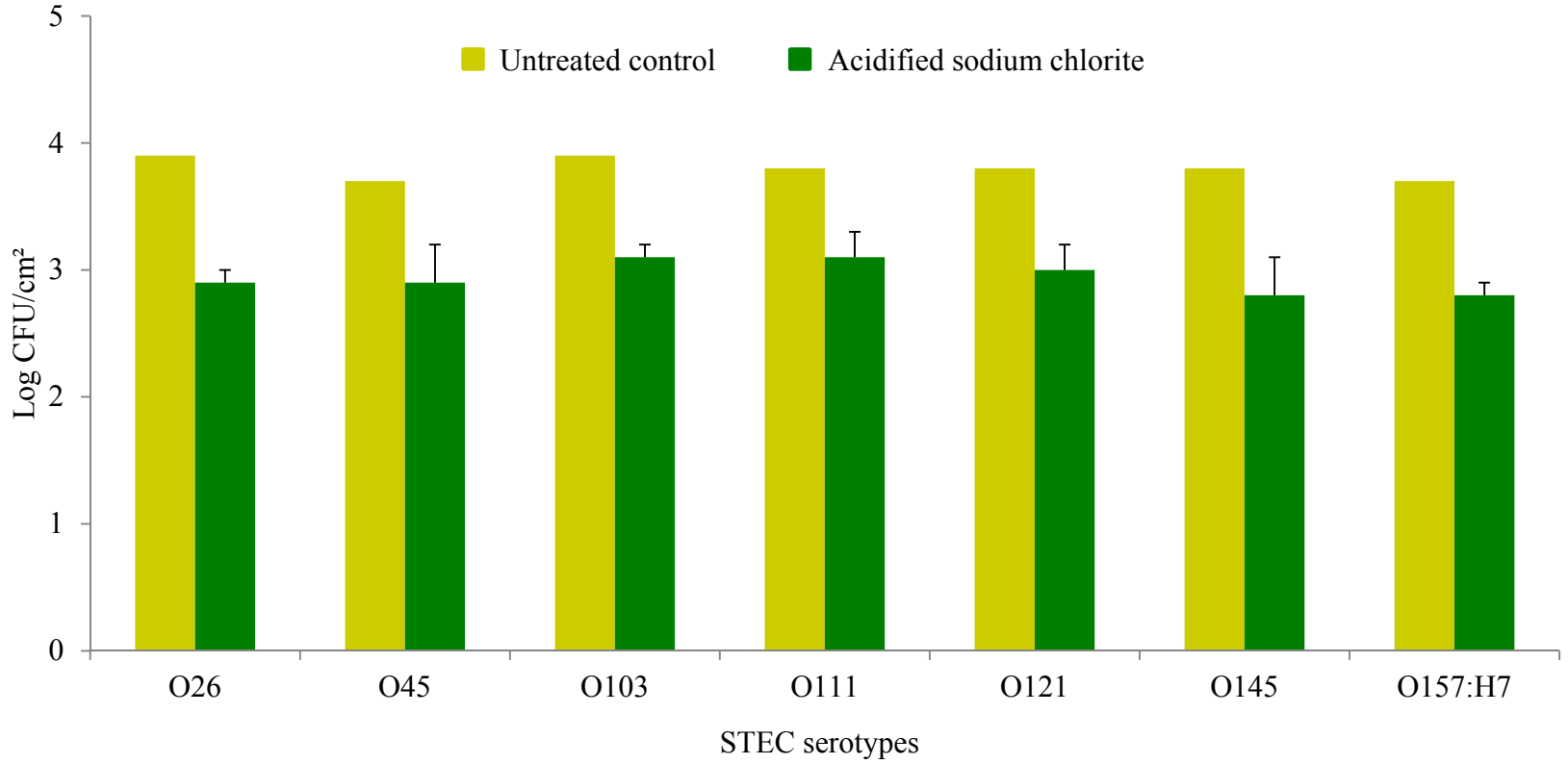

Figure 29. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 and six non-O157 STEC serotypes (recovered on tryptic soy agar + rifampicin) on beef trimmings before and after decontamination with acidified sodium chlorite (0.1%)

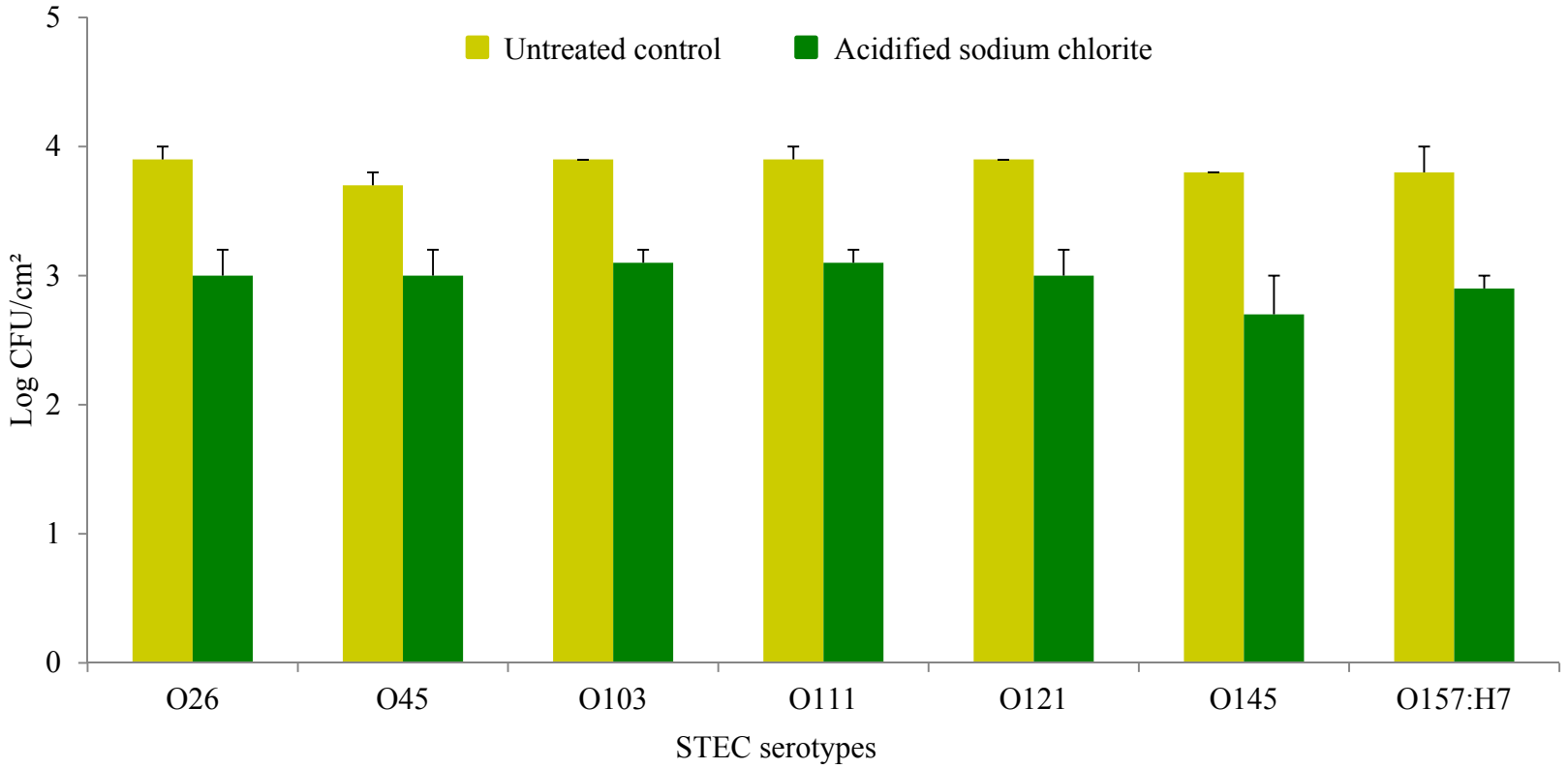

Figure 30. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or six non-O157 STEC serotypes) before and after decontamination with acidified sodium chlorite (0.1%)

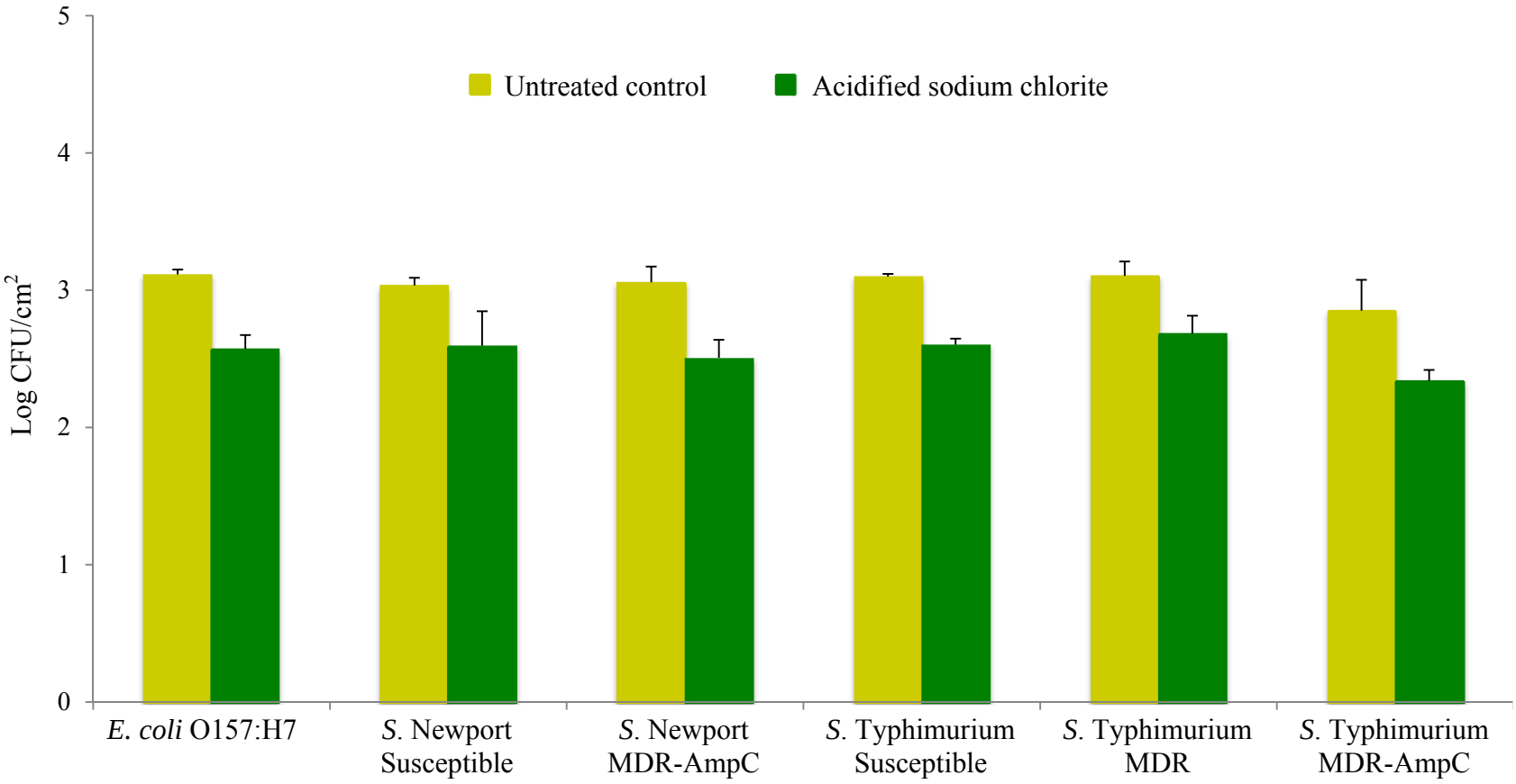

Figure 31. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on tryptic soy agar + rifampicin) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with acidified sodium chlorite (0.1%)

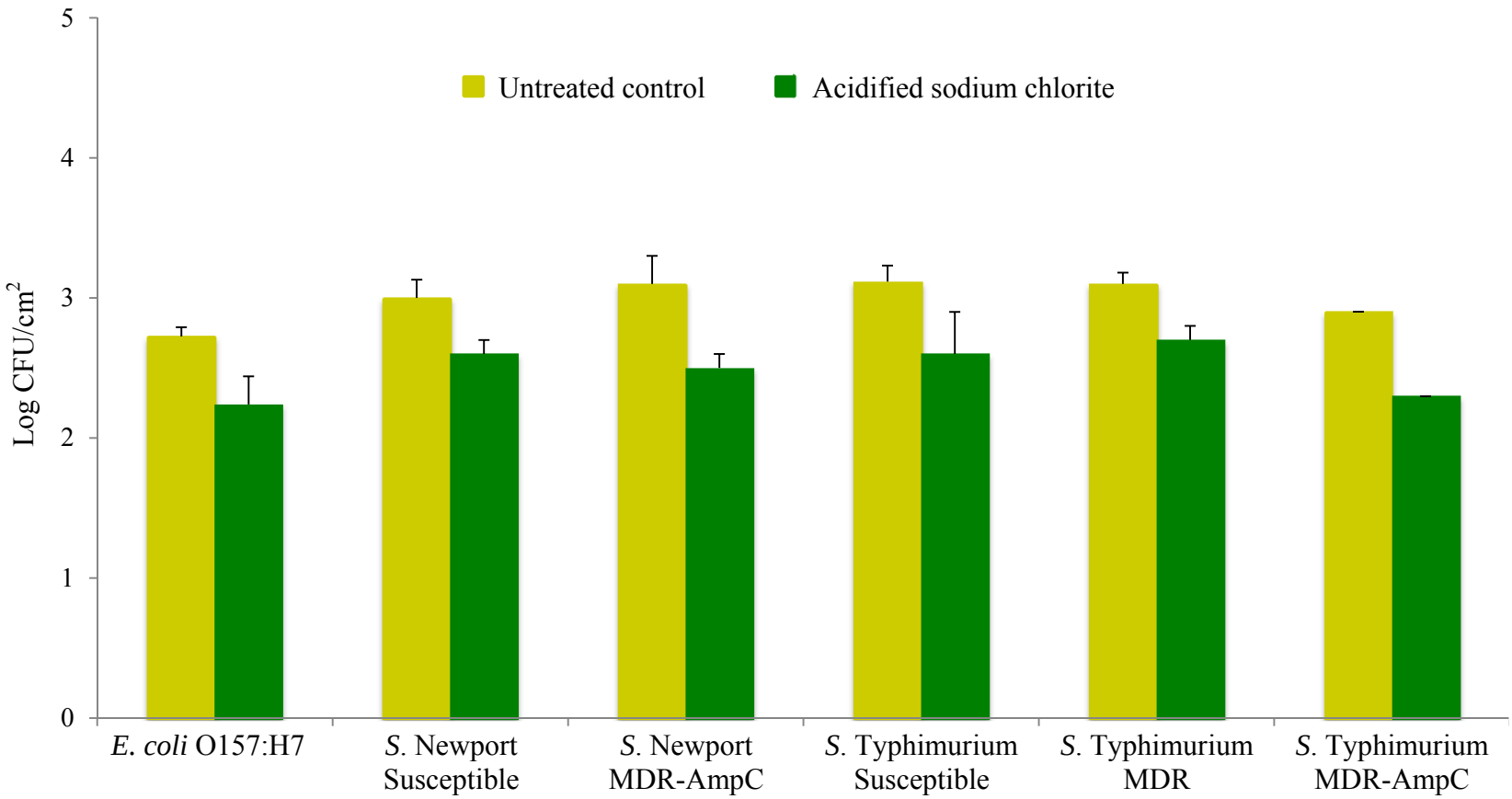

Figure 32. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on modified sorbitol MacConkey agar) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with acidified sodium chlorite (0.1%)

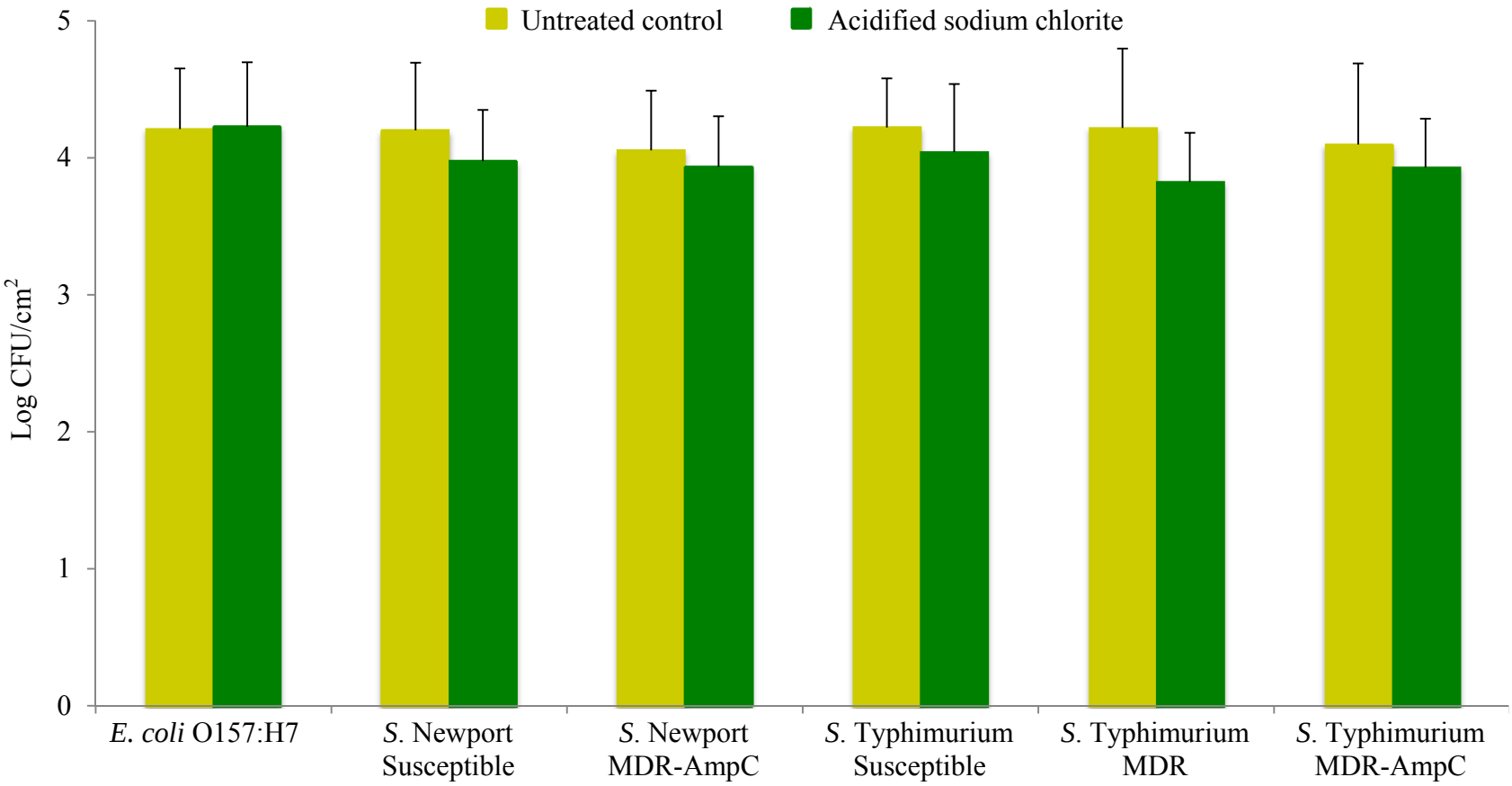

Figure 33. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or antibiotic susceptible or resistant [MDR and/or MDR-AmpC] *S.* Newport and *S.* Typhimurium) before and after decontamination with acidified sodium chlorite (0.1%)

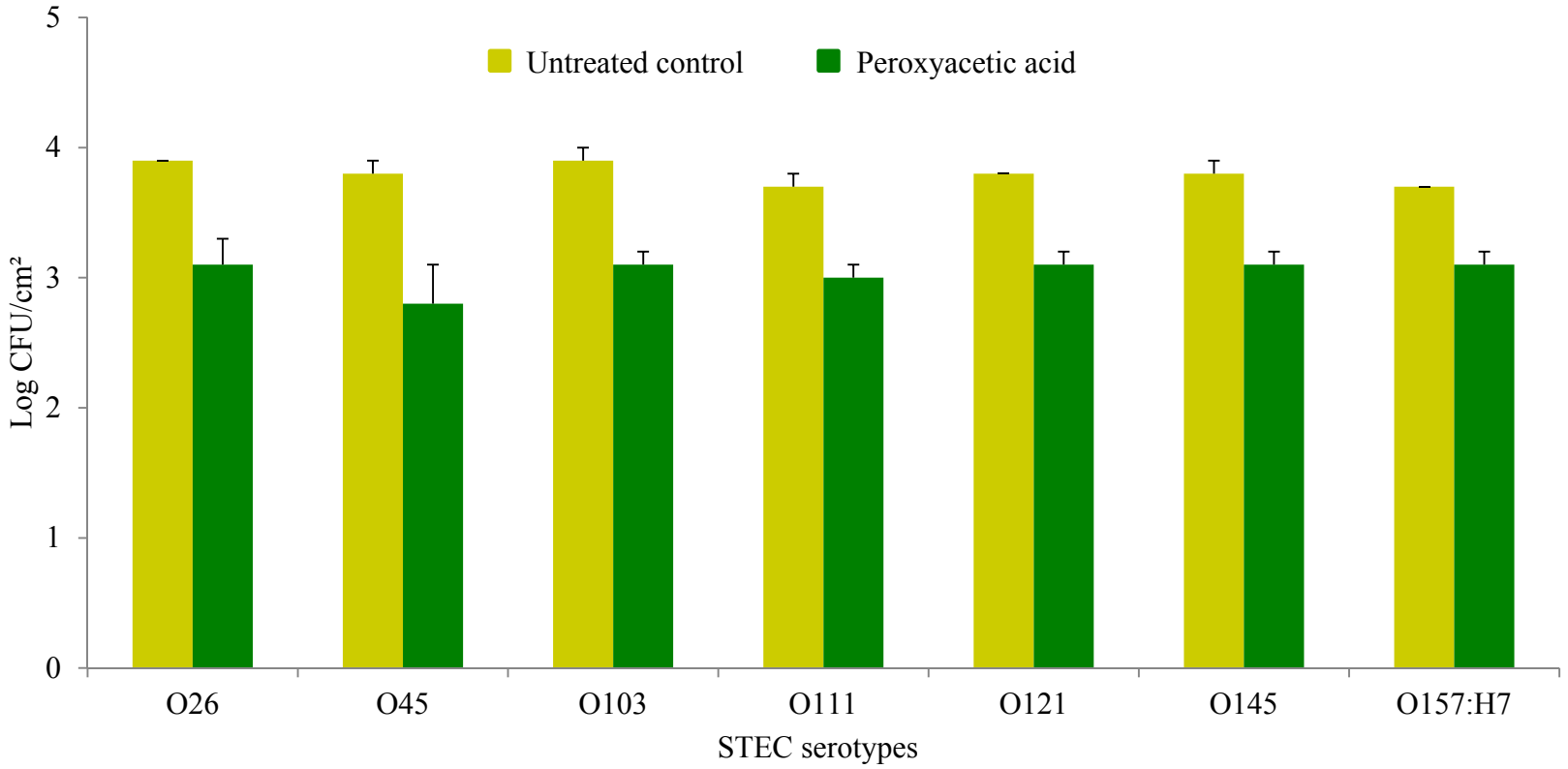

Figure 34. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 and six non-O157 STEC serotypes (recovered on tryptic soy agar + rifampicin) on beef trimmings before and after decontamination with peroxyacetic acid (0.02%)

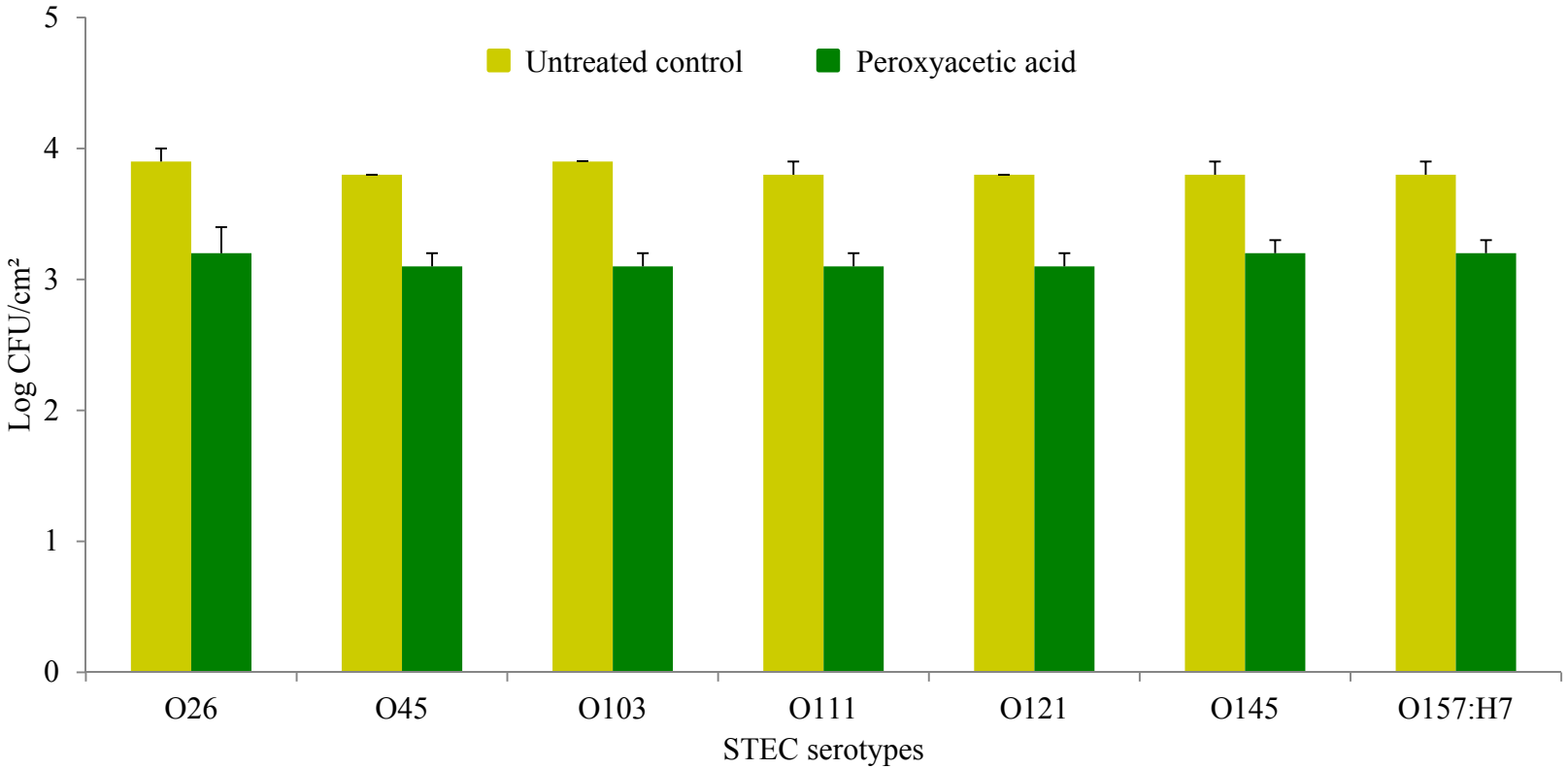

Figure 35. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or six non-O157 STEC serotypes) before and after decontamination with peroxyacetic acid (0.02%)

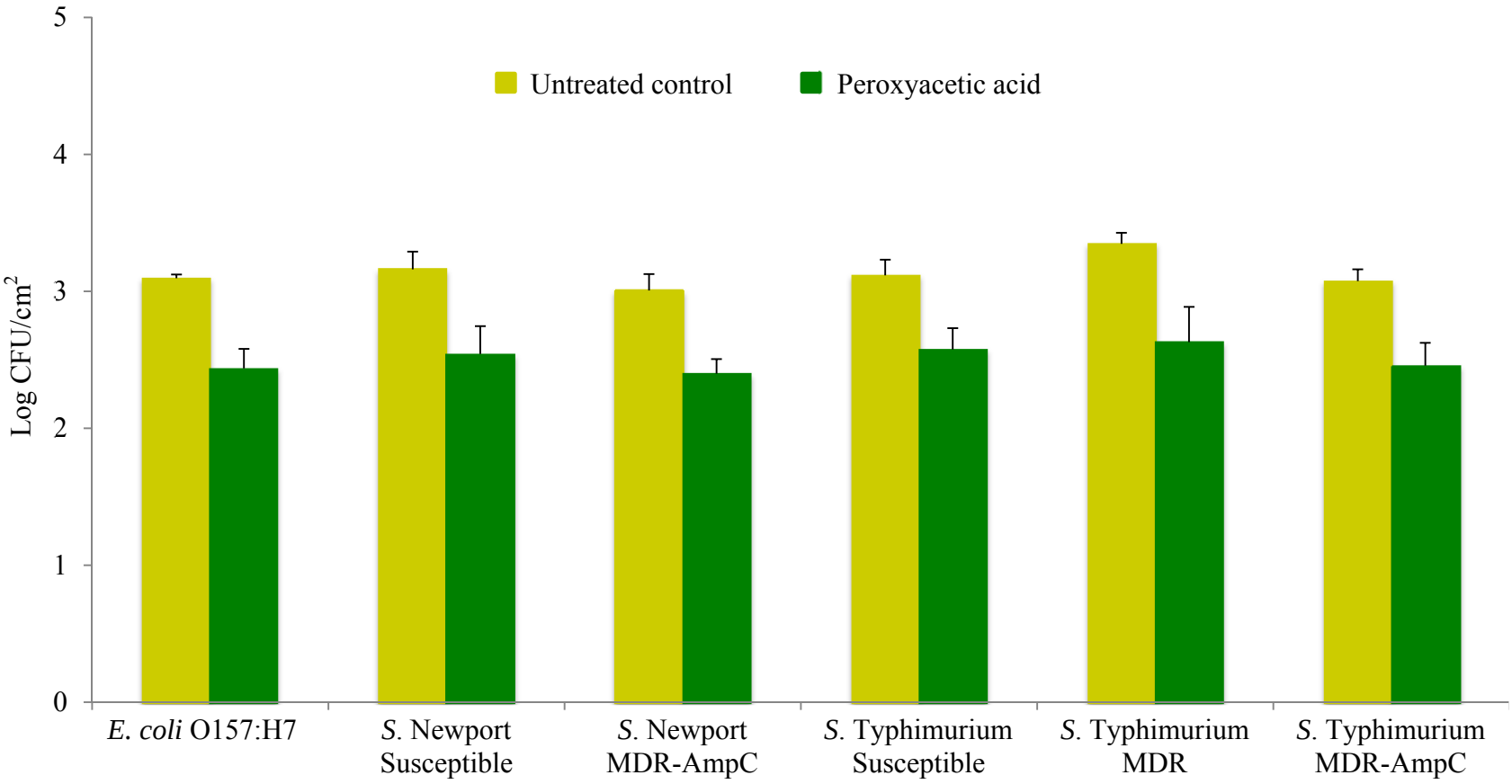

Figure 36. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on tryptic soy agar + rifampicin) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with peroxyacetic acid (0.02%)

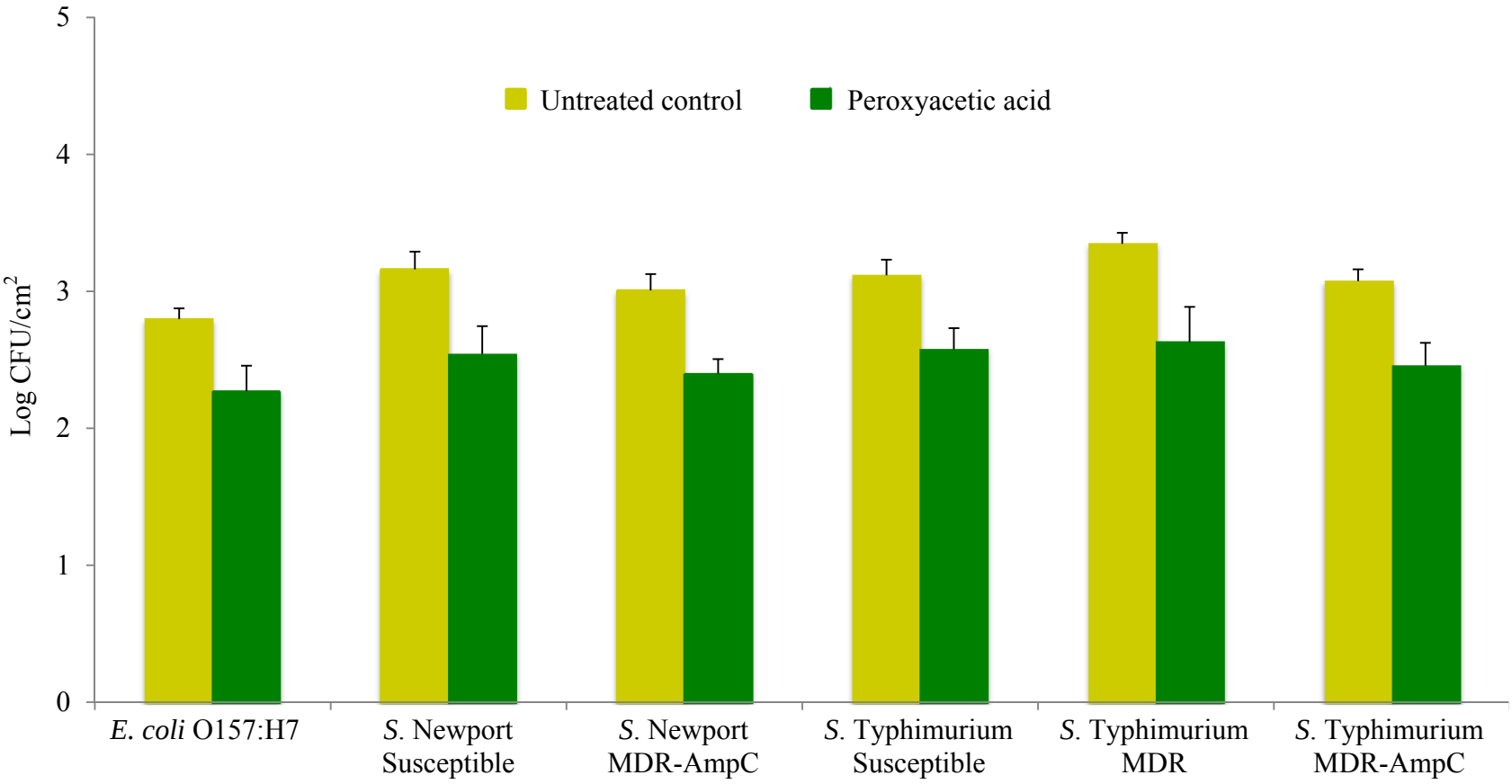

Figure 37. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on modified sorbitol MacConkey agar) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with peroxyacetic acid (0.02%)

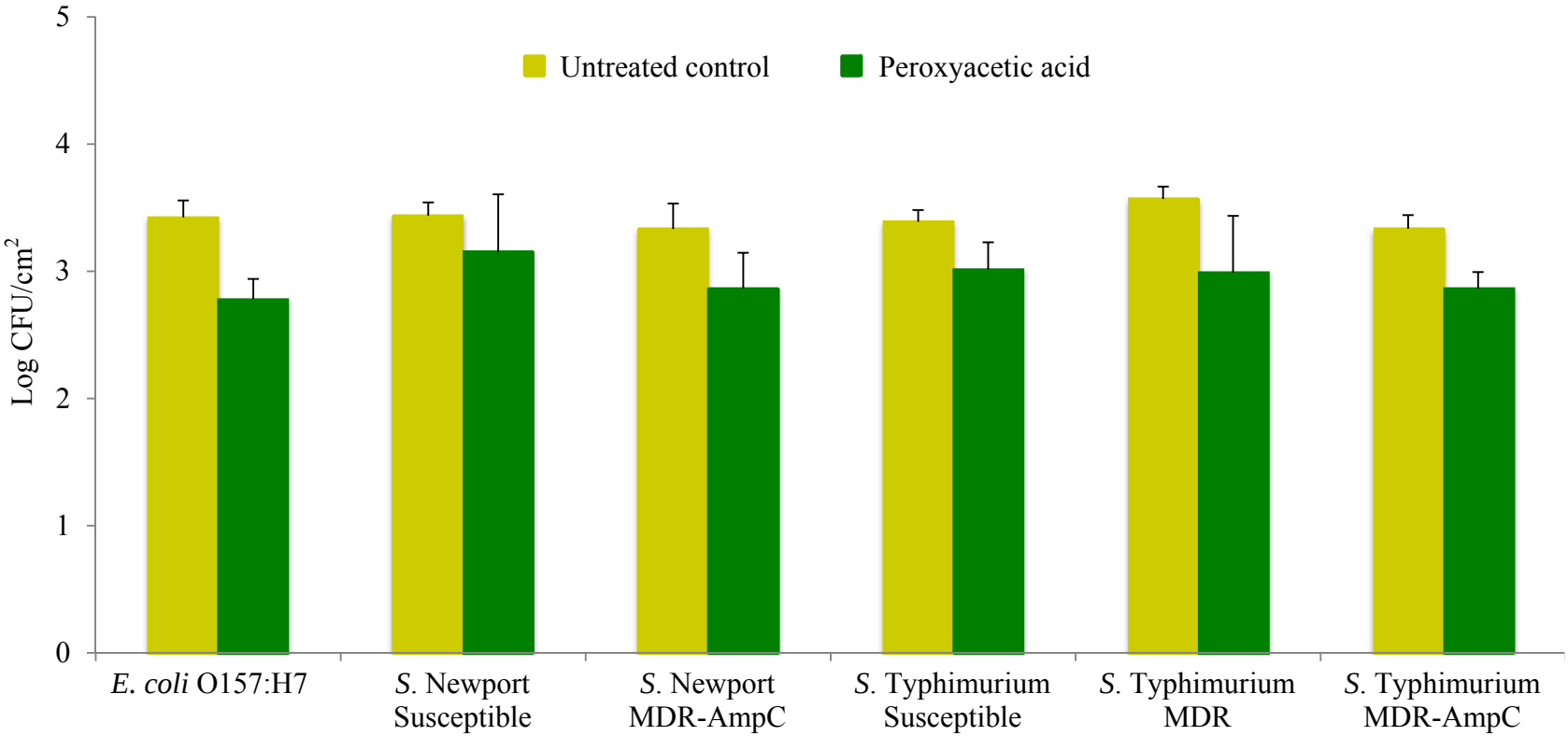

Figure 38. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or antibiotic susceptible or resistant [MDR and/or MDR-AmpC] *S.* Newport and *S.* Typhimurium) before and after decontamination with peroxyacetic acid (0.02%)

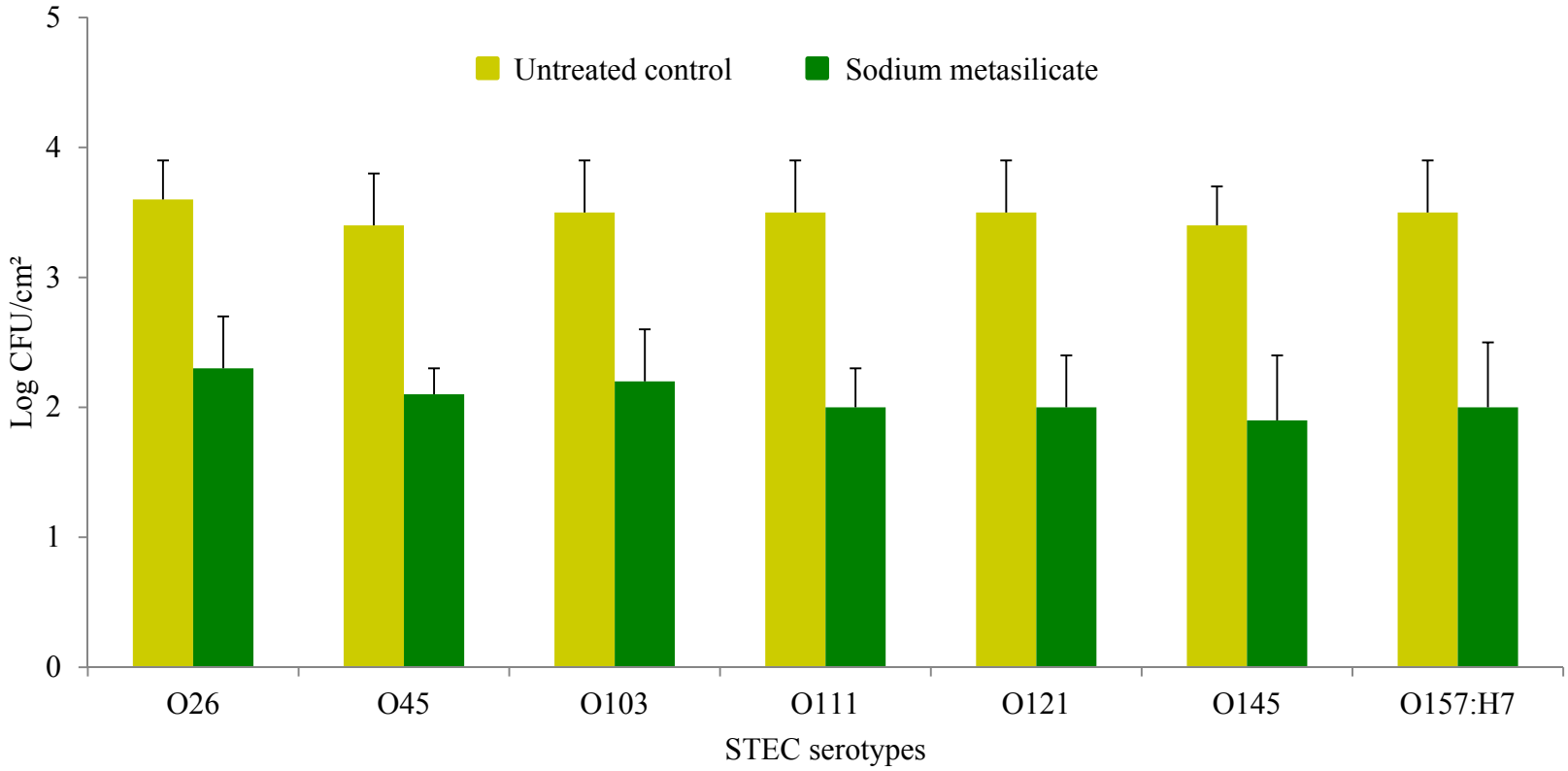

Figure 39. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 and six non-O157 STEC serotypes (recovered on tryptic soy agar + rifampicin) on beef trimmings before and after decontamination with sodium metasilicate (4%)

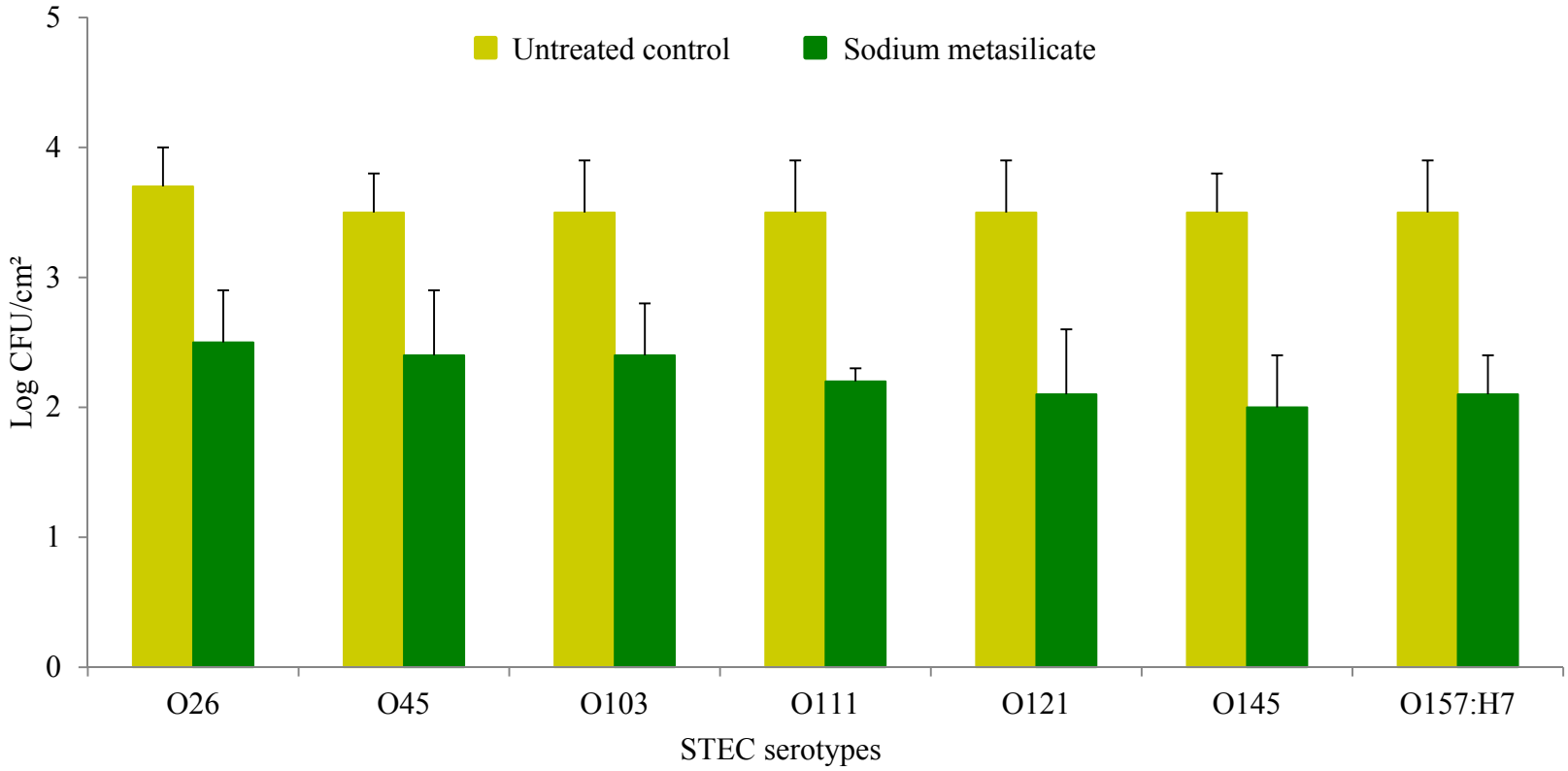

Figure 40. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or six non-O157 STEC serotypes) before and after decontamination with sodium metasilicate (4%)

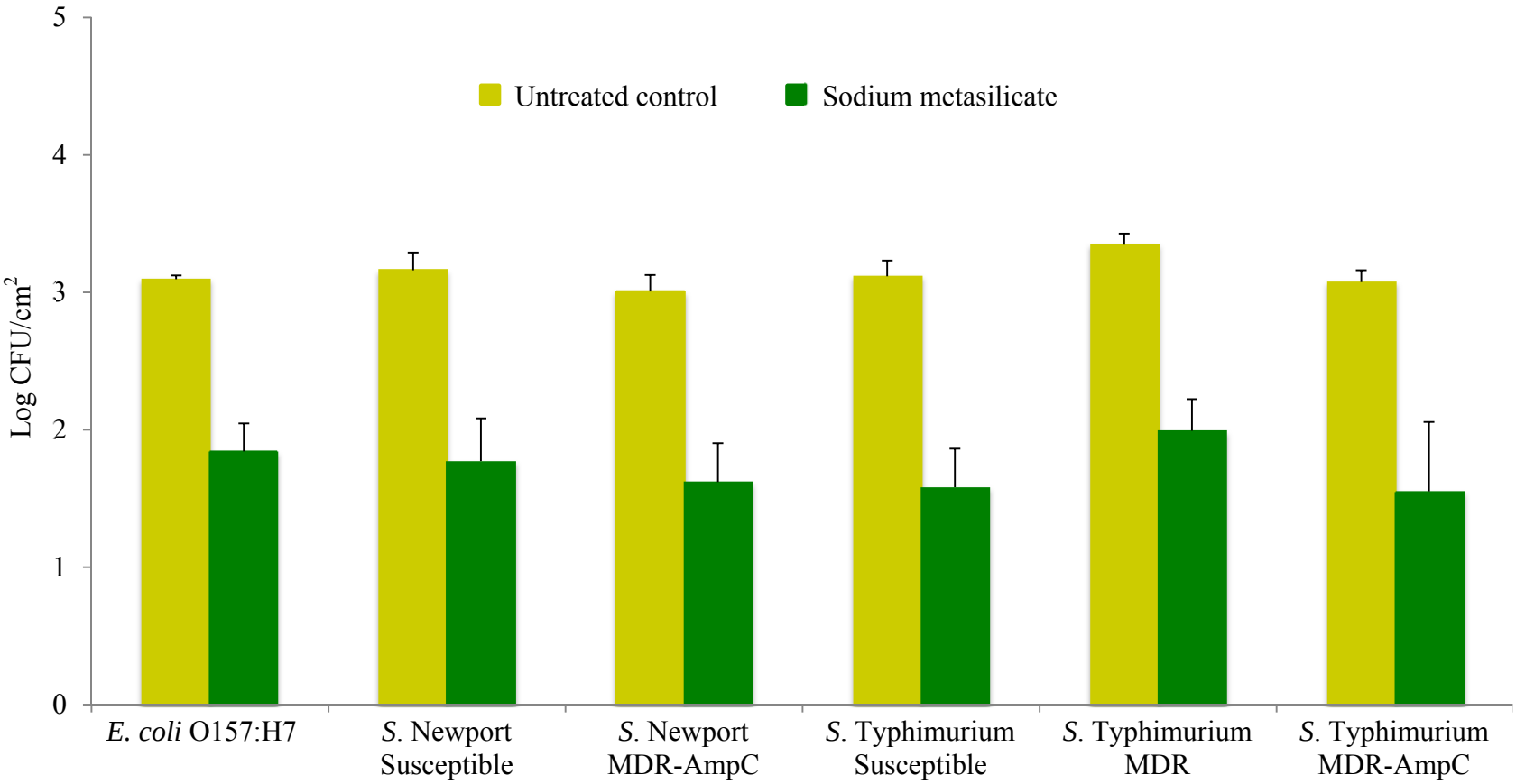

Figure 41. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on tryptic soy agar + rifampicin) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with sodium metasilicate (4%)

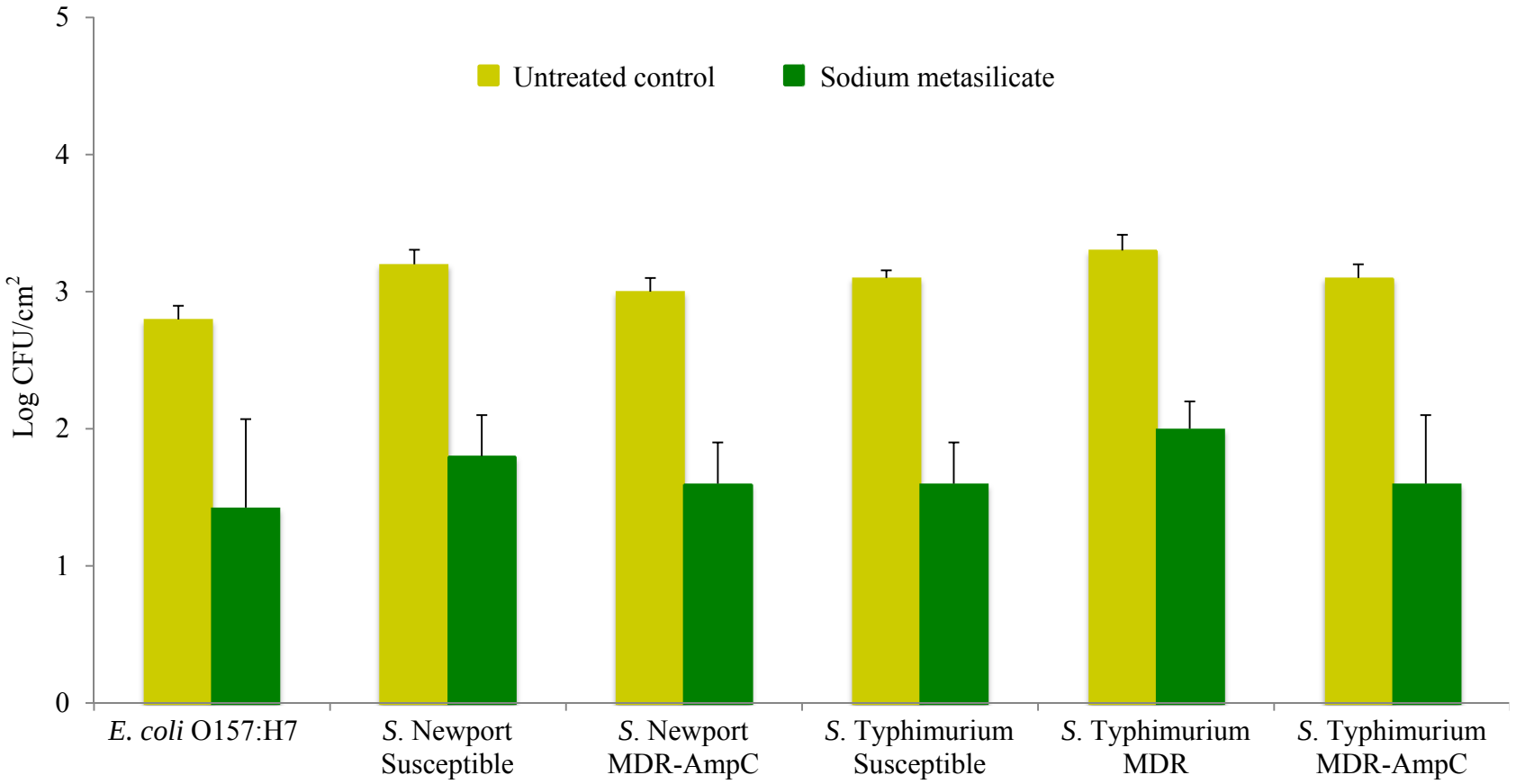

Figure 42. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on modified sorbitol MacConkey agar) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with sodium metasilicate (4%)

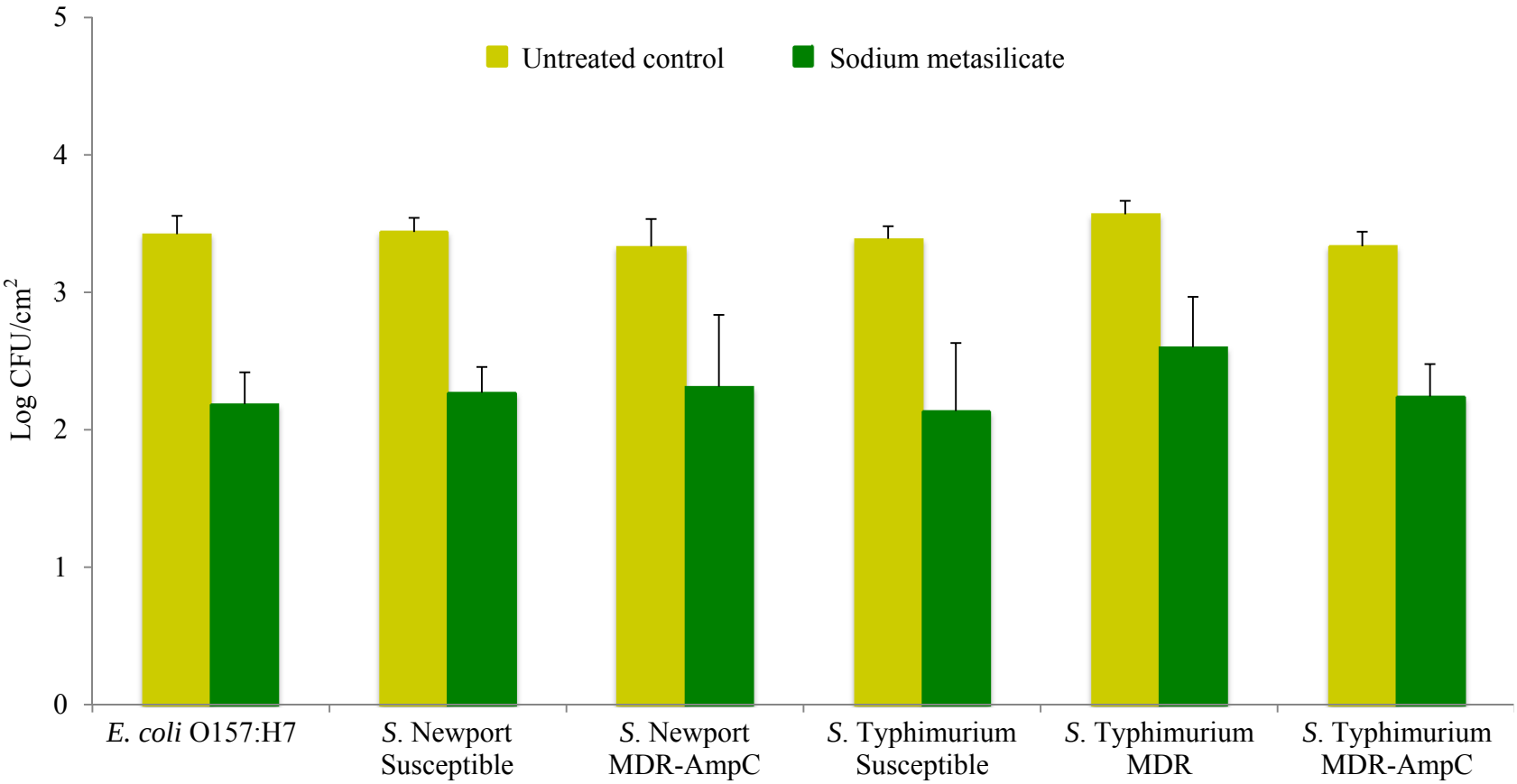

Figure 43. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or antibiotic susceptible or resistant [MDR and/or MDR-AmpC] *S.* Newport and *S.* Typhimurium) before and after decontamination with sodium metasilicate (4%)

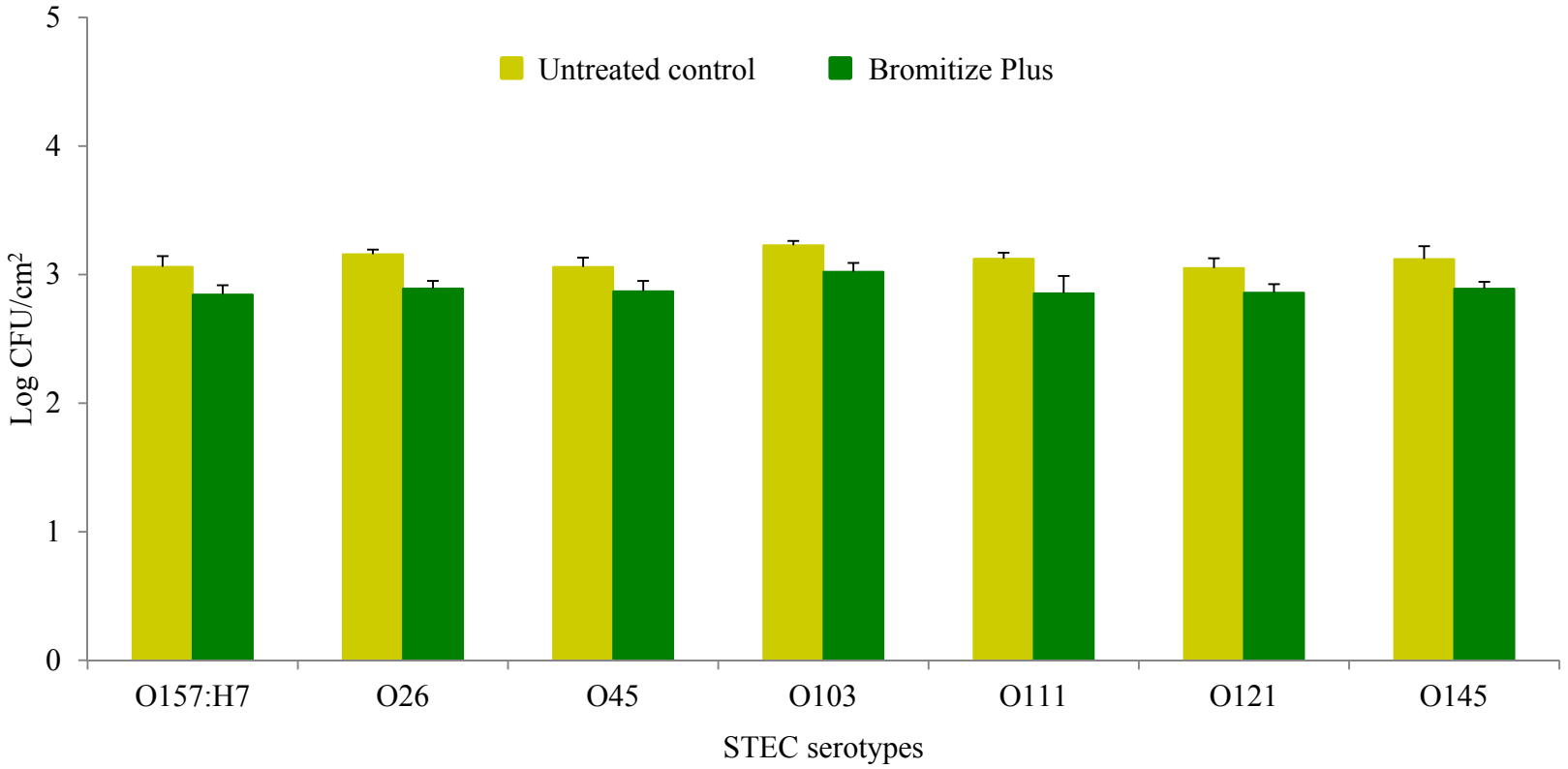

Figure 44. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 and six non-O157 STEC serotypes (recovered on tryptic soy agar + rifampicin) on beef trimmings before and after decontamination with Bromitize Plus (225 ppm active bromine)

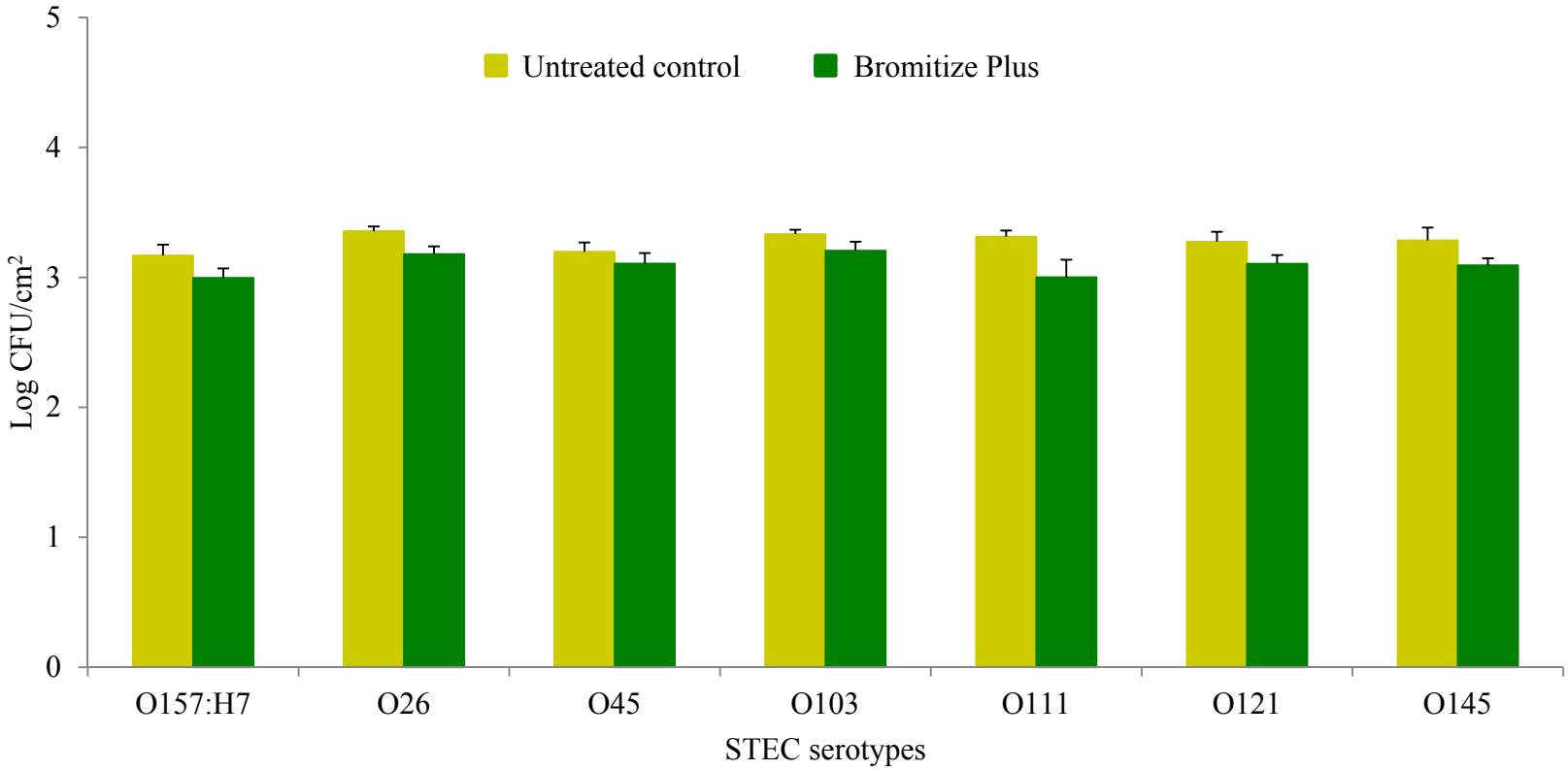

Figure 45. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or six non-O157 STEC serotypes) before and after decontamination with Bromitize Plus (225 ppm active bromine)

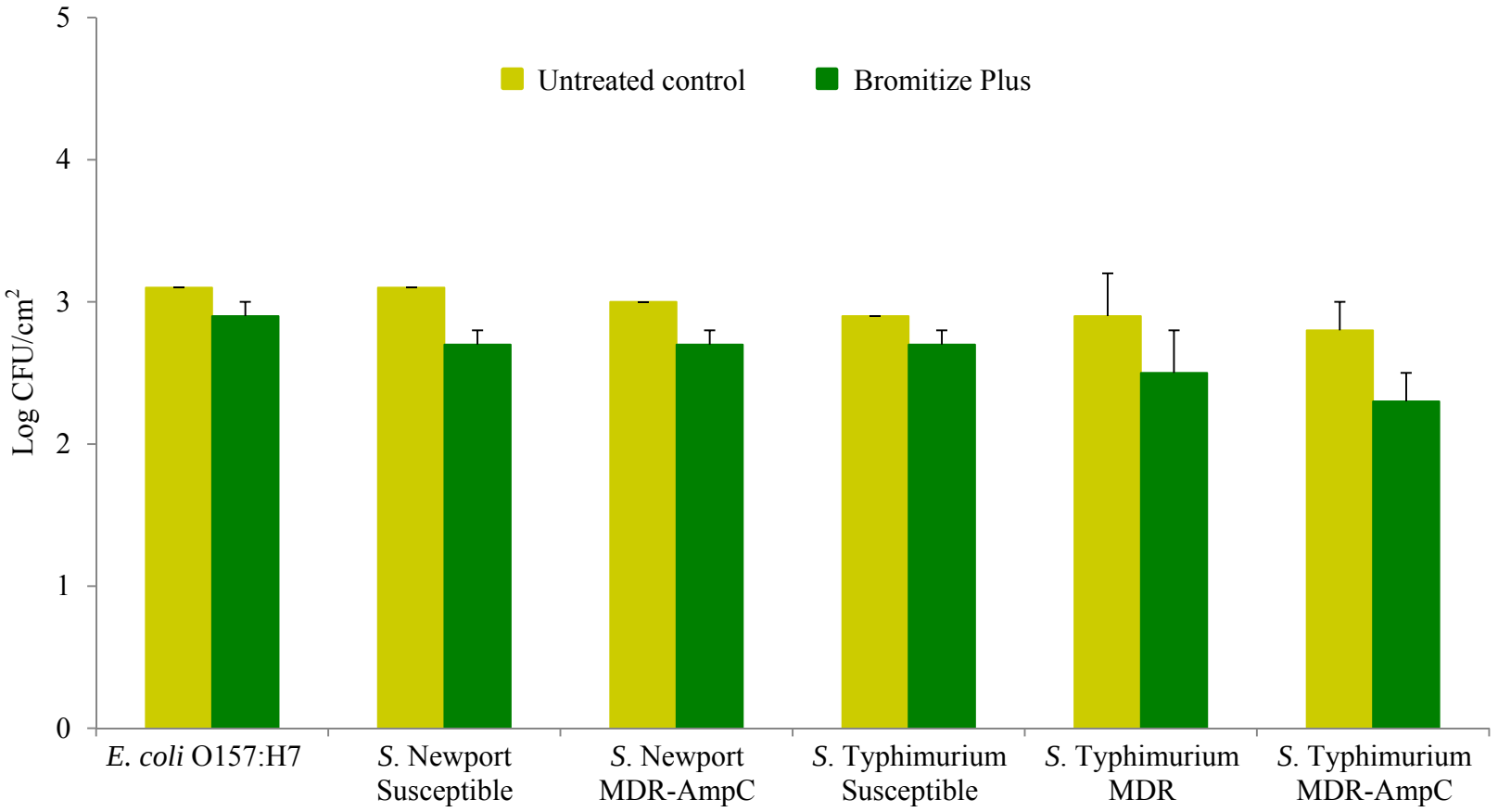

Figure 46. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on tryptic soy agar + rifampicin) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with Bromitize Plus (225 ppm active bromine)

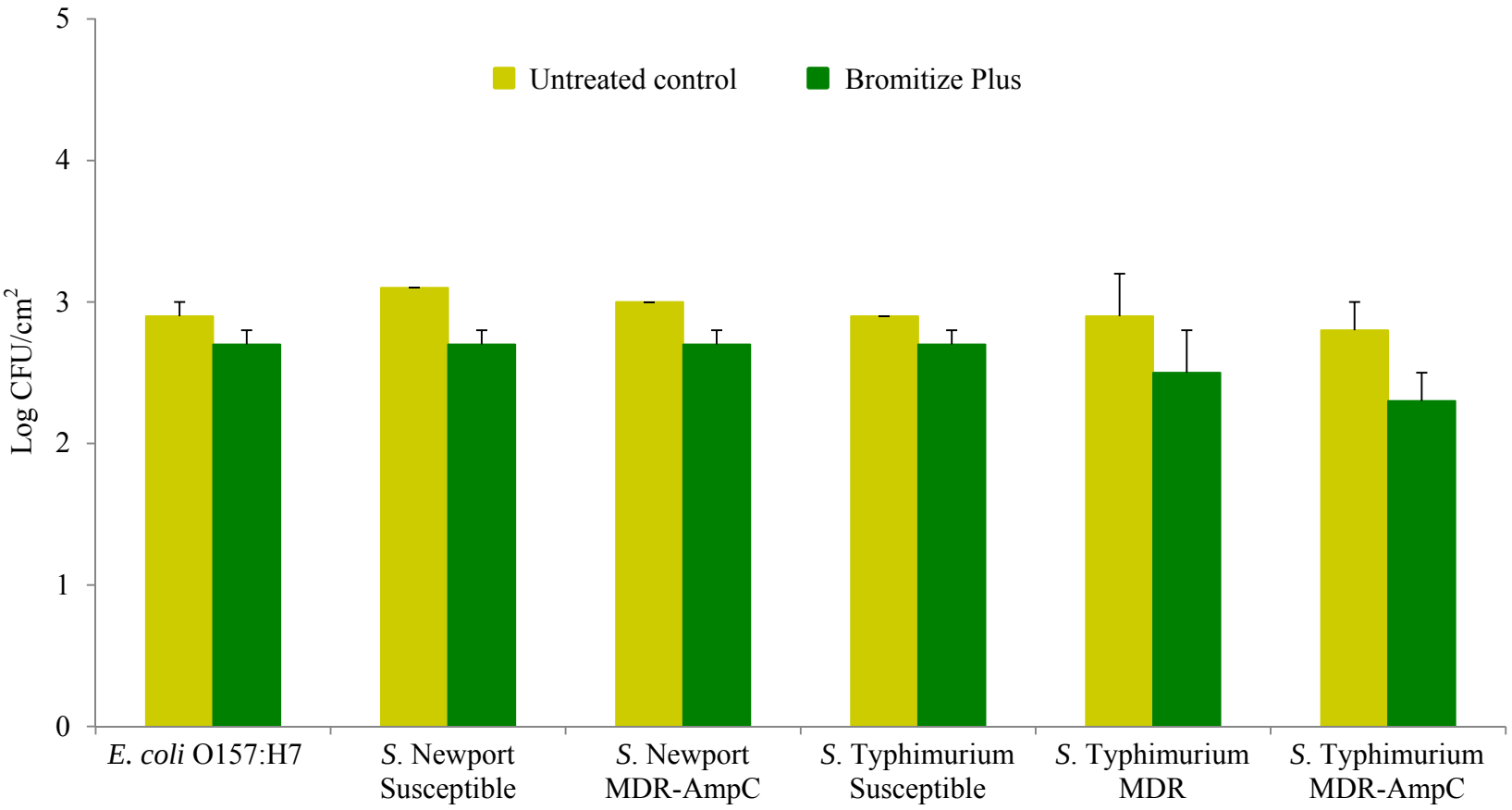

Figure 47. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on modified sorbitol MacConkey agar) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with Bromitize Plus (225 ppm active bromine)

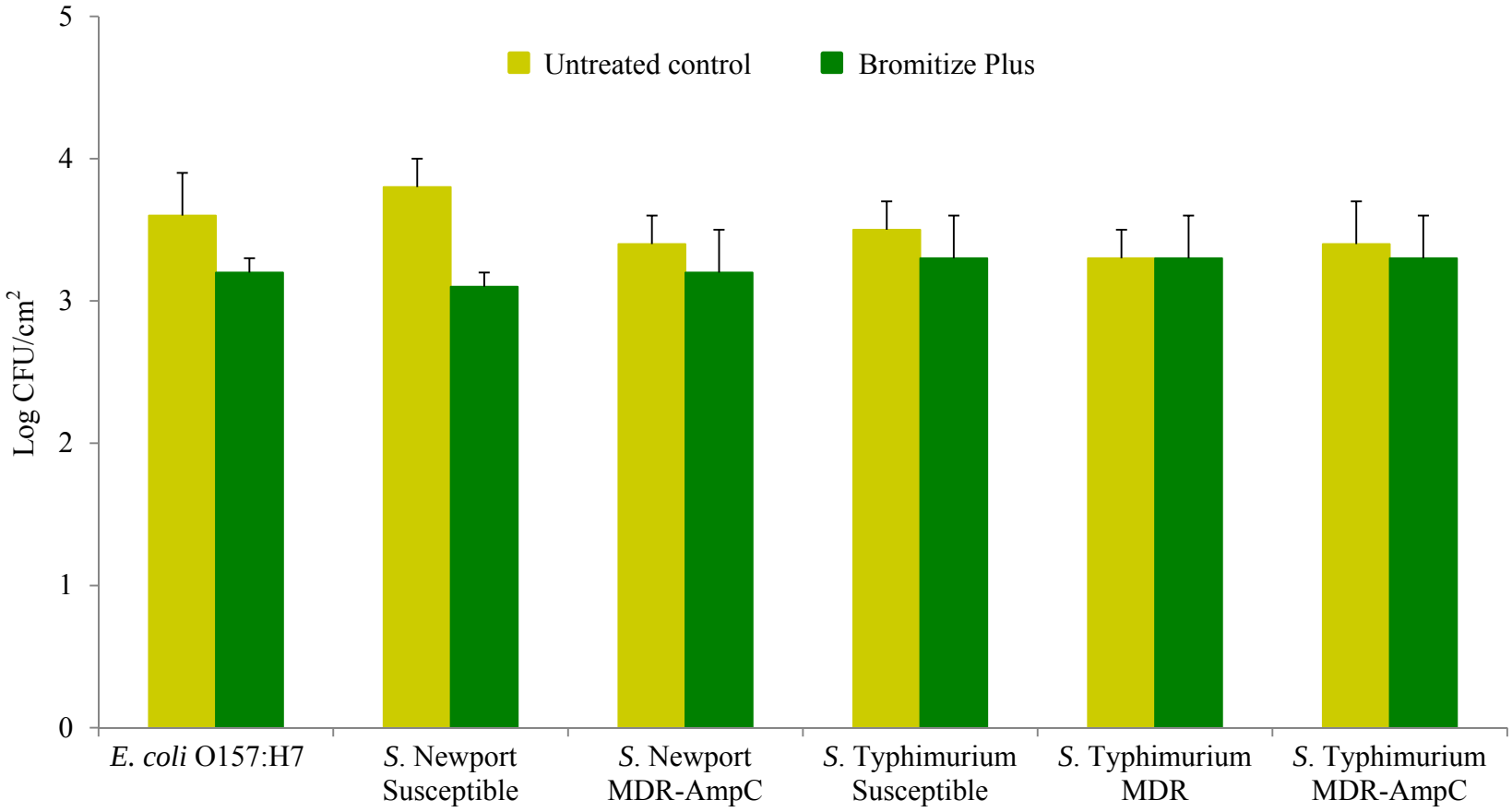

Figure 48. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or antibiotic susceptible or resistant [MDR and/or MDR-AmpC] *S.* Newport and *S.* Typhimurium) before and after decontamination with Bromitize Plus (225 ppm active bromine)

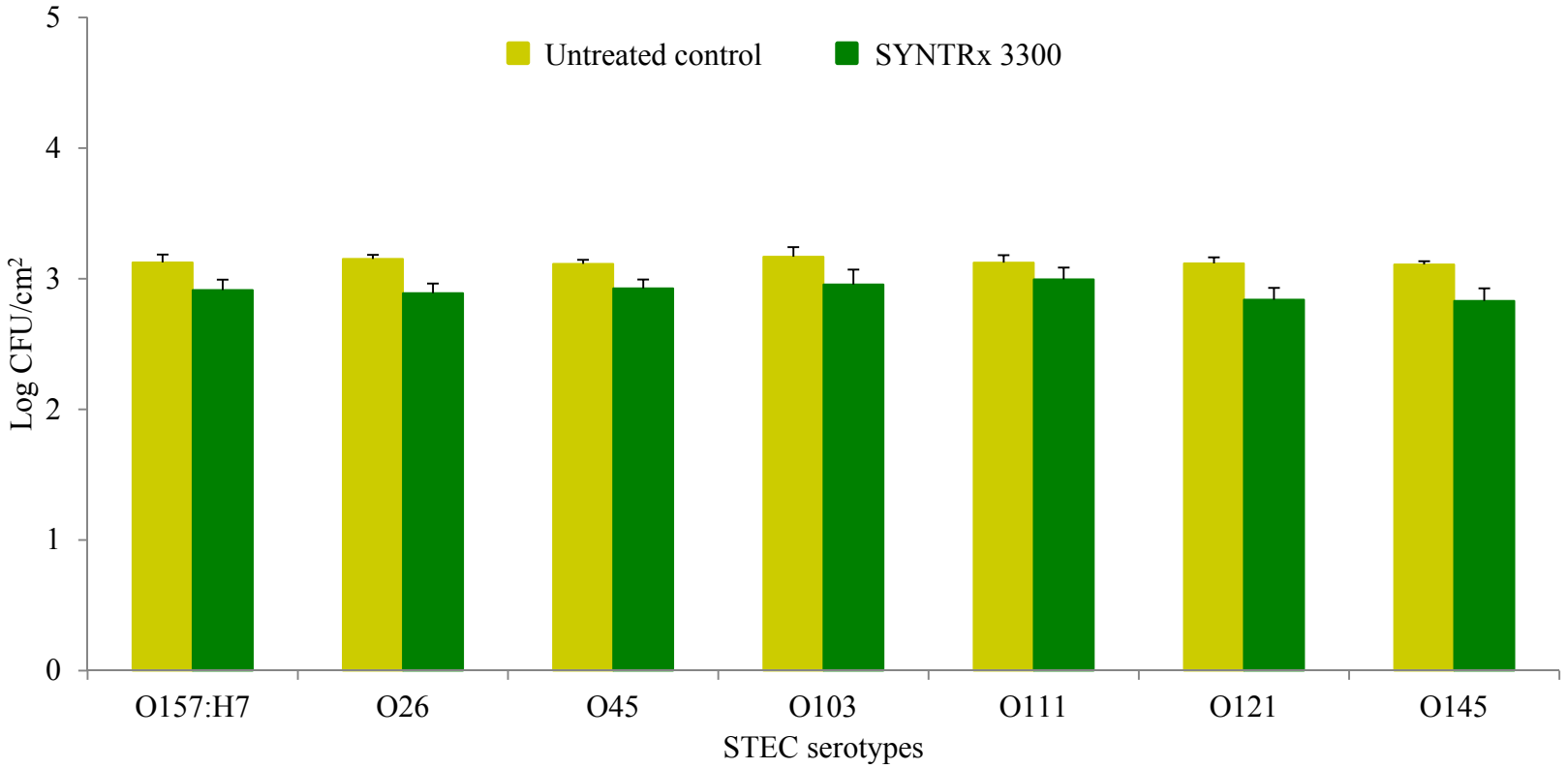

Figure 49. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 and six non-O157 STEC serotypes (recovered on tryptic soy agar + rifampicin) on beef trimmings before and after decontamination with SYNTRx 3300 (pH 1.0)

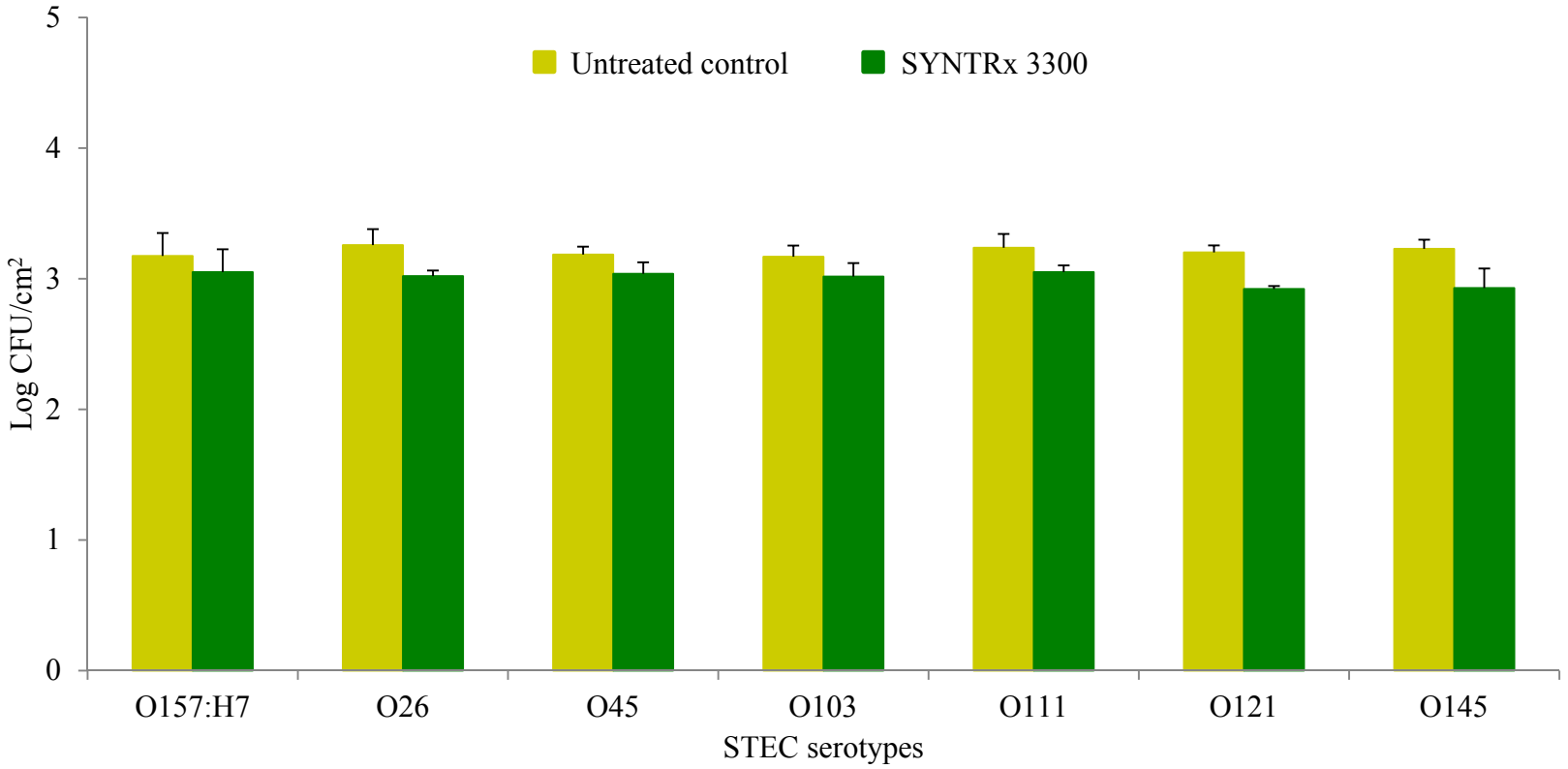

Figure 50. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or six non-O157 STEC serotypes) before and after decontamination with SYNTRx 3300 (pH 1.0)

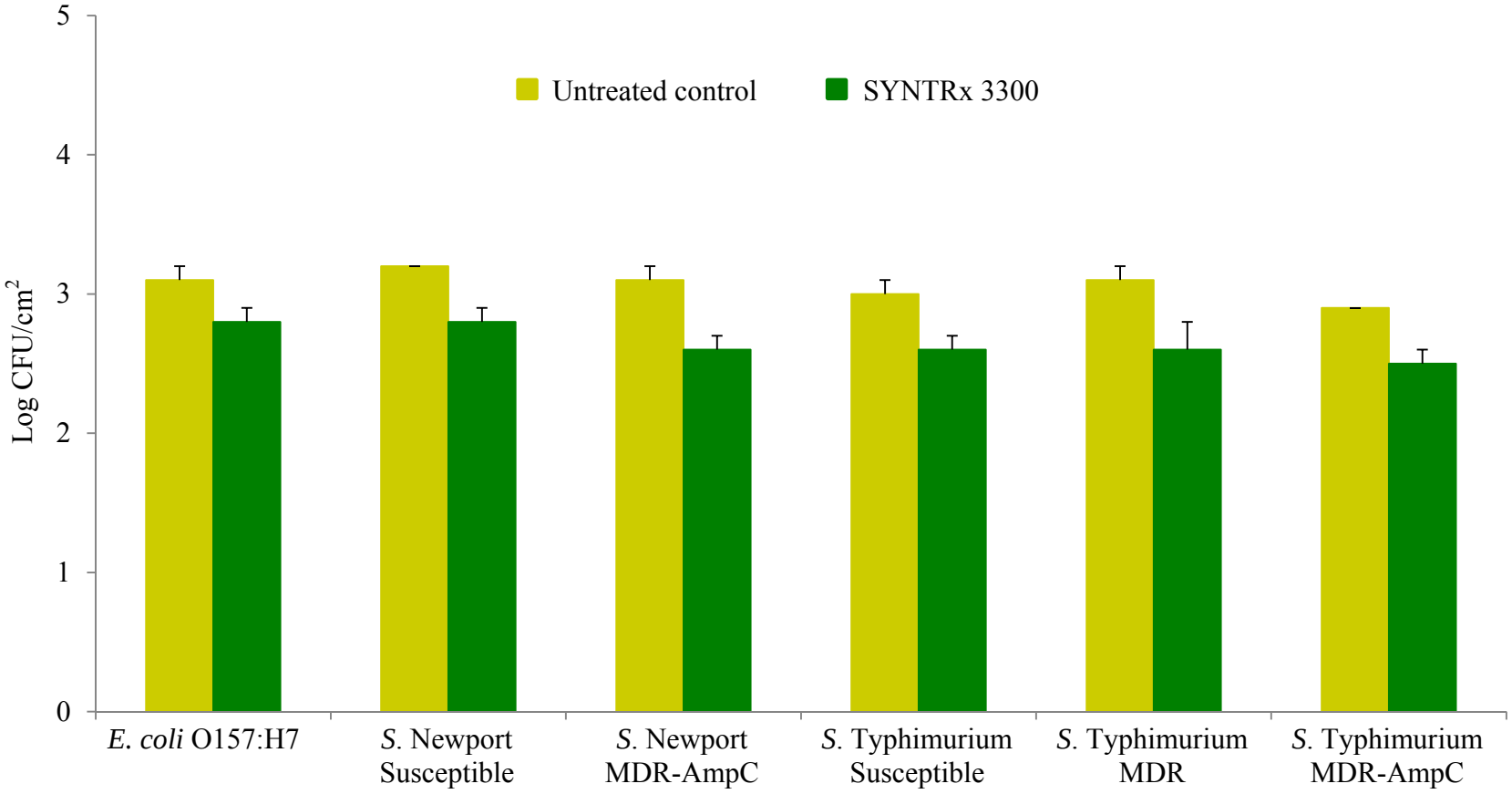

Figure 51. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on tryptic soy agar + rifampicin) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with SYNTRx 3300 (pH 1.0)

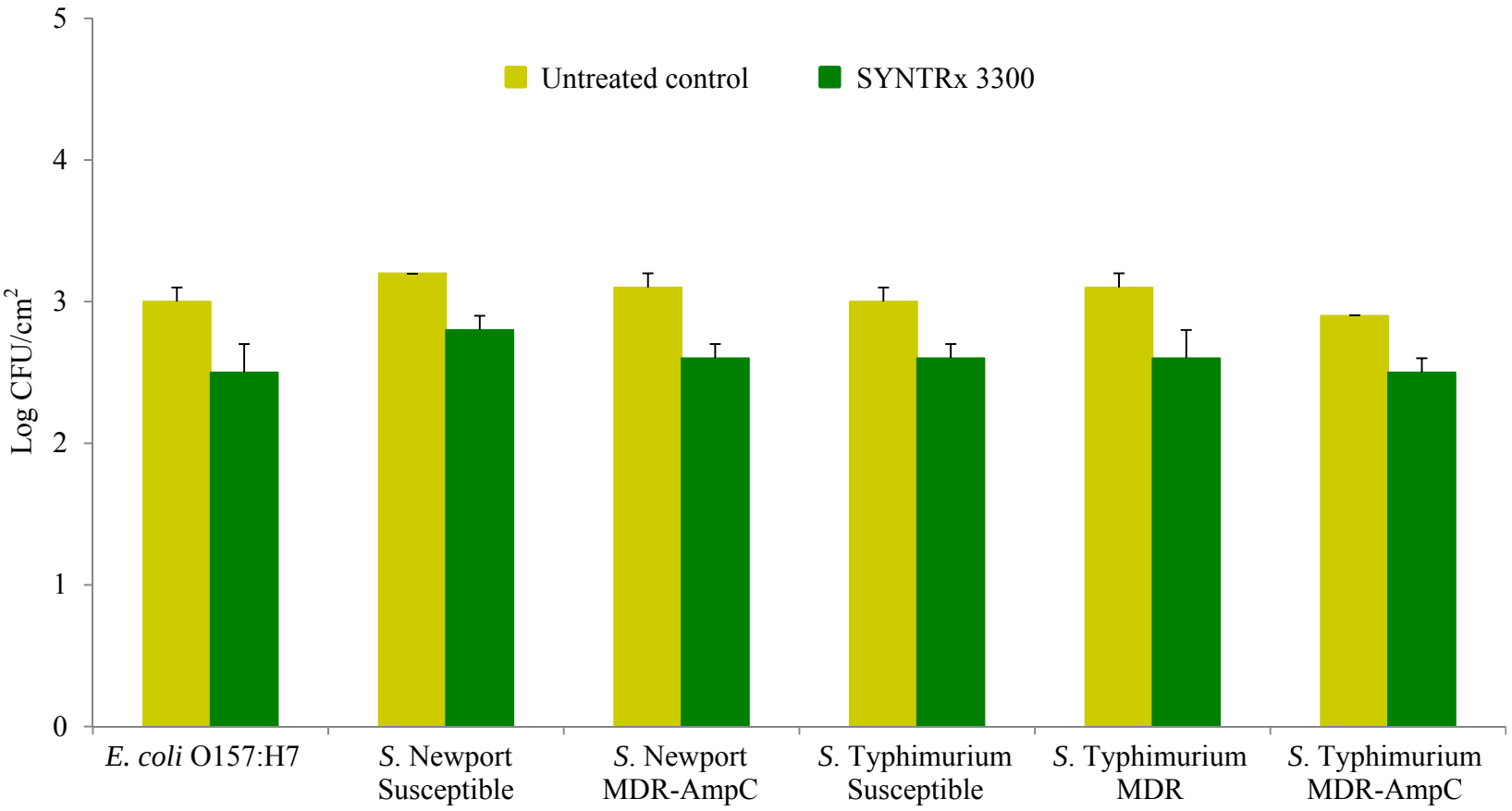

Figure 52. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on modified sorbitol MacConkey agar) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with SYNTRx 3300 (pH 1.0)

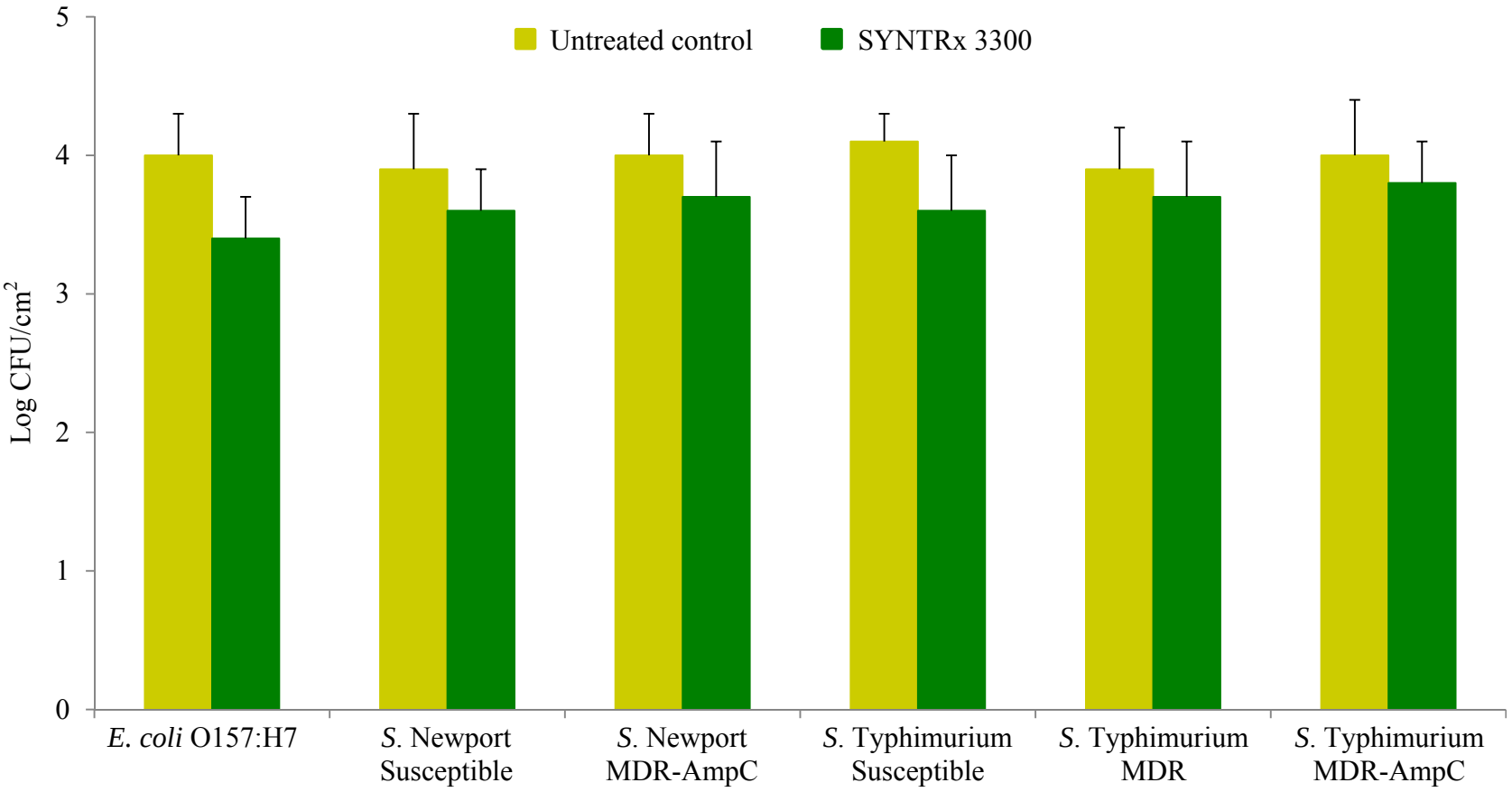

Figure 53. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or antibiotic susceptible or resistant [MDR and/or MDR-AmpC] *S.* Newport and *S.* Typhimurium) before and after decontamination with SYNTRx 3300 (pH 1.0 s)

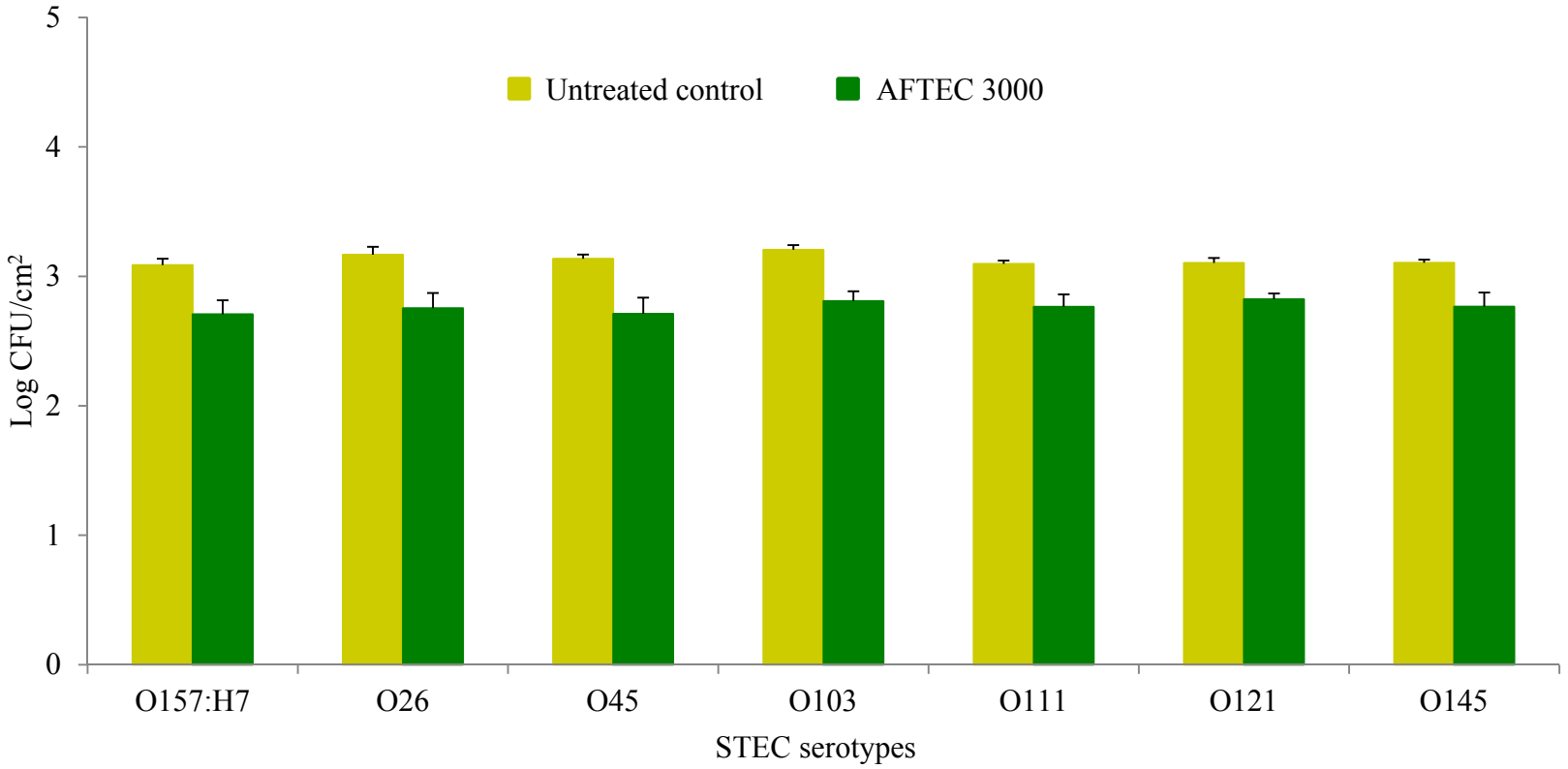

Figure 54. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 and six non-O157 STEC serotypes (recovered on tryptic soy agar + rifampicin) on beef trimmings before and after decontamination with AFTEC 3000 (pH 1.2)

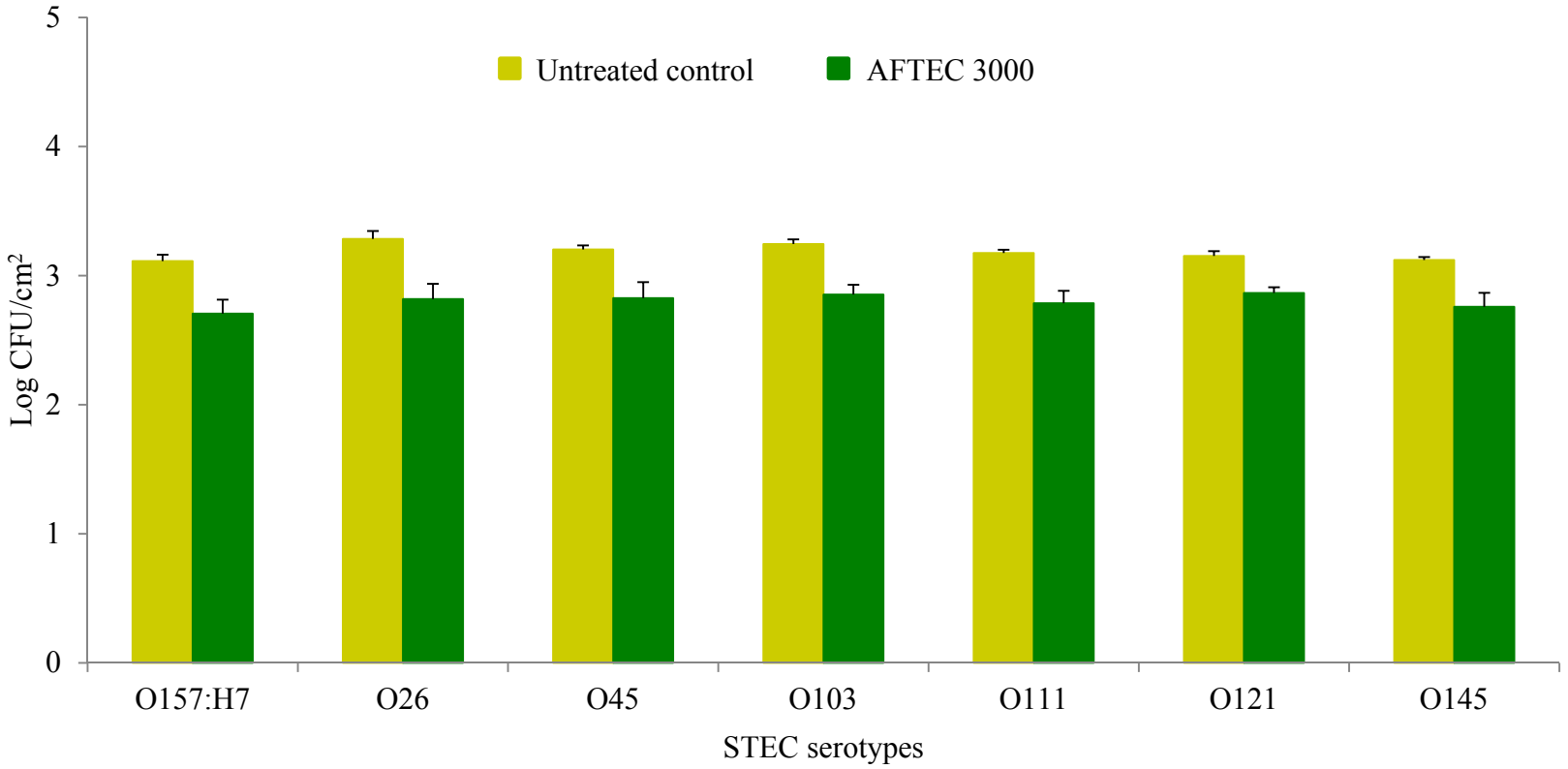

Figure 55. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or six non-O157 STEC serotypes) before and after decontamination with AFTEC 3000 (pH 1.2)

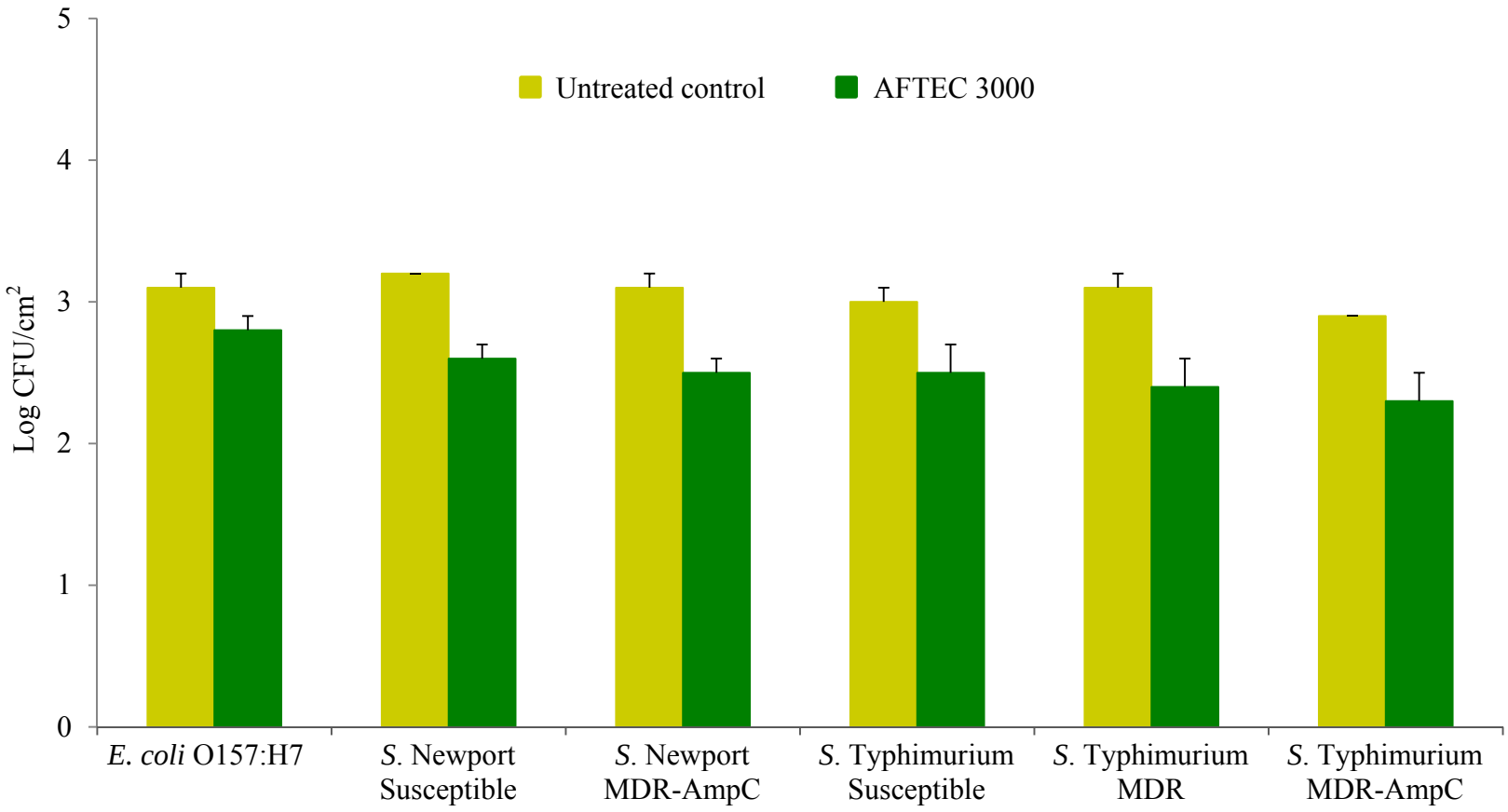

Figure 56. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on tryptic soy agar + rifampicin) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with AFTEC 3000 (pH 1.2)

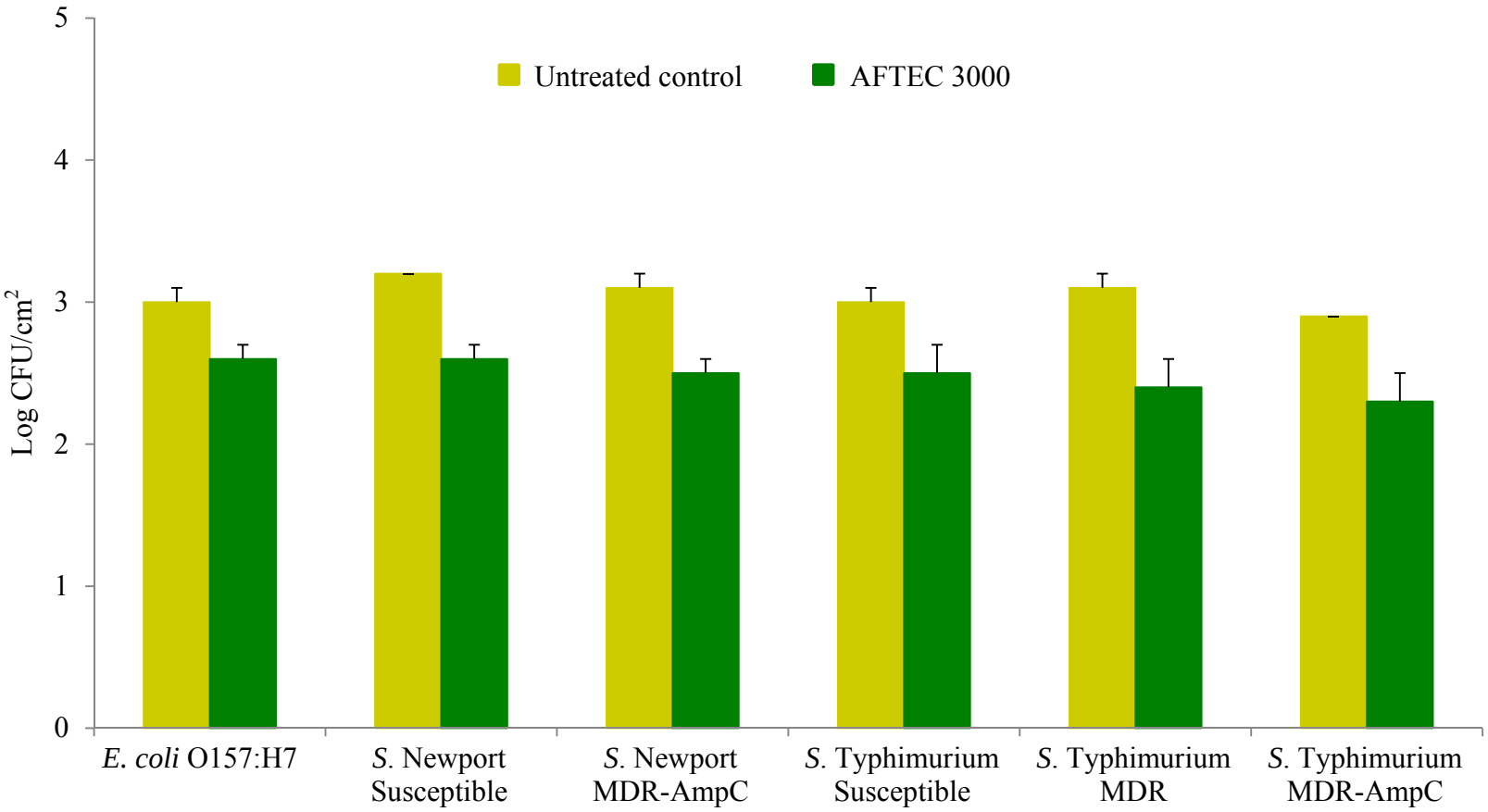

Figure 57. Populations (log CFU/cm^2) of rifampicin-resistant *E. coli* O157:H7 (recovered on modified sorbitol MacConkey agar) and antibiotic susceptible and resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium (recovered on XLD agar) on beef trimmings before and after decontamination with AFTEC 3000 (pH 1.2)

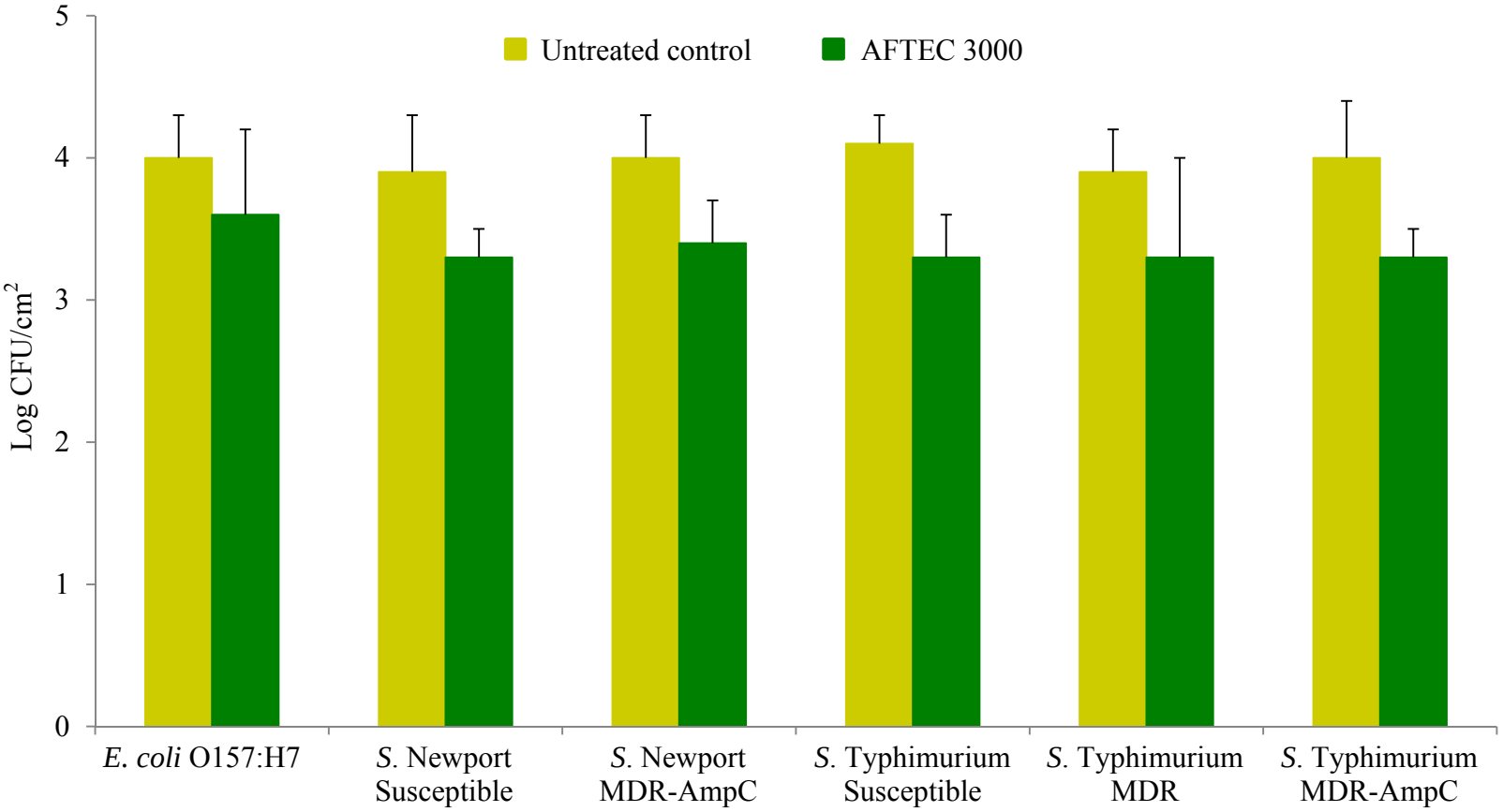

Figure 58. Total bacterial populations (log CFU/cm^2), recovered on tryptic soy agar, of beef trimmings (inoculated with rifampicin-resistant *E. coli* O157:H7 or antibiotic susceptible or resistant [MDR and/or MDR-AmpC] *S.* Newport and *S.* Typhimurium) before and after decontamination with AFTEC 3000 (pH 1.2)