International Journal of Food Microbiology 291 (2019) 25–34

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Effect of post-harvest interventions on surficial carrot bacterial community dynamics, pathogen survival, and antibiotic resistance☆

Vaishali Dharmarha[a], Natalie Pulido[a], Renee R. Boyer[a], Amy Pruden[b], Laura K. Strawn[a], Monica A. Ponder[a,*]

[a] Department of Food Science and Technology, Virginia Tech, Blacksburg, VA, USA
[b] Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA, USA

ARTICLE INFO

Keywords:
Produce
Escherichia coli O157
Pseudomonas
Sanitizer
Low temperature storage
Microbiota

ABSTRACT

Strategies to mitigate antibiotic-resistant bacteria (ARB), including human pathogens, on raw vegetables are needed to reduce incidence of resistant infections. The objective of this research was to determine the effectiveness of standard post-harvest interventions, sanitizer washing and cold storage, to reduce ARB, including antibiotic resistant strains of the human pathogen *E. coli* O15:H7 and a common spoilage bacterium *Pseudomonas,* on raw carrots. To provide a background inoculum representing potential pre-harvest carryover of ARB, carrots were dip-inoculated in dairy cow manure compost slurry and further inoculated with known ARB. Inoculated carrots were washed with one of three treatments: sodium hypochlorite (50 ppm free chlorine), peroxyacetic acid (40 ppm peroxyacetic acid; 11.2% hydrogen peroxide), tap water (no sanitizer), or no washing (control). Washed carrots were air dried, packaged and stored at 10 °C for 7d or 2 °C for up to 60 d. Enumeration was performed using total heterotrophic plate counts (HPC), HPCs on antibiotic-containing media ("ARBs"), *E. coli* O157:H7, and *Pseudomonas* sp. immediately after washing (0 d) and after 7 d of storage. In addition to the cultured bacteria, changes to the surficial carrot microbiota were profiled by sequencing bacterial 16S rRNA gene amplicons to determine the effect of sanitizer wash, storage temperature, and time of storage (0, 1, 7, 14 and 60 d). Storage temperature, addition of a sanitizer during wash, and duration of storage significantly affected the bacterial microbiota (Wilcoxon, $p < 0.05$). Inclusion of either sanitizer in the wash water significantly reduced the log CFU/g of *E. coli* O157:H7 and *Pseudomonas* sp., as well as HPCs enumerated on cefotaxime- (10 μg/ml), sulfamethoxazole- (100 μg/ml), or tetracycline (3 μg/ml) supplemented media compared to the unwashed control (ANOVA, $p < 0.05$). However, no significant reductions to bacteria resistant to vancomycin or clindamycin occurred after washing and storage. Members of the *Proteobactetria, Firmicutes, Actinobacteria, Planctomycetes*, and *Acidobacteria* comprised the bacterial carrot microbiota. The diversity of the carrot microbiota was significantly affected by the temperature of storage and by extended storage (60 d), when spoilage began to occur. There were no significant differences to the relative abundance of bacterial groups associated with the type of sanitizer used for washing. Results of this study indicate that inclusion of a sanitizer in wash water, followed by storage at 2 °C, might be an effective strategy to prevent re-growth of pathogenic *E. coli* O157:H7 and reduce levels of bacteria resistant to certain antibiotics on carrots.

1. Introduction

Fresh-vegetable consumption is sometimes associated with foodborne illness outbreaks, in part due to the lack of an inactivation step before consumption. In the United States, 46% of foodborne illness outbreaks have been attributed to consumption of fresh produce (Painter et al., 2013). Sometimes these outbreaks are caused by antibiotic resistant bacteria (ARB). (EHEC) have been implicated in produce-associated outbreaks. Recently, an outbreak of enterohemorrhagic *Escherichia coli* (EHEC) was traced back to fenugreek seed sprouts contaminated with antibiotic resistant *E. coli* O104:H4, resulting in 2987 illnesses, 855 cases of hemolytic uremic syndrome, and 53 deaths (Muniesa et al., 2012). Additionally, multidrug-resistant (MDR) *Shigella* strains have been isolated from raw salads and MDR isolates of *Pseudomonas* spp. have been recovered from carrots, cabbage, cucumbers, lettuce, and spinach (Allydice-Francis and Brown, 2012; Mokhtari et al.,

☆ Declaration of interest: None.

* Corresponding author.
E-mail address: mponder@vt.edu (M.A. Ponder).

https://doi.org/10.1016/j.ijfoodmicro.2018.11.006
Received 21 June 2018; Received in revised form 30 October 2018; Accepted 7 November 2018
Available online 10 November 2018
0168-1605/

2012; Raphael et al., 2011). While the proportion of infections caused by ARB attributed to produce consumption has not been determined, reports of multi-drug resistant bacteria isolated from fresh produce highlight the growing need for mitigation strategies.

Application of improperly composted or raw manure to soil or plants and use of contaminated water for irrigation or post-harvest washing are important sources from which raw vegetables may become contaminated with human pathogens, and antibiotic resistant bacteria (Alegbeleye et al., 2018; Berger et al., 2010; Ferens and Hovde, 2011; Marti et al., 2013; Rahube et al., 2014; Rangel et al., 2005). Rainfall and overhead irrigation may result in splashing of pathogen-contaminated manure particles from soil to vegetables (Alegbeleye et al., 2018). Thus, it has been recommended that manure is composted in a manner that assures reduction of human pathogens, in order to prevent carry-over of pathogens to food crops (FDA, 2018). However, studies have indicated differences in the effectiveness of composting for reducing different AR observed, particularly as a function of the various antibiotics to which they are resistant, as well asantibiotic resistance genes (ARG) (Qian et al., 2016; Sharma et al., 2009; Su et al., 2015; Wang et al., 2012; Wang et al., 2015). Application of composted manure enriched with ARGs to fields may increase the potential for dissemination of antibiotic resistant bacteria to humans through consumption of food crops (Williams-Nguyen et al., 2016). For example, antibiotic-resistant spoilage bacteria, such as *Pseudomonas*, may contaminate vegetables through soil resulting in spread of ARGs to other bacteria, as well as promote vegetable spoilage due to their pectinolytic capabilities (Cabot et al., 2016; Söderqvist et al., 2017). This highlights the need for processing interventions that may reduce contamination of vegetables with ARB, pathogens, and spoilage bacteria.

To improve quality and mitigate the risk of contamination with human pathogens, vegetable processors often employ post-harvest treatments such as sanitizer washing (López-Gálvez et al., 2009). Sanitizer washes, including chlorine or peroxyacetic acid, can be effective at reducing the presence of human pathogens on fresh produce, such as *E. coli* O157:H7 (Rodgers et al., 2004; Shen et al., 2013). However, effect of sanitizers on log reduction of pathogens depends on several factors, including vegetable type, washing method, type of sanitizer and concentration, contact time and temperature, organic carbon in the water (that may react with sanitizers) pathogen type, and microbiological method used (Prado-Silva et al., 2015). Sanitizer treatments with sodium hypochlorite may be associated with lower mean log reductions for washed leafy vegetables compared to other non-leafy vegetables such as carrots. Effectiveness of sanitizer differs with pathogen. For example, mean log reduction of *Salmonella* spp., by sodium hypochlorite washing was 5.9, however much smaller log reductions of 2.7 and 3.5 were reported for *E. coli* O157:H7 and *Listeria monocytogenes* (Prado-Silva et al., 2015). Therefore, the effect of sanitizer should be examined for different types of ARB, as it is evident that efficacy is variable among the bacteria.

In addition to post-harvest washing, it is also critical to consider subsequent storage conditions. To increase shelf life, washed vegetables are stored at low temperatures, typically 0–5 °C for carrots (Hazzard, 2013). Low temperature storage decreases the respiration rate of vegetables, and reduces microbiological growth during storage and distribution (Gil et al., 2015; Ragaert et al., 2007). On the other hand, temperature abuse, i.e., storage at elevated temperatures, can promote the growth of pathogens, such as was observed for *E. coli* O157:H7, which increased by 2.0 log CFU/g in fresh cut romaine and iceberg lettuce salads after 3 d storage at 12 °C (Francis and O'Beirne, 2001; Luo et al., 2010). For modified-atmosphere packaged spinach, removal of the native bacterial community and temperature abuse affected the growth, establishment, and physiology of *E. coli* O157:H7 and other members of the spinach phyllopshere (Lopez-Velasco et al., 2010). There is limited information is available regarding the influence of storage temperature and time on bacterial community dynamics of carrots or other root vegetables.

The objective of this study was to determine the effect of post-harvest interventions (sanitizer washing, storage temperature, and storage period) on the bacterial community composition of carrot surfaces and survival of antibiotic-resistant inoculated pathogens, spoilage-associated bacteria, and heterotrophic plate count (HPC) bacteria capable of growth on media containing antibiotics (operationally defined in this study as “ARB”). To provide a background microbiota that may represent carryover from a biological amendment, retail carrots were inoculated with composted dairy manure and further inoculated with two ARBs relevant to fresh produce, multi-antibiotic resistant human pathogenic *E. coli* O157:H7 and multi-antibiotic resistant spoilage-associated *Pseudomonas aeruginosa*. The survival and re-growth of known inoculated pathogens HPCs, ARBs, and *Pseudomonas* spp. present on carrot surfaces after washing with commercial food grade sanitizers was determined by serial dilution and spread plating. Culture-independent 16S rRNA gene amplicon sequencing was carried out to profile the microbiota among the different conditions, compare relative abundances of various bacterial populations (including non-culturable members), and gain insight into the effects of the sanitizers on the ARB.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Multi-drug resistant (MDR) *Pseudomonas aeruginosa* strain PAO1 (ATCC 47085) and MDR *Escherichia coli* O157:H7 strain SMS-3-5 (ATCC BAA-1743) were revived from freezer stocks stored at −80 °C by streaking onto Tryptic Soy Agar (TSA, Becton Dickinson, Franklin Lakes, NJ) and incubating for 24 h at 37 °C to obtain isolated colonies. An isolated colony of *P. aeruginosa* was streaked onto *Pseudomonas* Isolation Agar (PIA, Becton Dickinson, Franklin Lakes, NJ) and an isolated colony of *E. coli* O157:H7 was streaked on to Eosin Methylene Blue Agar (EMB, Becton Dickinson, Franklin Lakes, NJ) followed by incubation for 24 h at 37 °C. Separate single colonies from PIA and EMB were transferred to separate tubes of Tryptic Soy Broth (TSB, Becton Dickinson, Franklin Lakes, NJ) and incubated in a shaking incubator at 180 rpm for 24 h at 37 °C. Cells were washed two times in 0.1% (wt/vol) peptone (Becton Dickinson, Franklin Lakes, NJ) to remove any residual nutrients and media. Washed cells were suspended separately in 9 ml of 0.1% (wt/vol) peptone to prepare the inoculation solution.

2.2. Compost slurry preparation

In order to achieve a background carrot microbiota containing ARBs and representative of what could potentially be carried over during pre-harvest processes, compost generated from a companion experiment was slurried to prepare an inoculum for the carrots (Ray et al., 2017). In brief, manure from dairy cattle was collected from cows that did not receive antibiotics (control dairy manure), or dairy cattle prophylactically administered cephapirin and therapeutic levels of pirlimycin (dairy manure with antibiotics). Both control dairy manure and dairy manure with antibiotics were collected and composted at 55 °C for 3 d followed by storage at −20 °C. Prior to beginning of experiments, compost was thawed and mixed homogenously and stored at 4 °C for the duration of experiments (1–16 weeks). Compost slurry was prepared by blending 50 g of compost with sterile deionized water (450 ml for non-inoculated treatments or 440 ml for inoculated treatments) for 45 s using a blender (Oster®, Boca Raton, FL). The compost mixture was strained through a sieve into a sterile container and left at room temperature (23–25 °C) for 24 h to acclimate the compost microbiota. Experiments were conducted with three independent replicates of each type of slurry inoculum (originating from control and antibiotic-treated cattle) ($n = 3$), but because there was no statistically significant differences in the plate counts or microbial diversity parameters examined in this study (see Section 2.9), all compost treatments were combined for subsequent analysis as individual replicates ($n = 6$).

2.3. Sample procurement and dip inoculation

Pre-washed and peeled carrots were obtained from a wholesale club (Roanoke, VA). Carrots were subjected to dip inoculation using one of the two treatments: 1) Slurry generated from composted dairy manure (DC, dairy compost) ($n = 6$), 2) The same DC slurry further spiked with the above described cocktail of MDR *P. aeruginosa* and *E. coli* (n = 6). For the latter treatment, 10 ml of the above culture cocktail (containing 5 ml of *E. coli* O157:H7 and 5 ml of *P. aeruginosa*) was added to the compost slurry. Each experiment was performed six times using different lots of carrots that were refrigerated at 2 °C for 24 h prior to inoculation. After dipping, carrots were dried for 1 h at room temperature (23–25 °C) in a biological safety cabinet prior to washing.

2.4. Carrot washing and storage

Inoculated carrots were washed with agitation (125 rpm) with one of the three solutions (200 g each): **1**) Tap water wash (Blacksburg Municipal water, ~10 ppm free chlorine), **2**) Sodium hypochlorite, hereafter referred to as NaClO (XY-12 (EcoLab, Macquarie, Australia) prepared and applied as per manufacturer's recommendations (50 ppm free chlorine with a 2 min contact time followed by a 30 s rinse with tap water), and **3**) Peroxyacetic acid and hydrogen peroxide, hereafter referred to as POAA (Ecolab, St. Paul, Minnesota) prepared and applied as per manufacturer's recommendation (40 ppm free peroxyacetic acid with a 1.5 min contact time followed by a 30 s rinse with tap water). Washed carrots were dried for approximately 1 h at room temperature (23–25 °C) in a biological safety cabinet until they appeared visibly dry, packaged separately by wash type, and stored at 2 °C or 10 °C.

2.4.1. Wash water quality parameter measurements

Temperature and pH of the wash water were measured using a pH/conductivity meter (Fisher Scientific, Waltham, MA). Free chlorine was measured for the tap water and NaClO washes using chlorine test paper (Ecolab, St. Paul, MN). Free peroxyacetic acid was measured for the POAA wash using Hydrion® 0–160 ppm free peroxyacetic acid test strips (Micro Essential Laboratory, Brooklyn, NY).

2.5. Media preparation

R2A, Eosin Methylene Blue (EMB), and *Pseudomonas* Isolation Agar (PIA) were prepared according to manufacturer's direction (Becton Dickinson, Franklin Lakes, NJ). Antibiotic-supplemented media was prepared using stock solutions of antibiotics. Antibiotics were added to media to achieve the following concentrations in R2A: tetracycline (3 μg/ml, Dot Scientific, Burton, MI), cefotaxime (10 μg/ml, Sigma Life Sciences, St. Louis, MO), clindamycin (25 μg/ml, Sigma Aldrich, Laramie, WY), vancomycin (11 μg/ml, Sigma Life Sciences, St. Louis, MO), and sulfamethoxazole (100 μg/ml; Dot Scientific, Burton, MI). The only antibiotic supplemented into PIA was tetracycline (4 μg/ml) to select for the inoculated tetracycline-resistant *Pseudomonas*.

2.6. Microbiological analysis

Total aerobic bacteria, ARB, and inoculated *E. coli* O157:H7 and *Pseudomonas* sp. were enumerated by plate counts. Bacteria were removed from the washed and unwashed carrots using gentle methods to minimize the disruption of plant cells, which would result in large amounts of chloroplast sequences that may be amplified by 16S rRNA gene primers in the bacterial microbiota analysis (2.7.2–2.8). Carrot samples were weighed (10 g) into sterile filter bags and PT buffer was added in order to achieve a 1:10 dilution. The bag was then shaken at a speed of 220 rpm for 5 min using a bench top rotator, subsequently hand-massaged for 2 min and then shaken again using the bench top rotator for an additional 5 min. The liquid from filter bag was serial-diluted in sterile PT buffer and 100 μl aliquots were spiral-plated (Spiral Biotech Autoplate 4000®, Norwood, MA) onto the various media types and were incubated for 18–24 h at 37 °C.

2.7. DNA extraction and 16S rRNA gene quantification

2.7.1. DNA extraction

The remaining liquid contents of the bag were vacuum filtered through a 0.22 μm, 47 mm mixed cellulose ester membrane (Millipore Sigma, Burlington, MA) filter to collect bacterial cells from carrot surface. Filters were stored within sterile, DNase-free, O-ring screwcap tubes at −80 °C until DNA extractions were performed. DNA extractions were performed using FastDNA® Spin Kit for Soil (MP Biomedical™, Solon, OH) per manufacturer's instructions with an additional bead beating step and incubation for two hours to facilitate lysis of bacterial cells. The DNA was eluted in 100 μl of DNase/pyrogen free water and was subsequently treated with OneStep™ PCR Inhibitor Removal Kit (Zymo Research, Irvine, CA) per manufacturer's instructions. DNA was stored at −80 °C until qPCR was performed.

2.7.2. Quantification of 16S rRNA genes

Quantification of 16S rRNA gene copy numbers from carrot DNA samples was performed using quantitative real-time PCR (qPCR) according to the method by Suzuki et al., 2000. DNA extracts for unwashed samples were diluted to 1:10, while only few 1:10 dilutions were required for washed samples. Each reaction mixture (10 μl) was consisted of 2× SsoFast™ EVAgreen® Supermix (Bio-Rad Laboratories, Hercules, CA), 5-μM forward primer (400 nM), 5-μM reverse primer (400 nM), DNA template, and molecular grade water (Sigma-Aldrich, St. Louis, MO). The protocol on CFX Connect™ (Bio-Rad Laboratories, Hercules, CA) consisted of 1 cycle of 98 °C for 2 min, 40 cycles of 98 °C for 5 s and annealing temperature of 55 °C for 5 s followed by a melt curve. Standard curves were created using seven 10-fold dilutions of 16S rDNA standard (10^8–10^2 copies/μl).

2.8. 16S rRNA gene amplicon sequencing

DNA samples from carrots were diluted to 3×10^4 16S rRNA gene copies/μl to normalize 16S rRNA gene copy numbers. Bacterial 16S rRNA gene amplicon sequencing of each of the carrot samples associated with the different wash treatments, storage temperatures and times ($n = 4$) was based on Earth Microbiome Project protocol (modified from Caporaso et al., 2011). Each reaction mixture (25 μl) consisted of 2.5× 5PRIME HotMaster Mix (QuantaBio, Beverly, MA), 515fB primer (10-μM), 926r primer (10-μM), molecular grade water and template DNA. The protocol was run on the CFX Connect™ (Bio-Rad Laboratories, Hercules, CA) and thermocycler conditions were 1 cycle of 94 °C for 3 min, 35 cycles of 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s, and a final extension cycle at 72 °C for 10 min. Products obtained after PCR amplification were measured using Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA) and pooled equally at 240 ng each. Libraries were prepared at Virginia Tech Biocomplexity Institute (Blacksburg, VA) using the MiSeq® Reagent Kit v3 (Illumina) and 2 × 300-bp paired-end reads were produced using MiSeq (Illumina). Paired end reads were stitched using PANDASeq (Masella et al., 2012) and filtered based on the quality score (≥0.90) and sequence length (372–375 bp). Operational taxonomic units (OTUs) were picked de novo using QIIME from the GreenGenes database at 97% cutoff (Caporaso et al., 2010). Chimera sequences were removed using ChimeraSlayer with QIIME (Haas et al., 2011). Chloroplast and mitochondrial sequences were also removed. OTU libraries derived from each carrot sample were rarefied to 6000 reads using QIIME (single_rarefaction.py), equivalent to the sample with the shallowest sequencing depth, before performing diversity analyses. Shannon index and Chao1 were selected for the alpha diversity matrix, while Unifrac distances was selected for β-diversity (Chao, 1984; Lozupone and Knight, 2005; Shannon and Weaver, 1949).

2.9. Statistical analysis

The bacterial CFU/g were log transformed to achieve a normal distribution. Statistical analyses were performed using JMP statistical software (version 10, SAS, Cary, NC). The effect of storage temperature was compared using a one-way ANOVA to test for differences in the average log of all bacterial types (antibiotic-resistant *E. coli* O157:H7 and *Pseudomonas* sp., native *Pseudomonas* spp., HPC, and ARB on different antibiotic-supplemented media) of recovered CFU/g on carrots. The effect of day of storage and wash type were compared using an ANOVA analysis with a Tukey's post-hoc analysis to test for differences in the average log of all bacteria types (antibiotic-resistant *E. coli* O157:H7 and *Pseudomonas* spp., native *Pseudomonas* sp., HPC, ARB on antibiotic-supplemented media) of recovered CFU/g on carrots. Significance was determined at $P < 0.05$.

Relative abundances (percentage of total reads) of taxa at phylum, class, order, family, and genus level were determined. Comparisons were made among inoculation type (inoculated vs. non-inoculated), wash type, temperature, and days of storage. Due to non-normal distributions non-parametric Wilcoxon tests and Steel-Dwass tests were performed to determine significant differences in relative abundance among specific phylogenetic groups. $P \leq 0.05$ were used to designate significance. Analysis of β-diversity between samples was compared by plotting non-metric multidimensional scaling (NMDS) graphs using weighted Unifrac distances by each treatment type followed by analysis of similarity (ANOSIM) using Primer v6.1.13 (Plymouth, UK). R-value cutoffs as defined by Clarke and Warwick (2001) were used ($R > 0.75$, well separated; $0.75 < R > 0.25$, separated but overlapping; $R < 0.25$, barely separated). Because no significant differences between percent relative abundances of taxonomic groups on unwashed carrots dipped in dairy compost inoculated with antibiotic resistant strains of *E. coli* O157:H7 and *P. aeruginosa* and dairy compost not inoculated with ARB were found, therefore replicates were pooled to increase statistical power for subsequent analysis of surficial carrot microbiota ($n = 12$) changes associated with wash type, temperature and length of storage.

3. Results and discussion

3.1. Amplicon sequencing quality and depth

A total of 2,716,992 DNA sequence reads were retrieved across all carrot samples, with a mean of 22,454 and median of 21,407 reads per carrot. The range of sequences retrieved per carrot was 6071 to 53,553. OTU rarefaction curves were compared among the three washing treatments and unwashed control. Sequencing coverage was variable among treatments, as indicated by some treatments having rarefaction curves within exponential phase and others approaching plateau (Fig. S1). To account for these differences, the number of reads was rarefied to 6000 per sample for subsequent comparisons. Raw reads were deposited under NCBI Sequence Read Archive under SRA accession SRP150878.

3.2. Surficial carrot microbiota

The bacterial community of the unwashed carrot surfaces was characterized using 16S rRNA gene sequencing. This served as the background community and was used for comparison with all sanitizer and storage treatments. Overall, 12 phyla of bacteria were identified among the OTUs, with over 98% of sequences classified as belonging to only five phyla, ranking from most to least abundant: *Proteobactetria, Firmicutes, Actinobacteria, Planctomycetes,* and *Acidobacteria* (Table S1). *Proteobacteria* and *Firmicutes* have been reported as the most abundant phyla in bacterial communities in other fresh vegetables, including spinach, lettuce, tomatoes, and peppers (Leff and Fierer, 2013; Lopez-Velasco et al., 2011; Ottesen et al., 2013). Among the identified phyla, 33 bacterial classes were identified. The five most abundant classes identified, ranked from highest to lowest, were: *Gammaproteobacteria*, *Bacilli*, *Betaproteobacteria*, *Actinobacteria*, and *Alphaproteobacteria*. At the family level, 114 bacterial families were identified, with 78% sequences classified as *Pseudomonadaceae*, *Enterobacteriaceae*, *Oxalobacteraceae, Bacillaceae*, or *Paenibacillaceae.* These families have been previously reported on a variety of fruits and vegetables, including spinach, lettuce, tomatoes, peppers, apples, peaches, grapes, and mushrooms (Allard et al., 2016; Jackson et al., 2013; Leff and Fierer, 2013). Members of the *Pseudomonadaceae*, *Oxalobacteraceae* and *Bacillaceae* inhabit soil and plants. Some *Enterobacteriaceae* members are plant-associated while others are found in the gastrointestinal tract of mammals and find their way into the environment through manure or sewage (Baldani et al., 2014; Blaak et al., 2014; Mandic-Mulec et al., 2016; Palleroni, 1981). In this study, the genera identified within the *Enterobacteriaceae* detected on carrot surfaces inoculated with compost included *Escherichia* and *Yersinia.* The compost used in this study was found to contain 3–4 log MPN/g of *E. coli* (Williams, 2016). Other studies have also indicated the survival of *E. coli* following composting at 54–67 °C (Droffner et al., 1995; Inoue et al., 2005). Introduction of human pathogens onto produce through compost is of concern, especially since outbreaks of fecal-derived pathogens, such as *E. coli* O157:H7 and *Yersinia enterocolitica*, have been associated with fresh produce. *Y. pseudotuberculosis* caused gastrointestinal symptoms in schoolchildren after consumption of raw carrots in Finland (Kangas et al., 2008; Wendel et al., 2009).

In addition to the detection of potential human pathogens on the compost-inoculated carrots, the presence of genera associated with spoilage was detected. The compost used for the study may have contributed the microbes to the carrots, however several species of Pseudomonas have been detected on carrot root surfaces and are believed to be important in the spoilage of peeled packaged carrots (Surette et al., 2003). *Pseudomonas* sp. play important roles as plant pathogens and in post-harvest spoilage due to their pectinolytic capabilities (Söderqvist et al., 2017). *Pseudomonas* are intrinsically resistant to many antibiotic classes (e.g., β-lactams) and members of the genera have been documented to acquire resistance through horizontal gene transfer and mutations (e.g., cephalosporins) (Livermore, 2002). Presence of antibiotic resistant *P. aeruginosa* and *P. fluorescens/putida* has been identified in carrots for sale in Jamaica (Allydice-Francis and Brown, 2012) and raw salad vegetables in Canada (Bezanson et al., 2008) indicating the broad geographic distribution of antibiotic resistant *Pseudomonads* and their frequent associations with vegetables.

3.3. Effect of sanitizer wash on the bacterial community composition of carrots and recovery of bacteria on antibiotic media

Bacterial community structure of the carrot surfaces immediately after washing and drying were highly similar regardless of sanitizer inclusion (Fig. 1). Overall, the relative abundance of each of the taxonomic groups was not significantly affected immediately after sanitizer washing (Table S2). This result likely relates to the fact that live/dead bacteria cannot be directly distinguished using molecular methods.

Inclusion of sanitizers in the wash water resulted in small but statistically insignificant reductions in the HPCs recovered on R2A (Table 1). However, use of selective media and inclusion of antibiotics in the media revealed that sanitizer effectiveness varied. Larger reductions in the inoculated bacteria *E. coli* O157:H7 and *Pseudomonas* spp. resistant to tetracycline were achieved after washing in water including POAA compared to NaClO. *E. coli* O157:H7 was reduced by an average 2.61 log CFU/g after washing with POAA, 1.9 log CFU/g after washing with NaClO, and 0.61 log CFU/g after washing with tap water compared to the unwashed control (ANOVA, $p < 0.05$; Table 1). Results from this study are consistent with those of other studies, which have reported that inclusion of sanitizers, like chlorine and peroxyacetic acid (POAA), increased inactivation of pathogens compared to

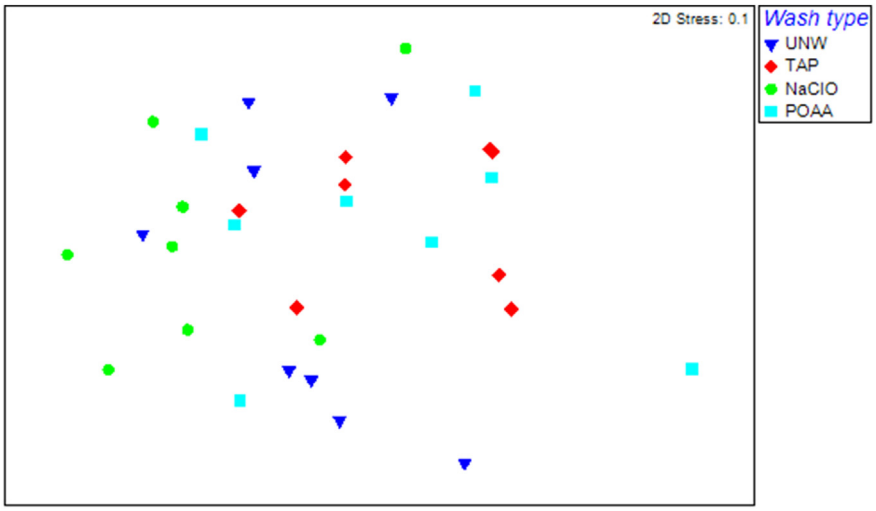

Fig. 1. Non-metric multidimensional scaling analysis of weighted Unifrac distance matrices of bacterial microbiota found on unwashed and washed carrot surfaces, immediately following the washing and air drying procedure. UNW-Unwashed, NaClO - Washed with sodium hypochlorite, POAA-Washed with peroxyacetic acid, TAP - Washed with tap water ($n = 8$ per wash type). Each point represents one carrot sample (ANOSIM, $R = 0.13$). ANOSIM, $R < 0.25$ indicates no separation in bacterial community taxonomic structures among the four wash treatments.

water washes only. Chlorine-based sanitizers when dissolved in water lead to the formation of hypochlorous acid (HOCl) formation, a strong oxidizing agent, which subsequently inactivate pathogens (Banach et al., 2015). POAA is a very strong oxidizing agent and exerts its antibacterial action primarily through the production of reactive oxygen species, subsequently leading to DNA and lipid damage (Vandekinderen et al., 2009). Specifically, 1–5 log reductions were observed in foodborne pathogens, like *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on tomatoes and lettuce (Beuchat et al., 2004; Chang and Schneider, 2012; Niemira, 2007). To the authors' knowledge, no prior study has specifically examined the effect of sanitizer washes on antibiotic-resistant bacterial populations on carrots.

Notably, inactivation of all groups of HPCs and ARBs as a function of the sanitizer wash conditions were not the same (Table 1). In this experiment, antibiotics were included in the media as a way to enumerate the populations of bacteria capable of growth in the presence of the antibiotic. In this study, we applied an operational definition to define ARBs, based on the capability of the HPCs to grow in the presence of a suite of antibiotics at concentrations selected to correspond to typical minimum inhibitory concentrations of clinically-relevant bacteria. It's important to acknowledge, though, that to truly confirm an ARB it would be necessary to further isolate, speciate, and assay the minimum inhibitory concentrations according to clinical standards. Otherwise, growth on antibiotic plates could be due to intrinsic resistance, i.e., not being a relevant target of the antibiotics, rather than due to being evolved or horizontally-acquired, which are more of concern to the potential for antibiotic resistance to spread (Livermore, 2002). Nevertheless, through applying the ARB enumeration method consistently in this study, it was evident that the number of HPCs capable of growth on media containing cefotaxime (10 μg/ml), sulfamethoxazole (100 μg/ml) and tetracycline (3 μg/ml) R2A plates were significantly reduced by an average of 1.3, 2.12 and 2.08 log CFU/g, respectively, after washing with POAA compared to unwashed carrots (Table 1). Washing with tap water resulted in smaller (~0.5 log CFU/g) reductions. Reduced capability of bacterial populations washed from carrot surfaces to grow on antibiotic-supplemented media suggests that inclusion of sanitizers in produce wash water might help reduce total numbers of antibiotic resistant aerobic heterotrophic bacterial populations immediately after washing.

3.4. Effect of storage temperature on the bacterial community composition of carrots, bacterial survival and re-growth

Sanitizer wash followed by 7 d refrigerated storage at two different temperatures, optimal (2 °C) and temperature abuse (10 °C), resulted in bacterial community structures that were separated but with some overlap ($R = 0.47$; Fig. 2). Overall, carrots stored at 2 °C had significantly greater bacterial community richness and evenness than carrots stored at 10 °C (Chao1, $p < 0.005$; Shannon, $p < 0.0001$; Wilcoxon, Table S3). The lower richness and evenness on carrots stored at 10 °C was characterized by higher levels of members of *Proteobacteria*, which were as much as 34% higher on washed carrots stored at 10 °C versus 2 °C (Table 2). Specifically, the relative abundance of *Pseudomonas* and *Janthinobacterium* were greater by 17% on POAA

Table 1
Effect of sanitizer wash on recovery of inoculated *E. coli* O157:H7 and *Pseudomonas* sp. and native antibiotic-resistant bacteria immediately after washing carrots dipped in inoculated dairy compost on different media types (n = 6). Values are CFU/g ± standard error from the mean. Connecting letters denote significant differences between wash types within media type (ANOVA, $p < 0.05$).

Wash type	Unwashed	Tap water	NaClO	POAA
Microorganism				
E. coli O157:H7	6.02 ± 0.07^{A}	5.41 ± 0.10^{B}	4.12 ± 0.07^{C}	3.41 ± 0.14^{D}
Pseudomonas sp.	5.72 ± 0.10^{A}	5.31 ± 0.11^{A}	4.32 ± 0.14^{B}	4.09 ± 0.29^{B}
Pseudomonas spp. resistant to tetracycline*	5.72 ± 0.14^{A}	5.19 ± 0.27^{A}	4.07 ± 0.16^{B}	3.49 ± 0.17^{B}
HPC bacteria	7.21 ± 0.26^{A}	7.07 ± 0.26^{A}	6.73 ± 0.36^{A}	6.75 ± 0.32^{A}
Bacterial colonies on clindamycin*plates	6.51 ± 0.11^{A}	6.46 ± 0.13^{A}	5.80 ± 0.26^{A}	5.71 ± 0.32^{A}
Bacterial colonies on cefotaxime*plates	5.51 ± 0.19^{A}	4.95 ± 0.17^{B}	4.49 ± 0.10^{BC}	4.21 ± 0.09^{C}
Bacterial colonies on sulfamethoxazole*plates	6.08 ± 0.08^{A}	5.46 ± 0.09^{B}	4.37 ± 0.10^{D}	3.96 ± 0.08^{D}
Bacterial colonies on tetracycline*plates	6.26 ± 0.09^{A}	5.89 ± 0.06^{A}	4.65 ± 0.11^{B}	4.18 ± 0.24^{B}
Bacterial colonieson vancomycin*plates	6.49 + 0.14^{A}	6.44 + 0.15^{A}	5.78 + 0.25^{A}	5.78 + 0.26^{A}

* *Pseudomonas* sp. resistant to tetracycline (4 μg/ml tetracycline); bacterial colonies on antibiotic-supplemented media - clindamycin (25 μg/ml); cefotaxime (10 μg/ml); sulfamethoxazole (100 μg/ml); tetracycline (3 μg/ml); vancomycin (11 μg/ml).

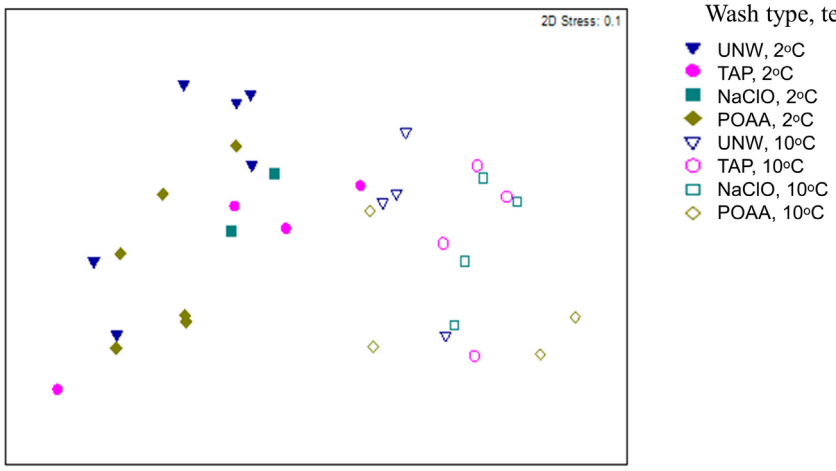

Fig. 2. Non-metric multidimensional scaling analysis of weighted Unifrac distance matrices of surficial bacterial microbiota found on carrots subject to the four washing treatments (UNW-Unwashed, NaClO - Washed with sodium hypochlorite, POAA-Washed with peroxyacetic acid, TAP- Washed with tap water) and stored at either 2 °C or 10 °C for 7 days (n = 6 UNW; n = 2 NaClO; n = 6 POAA; n = 4 TAP at 2 °C) and (n = 4 per wash type at 10 °C). Each point represents one carrot sample (ANOSIM, R = 0.47). ANOSIM, R > 0.25 indicates that refrigerated storage at two different temperatures, optimal (2 °C) and temperature abuse (10 °C), combined with sanitizer wash and storage for 7 d resulted in separated communities but with some overlapping.

washed carrots stored at 10 °C compared to 2 °C (Wilcoxon, $p < 0.05$; Table 2). This is not unexpected as some species of *Pseudomonas* and *Janthinobacterium* are psychrotrophic with faster growth rates at 10 °C (Ingraham, 1958; Gillis and De Ley, 2006). Members of the *Firmicutes*, chiefly the order *Bacillales* were significantly reduced by 18–21% when carrots were stored at 10 °C for 7 d with all wash types compared to 2 °C (Wilcoxon, $p < 0.03$; Table 2). Community analysis further supported that relative abundance of *Enterobacteriaceae* was reduced by sanitizer washing and low temperature storage. A small (2–5%) but not statistically significant increase in relative abundance of *Enterobacteriaceae* was observed when washed carrots were stored at 10 °C relative to 2 °C (Table 2). Other studies have reported a small increase in relative abundance of *Enterobacteriales* from < 5% to approximately 10–20% at 8 °C and 15 °C after 7 d of storage in baby spinach (Söderqvist et al., 2017). Members of the *Enterobacteriaceae* were more abundant on washed baby spinach stored at 4 °C for 15 d compared to 10 °C (Lopez-Velasco et al., 2011). However the levels of *Escherichia* were 23% greater on spinach stored at 10 °C for 15 d compared to freshly packaged spinach or spinach stored at 4 °C (Lopez-Velasco et al., 2011). Interestingly, in the present study, relative abundance of the genus *Escherichia* decreased by 3–4% on washed carrots at 10 °C after carrots were stored for 7 d, suggesting that other members of the bacterial community were preventing additional regrowth (Table 2). However, no reductions in *E. coli* O157:H7 were observed at 10 °C based on plate counts. Plate counts indicated that once these sanitizers initially reduced *E. coli* O157:H7 populations, they did not re-grow under optimal temperature (2 °C) or temperature-abuse (10 °C) conditions, indicating that the inclusion of sanitizers are effective at initially reducing and refrigerated storage prevented the re-growth of inoculated *E. coli* O157:H7 (Fig. 3). Another study indicated that average *E. coli* O157:H7 numbers reduced slightly (0.2 log CFU/g) after 15 d of storage at 4 °C, while a small increase (0.8 log CFU/g) was observed at 10 °C on inoculated spinach leaves (Lopez-Velasco et al., 2010). In shredded carrots, *E. coli* O157:H7 populations (log CFU/g) have been shown to increase during the first three days of storage at 12 and 21 °C, followed by significant reductions on Day 14, likely due to exhaustion of available nutrients and increased competition from other bacteria (Abdul-Raouf et al., 1993). In this study, the intact nature of the carrot may have restricted the amount of available nutrients.

Temperature abuse at 10 °C resulted in significant re-growth (1.4 log CFU/g and 0.8 log CFU/g) by Day 7, compared to day 0, of native *Pseudomonas* sp. in POAA and NaClO washed carrots, respectively (Fig. 3). Interestingly, there was a significant increase of ~2.0 log CFU/g on plates containing cefotaxime (10 μg/ml) from 0 to 7 d of storage at 10 °C, but only for carrots washed with POAA (Fig. 4). Impermeability of the cell membrane is a common intrinsic mechanism of resistance to several biocides and antibiotic classes (Russell, 2002). Much of the inactivation of bacteria by POAA is due to the generation of reactive oxygen species that lead to DNA and lipid damage and increased permeability of the cell wall (Vandekinderen et al., 2009). Bactericidal action of cefotaxime, a cephalosporin antibiotic, is by inhibition of bacterial cell well synthesis (LefFrock et al., 1982). We hypothesize that POAA washing selected for bacteria that were intrinsically resistant to cefotaxime or potentially some strains that may have possessed genes conferring resistance to extended spectrum β-lactam antibiotics that were then able to grow under the abuse storage temperature at 10 °C.

3.5. Effect of extended storage on bacterial community composition of carrots

Minimally processed and peeled carrots typically have a storage life of 2–3 weeks at 3–5 °C (Suslow et al., 2002). However, it is likely that they are stored and consumed beyond the recommended period as they may retain acceptable sensory characteristics for longer periods. This study also evaluated the effect of extended (60 d) storage on the carrot bacterial communities. The bacterial community structures of carrots based on weighted Unifrac distances were overall similar to each other and not highly separated based on the days of storage (R = 0.15, Fig. 5). Despite this similarity in overall membership of the community the carrots stored for 60 d had significantly reduced bacterial community richness and evenness compared to carrots stored for 0, 7, or 14 d (Shannon, $p < 0.005$; Chao1, $p < 0.002$; Steel Dwass; Table S1). Analysis of 16S rRNA sequencing results indicated significant changes to the relative abundance of the *Proteobacteria, Firmicutes*, *Actinobacteria* and *Planctomycetes* on carrots at 60 d of storage at 2 °C compared to 0 d (Table S4). In 60 d stored carrots at 2 °C, relative abundance of *Proteobacteria* was significantly greater in unwashed (24.5%), tap water (19.8%) and sodium hypochlorite (31.3%) washed carrots compared to 0 d ($p < 0.043$, Wilcoxon). In contrast the extended storage was associated with decreases in the relative abundance of *Firmicutes Actinobacteria* and *Planctomycetes* (Table S4). Significant increases in some bacterial genera were also seen at 60 d of storage. Relative abundance of *Pseudomonas* was 17.8% higher in POAA washed carrots and *Yersinia* was 11.2% higher in unwashed carrots than their corresponding 0 d carrots ($p < 0.045$, Wilcoxon, Table S4). Psychotropic strains of genus *Pseudomonas*, *Erwinia*, and *Enterobacter* are commonly associated with post-harvest spoilage of refrigerated vegetables, while *Yersinia* spp. have been implicated in disease outbreaks (Brackett, 1994). These results indicate that storing beyond the recommended period may compromise the safety and quality of carrots.

Multiple pre-harvest and post-harvest interventions are worthy of consideration for reducing the carriage of human pathogens and antibiotic resistant bacteria on fresh vegetables. To our knowledge, this is

Table 2
Comparison of relative abundance (%) of dominant taxa on carrots dipped in dairy compost with antibiotics (unwashed) and after washing and storage at 2 °C and 10 °C for 7 days (includes both inoculated and non-inoculated).

Wash type	UNW	UNW	UNW	TAP	TAP	TAP	NaClO	NaClO	NaClO	POAA	POAA	POAA
Storage temperature/days of storage Taxa	Day 0	2 °C Day 7	10 °C Day 7	Day 0	2 °C Day 7	10 °C Day 7	Day 0	2 °C Day 7	10 °C Day 7	Day 0	2 °C Day 7	10 °C Day 7
Phylum												
Proteobacteria	58.60	**57.29**	**78.51**	69.40	**60.65**	**87.40**	47.07	**57.32**	**84.65**	68.69	**52.12**	**85.70**
Firmicutes	29.83	**30.89**	**16.33**	22.62	**28.45**	**9.07**	36.84	**30.62**	**12.51**	22.34	**33.49**	**11.55**
Actinobacteria	4.99	**5.51**	**2.20**	3.50	**4.56**	**1.88**	6.59	**4.83**	**1.40**	3.47	**6.01**	**1.26**
Plantomycetes	2.83	**2.92**	**1.43**	1.95	**2.50**	**0.74**	3.41	**2.31**	**0.67**	2.08	**3.17**	**0.64**
Acidobacteria	1.42	**1.26**	**0.35**	0.95	**1.90**	**0.24**	3.08	**2.29**	**0.09**	1.71	**2.47**	**0.28**
Other	2.33	**2.13**	**1.19**	1.95	**1.94**	**0.67**	3.01	**2.62**	**0.69**	1.71	**2.75**	**0.57**
Class												
Gammaproteobacteria	45.97	48.73	50.43	54.81	53.88	57.73	39.47	47.11	52.05	54.67	41.62	57.21
Bacilli	28.52	**29.58**	**15.81**	21.56	**25.23**	**8.82**	37.05	**29.46**	**11.98**	21.45	**31.83**	**11.17**
Betaproteobacteria	9.54	**5.62**	**24.56**	12.95	**9.98**	**27.04**	5.76	**7.27**	**30.39**	11.48	6.58	23.90
Alphaproteobacteria	2.77	2.58	3.39	1.36	1.05	2.53	1.67	2.49	2.12	2.24	3.50	4.52
Actinobacteria	4.04	**4.39**	**1.77**	2.79	**2.60**	**1.52**	5.60	3.78	1.15	2.81	**4.76**	**1.11**
Planctomycetia	2.56	**2.72**	**1.36**	1.77	**2.02**	**0.66**	3.47	**2.08**	**0.63**	1.88	**2.72**	**0.60**
Other	6.60	**6.37**	**2.67**	4.76	**5.23**	**1.68**	6.98	**7.81**	1.69	5.48	**8.98**	**1.50**
Order												
Pseudomonadales	26.12	**19.73**	**27.28**	35.70	26.09	32.75	25.48	25.43	28.47	34.55	**21.29**	**37.18**
Bacillales	28.30	**29.49**	**15.49**	21.17	**27.13**	**8.76**	36.77	**29.26**	**11.88**	21.15	**31.76**	**10.81**
Enterobacterilaes	17.53	26.83	22.34	17.47	19.24	24.30	12.14	19.53	23.25	17.33	17.88	19.49
Burkholderiales	9.53	**5.59**	**24.55**	12.94	**11.28**	**27.03**	5.75	**7.25**	**30.37**	11.46	**6.51**	**23.89**
Actinomycetales	4.03	**4.37**	**1.76**	2.79	**3.59**	**1.52**	5.59	3.78	1.15	2.80	**4.73**	**1.11**
Rhizobiales	2.43	2.18	1.95	1.12	1.18	0.90	1.42	1.79	0.78	1.42	**2.71**	**0.80**
Xanthomonadales	1.76	**1.72**	**0.62**	1.25	**1.19**	**0.45**	1.22	**1.52**	**0.20**	2.34	**1.72**	**0.49**
Pirellulales	2.07	**2.10**	**1.09**	1.51	**1.77**	**0.57**	3.00	**1.63**	**0.52**	1.50	**2.07**	**0.50**
RB41	0.77	**0.54**	**0.08**	0.34	**1.28**	**0.14**	0.53	**1.42**	**0.04**	0.97	**1.44**	**0.10**
Other	7.37	**7.42**	**4.78**	5.66	**7.17**	**3.54**	8.05	**8.31**	**3.32**	6.37	**9.79**	**5.60**
Family												
Pseudomonadaceae	25.57	19.39	26.96	35.28	25.84	32.69	25.21	24.94	28.35	34.01	**21.00**	**37.11**
Enterobacteriaceae	17.53	26.83	22.34	17.47	19.24	24.30	12.14	19.53	23.25	17.33	17.88	19.49
Oxalobacteriaceae	9.14	**5.16**	**24.18**	12.72	**10.97**	**26.78**	5.52	**7.00**	**30.23**	11.14	**6.10**	**23.71**
Bacillaceae	14.80	**14.69**	**8.07**	10.77	**14.26**	**4.65**	19.85	14.47	5.89	10.09	**15.81**	**5.60**
Paenibacillaceae	4.60	**5.85**	**2.72**	3.15	**4.40**	**1.35**	5.33	4.11	2.05	2.84	4.97	2.08
Bacillales, other	3.54	**3.96**	**1.97**	2.84	**3.94**	**1.25**	5.53	**4.74**	**1.79**	3.16	**5.22**	**1.50**
Planococcaceae	3.94	3.19	1.72	3.18	**3.09**	**0.96**	4.00	**4.36**	**1.33**	3.96	**4.13**	**0.90**
Pirellulaceae	2.07	2.10	1.09	1.51	**1.77**	**0.57**	3.00	**1.63**	**0.52**	1.50	**2.07**	**0.50**
Ellin6075	0.77	**0.54**	**0.08**	0.34	**1.28**	**0.14**	0.53	**1.42**	**0.04**	0.97	**1.44**	**0.10**
Norcardiopsaceae	1.69	**1.63**	**0.72**	1.38	1.40	0.50	2.47	1.81	0.38	1.29	**1.80**	**0.41**
Other	16.36	**16.65**	**10.16**	11.38	**13.80**	**6.81**	16.44	**15.99**	**6.18**	13.71	**19.59**	**8.59**
Genus												
Pseudomonas	23.23	17.03	23.63	39.23	22.99	30.87	22.77	22.84	27.22	25.37	**18.32**	**35.31**
Yersinia	9.42	17.26	14.58	14.63	15.18	20.55	8.25	15.66	21.03	12.36	14.01	17.77
Janthinobacterium	9.03	**5.13**	**24.05**	14.88	**10.89**	**26.66**	5.44	**6.92**	**30.16**	9.22	**5.91**	**23.67**
Bacillus	8.88	**7.70**	**4.00**	3.93	**8.12**	**2.10**	10.53	**8.14**	**2.89**	6.74	**9.37**	**2.76**
Escherichia	5.47	6.42	3.84	4.05	5.57	2.31	8.50	5.82	2.81	4.84	5.86	2.60
Geobacillus	3.54	**3.96**	**1.97**	2.31	3.94	1.25	5.53	4.74	1.79	3.58	5.22	1.50
Bacillales, other	7.26	**8.08**	**6.15**	2.50	**3.07**	**2.20**	3.21	**2.34**	**0.94**	3.29	**2.37**	**0.81**
Pseudomonadaceae, other	2.34	2.36	3.33	1.57	2.85	1.83	2.44	2.09	1.13	4.22	2.68	1.80
Enterobacteriaceae, other	0.81	1.25	1.55	1.05	0.97	1.46	0.65	1.49	1.20	1.06	1.42	0.86
Ureibacillus	1.16	**2.06**	**0.77**	0.75	**1.56**	0.49	1.68	1.51	0.77	1.19	**1.94**	**0.55**
Other	28.62	**28.62**	**16.06**	15.04	**24.68**	10.23	30.82	**28.23**	**10.02**	27.85	**32.68**	**12.29**

Unwashed carrots (UNW), Tap water washed carrots (TAP), sodium hypochlorite washed carrots (NaClO), peroxyacetic acid washed carrots (POAA).
Bolded scripts, significant change in relative abundance with temperature change on day 7 ($p < 0.05$, Wilcoxon).
Plain scripts, no significant differences between Day 0 unwashed and sanitizer-washed carrots and no significant difference by change in temperature on Day 7 ($p > 0.05$, Wilcoxon).

the first comprehensive study of the effect of sanitizer washing, cold storage, and storage time on the survival and re-growth of ARB on carrots together with shifts in surficial bacterial community composition. Results of this study indicated that sanitizer washing in combination with storage at optimum temperature (2 °C) may offer strategies to reduce some ARB, including pathogenic and spoilage-associated bacteria, on fresh vegetables. Further, profiling effects of these post-harvest condition on surficial carrot bacterial communities and examination of responses of specific taxonomic groups provided insight into mechanistic actions of the sanitizers and microbial ecological factors at play in re-growth. At present, the possibility of cross-resistance between biocides, such as sanitizers, and resistance to antibiotics is recognized, however the mechanisms associated with such resistance is not well characterized. Future studies should examine these mechanisms and also determine the potential risks associated with ingestion and other exposures to antibiotic resistant bacteria in food.

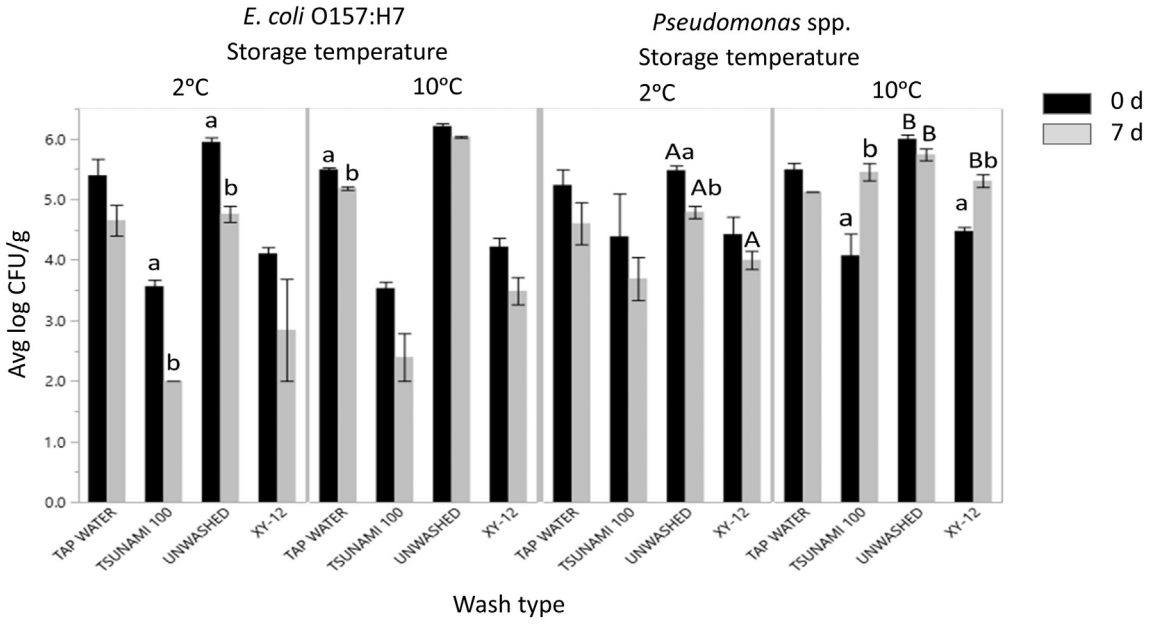

Fig. 3. Comparison of the effect of sanitizer wash on the average log CFU/g of *E. coli* O157:H7 and native *Pseudomonas* sp. recovered from inoculated dairy compost-dipped carrots at Day 0 and Day 7 of storage under both optimal temperature (2 °C) and temperature-abuse (10 °C) storage conditions (n = 6).
Error bars represent standard error from the mean.
Capital letters denote significant differences between storage temperature within the same wash type and day ($p < 0.05$). Lower-case letters denote significant differences between day within wash type and storage temperature ($p < 0.05$). Absence of letters denote there were no significant differences between storage temperature or day of storage within wash type.

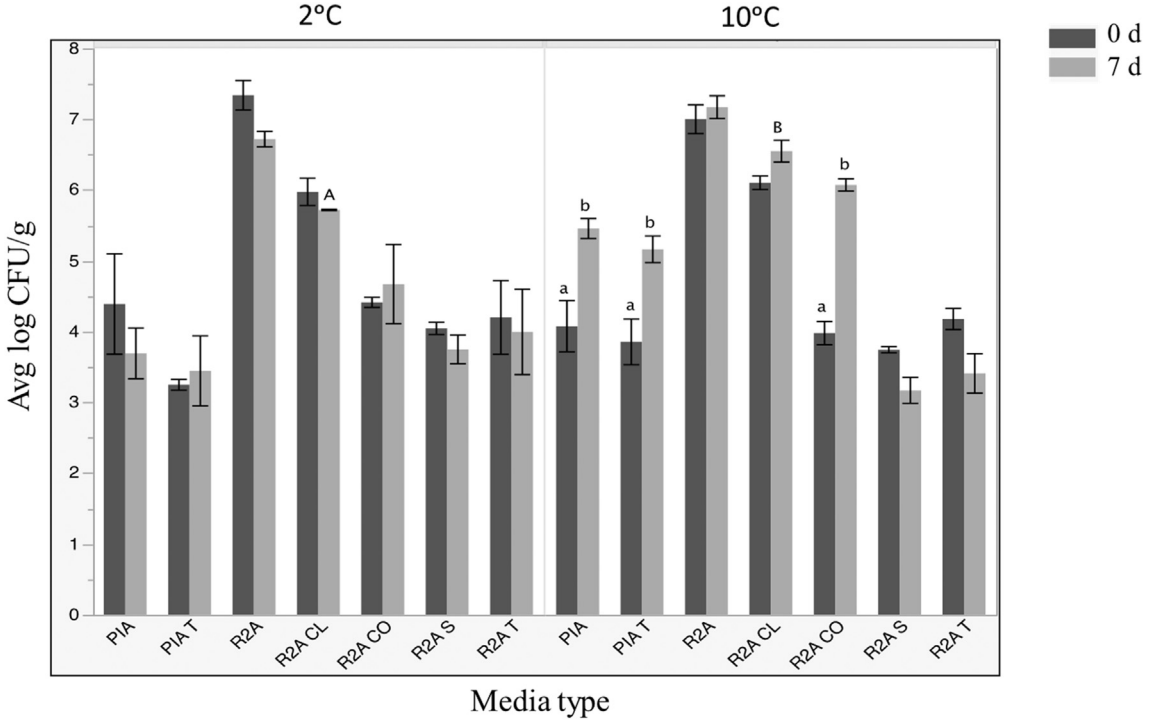

Fig. 4. Comparison of the average log CFU/g recovered from POAA-washed carrots dipped in tetracycline-resistant Pseudomonas sp. and antibiotic resistant *E. coli* O157: H7 inoculated dairy compost on different media types after different washes after 0 d and 7 d storage under both optimal (2 °C) and abuse (10 °C) temperature storage conditions (n = 6). PIA = native *Pseudomonas* sp., PIA T = *Pseudomonas* sp. resistant to tetracycline (4 μg/ml). HPC bacteria were enumerated on R2A media without antibiotics (R2A), with clindamycin (CL) (25 μg/ml), with cefotaxime (CO) (10 μg/ml) with (S) sulfamethoxazole (100 μg/ml), or with tetracycline (T) (3 μg/ml).
Error bars represent standard error from the mean.
Capital letters denote significant differences between storage temperatures on the same day (Day 7) of storage within the same media type (ANOVA, $p < 0.05$). Lower-case letters denote significant differences between days for the same storage temperature and media type (ANOVA, $p < 0.05$).

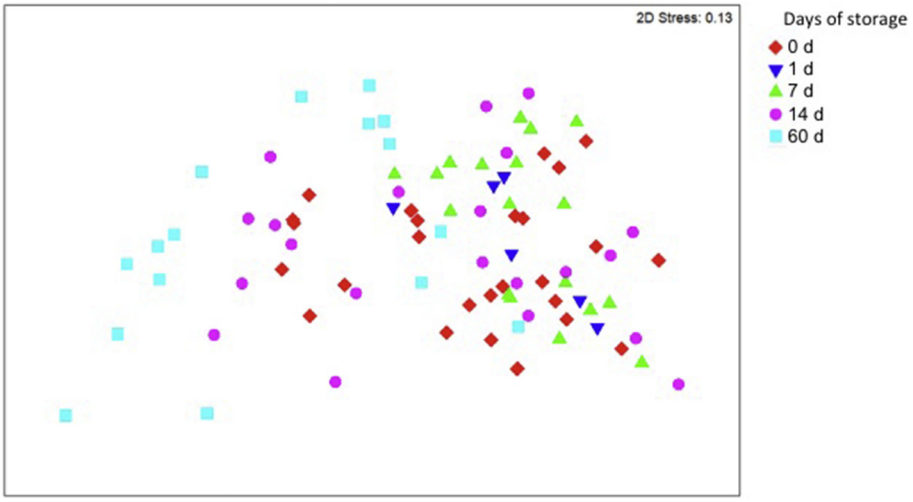

Fig. 5. Non-metric multidimensional scaling analysis of weighted Unifrac distance matrices of bacterial microbiota associated with carrot surfaces stored for 0, 1, 7, 14, and 60 days. Each point represents one carrot sample (ANOSIM, $R = 0.15$). ANOSIM, $R < 0.25$ indicates no separation of bacterial community taxonomic structures among days of storage.

Acknowledgements

The authors thank Dr. Katharine Knowlton, Dr. Partha Ray, and Chrissy Teets for providing the compost b. The authors also thank Dr. Giselle Guron, Kim Waterman, Kendall Fogler, Nicholas Poe, and Alvis Huynh for their assistance in the laboratory. Lastly, the authors thank Dr. Pang Du, Dr. Pan Ji, and Dr. Emily Garner for assistance with data analysis.

Funding

This work was supported by the USDA NIFA-AFRI #2014-05280 and the Virginia Agricultural Experiment Station and the Hatch Program of the National Institute of Food and Agriculture, U.S. Department of Agriculture. The funders had no role in the study design, collection of data, analysis or writing of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2018.11.006.

References

Abdul-Raouf, U., Beuchat, L., Ammar, M., 1993. Survival and growth of *Escherichia coli* O157: H7 on salad vegetables. Appl. Environ. Microbiol. 59, 1999–2006.

Alegbeleye, O.O., Singleton, I., Sant'Ana, A.S., 2018. Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: a review. Food Microbiol. 73, 177–208.

Allard, S.M., Walsh, C.S., Wallis, A.E., Ottesen, A.R., Brown, E.W., Micallef, S.A., 2016. *Solanum lycopersicum* (tomato) hosts robust phyllosphere and rhizosphere bacterial communities when grown in soil amended with various organic and synthetic fertilizers. Sci. Total Environ. 573, 555–563.

Allydice-Francis, K., Brown, P.D., 2012. Diversity of antimicrobial resistance and virulence determinants in *Pseudomonas aeruginosa* associated with fresh vegetables. Int. J. Food Microbiol. 2012, 426241. https://doi.org/10.1155/2012/426241. (7 pages).

Baldani, J.I., Rouws, L., Cruz, L.M., Olivares, F.L., Schmid, M., Hartmann, A., 2014. The family Oxalobacteraceae, the Prokaryotes. Springer, pp. 919–974.

Banach, J.L., Sampers, I., Haute, S.V., van der Fels-Klerx, 2015. Effect of disinfectants on preventing the cross-contamination of pathogens in fresh produce washing water. Int. J. Environ. Res. Public Health 12, 8658–8677.

Berger, C.N., Sodha, S.V., Shaw, R.K., Griffin, P.M., Pink, D., Hand, P., Frankel, G., 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environ. Microbiol. 12, 2385–2397.

Beuchat, L.R., Adler, B.B., Lang, M.M., 2004. Efficacy of chlorine and a peroxyacetic acid sanitizer in killing *Listeria monocytogenes* on iceberg and romaine lettuce using simulated commercial processing conditions. J. Food Prot. 67, 1238–1242.

Bezanson, G., MacInnis, R., Potter, G., Hughes, T., 2008. Presence and potential for horizontal transfer of antibiotic resistance in oxidase-positive bacteria populating raw salad vegetables. Int. J. Food Microbiol. 127, 37–42.

Blaak, H., van Hoek, A.H., Veenman, C., van Leeuwen, A.E.D., Lynch, G., van Overbeek, W.M., de Roda Husman, A.M., 2014. Extended spectrum ß-lactamase-and constitutively AmpC-producing Enterobacteriaceae on fresh produce and in the agricultural environment. Int. J. Food Microbiol. 168, 8–16.

Brackett, R.E., 1994. Microbiological spoilage and pathogens in minimally processed refrigerated fruits and vegetables. In: Minimally Processed Refrigerated Fruits & Vegetables. Springer, pp. 269–312.

Cabot, G., Zamorano, L., Moyà, B., Juan, C., Navas, A., Blázquez, J., Oliver, A., 2016. Evolution of *Pseudomonas aeruginosa* antimicrobial resistance and fitness under low and high mutation rates. Antimicrob. Agents Chemother. 60, 1767–1778.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. PNAS 108, 4516–4522.

Chang, A.S., Schneider, K.R., 2012. Evaluation of overhead spray applied sanitizers for the reduction of *Salmonella* on tomato surfaces. J. Food Sci. 77, M65–M69.

Chao, A., 1984. Nonparametric estimation of the number of classes in a population. Scand. J. Stat. 265-270.

Clarke, K.R., Warwick, R.M., 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 1st edn. Plymouth Marine Laboratory, Plymouth, UK.

Droffner, M.L., Brinton Jr., W.F., Evans, E., 1995. Evidence for the prominence of well characterized mesophilic bacteria in thermophilic (50–70 °C) composting environments. Biomass Bioenergy 8, 191–195.

FDA, 2018. FSMA final rule on produce safety. Standards for growing, harvesting, packing, and holding of produce for human consumption. Accessed June 8, 2017 from. https://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334114.htm.

Ferens, W.A., Hovde, C.J., 2011. *Escherichia coli* O157: H7: animal reservoir and sources of human infection. Foodborne Pathog. Dis. 8, 465–487.

Francis, G., O'Beirne, D., 2001. Effects of vegetable type, package atmosphere and storage temperature on growth and survival of *Escherichia coli* O157: H7 and *Listeria monocytogenes*. J. Ind. Microbiol. Biotechnol. 27, 111–116.

Gil, M.I., Selma, M.V., Suslow, T., Jacxsens, L., Uyttendaele, M., Allende, A., 2015. Pre- and postharvest preventive measures and intervention strategies to control microbial food safety hazards of fresh leafy vegetables. Crit. Rev. Food Sci. Nutr. 55, 453–468.

Gillis, M., De Ley, J., 2006. The genera *Chromobacterium* and *Janthinobacterium*. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., Stackebrandt, E. (Eds.), The Prokaryotes. Springer, New York, pp. 737–746.

Haas, B.J., Gevers, D., Earl, A.M., Feldgarden, M., Ward, D.V., Giannoukos, G., Ciulla, D., Tabbaa, D., Highlander, S.K., Sodergren, E., 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res. 21, 494–504.

Hazzard, R., 2013. UMass extension vegetable program. From field to storage: high quality carrots. In: New England Vegetable and Fruit Conference. Accessed Nov 7, 2017 from. https://ag.umass.edu/sites/ag.umass.edu/files/pdf-doc-ppt/postharvest_for_carrots_nevfc_12_16_2013.pdf.

Ingraham, J.L., 1958. Growth of psychrophilic bacteria. J. Bacteriol. 76, 75–80.

Inoue, K., Inanaga, S., Someya, T., 2005. Survival of pathogenic bacteria in compost with special reference to *Escherichia coli*. J. Environ. Sci. 17, 770–774.

Jackson, C.R., Randolph, K.C., Osborn, S.L., Tyler, H.L., 2013. Culture dependent and independent analysis of bacterial communities associated with commercial salad leaf vegetables. BMC Microbiol. 13, 274.

Kangas, S., Takkinen, J., Hakkinen, M., Nakary, U.-M., Johansson, T., Henttonen, Virtaluoto, L., Siitonen, A., Ollgren, J., Kuusi, M., 2008. *Yersinia pseudotuberculosis* O: 1 traced to raw carrots, Finland. Emerg. Infect. Dis. 14, 1959–1961.

Leff, J.W., Fierer, N., 2013. Bacterial communities associated with the surfaces of fresh fruits and vegetables. PLoS One 8, e59310.

LefFrock, J.L., Prince, R.A., Left, R.D., 1982. Mechanism of action, antimicrobial activity, pharmacology, adverse effects, and clinical efficacy of cefotaxime. Pharmacotherapy 2, 174–184.

Livermore, D.M., 2002. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? Clin. Infect. Dis. 34, 634–640.
López-Gálvez, F., Allende, A., Selma, M.V., Gil, M.I., 2009. Prevention of *Escherichia coli* cross-contamination by different commercial sanitizers during washing of fresh-cut lettuce. Int. J. Food Microbiol. 133, 167–171.
Lopez-Velasco, G., Davis, M., Boyer, R.R., Williams, R.C., Ponder, M.A., 2010. Alterations of the phylloepiphytic bacterial community associated with interactions of *Escherichia coli* O157: H7 during storage of packaged spinach at refrigeration temperatures. Food Microbiol. 27, 476–486.
Lopez-Velasco, G., Welbaum, G., Boyer, R., Mane, S., Ponder, M., 2011. Changes in spinach phylloepiphytic bacteria communities following minimal processing and refrigerated storage described using pyrosequencing of 16S rRNA amplicons. J. Appl. Microbiol. 110, 1203–1214.
Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235.
Luo, Y., He, Q., McEvoy, J.L., 2010. Effect of storage temperature and duration on the behavior of *Escherichia coli* O157: H7 on packaged fresh cut salad containing romaine and iceberg lettuce. J. Food Sci. 75, M390–M397.
Mandic-Mulec, I., Stefanic, P., van Elsas, J.D., 2016. Ecology of Bacillaceae, the bacterial spore: from molecules to systems. Am. Soc. Microbiol. 59–85.
Marti, R., Scott, A., Tien, Y.-C., Murray, R., Sabourin, L., Zhang, Y., Topp, E., 2013. Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. Appl. Environ. Microbiol. 79, 5701–5709.
Masella, A.P., Bartram, A.K., Truszkowski, J.M., Brown, D.G., Neufeld, J.D., 2012. PANDAseq: paired-end assembler for illumina sequences. BMC Bioinformatics 13, 31.
Mokhtari, W., Nsaibia, S., Majouri, D., Ben Hassen, A., Gharbi, A., Aouni, M., 2012. Detection and characterization of *Shigella* species isolated from food and human stool samples in Nabeul, Tunisia, by molecular methods and culture techniques. J. Appl. Microbiol. 113, 209–222.
Muniesa, M., Hammerl, J.A., Hertwig, S., Appel, B., Brüssow, H., 2012. Shiga toxin-producing *Escherichia coli* O104: H4: a new challenge for microbiology. Appl. Environ. Microbiol. 78, 4065–4073.
Niemira, B.A., 2007. Relative efficacy of sodium hypochlorite wash versus irradiation to inactivate *Escherichia coli* O157: H7 internalized in leaves of Romaine lettuce and baby spinach. J. Food Prot. 70, 2526–2532.
Ottesen, A.R., Peña, A.G., White, J.R., Pettengill, J.B., Li, C., Allard, S., Rideout, S., Allard, M., Hill, T., Evans, P., 2013. Baseline survey of the anatomical microbial ecology of an important food plant: *Solanum lycopersicum* (tomato). BMC Microbiol. 13, 114.
Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J., Griffin, P.M., 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerg. Infect. Dis. 19, 407.
Palleroni, N.J., 1981. Introduction to the family Pseudomonadaceae. In: The Prokaryotes. Springer, pp. 655–665.
Prado-Silva, L., Cadavez, V., Gonzales-Barron, U., Rezende, A.C.B., Sant'Ana, A.S., 2015. Meta-analysis of the effects of sanitizing treatments on *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* inactivation in fresh produce. AEM 81, 8008–8021.
Qian, X., Sun, W., Gu, J., Wang, X.-J., Sun, J.-J., Yin, Y.-N., Duan, M.-L., 2016. Variable effects of oxytetracycline on antibiotic resistance gene abundance and the bacterial community during aerobic composting of cow manure. J. Hazard. Mater. 315, 61–69.
Ragaert, P., Devlieghere, F., Debevere, J., 2007. Role of microbiological and physiological spoilage mechanisms during storage of minimally processed vegetables. Postharvest Biol. Technol. 44, 185–194.
Rahube, T.O., Marti, R., Scott, A., Tien, Y.-C., Murray, R., Sabourin, L., Zhang, Y., Duenk, P., Lapen, D.R., Topp, E., 2014. Impact of fertilizing with raw or anaerobically digested sewage sludge on the abundance of antibiotic-resistant coliforms, antibiotic resistance genes, and pathogenic bacteria in soil and on vegetables at harvest. Appl. Environ. Microbiol. 80, 6898–6907.
Rangel, J.M., Sparling, P.H., Crowe, C., Griffin, P.M., Swerdlow, D.L., 2005. Epidemiology of *Escherichia coli* O157: H7 outbreaks, United States, 1982–2002. Emerg. Infect. Dis. 11, 603.
Raphael, E., Wong, L.K., Riley, L.W., 2011. Extended-spectrum beta-lactamase gene sequences in gram-negative saprophytes on retail organic and nonorganic spinach. Appl. Environ. Microbiol. 77, 1601–1607.
Ray, P., Chen, C., Knowlton, K.F., Pruden, A., Xia, K., 2017. Fate and effect of antibiotics in beef and dairy manure during static and turned composting. J. Environ. Qual. 46, 45–54.
Rodgers, S.L., Cash, J.N., Siddiq, M., Ryser, E.T., 2004. A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157: H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. J. Food Prot. 67, 721–731.
Russell, A.D., 2002. Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. J. Appl. Microbiol. 92, 121S–135S.
Shannon, C.E., Weaver, W., 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana Illinois (117 pp).
Sharma, R., Larney, F.J., Chen, J., Yanke, L.J., Morrison, M., Topp, E., McAllister, T.A., Yu, Z., 2009. Selected antimicrobial resistance during composting of manure from cattle administered sub-therapeutic antimicrobials. J. Environ. Qual. 38, 567–575.
Shen, C., Luo, Y., Nou, X., Wang, Q., Millner, P., 2013. Dynamic effects of free chlorine concentration, organic load, and exposure time on the inactivation of *Salmonella*, *Escherichia coli* O157: H7, and non-O157 Shiga toxin–producing *E. coli*. J. Food Prot. 76, 386–393.
Söderqvist, K., Ahmed Osman, O., Wolff, C., Bertilsson, S., Vågsholm, I., Boqvist, S., 2017. Emerging microbiota during cold storage and temperature abuse of ready-to-eat salad. Infect. Ecol. Epidemiol. 7, 1328963.
Su, J.-Q., Wei, B., Ou-Yang, W.-Y., Huang, F.-Y., Zhao, Y., Xu, H.-J., Zhu, Y.-G., 2015. Antibiotic resistome and its association with bacterial communities during sewage sludge composting. Environ. Sci. Technol. 49, 7356–7363.
Surette, M.A., Sturz, A.V., Lada, R.R., Nowak, J., 2003. Bacterial endophytes in processing carrots (*Daucus carota* L. var. sativus): their localization, population density, biodiversity and their effects on plant growth. Plant Soil 253, 381–390.
Suslow, T.V., Mitchell, J., Cantwell, M., 2002. Carrots: Recommendations for Maintaining Post-harvest Quality. Department of Vegetable Crops, University of California, Davis, CA Accessed November 11, 2017 from. http://postharvest.ucdavis.edu/Commodity_Resources/Fact_Sheets/Datastores/Vegetables_English/?uid=9&ds=799.
Suzuki, M.T., Taylor, L.T., Delong, E.F., 2000. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5′-nuclease assays. Appl. Environ. Microbiol. 66, 4605–4614.
Vandekinderen, I., Devlieghere, F., De Meulenaer, B., Ragaert, P., Van Camp, J., 2009. Optimization and evaluation of a decontamination step with peroxyacetic acid for fresh-cut produce. Food Microbiol. 26, 882–888.
Wang, L., Oda, Y., Grewal, S., Morrison, M., Michel, F.C., Yu, Z., 2012. Persistence of resistance to erythromycin and tetracycline in swine manure during simulated composting and lagoon treatments. Microb. Ecol. 63, 32–40.
Wang, L., Gutek, A., Grewal, S., Michel, F.C., Yu, Z., 2015. Changes in diversity of cultured bacteria resistant to erythromycin and tetracycline in swine manure during simulated composting and lagoon storage. Lett. Appl. Microbiol. 61, 245–251.
Wendel, A.M., Johnson, D.H., Sharapov, U., Grant, J., Archer, J.R., Monson, T., Koschmann, C., Davis, J.P., 2009. Multistate outbreak of Escherichia coli O157: H7 infection associated with consumption of packaged spinach, August–September 2006: the Wisconsin investigation. Clin. Infect. Dis. 48, 1079–1086.
Williams, R.K., 2016. Effect of Composting on the Prevalence of Antibiotic Resistant Bacteria and Resistance Genes in Cattle Manure (Master's Thesis). Virginia Tech, Blacksburg, VA.
Williams-Nguyen, J., Sallach, J.B., Bartelt-Hunt, S., Boxall, A.B., Durso, L.M., McLain, J.E., Singer, R.S., Snow, D.D., Zilles, J.L., 2016. Antibiotics and antibiotic resistance in agroecosystems: state of the science. J. Environ. Qual. 45, 394–406.