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Salmonella inactivation and cross-contamination on cherry and grape tomatoes under simulated wash conditions

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ABSTRACT

Washing in chlorinated water is widely practiced for commercial fresh produce processing. While known as an effective tool for mitigating food safety risks, chlorine washing could also represent an opportunity for spreading microbial contaminations under sub-optimal operating conditions. This study evaluated *Salmonella* inactivation and cross-contamination in a simulated washing process of cherry and grape tomatoes. Commercially harvested tomatoes and the associated inedible plant matter (debris) were differentially inoculated with kanamycin resistant (KanR) or rifampin resistant (RifR) *Salmonella* strains, and washed together with uninoculated tomatoes in simulated packinghouse dump tank (flume) wash water. Washing in chlorinated water resulted in significantly higher *Salmonella* reduction on tomatoes than on debris, achieving 2–3 log reduction on tomatoes and about 1 log reduction on debris. Cross-contamination by *Salmonella* on tomatoes was significantly reduced in the presence of 25–150 mg/L free chlorine, although sporadic cross-contamination on tomatoes was detected when tomatoes and debris were inoculated at high population density. The majority of the sporadic cross-contaminations originated from *Salmonella* inoculated on debris. These findings suggested that debris could be a potentially significant source of contamination during commercial tomato washing.

1. Introduction

Salmonella enterica is responsible for over one million infections and the most hospitalizations related to foodborne illness each year in the United States (CDC, 2018a; Scallan et al., 2011). Several foodborne illness outbreaks have been associated with *Salmonella* contamination on tomatoes (Bennett et al., 2015; Jackson et al., 2013; Valadez et al. (2013)). The complex supply chain from farm-to-fork can expose produce to multiple opportunities for pathogen contamination (Sivapalasingam et al., 2004). Between 2004 and 2007, two *Salmonella* outbreaks associated with tomato were linked to potential contamination at packinghouse operations (Bennett et al., 2015), which led to industry-wide adoption of the current metrics targeting packinghouse sanitation. Currently, washing tomatoes, including cherry and grape varieties, in dump tanks or flumes of chlorinated water is commonly practiced at packinghouses in major Eastern U.S. production regions. This process has the potential to reduce bacterial populations of both public health and market disease significance, but can also provide a medium for pathogen cross-contamination if an insufficient concentration of sanitizer is present in the wash water.

Several studies examined the internalization of phytopathogens (e.g., *Pectobacterium carotovorum*) (Bartz, 1982, 1999; Bartz et al., 2015) and human pathogens (Xia et al., 2012; Zhou et al., 2014a) during tomato washing, and evaluated the efficacy of sanitizer concentrations in dump tank water in reducing microbial loads on tomato fruits (De et al., 2018; Schneider et al., 2017). In addition, several studies also examined cross-contamination of *Salmonella* during simulated flume washing (Gereffi et al., 2015; Sreedharan et al., 2017).

Guidelines pertaining to Florida food safety regulations, including Tomato Good Agricultural Practices (T-GAPS), and Tomato Best Management Practices (T-BMP), require 150 mg/L hypochlorous acid (free chlorine) or an appropriate level of other effective sanitizer in

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dump tank (or flume) water to prevent pathogen survival and cross-contamination. In the absence of abundant data on the survival and cross-contamination of human pathogens in tomato dump tank water at the time these guidance documents were developed, the 150 mg/L limit was chosen based on conditions needed to prevent tomato market disease (soft rot) caused by phytopathogens (Bartz, 1988; Bartz et al., 2001; Wei et al., 1995). In re-examining these guidelines, Gereffi et al. (2015) observed effective prevention of *S. enterica* cross-contamination in simulated tomato wash water containing 25 mg/L free chlorine. In another recent study, it was reported that 100 mg/L free chlorine effectively prevented cross-contamination in wash water with a moderate to high level of organic load (Sreedharan et al., 2017). In both these studies, mature green round tomatoes were washed, and sterilized commercial topsoils were used as the source of organic matter for modulating wash water chlorine demand.

It is well established that organic matter (often in the form of leaf debris, soil, and fruit waxes) can accumulate in commercially operated tomato dump tanks or flumes (Wei et al., 1995; Zhou et al., 2014b), which can reduce the efficacy of free chlorine for pathogen inactivation (Fu et al., 2018; Teng et al., 2018; Zhou et al., 2015). However, few studies have examined the role of plant matter in spreading potential *Salmonella* contamination. The purpose of this study was to examine the inactivation and cross-contamination of *S. enterica* during simulated cherry and grape tomato washing, with emphasis on the effect of the initial pathogen carrier. Organic matter was introduced by washing batches of fresh tomatoes in tap water, simulating commercial packinghouse dump tank operations. In addition to tomato fruit, debris (primarily non-edible leaf and stem matter co-harvested with tomatoes) was also examined as a *Salmonella* carrier for cross-contamination, owing to its common presence in commercial dump tank water and potential role in product contamination.

2. MATERIALS and METHODS

2.1. Tomatoes

Freshly harvested cherry (Trials I and II) and grape (Trial III) tomatoes were obtained from commercial tomato packinghouses located in Florida (Trial I and II) and Maryland (Trial III) at respective peak harvest seasons. Approximately 50 kg of tomatoes with associated plant matter such as tomato leaves and stems (termed henceforth as debris) were withdrawn randomly from the packinghouse production lines prior to dump tank washing, shipped overnight to the Food Quality Laboratory (FQL), Beltsville Agricultural Research Center (BARC), USDA ARS, in Beltsville, MD, and stored at 15 °C for up to 48 h before being used in washing experiments.

2.2. Bacterial strains and preparation of inoculum

All six *S. enterica* strains (Table 1) used for this study were from collections hosted at the Environmental Microbial and Food Safety Laboratory (EMFSL), BARC. FS3087 was derived from ATCC 14028 by P22 transduction of kanamycin resistance gene *npt*III. These strains, which were isolated from diverse food, animal, or clinical sources, represented five serovars and each was resistant to either kanamycin or rifampin. Pre-experimental testing (data not shown) indicated they were not significantly different in their capacity to bind to and detach from tomatoes and debris, and in sensitivity to chlorine treatment. Each individual *Salmonella* strain was streaked from a −80 °C freezer stock onto Xylose Lysine Tergitol 4 agar (XLT4; Becton-Dickenson [BD], Sparks, MD) supplemented with either 50 μg/ml kanamycin or 50 μg/ml rifampin, and grown for 24 h at 37 °C. Single colonies from each of these plates were inoculated into Tryptic Soy Broth (TSB; BD) supplemented with either 50 μg/ml kanamycin or 50 μg/ml rifampin and incubated overnight at 37 °C. Cells were pelleted by centrifugation at 4500 *g* for 5 min, washed once to remove residual nutrient broth, and re-suspended in phosphate buffered saline (PBS; Corning, Corning, NY). Bacterial cell density of each strain was determined by measuring OD_{600} with a spectrophotometer (Shimadzu, Kyoto, Japan), and adjusted to OD_{600} = 0.3. The three strains with common antibiotic resistance markers were combined in equal parts to form *Salmonella* cocktails of either kanamycin resistant strains (Se-KanR) or rifampin resistant strains (Se-RifR). The two cocktails were further diluted 100 times in sterile H_2O when necessary (for low inoculation) and used for differential inoculation of tomatoes and debris.

2.3. Inoculation of tomatoes and debris

Upon receipt of tomatoes, a portion was sorted to cull fruits that were overly or under-ripened, injured, or diseased, and to separate mature tomatoes with optimal visual quality from debris, which were subsequently inoculated with Se-KanR and Se-RifR cocktails, respectively. For tomato inoculation, 600 g of cherry tomatoes (average size 25 g/ea) or grape tomatoes (average size 13 g/ea) were individually marked with a waterproof marker and immersed in 1.2 L Se-KanR inoculation cocktail for 5 min with gentle agitation to allow for adherence of *Salmonella* to the fruits. Thereafter, liquid inoculum was carefully drained out of the container, and tomatoes were dried in a biosafety cabinet for 1 h at room temperature (Sapers and Jones, 2006). For debris inoculation, 7.5 g of debris was immersed in 37.5 ml Se-RifR inoculation cocktail in a Whirl-Pak bag lined with 0.3 mm filter (Nasco, Fort Atkinson, WI) for 5 min to allow for adherence of Se-RifR onto debris. Liquid inoculum, separated by hand-squeezing the debris, was drained out from the filter bag, and debris was transferred to a new filter bag for overnight storage. After inoculation, both tomatoes and debris were stored at 15 °C overnight (~18 h) prior to being used for washing experiments. Both tomatoes and debris were inoculated using inoculum cocktails at 8 log CFU/ml or 6 log CFU/ml, which resulted in recoverable populations of *Salmonella* on tomatoes and debris at high population density (HPD) or low population density (LPD), respectively.

2.4. Wash water preparation

To generate simulated wash water of commercial dump tank operations, water to be used for tomato washing in this study was prepared by soaking 30 kg of unsorted fresh cherry or grape tomatoes in 30 L of tap water for 30 min, with continuous moderate agitation. After removal of tomatoes and large pieces of debris, wash water was assessed by measuring various physicochemical parameters including

Table 1
Salmonella enterica strains used in this study.

Strain	Serovar	Antibiotic Resistance	Isolation source	Reference
FS3087	Typhimurium	Kanamycin	Animal tissue	ATCC 14028
SARA33	Heidelberg	Kanamycin	Human	Achtman et al. (2013)
SARB11	Derby	Kanamycin	Turkey	Achtman et al. (2013)
SL1344	Typhimurium	Rifampin	Calf	Hoiseth and Stocker (1981)
MDD314	Newport	Rifampin	Tomato	Greene et al. (2008)
USDA4559	Braenderup	Rifampin	Isolate # 95-682-997	Patel and Sharma (2010)

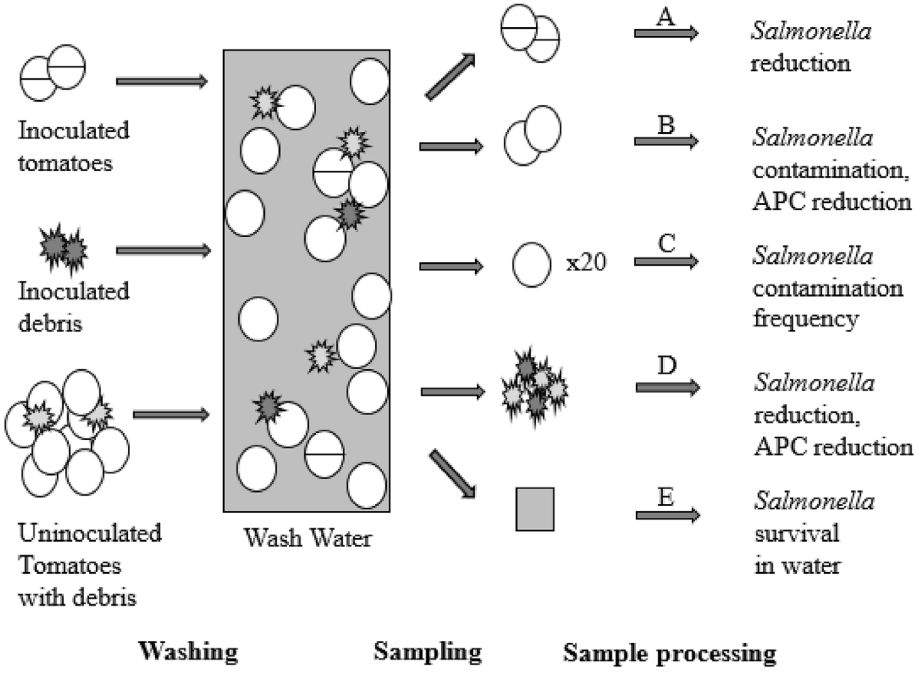

Fig. 1. Schematic presentation of experiment flow and sample assignment, as described in section 2.5 of materials and methods.

total dissolved solids (TDS), chemical oxygen demand (COD) and chlorine demand (CLD) (Luo et al., 2012; Zhou et al., 2014b). Then, wash water was chlorinated with 5.75% sodium hypochlorite (Clorox, Oakland, CA) to target concentrations of 25–150 mg/L, with pH adjusted to approximately 6.5 using hydrochloric acid as acidulant. Free chlorine was measured by the DPD (*N*,*N*-diethyl-*p*-phenyldiamine) method, using a free chlorine photometer (H.F Scientific, Fort Myers, Fl) and following manufacturer's instructions.

2.5. Tomato washing and sample collection

The flow of the washing experiment and the sample assignment are schematically summarized in Fig. 1. Each washing at different chlorine concentrations was conducted in a 2 or 4 L sterile propylene basin at a tomato to wash water ratio of 1:4 (w/v) with water depth 5–8 cm. Unsorted and uninoculated tomatoes (~550 g for cherry or ~360 g for grape tomatoes) were placed into a pre-determined volume of chlorinated or unchlorinated wash water, which was immediately followed by adding inoculated tomatoes (two cherry tomatoes, approximately 50 g; or three grape tomatoes, approximately 40 g), and inoculated debris (0.4–0.5 g). The inoculated and uninoculated tomatoes, along with inoculated debris, were washed together for 1 min at room temperature (23–25 °C) with continuous moderate (~60 RPM) agitation. Immediately following washing, samples were collected for analysis of *Salmonella* inactivation and cross-contamination. First, the marked inoculated tomatoes were removed from the wash water, and placed into a bag containing buffered peptone water (BPW; BD) supplemented with 0.3% sodium pyruvate and 0.1% sodium thiosulfate (Kemp and Schneider, 2000) (BPW + SS) in a tomato:BPW ratio of 1:2 (w/v) (Sample set A). Then, a matching number of uninoculated tomatoes to Sample set A were similarly removed and placed in BPW + SS (Sample set B), and concurrently, 20 uninoculated tomatoes were individually placed into 4-Oz Whirl-Pak bags containing 20 ml of BPW + SS (Sample set C), using sterile disposable chopsticks for retrieval of each tomato. Sample set B was to be used for a most probable number (MPN) based enumeration to determine the approximate population of cross-contamination, and sample set C was to be used for estimation of the frequency of *Salmonella* cross-contamination. After removal of all tomatoes, the spent wash water was filtered through a 4 L Whirl-Pak bag (0.3 mm perforation) to collect all debris (both inoculated and uninoculated), which was immediately neutralized with 50 ml of BPW + SS (Sample set D). An aliquot of 10 ml filtered spent wash water was neutralized with 125 μl 10% sodium thiosulfate (Sample set E). Another aliquot of spent water was collected to measure the post-washing free chlorine concentration.

2.6. Sample processing and enumeration

Inoculated tomatoes in BPW + SS (Sample set A) were massaged by hand 20 times, then sonicated in an ultrasonic water bath for 2 min at 40 kHz (Kim et al., 2012; Sanglay et al., 2004). After sonication, tomatoes were hand massaged for another 20 times. For enumeration, samples were diluted and directly plated on XLT4 with 50 μg/ml kanamycin (XLT4-Kan) and XLT4 with 50 μg/ml rifampin (XLT4-Rif) to determine populations of kanamycin and rifampin resistant *Salmonella* surviving on the tomatoes. The theoretic detection limit for direct plate count of tomato samples was 1.30 log CFU/g. For LPD inoculated tomatoes, MPN enumeration was also performed as described below to increase detection sensitivity. The calculated limit of detection for the MPN of LPD inoculated tomatoes was −1.44 log MPN/g.

Uninoculated tomatoes (Sample set B) were treated in the same manner as the inoculated tomatoes to dislodge bacteria. The rinsate was aliquoted into five tubes (10 ml) and decimally serial-diluted in BPW + SS for a 5-tube, 3-dilution MPN analysis. MPN tubes were incubated for 2 h at room temperature, followed by 16-h incubation at 42 °C. After incubation, contents in MPN tubes were plated on both XLT4-Kan and XLT4-Rif to determine Se-KanR and Se-RifR populations. The calculated limit of detection was −1.44 log MPN/g. An aliquot of the rinsate was also plated on tryptic soy agar (TSA; BD) to enumerate the surviving native microbiota as aerobic plate count (APC). For trials II and III, only those washed together with tomatoes and debris inoculated at lower population density level were used for APC enumeration.

The 20 uninoculated tomatoes (Sample set C) in individual 4-Oz Whirl-Pak bags in BPW + SS were incubated at room temperature for 2 h followed by 16-h incubation at 42 °C. After incubation, each sample was plated on both XLT4-Kan and XLT4-Rif to determine presence of Se-KanR and Se-RifR, and to calculate the cross-contamination frequency.

Total debris (Sample set D) was treated in the same manner as inoculated tomatoes to dislodge bacteria. For enumeration, the rinsate was decimally serial-diluted and directly plated on TSA for APC, and on XLT4-Rif for determination of Se-RifR populations on debris. The limit of detection for direct plate count was 3.0 log CFU/g. Water samples (Sample set E) were directly diluted and plated onto TSA for APC plate count, as well as XLT4-Kan and XLT4-Rif for *Salmonella* enumeration. The detection limit for direct plate count was 1.0 log CFU/ml.

All TSA plates were incubated at 30 °C for 48 h and XLT4 plates were incubated at 42 °C for 24 h. Colonies on plates were counted using a Flash and Go (IUL, Barcelona, Spain) automatic colony counter. Characteristic black colonies on XLT4-Kan and XLT4-Rif plates were counted as cells of *Salmonella* strains originally inoculated on tomatoes and on debris, respectively.

2.7. Statistical analysis

This study was carried out in three trials over one growing season. Two varieties of tomatoes grown in two production areas were used. In Trial I, a single inoculation level and four wash water chlorination levels were evaluated, while in Trials II and III, we used two inoculation levels and two wash water chlorination levels. Trial I was analyzed alone to assess for the effect of chlorine concentration on inactivation of *Salmonella* and APC, and trials II and III were analyzed together to determine the effect of pathogen carrier source on the inactivation of *Salmonella* and APC. Statistics were done with R 3.4.4. (R-foundation). Normality of data and equality of variance among groups were tested with the Shapiro-Wilk test and Levene's test, respectively. Analysis of Variance (ANOVA) was used to assess influence of experimental factors (free chlorine level, pathogen carrier source). Kruskal Wallis and Welch's unequal variance tests were used instead of ANOVA when, respectively, the assumptions of normality or equality of variance were violated. P values < 0.05 were considered statistically significant.

3. Results

3.1. Inactivation of Salmonella and native microbiota from tomatoes and debris by wash treatment

To simulate the commercial tomato washing process, tomatoes along with debris were soaked in unchlorinated water for 30 min to generate wash water that was comparable to commercial tomato wash water in multiple physicochemical characteristics including TDS, COD, CLD (Zhou et al., 2014b) (Table 2). This water was subsequently chlorinated as needed, and used to wash uninoculated cherry or grape tomatoes, along with tomatoes and debris that were differentially inoculated with *Salmonella* strains to evaluate the inactivation and cross-contamination of *Salmonella* during the washing process.

Trial I. Fig. 2 shows the effect of free chlorine concentrations (0, 25, 50, 100, and 150 mg/L) in wash water on inactivation of *Salmonella* and native microbiota (APC) on tomatoes and debris. Recoverable populations of *Salmonella* on unwashed inoculated tomatoes and debris were 4.82 ± 0.11 and 7.25 ± 0.08 log CFU/g, respectively. For inoculated tomatoes, exposure to chlorinated wash water resulted in significantly greater log reductions of *Salmonella* compared to unchlorinated wash water ($p < 0.02$). However, no significant differences in log reduction were observed among the chlorine levels tested (25, 50, 100, and 150 mg/L) ($p > 0.05$). For inoculated debris, wash treatment did not significantly impact the log reduction of *Salmonella*, irrespective of the presence or absence of chlorine in the wash water ($p = 0.36$).

Initial populations of native microbiota (measured as APC on TSA) recovered from tomatoes and debris before wash were 5.30 ± 0.37 and 9.18 ± 0.04 log CFU/g, respectively. Chlorination of wash water had a significant influence on the log reduction of APC ($p < 0.02$) on tomato surface, although the reduction efficacy did not show a strong correlation to free chlorine concentration ($R^2 = 0.006$, $p = 0.78$). Exposure to chlorinated wash water significantly reduced APC on debris when compared to treatment in unchlorinated wash water ($p < 0.005$), although there was no significant difference in log reduction of APC among the target free chlorine concentrations tested (25–150 mg/L) ($p > 0.05$). Similar studies have reported a lack of strong correlation between chlorine concentration and decontamination efficacy of resident microbiota from the surface of fresh produce (Mazollier, 1988; Poimenidou et al., 2016; Van Haute et al., 2013).

The inactivation of *Salmonella* on tomatoes and debris was not consistently correlated with chlorine concentration in the range of 25–150 mg/L. Because of this observation, subsequent trials in this investigation (trials II and III) tested only two free chlorine concentrations (50 and 150 mg/L), and utilized two different inoculation levels of *Salmonella* on both tomatoes and debris, to better evaluate the influence of initial *Salmonella* populations on the effectiveness of the wash process and probability of cross-contamination.

Trials II and III. Immersion inoculation of cherry and grape tomatoes with HPD *Salmonella* inoculum (Se-KanR, ~7.9 log CFU/ml) resulted in an initial inoculation of 4.17 ± 0.22 log CFU/g of recoverable *Salmonella*, while inoculation with LPD inoculum (~5.2 log CFU/ml) resulted in 1.03 ± 0.19 log CFU/g of recoverable *Salmonella* on tomato fruit. Debris was similarly inoculated by immersion in a cocktail of *Salmonella* strains (Se-RifR) at HPD and LPD inoculation levels. Immersion in the HPD inoculum (~7.8 log CFU/ml) resulted in an inoculation level of 7.58 ± 0.11 log CFU/g of recoverable *Salmonella* on the debris, while immersion in LPD inoculum (~4.9 log CFU/ml) resulted in an inoculation level of 5.38 ± 0.09 log CFU/g.

The reductions in *Salmonella* and native microbiota populations after wash treatment in 0, 50, and 150 mg/L free chlorine are shown in Table 3. For inoculated tomatoes, washing in 150 mg/L free chlorine resulted in the highest log reductions for both HPD (2.67 ± 0.23 log) and LPD (2.45 ± 0.16 log) inoculation levels, although this reduction

Table 2
Wash water physicochemical characteristics[a].

Trial	TDS (mg/L)	COD (mg/L)	CLD (mg/L)	Target FC (mg/L)	Prewash pH[b]	Prewash FC (mg/L)[b]	Postwash FC (mg/L)[b]
I	309	322	88	0	6.5	0.1	0.2
				25	6.5	27.0 ± 1.2	20.0 ± 0.7
				50	6.5	50.0 ± 0.2	41.7 ± 0.1
				100	6.5	107.3 ± 0.4	94.8 ± 2.3
				150	6.5	148.0 ± 7.7	143.4 ± 3.2
II	127.5	116.9	32	0	6.5	0.2	0.2
				50	6.5	52.1 ± 0.7	47.3 ± 1.5
				150	6.5	159.5 ± 6.2	153.0 ± 7.6
III	294	367.2	134.4	0	6.5	0.1	0.1
				50	6.4	52.8 ± 1.8	46.7 ± 1.5
				150	6.5	154.1 ± 2.7	148.6 ± 3.9

[a] TDS: total dissolved solids; COD: chemical oxygen demand; CLD: chlorine demand; and FC: free chlorine.
[b] pH and FC concentrations were averaged from 3 replicates for Trial I and 6 replicates for Trials II and III (for both high and low inoculation of *Salmonella*).

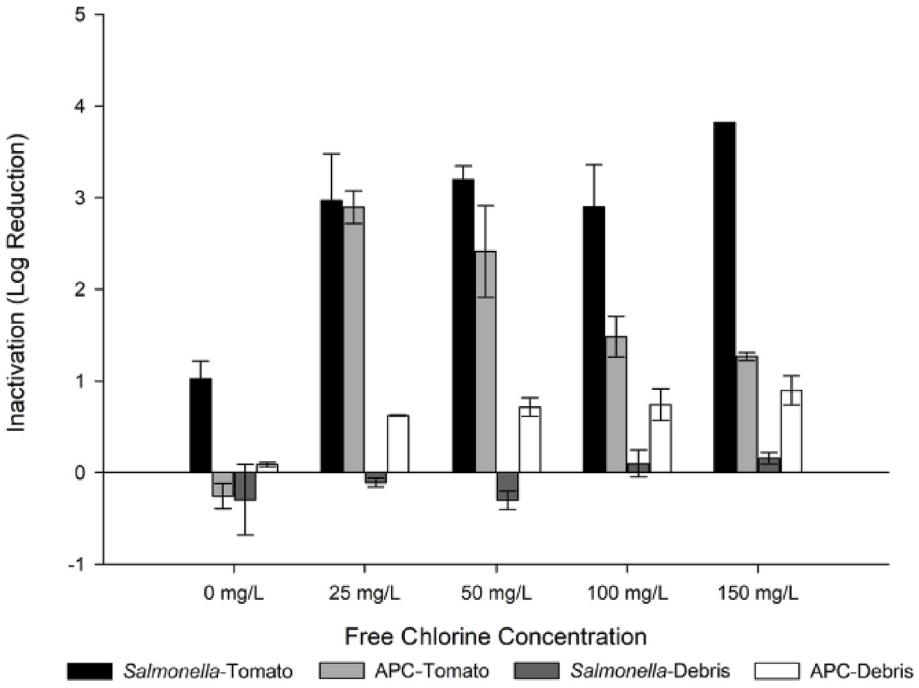

Fig. 2. Inactivation of *Salmonella* and native microbiota (APC) on tomato and debris after washing in chlorinated water. Bars show reduction (log) in *Salmonella* counts and in APC on washed tomatoes and debris in comparison to those without washing. Inactivation of *Salmonella* was determined using inoculated tomatoes and debris, while inactivation for APC was determined using uninoculated tomatoes and debris. Error bars represent standard errors.

Table 3
Inactivation (log reduction) of *Salmonella* (Se) and native microbiota (APC) on tomatoes (Se-KanR) and on debris (Se-RifR) after washing in water with different concentrations of free chlorine.

Bacterium and carrier	Population reduction of *Salmonella* and APC at given free chlorine level		
	0 mg/L	50 mg/L	150 mg/L
High Population Density			
Se on tomato	0.94 ± 0.27 Aa	2.29 ± 0.30 Ba	[a]2.67 ± 0.23 Ba
Se on debris	0.09 ± 0.12 Ab	0.54 ± 0.28 Ab	0.93 ± 0.15 Bb
Low Population Density			
Se on tomato	[a]1.22 ± 0.51 Aa	[a]2.23 ± 0.19 Aa	[a]2.45 ± 0.16 Aa
Se on debris	0.24 ± 0.11 Ab	1.51 ± 0.26 Bb	1.65 ± 0.19 Bb
APC on tomato	−0.58 ± 0.23 Aa	1.74 ± 0.39 Ba	[a]1.98 ± 0.46 Ba
APC on debris	−0.33 ± 0.10 Ab	0.49 ± 0.17 Bb	0.58 ± 0.06 Bb

Values are represented as arithmetic mean ± standard error ($n = 6$). Different capital letters in the same row indicate a significant difference ($p < 0.05$) in log reductions between treatments, and different lower-case letters in the same column indicate significant difference ($p < 0.05$) in log reductions between the paired carrier source (tomato vs debris).

[a] Value indicates that bacterial population was under detection limit for at least 1 replicate. For replicates under the detection limit, values of ½ detection limit (1 log CFU/g for direct plate count of high population density *Salmonella* and APC and −1.74 log MPN/g for MPN of low population density *Salmonella*) were used to calculate average population and statistical difference.

was only significantly different from washing in unchlorinated wash water for tomatoes inoculated at HPD ($p < 0.007$). When tomatoes were inoculated at LPD, *Salmonella* reductions in log scale were not significantly different for any of the wash treatments evaluated ($p = 0.13$). For inoculated debris, the highest log reductions of *Salmonella* were obtained after treatment with 150 mg/L free chlorine (0.93 ± 0.15 log for HPD and 1.65 ± 0.19 log for LPD), with both being significantly different from those obtained for washing in unchlorinated wash water ($p < 0.004$ and $p < 0.001$ for HPD and LPD, respectively).

The average reduction of *Salmonella* from inoculated tomatoes for HPD (1.97 ± 0.23 log) and LPD (1.97 ± 0.22 log) inoculums was significantly higher than that of HPD (0.52 ± 0.13 log) and LPD (1.14 ± 0.19) *Salmonella* from debris across all evaluated wash treatments ($p < 0.001$ and $p < 0.007$ for HPD and LPD, respectively). The diminished effect of washing treatment on the reduction of *Salmonella* from debris suggests that the surface of debris may provide more protection from the dislodging of inoculated *Salmonella* by the washing process.

Initial native microbiota populations of 4.39 ± 0.36 and 8.59 ± 0.12 log CFU/g were detected on unwashed tomatoes and debris, respectively. The addition of free chlorine to the wash system significantly impacted the reduction ($p < 0.001$) of APC from uninoculated tomato fruits and debris (Table 3). However, there was no significant difference in APC log reduction for tomatoes ($p = 0.89$) or debris ($p = 0.86$) when treated in wash water with free chlorine concentrations of 50 and 150 mg/L.

When comparing all wash treatments, reduction of APC from debris (0.25 ± 0.12 log) was consistently less than that from tomatoes (1.05 ± 0.34 log) ($p < 0.04$). These results not only demonstrate the incapacity of chlorine wash to inactivate bacteria harbored on debris, but are also evidence of the high capacity for bacterial retention in debris, which is consistent with the hypothesis that debris is a potential persistent source of microbial contaminants.

3.2. *Salmonella* cross-contamination onto uninoculated tomatoes

Since wash water is the most likely medium of cross-contamination during dump tank (flume) washing, we determined the number of *Salmonella* cells released into wash water from either inoculated tomato fruits or from inoculated debris by enumerating Se-KanR and Se-RifR populations in the unchlorinated spent wash water (data not shown). *Salmonella* cells released into unchlorinated wash water would represent the maximum available for cross-contamination under the worst scenario of processing control. When *Salmonella* was inoculated at HPD, average populations of 2.54 ± 0.08 log CFU/ml and 3.83 ± 0.16 log CFU/ml from tomatoes and debris, respectively, were recovered from the unchlorinated wash water. When inoculated at LPD, *Salmonella* from tomato was under the limit of detection (< 1 log CFU/ml), and *Salmonella* from debris was detected at 1.00 ± 0.25 log CFU/ml in unchlorinated wash water.

Table 4
Cross-contamination of *Salmonella* onto uninoculated tomatoes originating from either inoculated tomatoes (kanamycin resistant; Se-KanR) or debris (rifampin resistant; Se-RifR).

Target FC (mg/L)	*Salmonella* on uninoculated tomatoes (log MPN/g)		Cross-contamination frequency (Positive/Total)	
	Se-KanR	Se-RifR	Se-KanR	Se-RifR
High Population Density				
0	1.46 ± 0.16	1.89 ± 0.19	180/180	180/180
25[a]	LDL[b]	LDL	0/60	3/60
50	LDL	LDL	1/180	5/180
100[a]	LDL	LDL	0/60	1/60
150	LDL	-1.41 ± 0.04[c]	0/180	1/180
Low Population Density				
0	-1.42 ± 0.01[c]	-0.61 ± 0.28	7/120	79/120
50	LDL	LDL	0/120	0/120
150	LDL	LDL	0/120	0/120

Pooled data from trials I, II, and III and represented as mean ± standard error ($n = 9$ for high inoculation, $n = 6$ for low inoculation).

[a] Data collected from trial I only ($n = 3$).

[b] LDL: Less than limit of detection at −1.44 log MPN/g.

[c] Value indicates that bacterial population was under detection limit for at least 1 replicate. For replicates under the detection limit, the detection limit was used to calculate average population.

Two procedures were used to assess cross-contamination of *Salmonella* during the washing process. The MPN enrichment procedure (Sample set B), employing serially-diluted rinsates from uninoculated tomatoes for enrichment, was designed to estimate the numbers of *Salmonella* cells transferred onto uninoculated tomatoes from either inoculated tomatoes or inoculated debris. The whole tomato enrichment method (Sample set C), employing multiple tomatoes for individual enrichment, was designed to estimate the frequency of *Salmonella* transference onto uninoculated tomatoes (cross-contamination events) during washing. The latter allowed detection of *Salmonella* cells that survived chlorine treatment even when they were not detached by sonication (as used in this study) due to firm attachment to the stem scar (Fan et al., 2018; Mukhopadhyay et al., 2014), and hence not recoverable by examination of the rinsate.

Table 4 shows the frequency and estimated number of *Salmonella* cells transferred to uninoculated tomatoes after washing. Without wash water chlorination, cross-contamination from tomatoes and debris inoculated at HPD was detected on 100% (180/180 total across all three trials) of uninoculated tomatoes, with estimated populations reaching 1.46 ± 0.16 log MPN/g and 1.89 ± 0.19 log MPN/g for contamination originating from tomatoes and debris, respectively. Under the same conditions, cross-contamination originating from tomatoes inoculated at LPD (trials II and III) was detected in 6% (7/120) of uninoculated tomatoes at approximately −1.42 ± 0.01 log MPN/g, whereas cross-contamination originating from debris was detected in 66% (79/120) of uninoculated tomatoes at approximately −0.61 ± 0.28 log MPN/g.

When wash water was chlorinated, sporadic cross-contamination on uninoculated tomatoes was observed from tomatoes and debris inoculated at HPD, but not at LPD. With enrichment, one single cross-contamination event originating from inoculated tomatoes was observed, when tomatoes were washed in 50 mg/L chlorinated wash water. All other 10 cross-contamination events (among 720 uninoculated tomatoes) detected in this study were scattered among different washes of the three trials and all originated from debris inoculated with HPD *Salmonella* inoculum. Only one quantifiable cross-contamination event was detected using MPN procedures when tomatoes were washed in 150 mg/L chlorinated water. This cross-contamination originated from HPD inoculated debris, with estimated contamination of −1.41 ± 0.04 log MPN/g.

4. Discussion

Several outbreaks of salmonellosis associated with tomatoes have been reported, which in some cases implicated tomato packinghouse operations (Bennett et al., 2015; Gurtler et al., 2018). Most of these reported cases, and hence studies on *Salmonella* survival, involved round or Roma tomatoes, rarely implicating the smaller varieties, such as cherry and grape tomatoes (Bartz et al., 2015; Greene et al., 2008; Gupta et al., 2007). One multistate outbreak of *Samonella* SaintPaul in 2013, which was responsible for 131 illnesses and 23 hospitalizations, was associated with consumption of cherry/grape tomatoes (CDC, 2018b). The increasing popularity of cherry/grape tomatoes among consumers (Casals et al., 2019; Coker et al., 2018) could also be accompanied by greater potential food safety risks (CDC, 2018b).

Apart from the differences in size and shape, a major distinction between round/Roma and cherry/grape tomato varieties is the maturity level at which the fruits are harvested. While round and Roma tomatoes are typically harvested at the breaker stage, which makes it necessary for a commercial ripening prior to marketing, cherry and grape tomatoes are harvested and processed at full maturation, and are market-ready post washing. Nevertheless, cherry and grape tomatoes share many similarities in growth and processing with round and Roma varieties. They are grown in approximately the same seasons, often in adjacent fields, using very similar farm management practices. Although dedicated dump tanks (or flumes) are used for different tomato varieties, similar washing processes for round/Roma tomatoes are often also employed for cherry/grape varieties in packinghouses under the same managements. In this study, we examined *Salmonella* inactivation and cross-contamination of cherry/grape tomatoes during simulated washing processes, taking advantage of their smaller fruit size and thus increased feasibility to analyze larger numbers of individual fruit samples. Findings in this study provided useful information for risk assessment of *Salmonella* contamination of cherry/grape tomatoes. The information is also relevant for other tomato varieties owing to their commonalities in growth and processing.

Salmonella is the primary target for antimicrobial interventions during commercial tomato packinghouse operation, as it has been the foodborne bacterial pathogen most frequently associated with tomato consumption (Anderson et al., 2011; United Fresh, 2018). *Salmonella* can survive, internalize, and grow on tomato surface and internal tissues post-harvest (Gu et al., 2011, 2018; Zheng et al., 2013; Zhou et al., 2014a). Current guidelines for tomato packinghouse operations recommend 150 mg/L of free chlorine or equivalent concentration of other approved wash water antimicrobials to prevent cross-contamination (Florida Department of Agriculture and Consumer Services, 2018; United Fresh, 2018). In a laboratory setting, over 4.5 log reduction of *Salmonella* was achieved when inoculated round tomatoes were washed in the presence of free chlorine in wash water with commercial topsoil as organic load simulator (Sreedharan et al., 2017). In this study, we observed approximately 2–3 log reduction on tomatoes in the presence of free chlorine ranging from 25 to 150 mg/L, when tomatoes were inoculated with a high concentration of *Salmonella*. However, no significant difference on *Salmonella* inactivation was observed among the chlorine concentrations tested.

The commodity specific guidelines for tomatoes recommend that leaves and other debris be removed to a practicable degree in the field, and not accumulate in the flume (United Fresh, 2018). In practice, field debris in harvested tomato bins varies considerably depending on multiple factors, including tomato variety, growing conditions, and worker training. The presence of debris in tomato dump tank (flume) water contributes to the significant increase in wash water organic load and could represent a significant source of pathogen contamination. In this study, debris was also inoculated with *Salmonella*, and proportionally added to the simulated washing process to determine *Salmonella* inactivation on debris and to assess its significance as a source of cross-contamination.

When inoculated by submersion at comparable levels, debris was highly efficient in inoculum retention in comparison to tomatoes, contributing more heavily to the total *Salmonella* counts (at least 1 log higher) in the wash load despite making up a small percentage by weight in each simulated wash. Preferential adherence of *Salmonella* on debris may have been associated with inherent structural surface features, such as leaf veins and trichomes (Cevallos-Cevallos et al., 2012; Monier and Lindow, 2004). Several studies have shown high potential for *Salmonella* survival on tomato leaves (Barak et al., 2011; Gu et al., 2013; Potnis et al., 2015) compared to tomato fruit (Deering et al., 2015; Gu et al., 2018; Kumar and Micallef, 2017). In addition, *Salmonella* has been shown to survive for a longer duration on leaves than on fruits when directly applied in the field (Guan et al., 2005). Hence it would be plausible to hypothesize that in a naturally occurring contamination event in the field, the leaves and debris would also be a more likely carrier of viable pathogens than tomatoes. Considering that the inactivation of *Salmonella* inoculated on debris was consistently less than that on tomatoes at each chlorine treatment condition tested, debris may represent a feasible source for harboring potential cross-contaminating foodborne pathogens in the washing system.

Sreedharan et al. (2017) showed that free chlorine at 100 mg/L could sufficiently prevent cross-contamination of *Salmonella* during washing of round tomatoes. They observed a single cross-contamination event at 75 mg/L free chlorine under high inoculation conditions in the presence of high organic load. In this study, both frequency and magnitude of cross-contamination were estimated. Overall, the occurrence of *Salmonella* cross-contamination was very low at the free chlorine levels tested in this study (25–150 mg/L), and it was only observed when uninoculated tomatoes were washed with either tomatoes or debris that were inoculated with high population density of *Salmonella*. A single cross-contamination event, originating from debris inoculated at high population density, was quantifiable by MPN procedure. Among 12 sporadic cross-contaminations in the presence of free chlorine, 11 were originated from inoculated debris. It is noteworthy that, in a given wash, the quantity of *Salmonella* cells released into wash water from inoculated debris was over 10-fold greater than that released from inoculated tomato fruits, which likely contributed to the higher cross-contamination of uninoculated tomatoes from debris. This observation could be a reasonable reflection of a scenario of a potential contamination event in the field, where chances of *Salmonella* contamination and survival/growth on tomato canopy would be higher than on tomato fruits. Data presented here is consistent with this view.

Salmonella survival in spent wash water at the tested free chlorine levels (25–150 mg/L) was not observed. This is in agreement with Luo et al. (2011) which found no pathogen survival in wash water associated with fresh-cut leafy greens after free chlorine levels reached 5 mg/L. These observations were consistent with the low incidence of cross-contamination when tomatoes were washed together with heavily contaminated tomatoes and debris. While the presence of free chlorine significantly reduced *Salmonella* cross-contamination overall, sporadic cross-contamination was detected irrespective of free chlorine concentration between 25 and 150 mg/L. This suggests that additional factors, other than contaminated water, might have played a role in mediating cross-contamination. The frequent occurrence of debris-originated *Salmonella* strains among these rare cross-contamination events suggests that debris might play a role in direct *Salmonella* transference without cells being released into the wash water. Such direct transference could occur by direct contact between tomatoes and debris, or could be mediated by the adherence of small pieces of debris to tomato surface.

This study was undertaken with the primary objective of assessing the risk associated with *Salmonella* survival and cross-contamination from different potential pathogen carrier sources at a range of free chlorine concentrations. Data presented here showed that chlorination of wash water had a differential effect on the inactivation of *Salmonella* inoculated on debris and on tomatoes. Additionally, a low frequency of *Salmonella* cross-contamination during tomato washing in chlorinated wash water was observed sporadically, only when tomatoes and debris were inoculated at a level that would be extremely unlikely to be encountered under current production practices. Nevertheless, the data presented here supports the notion that debris represents a potentially significant risk for harboring foodborne pathogens, since the cross-contamination observed in this study primarily originated from inoculated debris, rather than inoculated tomatoes. Future work should determine whether minimizing acquisition of debris during harvest and accumulation during processing improves food safety outcomes. Restricting debris could improve wash water quality by lowering the input of chlorine demand, while also eliminating a potential source of undesirable human and plant pathogens, improving the microbial safety and shelf life of tomatoes after processing. Presently, many packinghouses institute an overhead spray of an alternative sanitizer such as chlorine dioxide or peracetic acid solution at the exit of wash tanks. The efficacy of this step on removing fine pieces of debris and reducing the occurrence of tomato contamination are also worth additional studies.

Declaration of competing interest

The authors declare no financial or professional conflict of interest pertaining to the research described in this manuscript.

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