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The transfer rate of *Salmonella* Typhimurium from contaminated parsley to other consecutively chopped batches via cutting boards under different food handling scenarios

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The transfer rate of *Salmonella* Typhimurium from contaminated parsley to other consecutively chopped batches via cutting boards

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Abstract

In many Mediterranean and Middle Eastern countries, leafy green parsley is typically eaten raw and prepared by fine chopping several batches. This study aimed to quantify the transfer rate of *S.*Typhimurium (Tr) across all chopped batches in scenarios that resemble normally occurring operations in restaurants and home kitchens. Fresh parsley leaves were inoculated at concentrations of 6 and 3 log CFU/g and chopped on a polyethylene cutting board (CB). Uninoculated parsleys were sequentially chopped in individual batches on the same cutting surface, 1) instantly (CB Instant), 2) after washing in water and holding at 30°C 24 h (CBWW), 3) after washing in soapy water, sponge scrubbing and holding at 30°C 24 h (CB SW). Using the high inoculum levels, the mean Tr was 0.012 ± 0.04 , 0.014 ± 0.02 and 0.010 ± 0.008 , via CB Instant, WW and SW, respectively. Comparatively, the Tr mean values were significantly higher with the low inoculum levels, 0.60 ± 0.65 and 0.64 ± 0.46 , via CB Instant and CB WW respectively, and transmissions of *S.*Typhimurium significantly decreased across consecutively chopped batches on both washed CBs ($P < 0.05$). These results demonstrated continuous transfer of *Salmonella* cells, from contaminated parsley to cutting boards and subsequently re-contaminating up to 6 batches of parsley chopped consecutively on the same surface. A greater cross-contamination rate was recorded during the initial phases of chopping and remained at 24 h at 30°C. Vigilant cleaning and sanitation procedures

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on cutting surfaces should be a fundamental requirement after use with fresh produce, particularly if there is a likelihood of insufficient food safety measures at harvest and post-harvest stages

Keywords: *S. Typhimurium*; parsley; transfer rate; cross-contamination; cutting surfaces

1. Introduction

Many strains of *Salmonella* pose a global health threat for foodborne disease including *Salmonella enterica* serovar Typhimurium (CDC., 2006; European Food Safety Authority, 2010). At the same time, the health concerns are becoming significant given the increasing prevalence of multidrug-resistant *S. Typhimurium* infections in many parts of the world (Dutta et al., 2014; Kumar et al., 2008). It is actually becoming more evident that *Salmonella*-associated outbreaks are not limited to contaminated foods of animal origin; they are periodically linked to consumption of fresh produce (Jackson et al., 2013), including parsley and lettuce (Berger et al., 2010) and *S. Enteritidis* and *S. Typhimurium* have been commonly isolated from fresh vegetables (Kisluk et al., 2012; Rana et al., 2010).

Salmonella spp. can be transferred to the food chain directly from human or animal faecal sources, run-off of nearby farms, untreated manure (Islam et al., 2004), or from contaminated irrigation water (Kroupitski et al., 2009). Additionally, various routes for cross-contamination in the kitchen and processing environments, where mishandling practices and improper hygienic practices are prevalent, have been reported to contribute significantly in the transmission of foodborne pathogens to food (Chen et al., 2001; Kusumaningrum et al., 2004; Lubber et al., 2006). More specifically, the transmission of pathogens to food is often facilitated by poor personal hygiene of food handlers, inadequate storage or processing food on equipment, and contact surfaces that were not properly cleaned and disinfected (de Jong et al., 2008). Of food contact surfaces, cutting boards were shown to represent critical risk

factors of cross-contamination and recontamination events (Redmond et al., 2003; Tang et al., 2011; van Asselt et al., 2008), as vectors of pathogens to food (Chen et al., 2001; Moore et al., 2003).

In our previous study on the assessment of food handlers' knowledge, attitudes and practices (Faour-Klingbeil et al., 2015), the majority of small restaurants used plastic cutting boards, half of which relied on washing in water with or without soap with no sanitizer used thereafter. When inadequately cleaned such as after cutting raw meat and poultry they can harbour pathogenic microorganisms leading to hazardous events, even so in some circumstances with one single bacteria adhered to the surface (Ravishankar et al., 2010; Soares et al., 2012).

Many reports have focused on survival and transfer of pathogens including *S. Typhimurium* from food of animal origins to surfaces or other food types in meat preparation (Kusumaningrum et al., 2004; Moore et al., 2007; Ravishankar et al., 2010).

Although much research has shown that cross-contamination can occur between food contact surfaces and foods, the studies mostly focused on bacterial residence time, types of equipment surfaces and other conditions, typically using a single food being sliced (one-time food-slicing scenarios). Limited information exists on cross-contamination events from food of plants origin (Wachtel et al., 2002), and the order of magnitude or trend in cross-contamination of the same type of food sliced subsequently on same contaminated surfaces. Only recently, Zilelidou et al. (2015) described the bacterial transfer during consecutive knife cutting of lettuce leaves and its distribution between cutting knives and lettuce. In many Mediterranean and Middle Eastern countries, parsley is typically eaten raw and prepared by finely chopping several batches of the leaves for processing into appetizers, ready-to-eat salads, and garnishes served in foodservice and home settings. Because of the convoluted nature of parsley leaves and no precedent for transfer studies with this vegetable, we would expect that this work will complement other studies on bacterial transfer rate (Zilelidou et

al.2015; Rodríguez et al., 2011). Therefore, in this study we chose parsley to evaluate the transfer rate of *S. Typhimurium* in scenarios that resemble normally occurring operations in restaurants and home kitchens. The aim was to quantify the transfer rate of *Salmonella* across all chopped batches from one originally contaminated bundle of parsley. The transfer rates would be expected to be lower upon consecutive chopping of each new batch of parsley on the same contaminated surface. The data of this study could be used in quantitative microbial risk assessment of *S. Typhimurium* on parsley under different handling conditions.

2. Materials and Methods

2.1 Strains and cells suspension preparation

Salmonella enterica serovar Typhimurium LT2 (*S. Typhimurium*) was adapted to grow in the presence of 50 mg/ml nalidixic acid (Sigma-Aldrich), through stepwise exposure to nalidixic acid (Bio-rad laboratories Ltd, Hemel Hempstead, UK), i.e., 10, 20, 30, 40 and 50 µg/ml (Parnell et al., 2005). A loopful of culture was streaked onto plate count agar (Bio-rad laboratories Ltd, Hemel Hempstead, UK) supplemented with 50 µg/ml nalidixic acid (PCAN) and incubated at 37°C for 24 h. One to two colonies of this strain were grown overnight at 37°C in 10 ml of tryptic soy broth (TSB; Conda, Spain) supplemented with 50 µg/ml nalidixic acid (TSBN), and incubated at 37 °C for 18-20 h to yield 10⁹cfu/ml which was initially verified by direct plating. Target concentrations of 3 and 6 log CFU/g were prepared by suspending 1 ml of an overnight culture of appropriate dilution in 1 l of 0.1% peptone water (PW).

2.2 Contamination of parsley

Bundles of fresh parsley were purchased from a local grocer on the day of the experiment. They were washed with running tap water for 10 s to remove dirt and soils. Only green fresh leaves with 5 cm stalks (100g) were used and inoculated by dipping into inoculum at the target concentrations of *S. Typhimurium* for 30 min to allow attachment. Thereafter, inoculated parsley leaves were placed on sterile paper in a laminar airflow for approximately 60 min (Ruiz-Cruz et al., 2007).

The immersion process of fresh produce is a possible point of contamination at post-harvest based on our observations during post-harvest washing (Faour-Klingbeil et al., 2016); hence, dip-inoculation was considered to be a suitable method for simulating contamination in commercial fresh produce operations (Beuchat et al., 2001).

2.3 Cutting boards preparation and cross -contamination scenarios

Polyethylene domestic cutting boards (CB) were purchased from a local kitchenware store and were disinfected by soaking in 0.30 % sodium hypochlorite (Clorox® Bleach) overnight before use. Cutting board surfaces were thoroughly rinsed by immersion in hot sterile water (ca.80°C) to remove any remaining disinfectant prior to use (Pui et al., 2011) , then soaked in 70% ethanol for 1 h and air-dried in the laminar flow cabinet prior to use in each experiment (Kusumaningrum et al., 2003). The cleaning process was validated by a swab test, which confirmed the absence of *S. Typhimurium* after each experiment.

The different scenarios performed in the laboratory experiments were designed to determine 1) the transfer rate of *S.Typhimurium* each time a new batch is chopped after one contaminated bundle of parsley that typically weighs 100g and 2) to quantify the remaining cells on the cutting board each time we chopped a new batch, as follow:

Scenario 1 (CB Instant): Inoculated bundle of parsley (100g) was initially chopped on a clean disinfected CB. Afterwards, 5 batches of 40g parsley were chopped consecutively and instantly on the same cutting board.

The size of consecutive batches was set to 40g for all scenarios to ensure consistency in the experiments. This was the quantity a hand grabbed wholly within the defined area of the CB, but few leaves remained adhered on the board after chopping.

Scenario 2 (CB WW): After initially chopping 100g of inoculated parsley, the cutting board was placed at room temperature (21-22°C) for 1h with some remaining exudates and leaves to mimic busy food operations and intermittent chopping practices; then the CB was rinsed under running tap water for 5 s to remove any adhering leaves and stored at 30°C for 24 h, a typical holding temperature in small eateries during summer. The next day, clean batches of parsley (ca.40g) were instantly chopped on that same CB.

Scenario 3 (CB SW): Similar to Scenario 2 except that water washing was combined with three manual scrubblings in one direction along the defined chopping area using a soft sponge containing kitchen soap detergent (15-20% anions surfactants). Overall contact time was estimated to be about 10 seconds, although sometimes this was shorter during peak food preparation occasions. The sponge was disinfected before use in replicated experiments by soaking in 0.30% hypochlorite for 5 min, followed by thorough rinse in hot water (ca.80 °C) and air drying.

Scenario 4 (Changing gloves): This scenario was designed to observe variation in cross-contamination rates which could be attributed to a person's hand coming in contact with contaminated surfaces and later to un-inoculated batches during chopping. For this, gloves were regularly replaced by a fresh sterile pair before holding and chopping each clean batch.

A total of 6 batches, with similar weights as in previous scenarios, were chopped in succession and analyzed in triplicates., to allow observation in trend differences.

To determine the number of parsley bundles to chop, the inoculum level was considered in context, with previous observations in small restaurant (Faour-Klingbeil et al., 2015) and understanding of home kitchens practice. Thus, up to 6 bundles (N=6) would cover what a small restaurant may prepare for the day, even when it likely exceeds what is prepared in home kitchens. At low inoculum levels, the number of bundles was reduced to 3 for observations of bacterial transfer and for microbial detection.

Experiments with low inoculum levels were conducted in two scenarios only, CB Instant and CB WW, for comparison with high inoculum size. All experiments were repeated 3-5 times, except for Scenario 4 which was conducted once as an additional validation step, and the values represent the means of replicated experiments.

To remove any potential effect of utensil characteristics and transfer, autoclaved scalpels instead of knives were used for chopping. The same scalpel and hand gloves were used in each experiment to mimic what typically occurs in foodservices. A standard fixed duration for chopping each batch of parsley was 1 min over a 21 cm² area.

2.4 Analysis of samples and quantification of *S. Typhimurium*

2.4.1 Analysis of parsley batches after chopping

For detection of direct presence of *Salmonella* spp. non-selective and selective enrichment steps were performed according to ISO 16140. Parsley samples that had not been inoculated (control) were confirmed for the absence of *Salmonella*.

Enumeration of *S. Typhimurium* was determined on each triplicate batch of chopped parsley. From each, 10g were individually weighed and homogenised in TSB stomaching for 2 min at 230 rpm. Aliquots of 0.1 ml were spread-plated in duplicate onto Rapid' *Salmonella* agar (Bio-rad laboratories Ltd, Hemel Hempstead, UK) supplemented with nalidixic acid and incubated at 37° C for 24 h. To avoid over or underestimation of counts, average detection limit was set to 0.7 log CFU/g for <10 CFU/g - enumerated on the lowest dilution and when detection results are positive.

2.4.2 Procedures for the recovery of *S. Typhimurium* cells from CBs after initial contaminated parsley and subsequent chopping

CB was divided, by marking, into 6 sections of 7cm x 3 cm area for experiments with high inoculum levels, and 7 sections areas for the low inoculum levels in view of the lower number of batches to chop (n=3), hence allowing duplicate swabs after chopping each batch. An initial swabbing of the CB was performed after chopping the 100 g contaminated parsley, and the 24 h post-wash incubation. Successive swabbing was taken after chopping each new batch of uncontaminated parsley. The last swab of the 6th section was taken over the entire area (126 cm²) at the end of the chopping process. For the low inoculum level, one section was swabbed twice, once after the first batch and another at the end of the chopping process. The partitioning of CB was made to avoid swabbing the same site more than once during 6 successive chopping, hence avoiding errors of underestimating the actual numbers of cells remaining on CB after each new single batch. Sections were swabbed with cotton-tips moistened in buffered peptone water (Bio-rad laboratories Ltd, Hemel Hempstead, UK) (BPW) in three different directions: left to right, top to bottom, and diagonal (Pui et al, 2011). Each swab was placed into a tube with 9 ml BPW and vortexed vigorously for 1 min. and tenfold serial dilutions were spread-plated on duplicate plates of Rapid' *Salmonella* agar

supplemented with nalidixic acid and incubated for 24h at 37 °C. Counts were expressed as log CFU/cm², calculated according to the formula:

$$\frac{\text{Average log}_{10} \text{ cfu/plate} \times (\text{volume of original suspension})}{(\text{Total surface area} \times 1 \text{ swab}) \times (\text{dilution factor}) \times (\text{volume applied to plate})}$$

2.4.3. Data presentation

The quantitative data was expressed as means \pm standard deviation.

To determine the trend of cross-contamination and transfer rate of *S. Typhimurium* (Tr) from one contaminated chopped bundle of parsley to each consecutively chopped clean batch, the Tr was estimated by dividing CFU on non-inoculated samples (receiver) with CFU on inoculated samples chopped on the same cutting board surface (Chen et al., 2001; Goh et al., 2014; Pérez-Rodríguez et al., 2008), i.e., [(CFU on the clean parsley (recipient)/ CFU on contaminated parsley(donor)]. Transfer rates are multiplied by 100 to be presented as % Transfer rate.

Additionally, the Tr data were log₁₀ transformed, i.e., (log₁₀ ratio of [CFU/g (receiver)/CFU/g (donor)]) for easier understanding and presentation at top scale (as log reduction) (Chen et al., 2001).

3. Data analysis.

All statistical analysis, distributions and data presentation in frequency histograms of transfer rates and log Tr were performed using SPSS version 22.

Differences between distributions as affected by different handling conditions of CBs were determined using a one-sided Wilcoxon's matched pairs signed rank test for two related groups and Friedman test for more than two related groups on the same continuous, dependent variable.

Statistical significance among mean values of log CFU/g of *S. Typhimurium* for the different batches chopped on same cutting board was determined using Kruskal Wallis test. Mann-

Whitney U test was performed to determine the statistical differences between means distribution of the transfer rates between high and low inoculum levels. Spearman's rho correlation was performed to determine association between inoculum size and transfer rate of *S. Typhimurium* to parsley samples. Statistically significant difference was set at $p < 0.05$.

4. Results and discussion

4.1 Transfer rate of *S. Typhimurium* from one contaminated parsley to all processed parsleys on same cutting board surface

Results of transmission of *S. Typhimurium* populations from artificially contaminated parsley to all processed uncontaminated batches via cutting boards are presented in Table 1.

After chopping parsley inoculated at low concentration levels with *S. Typhimurium*, the recovered cells on un-inoculated samples instantly chopped on the same surface (CB Instant) ranged from 2.00 to 3.85 log CFU/g and mean value was significantly higher than on samples chopped on the CB held for 24 h at 30°C after a water wash (CB WW) ($p < 0.05$) (Table 1).

Wilcoxon Signed Rank test showed that the median of differences of recovered *S. Typhimurium* was significantly different between CB Instant and CB WW ($p < 0.001$). The

Tr of bacterial cells to parsley chopped on CB Instant and CB WW recorded high values, 60.0% and 64.0% with a Tr magnitude ranging from 2-100% (Table 1), respectively.

Conversely, at high inoculum level, Tr data did not differ significantly among the 3 cutting boards scenarios ($p > 0.05$). The concentration of *S. Typhimurium* cells ranged from < 1.00 on

CB Instant and CB SW, to maximum 5.51, 4.69 and 4.72 log CFU/g on CB Instant, CB WW and CB SW respectively (Table 1) as a result of a substantially lower cross-contamination

rate. Bacterial Tr to parsley were highly variable being as low as low as 0.01 to as high as 25.00% via CB Instant. Washing CBs with water and with water and soap combined with

sponge scrubbing did not effectively reduce the bacterial transfer to parsley although maximum values diminished to 7.50% and 2.83% via CB WW and CB SW, respectively

(Table 1). Statistical analysis showed that Tr of *S. Typhimurium* to un-inoculated parsley was significantly higher with the initial chopped samples (source) inoculated with low levels than with high contamination levels ($p < 0.05$), on both, CB Instant and CB WW. The CB SW scenario was not tested at low inoculum level (Table 1). Spearman's rank-order confirmed a strongly negative correlation indicating that the initial bacterial low density on the source is a critical factor that has substantially heighten the cross-contamination process ($p < 0.001$). However, the correlation was particularly stronger for CB Instant ($r_s = -0.846$, $n=110$) than for washed CB (CB WW, $r_s = -0.676$, $n=68$). Our results corroborate with several studies that demonstrated an inverse relationship between inoculum size and transfer rate of pathogens (Montville et al., 2003); in this context, our results concur with Ravishankar et al. (2010) who reported a high transfer rate of 75% as a result of low inoculum size on the cutting boards surfaces and knives. Likewise, Fravallo et al. (2009) found that the percent transfer rate of *Campylobacter* from contaminated chicken to cutting boards was found to be inversely related to the initial inoculum level.

While the precise mechanism underlying this relationship is not well established, Montville and Schaffner (2003), suggested that the reduction in transfer rate at high inoculum level could be attributed to enhancement in cell adherence to the donor surface when bacterial concentrations are high; inferring from findings of Takeuchi et al. (2000) who highlighted the improved attachment of *E. coli* O157:H7 to lettuce leaves due to higher inoculum levels.

Frequency histograms at a logarithmic scale in Figure 1 and 2 represent merged data of all batches processed in each cutting board to examine the general cross-contamination events when all batches would be processed and mixed together. The log reduction extended with high frequency from 0.57 to -1.7 log Tr on CB Instant, and slightly broader to -2.00 on CB WW. The value “zero” represents the limit of the transfer rate, i.e., $Tr=1$ (100%); values above 0 were encountered in some samples when the recovered population on parsley was higher than the averaged values of concentration originally on contaminated parsley (CFU)

i.e., the denominator of the Tr fraction. This was similarly encountered by Zilelidou et al. (2015) as they reported a high variability in log Tr for *L. monocytogenes* from knife to lettuce ranging between -1.0 and -0.5 on day 0 with log reduction reaching in some occasions below -1.00 log CFU during the first cuts.

At high inoculum level, the range of log Tr data shifted higher the scale (<0.00) than that observed at low inoculum level (Figure 2) due to lower cross-contamination rate. Despite the application of water (CB WW) and soap coupled with scrubbing (CB SW), the distribution of data didn't differ greatly ($p>0.05$), as aforementioned.

4.2. Distribution data of *S. Typhimurium* to individual batches of parsley chopped consecutively on the same surface

Patterns in log reduction histograms at batch levels in relation to different CBs paralleled those observed with merged data; for instance, at low inoculum level, log Tr data were generally distributed from 0.57 to -0.90 on the first batch (B1) chopped on CB Instant; cross-contamination was significantly reduced by the third (B3) as the distribution extended further down to -1.00 and more than -1.50 log reduction ($p<0.05$) (Figure 3). There was significant reduction in mean values of recovered bacterial cells from B1 to B3, 3.12 ± 0.50 , and 2.78 ± 0.60 , respectively (Figure 4); on the contrary, the Tr was similar across all batches when the CB was washed with water ($p>0.05$) as shown in mean counts of 2.80 ± 0.22 and 2.47 ± 0.37 log CFU/g recovered from B1 and B3, respectively. (Figure 4), obviously explaining the significant differences in Tr and log CFU values between CBs when data of all batches were merged (Table 1).

At high inoculum level, Tr of *S. Typhimurium* was significantly the highest to B1, and considerably dropped by the third batch on CB Instant and CB WW ($p<0.05$), however, the distribution of bacterial cells was similar across all batches chopped on CB SW ($p>0.05$) (Table 2, Figure 4).

The results in Tr were not significantly different when the hand gloves were regularly changed with the chopping of each un-inoculated batch (Figure 5). In both scenarios (1 and 4), Tr to B1 was constantly higher than to all successive batches ($p < 0.05$) with a remarkable difference at the last batch, B6. This may imply that although gloves changing still have contributed to pathogen transmission onto the last batches, the contaminated surface of the CB is relatively the key contributing factor to constant transmission of pathogens to all parsley batches.

Our results concur with Pérez-Rodríguez et al. (2011) where a risk mathematical model showed *Escherichia coli* O157:H7 was able to survive and contaminate final bags of fresh-cut lettuce in all simulated interventions scenarios. They are also consistent with a study by Soares et al. (2012) where an average of 2.71 log CFU was recovered from tomatoes cross-contaminated via cutting boards that were formerly contaminated with artificially inoculated chicken skin with *S. Enteritidis* (5 log CFU/g). Several studies examining bacterial Tr between surfaces and single sliced foods reported a substantially high variability in data; Chen et al. (2001) recorded a Tr of *E. aerogenes* between various surfaces ranging from 0.0005% to 100%, and between washed hand previously contaminated with 10^6 cells and lettuce (0.003 to 100%). This variable pattern was observed from individual to individual despite that all participants followed the same experimental protocol. In comparison to above studies, Tr values to single sliced batch (B1) was generally lower and varied to a lesser extent from 0.76 to 25% on CB Instant and 0.06-7.5% on CB WW (Table 2). This difference and variations in Tr data are likely to be attributed to inner folds of parsley leaves, level of *S. Typhimurium* originally on parsley, and experiment set-ups, e.g., post-inoculation holding time before chopping. In other studies, bacterial transmissions occurred from inoculated abiotic to biotic surfaces (Soares et al., 2013; Moore et al., 2003; Ravishankar et al., Chen et al., 2001; Zilelidou et al., 2015), whereas in the present work, cross-contamination events were studied from contaminated parsley to clean uncontaminated batches by means of CB. In this case,

heterogeneity in attachment levels of cells to CBs could occur as affected by angle of contact on CB, thus the variations in Tr to batches of un-inoculated leaves. Nevertheless, the scenarios in the present work may closely reflect the real and natural variability expected among individuals during chopping parsley in restaurants and homes settings.

Moore et al. (2003) also reported a wide range in Tr data from stainless steel surfaces to one-time sliced lettuce for *S. Typhimurium*, 13.15-67.63% and *Campylobacter*, 0.19-43.97%.

It is maintained that large variations in Tr data are a consequence to errors inherent to microbial collection from surfaces (Carrasco et al., 2012), methodological differences and difficulty in controlling all factors involved in bacterial transfer phenomena which in this case would not allow for easy comparisons among different cross contamination studies (Zilelidou et al., 2015).

In general, *S. Typhimurium* was apparently readily transferred into cutting boards, and later was capable of contaminating chopped parsley both at instant contact and at 24 h after washing, with the ability to cross-contaminate the entire batches of leafy greens most outstandingly at low contamination level.

The survival of bacteria for long time on surfaces is documented in various works where bacterial counts increased over time and wet surfaces played an important role in bacterial transfer to food (Scott et al., 1990). Many other factors were suggested to influence pathogens transmissions between surfaces such as the topography of different kind of cutting boards (Goh et al., 2014) and temperature of food (Goh et al., 2014; Tang et al., 2011). Nonetheless, we believe that the survival of *S. Typhimurium* for prolonged time (24 h) has been probably sustained by remaining substrates from parsley juice within knives-scars and fissures on the plastic boards surfaces which have been shown to be very difficult to clean and disinfect, although this may vary among the types of plastic cutting boards (Cliver, 2006). It was evident in this study that the density of bacteria can remain constant up to 24 h supported by nutrients abundance (Dawson et al., 2007). Although we have not tested the

moisture levels on cutting board surfaces, CBs were apparently dried out after 24 h incubation at 30°C, and still was found to harbour microorganisms although at constant and in other conditions at reduced levels. There is a wide recognition that *S. Typhimurium* is capable of persistent survival on dry surfaces for up to 4 weeks and that the transfer rate to food was reduced as the bacterial exposure time on the surface increased up to 24 h (Dawson et al., 2007). Other authors proposed that higher temperatures enhance the drying process resulting in a decrease of cultivable bacteria (Milling et al., 2005).

The plausible explanation for the reduced transfer rate observed in our study is that *S. Typhimurium* might have been stressed or injured during the washing process (Dawson et al, 2007) and more likely that there is a threshold value of cells that can be transferred depending on the capacity of cutting board surface to harbour attached cells under the conditions of the study. Thus, even if 10^6 cells are present on surface, only a magnitude of 10^3 can be transferred from the contaminated surface to fresh (un-inoculated) batches as a result of simple contact.

Our work paralleled several studies that revealed the inefficiency of water or water and soap in eliminating pathogens from cutting board surfaces, hence the limited reduction in transfer rate (Cogan et al., 2002; Ravishankar et al., 2010; Soares et al., 2012). It showed that without appropriate disinfection procedures for cutting boards used with contaminated fresh leafy greens, the risk of cross-contamination remains regardless of the number of batches processed at one time, particularly when the infective dose can be as low as 10 cells.

4.2 Recovery of *S. Typhimurium* cells from the cutting board surfaces

Table 3 and 4 show the numbers of *S. Typhimurium* recovered from CBs after initial chopping of inoculated samples (S0) and after each subsequent chopping of a new batch (S1-S6).

At high inoculum level, mean values of recovered *S. Typhimurium* (log CFU/cm²) showed a decreasing trend as more batches were sequentially placed on the same surface.

Overall, the number of recovered cells ranged from below detection limit (10 CFU/ cm²), observed towards the last chopped batches, to a maximum mean of 0.23 log CFU/cm² at the early stage of chopping, which is equivalent to max. 4.14 log CFU/swabbed area. Whereas at low inoculum levels, fewer organisms were recovered from CB Instant (0.10-0.12 log CFU/cm²) ranging from below detection limit to 0.17 log CFU/cm². Washing CB with water significantly decreased the number of microorganisms on cutting boards to mean values of 0.09 to 0.11 log CFU/cm² (P<0.05) with a maximum level of 2.73 log CFU/swabbed area. Mann-Whitney U test indicated a significantly lower level recovered from CB Instant exposed to low inoculum levels than from that exposed to high inoculum levels. Results on surface swabs support those obtained on the Tr to parsley, and the assumption that with high inoculum size at the source, the adherence of bacterial cells to the surface is enhanced and vice-versa (Montville & Schaffner, 2003).

Our results are in accordance with other studies where bacteria were recovered from plastic cutting boards at 5 min resident drying times and 24 h following cold wash water (Abrishami et al., 1994) and where low counts (<1 log CFU/g or cm²) of *S. Newport* remained on the plastic surface previously exposed to contaminated poultry, and tested after washing with soap, warm water, and vigorous scrubbing (Ravishankar et al., 2010).

Although some swabs had counts below detection limits, detection tests recorded a positive presence of the pathogen. As discussed earlier, *S. Typhimurium* has the propensity to survive in high levels depending on nutrient and water availability (Pui et al., 2011) within the

cutting boards crevices for a prolonged period in stressful conditions. In such conditions, microorganisms may enter a state of metabolic inactivity, resulting in viable and non-cultivable cells that are able to grow again under favourable conditions (De Boer et al., 1990).

There is the limitation that part of the inoculum within the knives-scarred plastic surfaces could possibly become unavailable to the swabs used to recover it. Besides, the disadvantage of cotton swabs being limited to recover 100% of the resident microorganisms is known as the pressure applied to the surface during sampling could be too light (Moore et al., 2007). Despite this limitation, our results confirmed that bacterial cells can be transmitted from contaminated leafy greens to a sterile surface and subsequently contaminating a number of individually chopped parsley portions providing valuable information for future risk assessments of cross-contamination associated with the preparation of fresh parsley.

5. Conclusion

This study demonstrated how *S. Typhimurium* is transferred by common operations from contaminated parsley to cutting boards and it would subsequently re-contaminate several batches of parsley, in this case to up to 6 sets when chopped consecutively on the same surface. More concerning was the recovery of presumably more resilient pathogen cells from cutting boards at 24 h at 30°C after washing. Apparently, the simple domestic washing methods applied in restaurants using water and soapy water with sponge scrubbing reduced the transfer rate to all batches of parsley chopped subsequent to the contaminated samples on the same surface, but it did not effectively eliminate the risk of cross-contamination at instant and 24 h exposure to bacteria. The results of this study also confirmed that even at low contamination level on the source, considerable amounts of bacteria were transferred to parsley, as significantly higher transfer rates occurred at low inoculum level than at high initial level.

Therefore, the application of additional sanitation procedures such as hypochlorite are needed on cutting surfaces not only after use with raw meat and poultry, but also with fresh produce especially as parsley is not further treated (ready-to-eat). Research on the efficiency of the FDA recommended practice for cleaning cutting boards with soap, hot water and mechanical scrubbing on *S.Typhimurium* merits investigation.

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7. References

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Table 1. *S.Typhimurium* transferred to un-inoculated parsley chopped subsequently to inoculated batches

Inoculum level	Cutting board handling	Initial inoculated batch [‡]	N [†]	Mean (min.-max.)	Median	Transfer rate (%) Mean (min.-max.)
Low	CB Instant	3.30	64	2.94 ^a (2.00 - 3.85)	2.87	60 ^b (2.00-100.00)
	CB WW	3.20	55	2.67 ^a (2.00 - 3.48)	2.70	64 ^c (2.00- 100.00)
High	CB Instant	6.08	50	3.41 (<1.00 [†] - 5.51)	3.54	1.2 ^b (0.01-25.00)
	CB WW	5.95	15	3.67 (2.78 - 4.69)	3.64	1.4 ^c (0.05-7.50)
	CB SW*	6.23	26	3.50 (<1.00- 4.72)	4.07	1.0 (0.05-2.83)

[‡]The inoculated parsley (100g) was initially chopped as the very first batch. Low inoculum level range= 2.85 -4.00

Log CFU/g. High inoculum range= 5.80 – 6.32 Log CFU/g

[†] The number of analyzed samples of parsley

*CB SW scenario was not tested with low inoculum levels

Similar superscript letters in the same column indicate significant difference at p<0.05

Table.2. *S.Typhimurium* transfer rates (%) to three consecutively chopped un-inoculated parsley subsequent to contaminated samples on same cutting board surface

Cutting board handling	Parsley batches					
	Mean (min-max)					
	Low inoculum			High inoculum		
	B1	B2	B3	B1	B2	B3
CB Instant	81.0 ^a (23.0-100)	58.5 (10.0-100)	53.1 ^a (2.0-100)	5.5 ^{ab} (0.76-25)	0.7 ^a (0.09-2.0)	0.5 ^b (0.09-1.0)
CB WW	61.0 (9.0-100)	55.0 (3.0-100)	30.0 (2.0-100)	3.2 ^a (0.06-7.5)	0.4 (0.08-0.7)	0.08 ^a (0.05-0.08)

Similar superscript letters in the same row at each inoculum level indicate significant difference at p<0.05

B1=first batch; B2=second batch;B3=third batch

Table 3. Recovery of *S. Typhimurium*[‡] from CB Instant after consecutively chopped batches of parsley subsequent to inoculated sample with high inoculum levels

Sequence of swabs [†]	N	Mean (min.-max.)
S0	3	0.22 (0.18 - 0.25)
S1	3	0.23 (0.20 - 0.24)
S2	3	0.21 (0.16 - 0.25)
S3	3	0.20 (0.16 - 0.23)
S4	3	0.14 (<1.0 - 0.15)
S5	3	0.16 (<1.0 - 0.20)
S6	3	0.03 (<1.0 - 0.03)

[‡]Log CFU/cm²

[†]Swabbing after chopping parsley. S0 is the first swab taken after initial chopping of 100g parsley inoculated with a mean population size of 6.13 Log CFU/g, followed by 6 swabs (S1-S6), each taken after chopping un-inoculated batch (for the additional 6 batches).

Table 4. Recovery of *S. Typhimurium*[‡] from CB Instant after consecutively chopped batches of parsley subsequent to inoculated sample with low inoculum levels

Sequence of swabs [†]	CB Instant		CB Water Wash (WW)	
	N	Mean (min.-max.)	N	Mean (min.-max.)
S0 ^a	12	0.10 (<1 - 0.17)	10	0.09 (0.09 – 0.09)
S1	12	0.12 (<1 - 0.14)	10	0.10 (<1– 0.11)
S2	12	0.12 (<1- 0.14)	10	0.11 (<1– 0.12)
S3	12	0.12 (<1- 0.14)	10	0.11 (<1– 0.13)
S4	6	0.11 (<1- 0.12)	5	0.10 (<1– 0.12)

[‡] Log CFU/cm²

[†] Swabbing after chopping parsley. S0 is the first swab taken after initial chopping of 100g parsley inoculated with a mean population size of 3.73 and 3.15 Log CFU/g for CB Instant and CB WW, respectively, followed by 4 swabs (S1-S4), each taken after chopping un-inoculated batch (for the additional 4 batches).

In the case of CB WW, the swab S0 was taken at 24 h after water wash and incubation at 30°C.

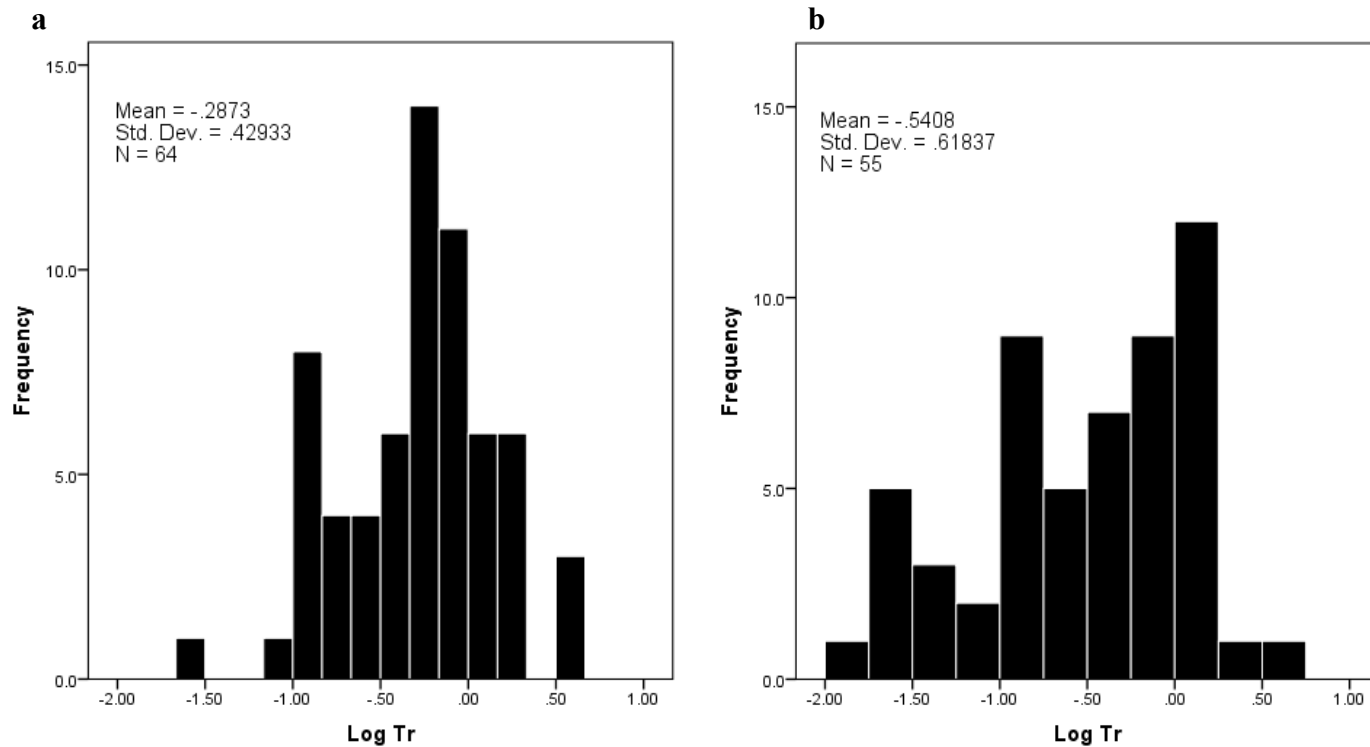


Figure 1. Frequency histograms of Log transfer rate of *S. Typhimurium* on all un-inoculated chopped batches

A comparison of Log transfer rate of *S. Typhimurium* from inoculated parsley to un-inoculated batches chopped (merged data), (a) instantly on same cutting board (CB Instant), (b) after simple water wash of same cutting board and 24 h holding at 30°C (CB WW), subsequent to initially chopped 100g parsley inoculated with 3.26 Log CFU (a), and 3.16 Log CFU (b).

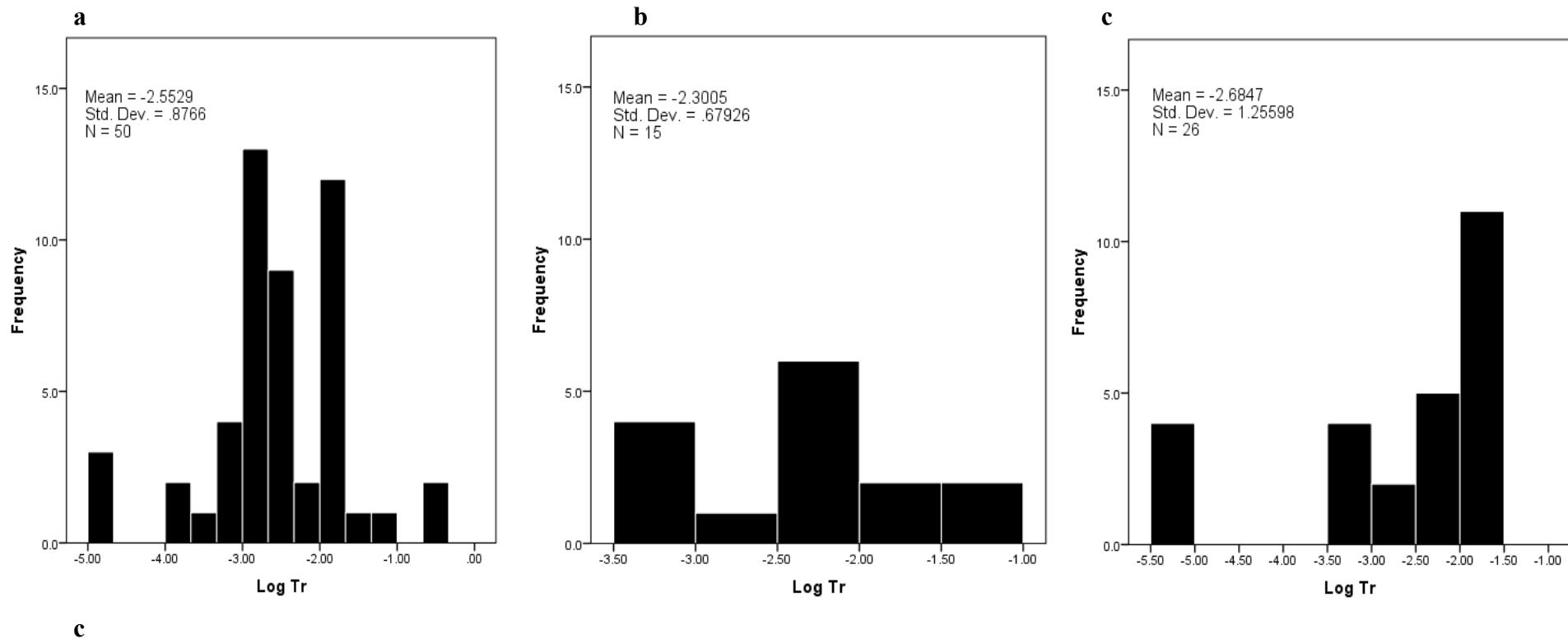


Figure 2. Frequency histograms of Log Transfer rate of *S. Typhimurium* on all un-inoculated chopped parsley

A comparison in Log transfer rate of *S. Typhimurium* to un-inoculated parsley chopped, (a) instantly on same cutting board (CB Instant), (b) after water wash of same cutting board and holding for 24 h at 30°C (CB WW), (c) after soap and water wash combined with soft sponge rubbing and holding for 24 h at 30°C (CB SW), subsequent to initially chopped 100g parsley inoculated with high inoculum level of 6.08 (a), 5.95 (b), and 6.23 Log CFU/g (c)

The outliers' data values >-4.00 represents samples with bacterial counts below detection levels (10 CFU) (n=8)

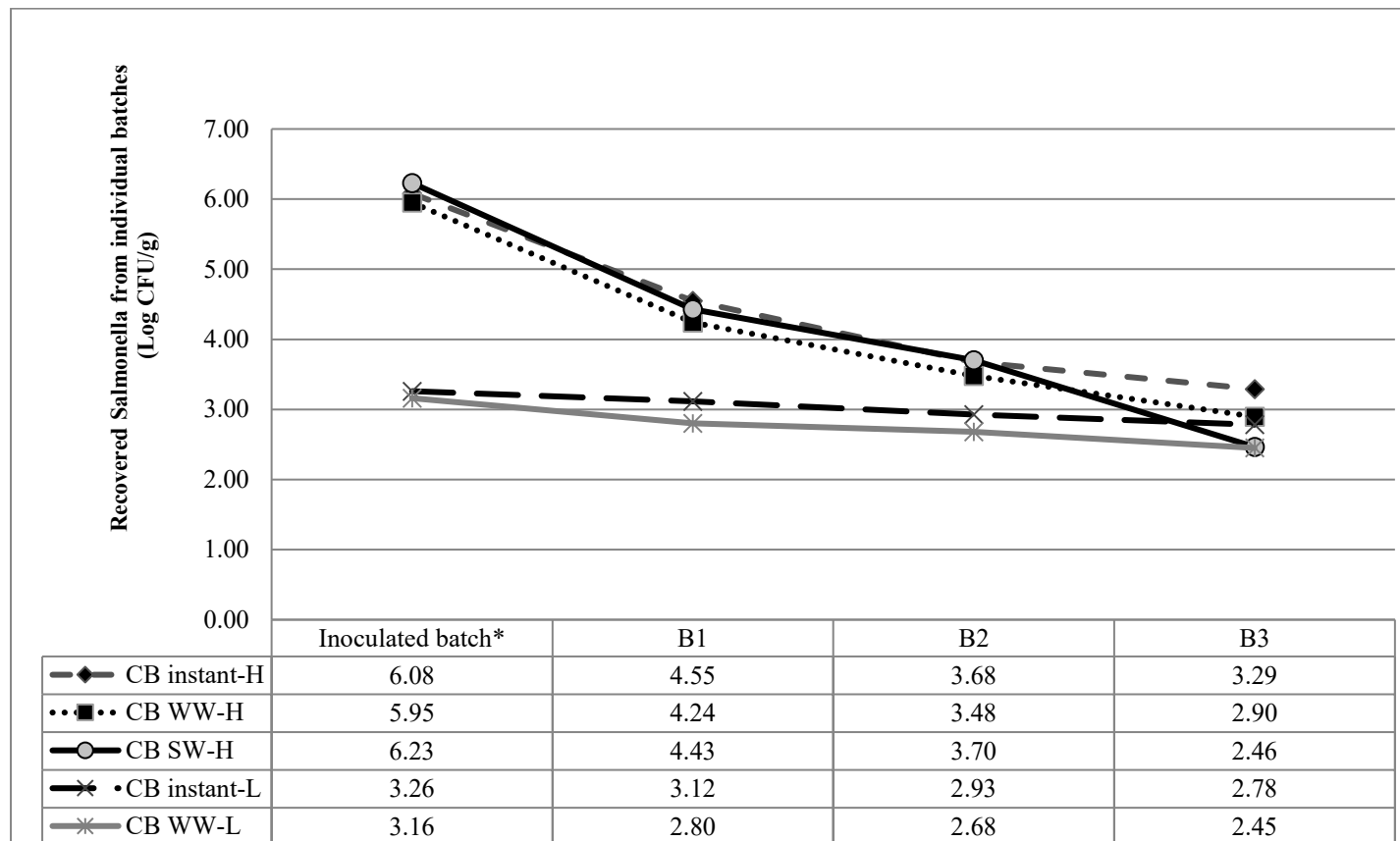


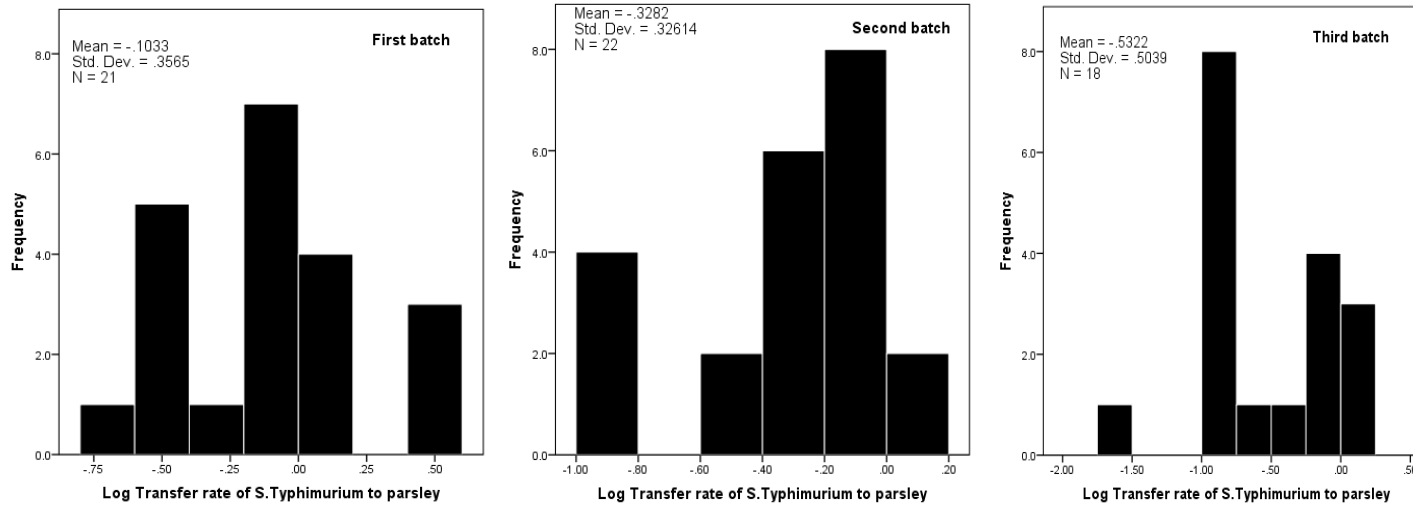
Figure 3. The population size of *S. Typhimurium* on successively chopped batches of parsley after initial chopping of artificially contaminated samples

A decreasing trend in the recovery of *S. Typhimurium*, particularly with high inoculum levels on source, along the three successive batches of un-inoculated parsley chopped subsequent to initially chopped 100g parsley inoculated, (A) instantly on same cutting board (CB Instant), (B) after water wash of same cutting board and holding for 24 h at 30°C (CB WW), (C) after soap and water wash combined with soft sponge rubbing and holding for 24 h at 30°C (CB SW). The latter scenario was not tested with low inoculum levels.

*Initially chopped inoculated parsley (Log CFU/g)

L = low inoculum; H= High inoculum.

a. CB Instant



b. CB WW

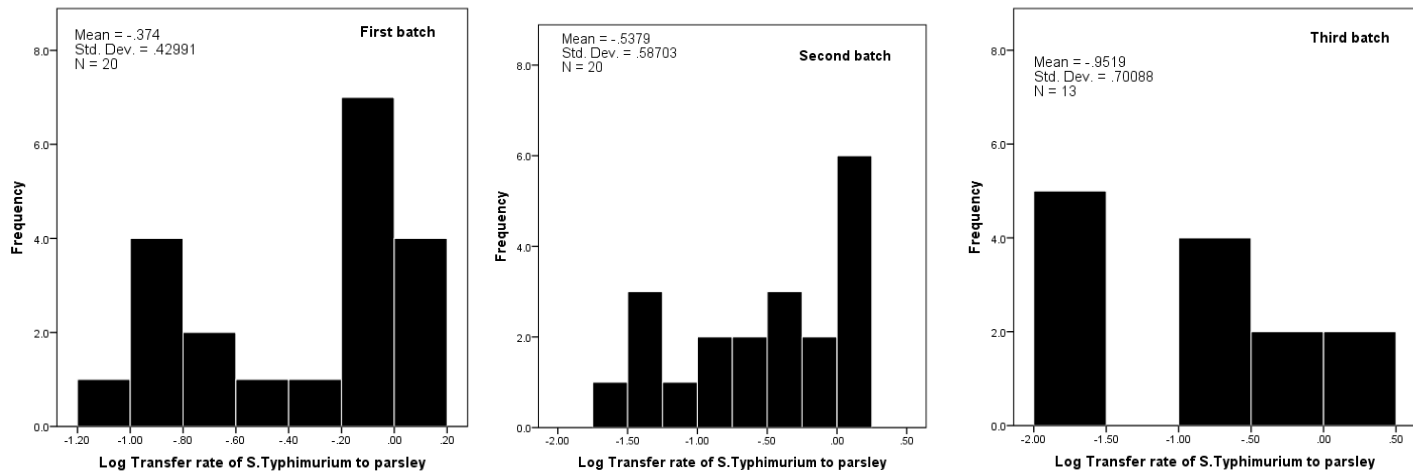


Figure 4. Frequency histograms of Log Transfer rate of *S.Typhimurium* on individual chopped batch
 Comparison of Log transfer rate of *S.Typhimurium* from initially chopped inoculated parsley (100g) with ca. 3 Log CFU/g to three consecutively chopped batches of un-inoculated parsley (30g) on, (a) CB Instant (b) CB WW.

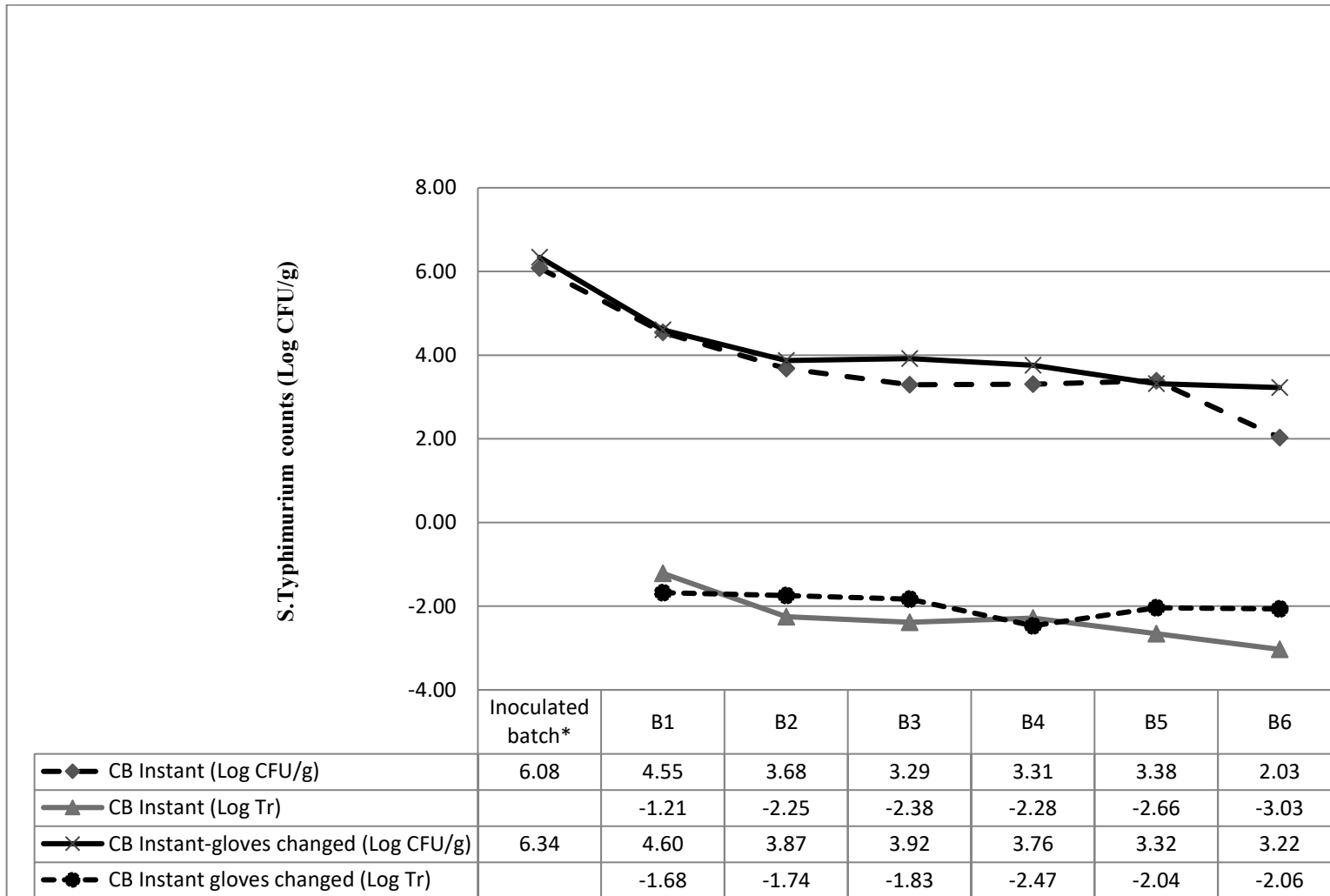


Figure 5. Log transfer rate and *S.Typhimurium* counts recovered from consecutively chopped batches of parsley on CB Instant with and with no changing gloves

The reduction pattern in cells recovery and Log transfer rate of *S.Typhimurium* was consistent through the first five batches of parsley chopped instantly subsequent to inoculated samples (CB Instant), with and without changing gloves.

B1 to B6 is the order of chopping order of each batch following the first 100g inoculated samples

*Initially chopped inoculated parsley (Log CFU/g)