



# Survival and inactivation of *Salmonella enterica* serovar Typhimurium on food contact surfaces during log, stationary and long-term stationary phases



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## ABSTRACT

One of the most important transmission routes of foodborne pathogens is through contaminated food contact surfaces. In this study, we investigated the survival and inactivation of *Salmonella enterica* serovar Typhimurium on frequently used food contact surfaces in household settings, including plastic cutting board (CB), formica laminate (LA), and stainless steel (SS) surfaces. *S. Typhimurium* at log (6 h), stationary (24 h), or long-term stationary (LTS) (14 days) phases were evaluated. Results showed that, at medium and high microbial loads, LTS phase cells had significantly higher survivor population compared to log and stationary phase cells at 24 h after inoculation ( $p < 0.05$ ). Disinfection study showed that LTS phase cells were very resilient to sodium hypochlorite when the initial microbial load was high with disinfection efficacy ranging from 26 to 35%. Scanning electron microscopy of these surfaces at 24 h after inoculation with a high microbial load revealed an early biofilm structure.

## 1. Introduction

Foodborne illness is an important public health problem in the United States. According to the Centers for Disease Control and Prevention (CDC), about 1.2 million illnesses, 19,000 hospitalizations and 450 deaths are caused annually by non-typhoidal *Salmonella* in the U.S. (Scallan et al., 2011). *Salmonella enterica*, an important pathogen closely related to food-producing animals especially poultry, is one of the most frequent causes of foodborne gastroenteritis in humans (Ibarra and Steele-Mortimer, 2009). The annual cost of foodborne illnesses associated with non-typhoidal *Salmonella* is estimated to be 3.7 billion dollars (Hoffmann et al., 2015). These costs include medical expenses, productivity loss, and valuation of premature mortality. Not only foodborne illnesses harm the consumers, they also cost the food industry about 7 billion dollars yearly in consumer notifications, the removal of food from shelves, and the settlement of damages as a result of lawsuits (Hussain and Dawson, 2013).

Contaminated food contact surfaces play a major role in the transmission of bacterial and viral pathogens to food (Martinez-Gonzales et al., 2003; Tuladhar et al., 2012). In the case of *Salmonella*, food contact surfaces can be contaminated through direct or indirect contact with raw meat and poultry products (Adak et al., 2005; Wilson, 2002). Adequate cleaning and disinfection procedures are essential to reduce or eliminate microbial contamination from surfaces and to prevent the

formation of biofilm on food contact surfaces (Abushelaibi et al., 2012).

Several types of disinfectants are available on the market such as oxidizing disinfectants, alcohols, and tenside-based disinfectants. Multiple factors, such as temperature, presence of organic matter, type of surface to be disinfected, exposure time, user-concentration, and target organism could influence disinfectant's effect (Møretro et al., 2009). The choice of both disinfectant and disinfection strategy should be based on the antimicrobial effect required for a specific application (Møretro et al., 2009).

Bacteria can survive for a long period of time without the addition of nutrients on food contact surfaces where they exist in long-term stationary phase (LTS). During this time, their stress responses to starvation may also increase pathogenicity and enhance resistance to secondary stresses (Lisle et al., 1998). Insufficient or ineffective removal of organic material from spills or carcass eviscerations run off provides the bacteria with an ideal medium to survive and prosper on food contact surfaces (Brown et al., 2014). This represents a serious problem in not only food processing facilities but also the home kitchen settings. To our knowledge, however, no study has been published on the survival and inactivation of *S. Typhimurium* on food contact surfaces during log, stationary, and LTS phases. Therefore, it is interesting to evaluate the persistence of *S. Typhimurium* collected during these three growth phases on three food contact surfaces as well as their resistance to household disinfectants with LTS phase being the focal point of this

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study.

## 2. Materials and methods

### 2.1. Bacterial strain and inoculum preparation

For each experimental trial, 10  $\mu\text{L}$  of *S. Typhimurium* (ATCC, 19585) frozen glycerol stock was used to inoculate 10 mL fresh tryptic soy broth (TSB) (Remel, Lenexa, KS) tube. The culture was incubated for 24 h at  $37 \pm 2^\circ\text{C}$  and second transferred to a 10 mL fresh TSB tube to yield an approximate population of  $10^9$  CFU/mL after overnight incubation at  $37^\circ\text{C}$ . Twenty-five microliters of the 1:100 diluted overnight culture of *S. Typhimurium* was used to inoculate 25 mL TSB. Cultures were incubated at  $37^\circ\text{C}$  under shaking (180 r/min) for up to 14 days.

### 2.2. Food contact surfaces preparation and inoculation

Three typical food contact surfaces—plastic cutting board (CB), formica laminate (LA), and stainless steel (SS) were purchased from a chain hardware store. Surfaces were cut into  $7.6\text{ cm} \times 1.2\text{ cm}$  ( $9.12\text{ cm}^2$ ) coupons. Surface coupons were sterilized by autoclaving at  $121^\circ\text{C}$  for 30 min and placed in sterile petri dishes with sterile tweezers in a biosafety hood until use. Bacterial cells were collected at different growth phases (log (6 h), stationary (24 h), and LTS (14 days)). Cells were centrifuged and re-suspended in 1 mL of chicken juice to simulate real-life contamination with high organic load. Chicken juice was collected from packages of whole chicken and stored in sterile tubes at  $-25^\circ\text{C}$  until use. The presence of organic load on the surface has been reported to benefit bacterial adhesion and to improve its survival on surfaces (de Kerchove and Elimelech, 2007; Helke and Wong, 1994; Hwang et al., 2013). Each surface coupon was spot inoculated with 100  $\mu\text{L}$  of the inoculum where final cell concentrations were approximately 2, 4, or 6  $\log_{10}$  CFU/ $\text{cm}^2$  to simulate high, medium, and low microbial load, respectively.

### 2.3. Disinfection study

Surfaces were either disinfected immediately (0 h) or after 2 or 24 h, during which surface coupons were kept in the biosafety hood. One of the two commercial disinfectants, including sodium hypochlorite (0.0095%) or  $\text{H}_2\text{O}_2$  (0.88%) was applied according to manufacturer's instructions. After required contact time (2 min for sodium hypochlorite or 10 min for  $\text{H}_2\text{O}_2$ ), sterilized wipes held by sterilized tweezers were used to wipe the surface one time. The wiped surface was transferred with sterilized tweezers to a sterile stomacher bag with 50 mL Dey/Engley (D/E) neutralizing broth, (BD Difco, Sparks, MD) and massaged vigorously for 2 min. Recovered bacteria from surfaces were determined by direct plating method on xylose lysine deoxycholate (XLD) agar (BD Difco, Sparks, MD). *S. Typhimurium* colonies were enumerated after incubation for 24 h at  $37^\circ\text{C}$ . After plating, all stomacher bags containing neutralizer broth and surface coupon were incubated as enrichment for 24 h at  $37^\circ\text{C}$ .

In case of zero plate count, enrichment was streaked in duplicates on XLD plates and incubated for 24 h at  $37^\circ\text{C}$ . The detection limit ( $0.73 \log_{10}$  CFU/ $\text{cm}^2$ ) was assigned for positive samples after enrichment. A surface coupon without disinfection served as control at each time point where recovered bacteria were used to assess *Salmonella* survival on the CB, LA, and SS as well as to calculate log reduction in *S. Typhimurium* population after disinfection. Disinfection study results were also expressed in percentage disinfection efficacy as it was a better indicator of disinfection effectiveness than log reduction. Each log reduction value was normalized to its correspondent control as shown in this equation: Disinfection Efficacy (%) =  $100 \times (A-B)/A$ ; With A: *S. Typhimurium* control population and B: *S. Typhimurium* survivor population after disinfection.

### 2.4. Scanning electron microscopy (SEM) of surfaces inoculated with *S. Typhimurium* collected at LTS phase

*S. Typhimurium* cells were collected at day 14 and prepared as abovementioned. The final cells concentration was at  $6 \log_{10}$  CFU/ $\text{cm}^2$ . CB, LA, and SS coupons were spot inoculated with a 100  $\mu\text{L}$  inoculum and allowed to dry out for 2 h in a biosafety hood. At 24 h after inoculation, samples were fixed in 1.25% glutaraldehyde for 2 h and prepared for SEM. Preparation steps included washing three times with 0.1 M sodium cacodylate (CAC) buffer with 5% sucrose (pH 7.4) at room temperature. Then, a solution of 1% of osmium tetroxide in 0.1 M CAC buffer (pH 7.4) was applied to wash samples at room temperature for 1 h. After washing with water, the critical point drying was performed. Finally, samples were sputter coated with platinum for 4 min. Samples were observed under Quanta™ 3D Dual Beam™ FEG FIB-SEM microscope (FEI, Hillsboro, OR). Pictures were acquired at magnifications of 6.5 K and 20.00 K.

### 2.5. Statistical analysis

All experiments were repeated three times with duplicate surface coupons. Results were reported as Mean  $\pm$  SD. Statistical comparisons in the results section were performed using Tukey's Honest Significant Difference (HSD) test in JMP®, version Pro 13 (SAS Institute Inc., Cary, NC). A *p*-value of less than 0.05 was considered statistically significant.

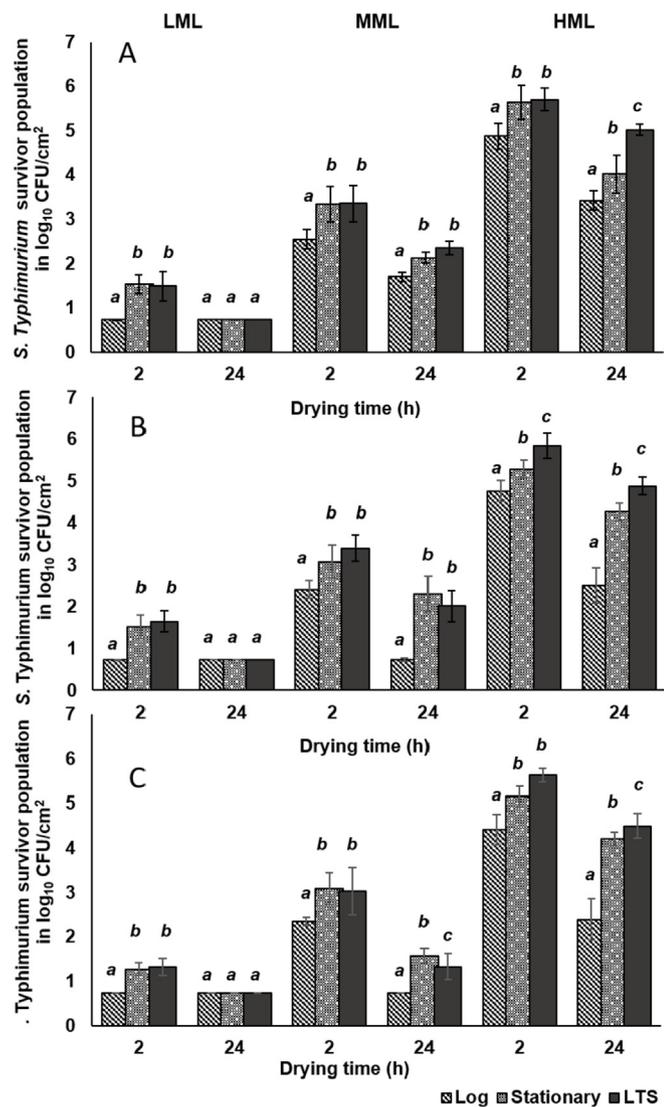
## 3. Results and discussion

### 3.1. Survival of *S. Typhimurium* on plastic cutting board (CB), laminate (LA) and, stainless steel (SS) surfaces

The survival of *S. Typhimurium* on three food contact surfaces in home kitchen settings was illustrated in Fig. 1. The overall survival population of *S. Typhimurium* ranged from the detection limit ( $0.73 \log_{10}$  CFU/ $\text{cm}^2$ ) to  $5.8 \log_{10}$  CFU/ $\text{cm}^2$  varying by the surface type and initial microbial load.

To evaluate the survival of *S. Typhimurium*, besides surface type and growth phase of bacteria, factors such as initial microbial load and drying time before recovery were also taken into consideration. Overall, regardless food contact surface types, LTS phase cells survived better than log phase cells at all drying times in the case of high and medium microbial loads, as well as at 2 h for low microbial load. Compared to stationary phase cells, LTS phase cells had greater survival population only at high microbial load (at 24 h on CB surfaces, at 2 and 24 h on LA surface, and at 2 h on SS surface). The resilience showed by both stationary phase and LTS phase cells to drying compared with log phase cells may be explained by the expression of the alternative sigma factor *rpoS* triggered by bacteria entry into stationary phase (Wesche et al., 2009). The *rpoS* sigma factor in gram-negative bacteria prepares the cell for survival under minimal nutrition and cell division (Wesche et al., 2009). In *Salmonella*, the expression of *rpoS* was reported to be crucial for optimal drying and desiccation tolerance (Jørgensen et al., 2000).

*S. Typhimurium* showed different survival population on different food contact surfaces, especially between 2 and 24 h after inoculation. Greater survivor population was observed on CB and LA (hydrophobic surfaces) than on SS (hydrophilic surface) at 24 h drying time at medium ( $4 \log_{10}$  CFU/ $\text{cm}^2$ ) and high ( $6 \log_{10}$  CFU/ $\text{cm}^2$ ) microbial loads. According to De Cesar et al. the evaporation rate of the cellular suspending media varies depending on the surface hydrophobicity, which may further affect bacterial survival (De Cesare et al., 2003). Hydrophilic surfaces such as SS yield a larger droplet surface area causing them to dry out faster, while hydrophobic surfaces such as CB and LA yield droplets with smaller diameter and a larger height resulting in a slower drying rate, allowing therefore the bacterial cells to remain viable in a hydrated environment for a longer period of time (De



**Fig. 1.** Survival of *S. Typhimurium* cells collected at log, stationary, and LTS phases on food contact surfaces. Plastic cutting board (A), laminate (B), and stainless steel surfaces (C) were inoculated with low (LML, 2 log<sub>10</sub> CFU/cm<sup>2</sup>), medium (MML, 4 log<sub>10</sub> CFU/cm<sup>2</sup>), and high (HML, 6 log<sub>10</sub> CFU/cm<sup>2</sup>) microbial loads of *S. Typhimurium* collected at the appropriate growth phase. Samples were taken at 2 and 24 h after surface drying under biosafety hood. Experiments were performed in triplicate, and error bars indicate standard error. Different letters show statistically significant differences (*p* < 0.05) when comparing *S. Typhimurium* survivor population between log, Stationary, and LTS phases in log<sub>10</sub> CFU/cm<sup>2</sup> within each microbial load.

Cesare et al., 2003).

### 3.2. Efficacy of two household disinfectants against *S. Typhimurium* at log, stationary and LTS phases

The disinfection efficacy of two commonly used disinfectants: sodium hypochlorite and H<sub>2</sub>O<sub>2</sub> to eliminate *S. Typhimurium* from three food contact surfaces (CB, LA, and SS) were evaluated in this study. Decontamination was performed immediately (0 h), and after 2 or 24 h following surface inoculation to mimic three common cleaning/sanitizing behaviors.

Sodium hypochlorite and H<sub>2</sub>O<sub>2</sub> are both widely used disinfectants in home kitchens. The antimicrobial activity of both disinfectants include attacking essential bacterial cell components such as DNA and proteins through their active form (OCl<sup>-</sup> and <sup>•</sup>OH in the case of sodium

hypochlorite and H<sub>2</sub>O<sub>2</sub>, respectively) (Block, 2001). Tables 1–3 show decontamination results for sodium hypochlorite disinfectant on CB, LA, and SS, respectively. Since H<sub>2</sub>O<sub>2</sub> achieved 100% decontamination efficacy, data was not shown in the paper. Decontamination results were reported in log reduction in *S. Typhimurium* after surface decontamination with the correspondent disinfectant as well as in percentage disinfection efficacy.

Sodium hypochlorite reduced the microbial population significantly compared to the control (no disinfectant) on all three surfaces and over all three microbial loads. On CB and SS surfaces (Tables 1 and 2), when the initial microbial load was high, LTS phase cells were significantly more resilient to sodium hypochlorite decontamination compared to stationary phase cells (*p* < 0.05). Disinfection efficacy percentages against LTS phase cells were at 31–32% and 34–35% at 2 and 24 h, on CB and SS surfaces, respectively. On LA surface, sodium hypochlorite's disinfection efficacy against LTS phase cells was at its lowest (around 26%) at all times when the initial microbial load was high, which may be because of the textured and non-smooth surface structure (Table 3). However, no statistical significant difference was observed between LTS and stationary phase cells (*p* > 0.05).

Mixed results were observed at medium and low microbial loads. On CB surface, significant difference between disinfection efficacy of stationary and LTS phase cells was found only when disinfection occurs immediately after surface inoculation (0 h) at high and medium microbial load. The same trend was observed on SS surface but only at high microbial load. On LA surface, LTS phase cells were more resilient to disinfection than stationary phase cells at all times except at 0 h when starting with a medium microbial load (*p* < 0.05). Whereas, at low microbial load, disinfection efficacy for both stationary and LTS were only similar at 24 h (*p* > 0.05). Log phase cells were the least resistant to sodium hypochlorite disinfection throughout this study compared to stationary and LTS phase cells (*p* < 0.05).

The resilience showed by LTS phase cells to sodium hypochlorite at 24 h after inoculation when the initial microbial load was high (6 log<sub>10</sub> CFU/cm<sup>2</sup>) may be explained by the high cell density on the surface leading to a potential early biofilm formation, the presence of organic matter (chicken juice), and the expression of bolA, a transcription factor induced at the onset of stationary phase (Mil-Homens et al., 2018).

SEM micrographs as illustrated in Fig. 2 show that *S. Typhimurium* LTS phase cells look enclosed in a matrix and interconnected with rod like structures, which indicate an early biofilm structure formation on these surfaces. Biofilm structure was more prominent however on hydrophobic surfaces (CB and LA) compared to hydrophilic surface (SS). This finding was in accordance with numerous studies reported that once attached to abiotic surfaces, planktonic bacteria cells aggregate in a hydrated extracellular polymeric substance of their own synthesis which may consist of proteins, polysaccharides, and nucleic acids (Aparna and Yadav, 2008; Reysenbach and Cady, 2001; Stoodley et al., 2002). This interconnected matrix linking bacteria cells together comes as a first step in preparation of forming biofilms where cells begin to establish an organized community (Aparna and Yadav, 2008; Reysenbach and Cady, 2001; Stoodley et al., 2002). The presence of such early biofilm structure would decrease the diffusion rate of the disinfectant leading to a lower disinfection efficacy, which was indeed observed in this study.

In addition, the presence of organic matter on the surface may have contributed to the observed sodium hypochlorite resistance. According to Randtke (2010), redox reactions can take place between chlorinated compounds and organic matter converting HOCl, the reactive free available chlorine species (at neutral pH) to less reactive chemicals including mono, dichloramines, and N-chloro compounds (Randtke, 2010). Finally, bolA protein has been shown to contribute to the survival of the pathogen in hostile conditions. In *S. Typhimurium*, Mil-Homens et al. (2018) showed that bolA influenced pathogen fitness, conferred its resistance to oxidative stress, and increased its

**Table 1**  
Inactivation of *S. Typhimurium* on plastic cutting board (CB) surface using sodium hypochlorite

Microbial load	Time waited before disinfection (h)	Log	Stationary	LTS
		Log <sub>10</sub> reduction in <i>S. Typhimurium</i> (Disinfection efficacy (%))		
<sup>a</sup> HML	0	3.79 ± 0.26 (63%) <sup>ax</sup>	3.22 ± 0.11 (56%) <sup>ay</sup>	2.83 ± 0.31 (47%) <sup>az</sup>
	2	3.43 ± 0.20 (67%) <sup>ax</sup>	2.03 ± 0.42 (40%) <sup>by</sup>	1.82 ± 0.33 (31%) <sup>bz</sup>
	24	2.99 ± 0.24 (93%) <sup>bx</sup>	1.47 ± 0.40 (43%) <sup>by</sup>	1.34 ± 0.22 (35%) <sup>bz</sup>
<sup>b</sup> MML	0	3.10 ± 0.34 (65%) <sup>ax</sup>	1.73 ± 0.11 (44%) <sup>ay</sup>	2.11 ± 0.25 (56%) <sup>az</sup>
	2	2.01 ± 0.09 (87%) <sup>bx</sup>	2.27 ± 0.45 (73%) <sup>by</sup>	2.17 ± 0.10 (69%) <sup>by</sup>
	24	1.70 ± 0.11 (100%) <sup>cx</sup>	2.16 ± 0.11 (100%) <sup>cx</sup>	2.48 ± 0.15 (100%) <sup>cx</sup>
<sup>c</sup> LML	0	1.96 ± 0.12 (80%) <sup>ax</sup>	1.77 ± 0.03 (71%) <sup>ay</sup>	1.75 ± 0.26 (70%) <sup>ay</sup>
	2	0.73 ± 0.00 (100%) <sup>bx</sup>	1.53 ± 0.09 (100%) <sup>bx</sup>	1.49 ± 0.00 (100%) <sup>bx</sup>
	24	0.73 ± 0.00 (100%) <sup>bx</sup>	0.73 ± 0.00 (100%) <sup>bx</sup>	0.73 ± 0.00 (100%) <sup>bx</sup>

Different letters (a, b, c) show statistically significant differences ( $p < 0.05$ ) when comparing disinfectant efficacy in percentage by time within the same phase (within column). Different letters (x, y, z) show statistically significant differences ( $p < 0.05$ ) when comparing disinfectant efficacy in percentage by phase within the “time waited before disinfection”.

<sup>a</sup> HML denotes high initial microbial load (6 log<sub>10</sub> CFU/cm<sup>2</sup>).

<sup>b</sup> MML denotes medium initial microbial load (4 log<sub>10</sub> CFU/cm<sup>2</sup>).

<sup>c</sup> LML denotes low initial microbial load (2 log<sub>10</sub> CFU/cm<sup>2</sup>).

**Table 2**  
Inactivation of *S. Typhimurium* on stainless steel (SS) surface using sodium hypochlorite.

Microbial load	Time waited before disinfection (h)	Log	Stationary	LTS
		Log <sub>10</sub> reduction in <i>S. Typhimurium</i> (Disinfection efficacy (%))		
<sup>a</sup> HML	0	3.35 ± 0.18 (62%) <sup>ax</sup>	2.80 ± 0.19 (52%) <sup>ay</sup>	2.40 ± 0.17 (43%) <sup>az</sup>
	2	3.85 ± 0.15 (81%) <sup>bx</sup>	2.37 ± 0.14 (44%) <sup>by</sup>	1.77 ± 0.19 (32%) <sup>bz</sup>
	24	2.39 ± 0.46 (100%) <sup>cx</sup>	2.30 ± 0.10 (41%) <sup>by</sup>	1.50 ± 0.24 (34%) <sup>bz</sup>
<sup>b</sup> MML	0	2.69 ± 0.31 (71%) <sup>ax</sup>	2.88 ± 0.27 (61%) <sup>ay</sup>	2.70 ± 0.20 (65%) <sup>ay</sup>
	2	2.47 ± 0.21 (100%) <sup>bx</sup>	2.17 ± 0.18 (72%) <sup>by</sup>	2.43 ± 0.31 (78%) <sup>by</sup>
	24	0.73 ± 0.00 (100%) <sup>bx</sup>	1.20 ± 0.21 (100%) <sup>cx</sup>	0.68 ± 0.04 (100%) <sup>cx</sup>
<sup>c</sup> LML	0	1.80 ± 0.20 (76%) <sup>ax</sup>	1.45 ± 0.13 (66%) <sup>ay</sup>	1.29 ± 0.08 (63%) <sup>ay</sup>
	2	0.73 ± 0.00 (100%) <sup>bx</sup>	1.27 ± 0.14 (100%) <sup>bx</sup>	1.30 ± 0.21 (100%) <sup>bx</sup>
	24	0.73 ± 0.00 (100%) <sup>bx</sup>	0.73 ± 0.00 (100%) <sup>bx</sup>	0.73 ± 0.00 (100%) <sup>bx</sup>

Different letters (a, b, c) show statistically significant differences ( $p < 0.05$ ) when comparing disinfectant efficacy in percentage by time within the same phase (within column). Different letters (x, y, z) show statistically significant differences ( $p < 0.05$ ) when comparing disinfectant efficacy in percentage by phase within the “time waited before disinfection”.

<sup>a</sup> HML denotes high initial microbial load (6 log<sub>10</sub> CFU/cm<sup>2</sup>).

<sup>b</sup> MML denotes medium initial microbial load (4 log<sub>10</sub> CFU/cm<sup>2</sup>).

<sup>c</sup> LML denotes low initial microbial load (2 log<sub>10</sub> CFU/cm<sup>2</sup>).

**Table 3**  
Inactivation of *S. Typhimurium* on laminate (LA) surface using sodium hypochlorite.

Microbial load	Time waited before disinfection (h)	Log	Stationary	LTS
		Log <sub>10</sub> reduction in <i>S. Typhimurium</i> (Disinfection efficacy (%))		
<sup>a</sup> HML	0	1.62 ± 0.18 (27%) <sup>ax</sup>	1.35 ± 0.45 (25%) <sup>ax</sup>	1.56 ± 0.26 (28%) <sup>ax</sup>
	2	2.74 ± 0.50 (54%) <sup>bx</sup>	1.40 ± 0.21 (27%) <sup>ay</sup>	1.51 ± 0.25 (27%) <sup>ay</sup>
	24	2.30 ± 0.75 (93%) <sup>cx</sup>	1.27 ± 0.38 (43%) <sup>ay</sup>	1.29 ± 0.36 (26%) <sup>az</sup>
<sup>b</sup> MML	0	2.25 ± 0.36 (48%) <sup>ax</sup>	1.67 ± 0.11 (38%) <sup>ay</sup>	1.46 ± 0.25 (36%) <sup>ay</sup>
	2	1.80 ± 0.37 (87%) <sup>bx</sup>	1.55 ± 0.24 (52%) <sup>by</sup>	1.40 ± 0.25 (42%) <sup>bz</sup>
	24	0.73 ± 0.00 (100%) <sup>cx</sup>	1.20 ± 0.21 (100%) <sup>by</sup>	0.68 ± 0.04 (100%) <sup>cz</sup>
<sup>c</sup> LML	0	1.64 ± 0.12 (69%) <sup>ax</sup>	1.59 ± 0.13 (60%) <sup>ax</sup>	1.12 ± 0.08 (39%) <sup>ay</sup>
	2	0.73 ± 0.00 (100%) <sup>bx</sup>	0.82 ± 0.09 (54%) <sup>ay</sup>	0.63 ± 0.11 (42%) <sup>az</sup>
	24	0.73 ± 0.00 (100%) <sup>bx</sup>	0.73 ± 0.00 (100%) <sup>bx</sup>	0.73 ± 0.00 (100%) <sup>bx</sup>

Different letters (a, b, c) show statistically significant differences ( $p < 0.05$ ) when comparing disinfectant efficacy in percentage by time within the same phase (within column). Different letters (x, y, z) show statistically significant differences ( $p < 0.05$ ) when comparing disinfectant efficacy in percentage by phase within the “time waited before disinfection”.

<sup>a</sup> HML denotes high initial microbial load (6 log<sub>10</sub> CFU/cm<sup>2</sup>).

<sup>b</sup> MML denotes medium initial microbial load (4 log<sub>10</sub> CFU/cm<sup>2</sup>).

<sup>c</sup> LML denotes low initial microbial load (2 log<sub>10</sub> CFU/cm<sup>2</sup>).

pathogenicity.

Three common cleaning and sanitizing behaviors—cleaning right away (0 h), cleaning after each meal (2 h), as well as cleaning daily (24 h) were mimicked in this study. Time waited before

decontamination had a significant effect on sodium hypochlorite disinfection efficacy mainly at medium microbial load, regardless of phase, except for stationary cells on LA (52% at 2h; 55% at 24 h) and log cells on SS (100% at 2 and 24 h). On CB and SS surfaces, at high microbial

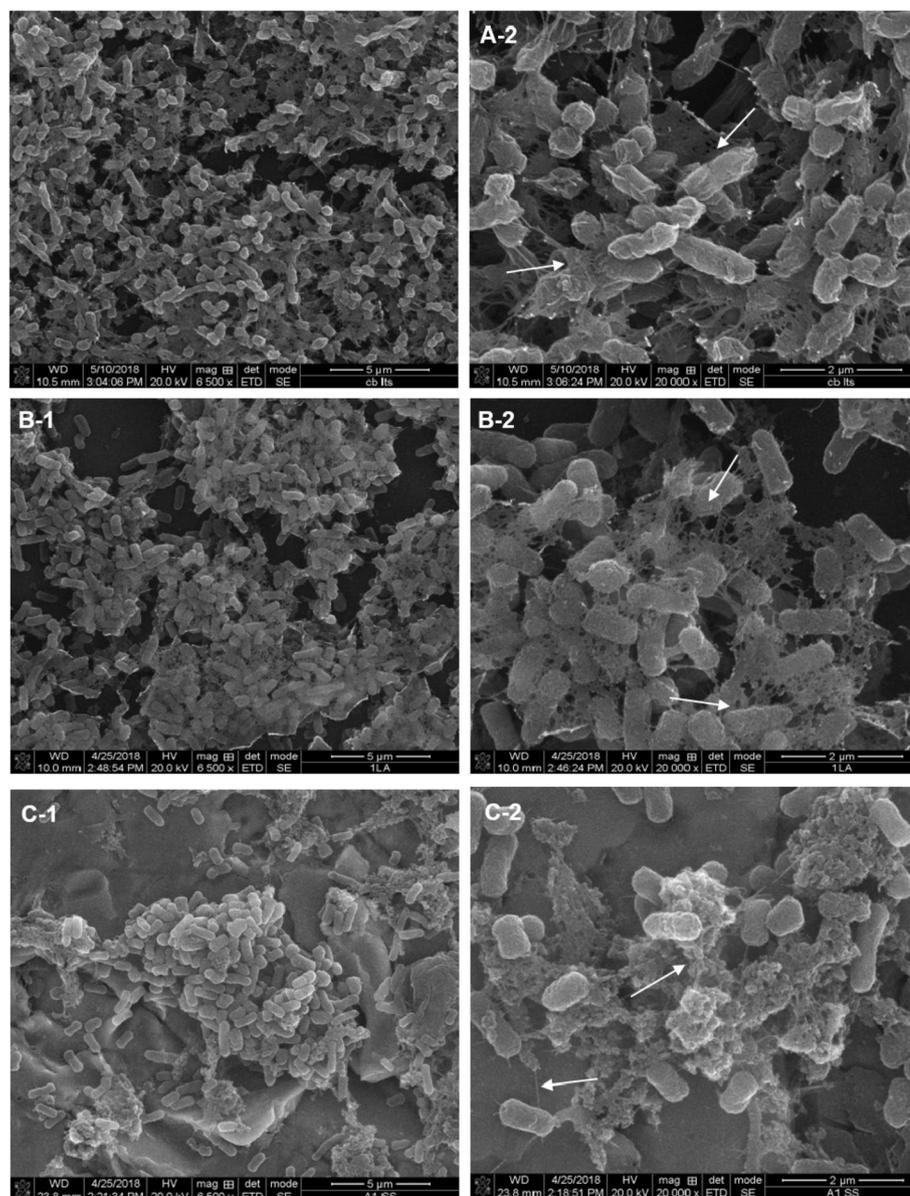


Fig. 2. Scanning electron micrographs of *S. Typhimurium* LTS cells on plastic cutting board, laminate, and stainless steel surfaces. SEM micrographs of *S. Typhimurium* on surface coupons were taken 24 h after inoculation with a high microbial load inoculum ( $6 \log_{10}$  CFU/cm<sup>2</sup>) on plastic cutting board (A1 and A2), laminate (B1 and B2), and stainless steel (C1 and C2) surfaces. A-1, B-1, and C-1 show the early biofilm structure where bacteria cells looked enclosed in an extracellular interconnected matrix. A-2, B-2, and C-2 micrographs taken at a higher magnification show that this early biofilm structure is dominated by short rods linking the bacteria cells together and to the extracellular matrix.

load, higher efficacy was achieved at 0 h compared to 2 and 24 h for stationary and LTS phase cells ( $p < 0.05$ ). However, at medium microbial loads, the opposite was observed where the disinfection efficacy significantly increased with the increase in drying time ( $p < 0.05$ ). This can be explained again by the protective effect provided by the high cell density (more bacterial aggregation, thicker barrier for the disinfectant to diffuse in) which would be greater at high microbial load ( $6 \log_{10}$  CFU/cm<sup>2</sup>) compared to the medium one ( $4 \log_{10}$  CFU/cm<sup>2</sup>). On all three surfaces, and for log phase cells, an increase in drying time resulted in a significant increase in disinfection efficacy ( $p < 0.05$ ) reaching 100% at 2 h for low microbial load. This finding is similar to the one reported by [Cherchi and Gu \(2011\)](#) who found that log phase cells were more susceptible to chlorine disinfection compared to stationary phase cells, with inactivation rates of 63–69% versus 19–32% for stationary cells.

Although hydrogen peroxide is also considered an oxidative agent, its 100% efficacy can be explained by its high concentration (0.88% equivalent to 258 mM) compared to what it is used in food industry sanitation procedures (5.88 mM) ([FDA, 2015](#)). Additionally, this product was applied for a contact time of 10 min (according to manufacturer's instruction). In this regard, it is important to point out that

failing to comply with the manufacturer's instruction is expected to lower the disinfection efficacy of antimicrobial agents.

### 3.3. Conclusion

*Salmonella* is one of the most frequent causes of foodborne gastroenteritis in humans worldwide. Contaminated surfaces play a major role in the transmission of bacterial pathogens to food. This study showed that overall *S. Typhimurium* LTS phase cells have high environmental persistence on consumer contact surfaces with the highest survivor population being ( $5.8 \log_{10}$  CFU/cm<sup>2</sup>) recorded on LA surface at 2 h after inoculation. The use of adequate disinfectants is critical in order to reduce bacterial load on food contact surfaces and prevent cross-contamination.

In this study, H<sub>2</sub>O<sub>2</sub> was effective at eliminating *S. Typhimurium* completely from all surfaces, at all microbial loads and at all disinfection times, while sodium hypochlorite was less effective, especially on surfaces inoculated with high microbial load of LTS phase cells.

In a food manufacturing or home kitchen settings, surface disinfection should take place only after a cleaning step. Cleaning removes visible food debris from surfaces and allows direct contact of

disinfectant and target bacteria. However, in a food service setting such as commercial kitchens, restaurants, as well as in home kitchen settings, cleaning and disinfection are generally performed as one single step, which would make disinfection challenging (Meyer et al., 2010).

In addition, although reports on the disinfection efficiency of various disinfectants showed mixed results, studies have agreed that conventional cleaning and sanitation may not be efficient when bacterial cells adhere to food contact surfaces. Bacterial adhesion to surface represents the first step in biofilm formation, a major challenge for the sanitation process as they represent a persistent harborage for native microbes and in the worst case scenario, a source of ongoing contamination with pathogenic bacteria such as *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* (Carpentier and Cerf, 1993; Sharma and Anand, 2002).

In this study, all five main factors (bacterial growth phase, type of surface, inoculum concentration, disinfectants, and drying time) showed significant impact on disinfection efficacy. These factors should be considered by consumers when simply choosing disinfectants in order to achieve effective cleaning and sanitation.

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