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Impact of wiping materials on the elimination from surfaces of dry surface biofilm of bacteria of food safety concern

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Running title: Wipe material impact on sanitizers efficacy

Keywords: *Salmonella, Listeria*, dry surface biofilm, disinfection, mechanical removal, transfer

Highlights

- *Listeria monocytogenes* is able to form environmental dry surface biofilms (DSBs)
- Planktonic bacteria dried on surfaces were easier to eliminate than DSBs
- Not all wiping materials used with no-rinse QAC sanitizers performed equally
- Paper towels performed significantly better when used with no-rinse QAC sanitizers
- Wiping materials and no-rinse sanitizers must be carefully paired to ensure efficacy

ABSTRACT

Salmonella spp. and *Listeria monocytogenes* are common foodborne pathogens that easily contaminate food preparation surfaces. Salmonella's ability to form dry surface biofilms (DSBs) likely exacerbates surface persistence, making effective removal from food contact surfaces essential. This study is the first to evaluate the efficacy of food contact surface sanitizers against artificial *L. monocytogenes* DSBs, with comparisons to hydrated biofilms and dried planktonic cells. We hypothesized that the effectiveness of no-rinse, quaternary ammonium compound (QAC)-based sanitizers depends on both the wiping material used and the bacterial strain present.

Two pre-formulated no-rinse QAC sanitizers and one QAC spray were tested with six commercial wiping materials against three dried planktonic Salmonella spp. and one *L. monocytogenes*, as well as their DSBs, on stainless steel surfaces. Dried planktonic cells were more easily eliminated than DSBs, achieving approximately 4 $\log_{10}$ versus 2 $\log_{10}$ reductions, respectively. Although no-rinse QAC sanitizers are designed to reduce bacterial levels to acceptable limits, formulation constraints may limit their cleaning efficacy, particularly against DSBs in the presence of organic matter.

Pre-formulated QAC wipes were less effective than spraying the sanitizer followed by wiping. Wiping material type significantly influenced efficacy: paper towels significantly outperformed cloths, though performance varied among brands, and one sponge was the most effective overall.

This study underscores the need to carefully select wiping materials and no-rinse food contact surface sanitizers to eliminate *Salmonella* and *Listeria* DSBs, ensuring effective sanitation practices in foodservice settings.

Introduction

Salmonella enterica is a pathogen of major concern within the food preparation industry. It is causing millions of cases of gastroenteritis worldwide, most of which associated with the ingestion of contaminated food. (Chlebicz & Śliżewska, 2018). The primary route of infection for *S. enterica* is via fecal-oral transmission or through the ingestion of contaminated food (Mkangara, 2023). For this reason, food preparation facilities must be kept sanitary, and cleaning regimes need to be effective to minimize the risk of food contamination and the transmission of these pathogens (Wang et al., 2017).

Additionally, it has been demonstrated that *Salmonella* spp. can reside on dry substrata as a biofilm, which has been shown to be more difficult to eradicate than hydrated biofilm counterparts (Alonso et al., 2023; Duggan et al., 2024). Dry Surface Biofilms (DSBs) are a concern. Their biological structure, which includes an exopolysaccharide (EPS) matrix, allows them to adhere strongly to surfaces and survive for extended periods (Morita et al., 2011; Alonso et al. 2023), making DSBs hard to remove with conventional cleaning methods (Alonso et al. 2023). Furthermore, because dry biofilms are arranged in layers, conventional cleaning may remove only the top layers of the biofilm, potentially releasing more organisms. Indeed, it has been reported that when disturbed following cleaning and mechanical action, bacteria in DSBs became transferrable (Chowdhury et al., 2018; Ledwoch et al., 2021a). Strain-specific factors, including persistence and tolerance to biocides, are significant considerations for bacterial persistence on surfaces. It has been observed that *Salmonella* strains attach differently to substrata during biofilm formation, depending on temperature and surface type, which may influence persistence in food processing environments (Obe et al. 2022). Listeria infection caused by the consumption of contaminated product is less common than Salmonella ones, but illnesses are more severe demanding stricter food safety control (Datta & Burall, 2018). *Listeria monocytogenes* can form hydrated biofilms on various substrata (di Bonaventura et al., 2008). Hydrated biofilms of *L. monocytogenes* have been shown to be less susceptible to sanitizers than planktonic cells (Chavant et al., 2004; Pan et al. 2006). The propensity of *L. monocytogenes* to form DSBs and their susceptibility to sanitizers have not yet been reported.

Food contact surface sanitizers containing quaternary ammonium compounds (QACs) are routinely used to achieve sanitation of surfaces within food preparation facilities. In the US, no-rinse required, food contact sanitizers (NR-FCS) are used instead of disinfectants on food contact surfaces for non-emergency sanitation compliance (FDA, 2022). NR-FCS are simple formulations with no effective cleaning ingredients since the product is left on the surface. The recommended standard test efficacy requirement (e.g. EPA OCSPP 810.2300) for such product is 99.999% (i.e. 5 $\log_{10}$) reduction in bacteria within 30 seconds. The use of QACs, such as didecyl dimethyl ammonium chloride (DDAC) and benzalkonium chloride, has been shown to be effective in controlling surface contaminants (Pablos et al., 2022). Their efficacy is linked to the chain length of the alkyl groups, impacting on the overall positive charge of the molecule, and to the degree of C–C saturation (Gilbert & Moore, 2005; Yoshimat & Hiyama, 2007). Due to their chemical structure, QACs are easily absorbed by bacterial cells (Denyer & Maillard, 2021). QACs are membrane-active substances; they work by binding irreversibly to phospholipids and proteins in microbial cell membranes. At the cell membrane, QACs cause disruption and dissociation of lipid bilayers, impairing membrane permeability and leading to leakage of vital cellular components (Denyer & Maillard, 2021). Due to these mechanisms of action, QACs have a broad spectrum of activity against a wide range of gram-negative and gram-positive bacteria, as well as enveloped viruses (Denyer & Maillard, 2021; Alajlanet al., 2022).

The application of a disinfectant on surfaces is usually combined with the use of a material or wipe (Sattar & Maillard, 2013). The type of material is a significant factor affecting the efficacy of QAC disinfectants in removing contaminated bioburden from surfaces (Siani et al., 2011).

In addition, QACs can adsorb to, and be sequestered by, cellulosic materials, such as viscose, hindering microbicidal efficacy (Bloss et al., 2010; Hinchliffe et al., 2018; Pascoe et al., 2022). The type of the wipe materials is particularly important to consider when the product formulation does not contain a cleaning agent, since the wipe would particularly contribute to removing a microbial bioburden from the treated surface. While previous studies have examined sanitizers against hydrated biofilms, no studies have systematically evaluated the combined effect of wiping materials and QAC-based NR-FCS against bacterial DSBs. This study aims to understand the impact of wiping materials used with NR-FCS to control *Salmonella* spp. and *Listeria monocytogenes* DSBs.

Materials and methods

Bacterial strains. Three *Salmonella enterica* and one *Listeria monocytogenes* isolates were used to produce DSBs (Table 1). *S. enterica* serovar Typhimurium SL1344 is commonly used as a reference strain in studies on disinfection and biofilm formation (Guest et al., 2022). *S. enterica* serovars Agona and Havana have been linked to persistence in food and food production environments and have been associated with heavy biofilm production (Diez-Garcia et al., 2012; Guerrero et al. 2022; Guest et al., 2022). *L. monocytogenes* NCTC11994 serovar 4b is a food isolate, commonly used in studies investigating antimicrobial efficacy or thermal processing. *L. monocytogenes* NCTC11994 was only used in relation to DSBs in this study.

Bacterial isolates were propagated aerobically in tryptone soy broth (TSB) at 37°C in an orbital shaker (120 rpm) overnight. The bacterial suspension was then centrifuged at 3,000 × g for 10 minutes at 20°C, and the pellet was resuspended in TSB. Working stocks were maintained on tryptone soy agar (TSA) and stored at 4°C for up to 2 months. For long-term storage, bacterial cultures were washed and resuspended in TSB with a cryoprotectant (20% glycerol) in cryovials. Vials were stored at both -20°C and -80°C for short-term (< 1 year) and long-term (> 1 year) storage.

DSB production. DSB formation was based on sedimentation biofilm, alternating wet and dry phases over a 12-day period (Ledwoch et al., 2019). Briefly, 3 - 4 bacterial colonies were used to inoculate TSB, and after 24 hours of incubation at 37°C, bacterial suspensions were pelleted by centrifugation at 3,000 × g for 10 minutes and resuspended in 10 mL tryptone saline chloride (TSC) (peptone, pancreatic digest of casein: 1 g; NaCl: 8.5 g; water: 1 L; pH 7.0 ± 0.2). A 10-fold dilution of the inoculum was performed using TSC as the diluent. A further 10-fold dilution step was performed in TSB supplemented with bovine serum albumin (BSA) at a final concentration of 0.3 g/L for *S. enterica* isolates, and 1% skim milk media for *L. monocytogenes*. Skim milk was used here since *Listeria* is a common contaminant in dairy processing plants. Skim milk may also simulate better the protective matrix of food residues. Following these dilutions, the bacterial inoculum concentration was 1–5 × 10^6 cfu/mL. The addition of organic load during DSB formation has been shown to increase the viability of bacteria in DSBs (Ledwoch et al., 2019) and was not intended to mimic a dirty soiling condition during testing, although it might decrease a sanitizer bactericidal efficacy.

Sterile stainless-steel coupons (10 mm, grade 2B finish) were placed into each well of a 24-well plate, and 1 mL of the bacterial inoculum with BSA or 1% skim milk media was added

(wet phase). The plate was incubated at 21 ± 1°C for 48 hours with orbital shaking, followed by complete removal of the inoculum via pipetting and incubation of the plates at 37 ± 1°C (21 ± 1°C for *L. monocytogenes*) for 3 days (dry phase). The wet and dry phases were repeated until 3 cycles had been completed. Biofilms were used for testing after the final dry phase.

Formulation preparation. One formulation (Formulation A) and two pre-formulated wipe products (wipe products A & B) (Table 2) were prepared according to the manufacturers' instructions in deionised water. All products underwent neutralizer validation according to BS EN 13727 (2015) (data not shown). The neutralizer used was composed of L-histidine (1 g/L), L-α-lecithin (3 g/L), sodium chloride (8.5 g/L), tryptone (1 g/L), sodium thiosulfate (3 g/L), saponin (30 g/L), and polysorbate-80 (30 g/L).

Quantification of DDAC concentration (DDAC equivalent concentration). DDAC concentration from formulations or extracted liquid from wipes was quantified using the colorimetric disulphine blue active substance assay (DBAS) (Nozière et al., 2017). Pre-formulated wipes were inserted into the barrel of a 20 mL syringe, and the liquid formulation was extracted by pressing the plunger. Extracted formulations were diluted 2,000-fold in ultrapure water to achieve a QAC concentration within the detection range of the assay. 25 mL of each diluted sample were placed in 50 mL tubes, where 2.5 mL of buffer (115 g/L anhydrous sodium acetate and 35 mL/L glacial acetic acid in deionized water), 1 mL of dye (0.64 g/L disulphine blue, 8 mL/L ethanol in deionized water), and 7.5 mL of chloroform were added. Each tube was agitated vigorously for 2 minutes and then left to separate for a minimum of 5 minutes. A glass Pasteur pipette was used to remove the organic phase from the bottom of each tube and transfer it into quartz cuvettes. The OD_{628nm} of each sample was measured spectrophotometrically. Formulation A and extracted formulations from wipe products A and B were compared against an adjusted calibration curve of prepared DDAC solutions (0, 0.1, 0.5, 1 g/L). The QAC concentration of formulation extracts was recorded as DDAC equivalent (ppm).

Product efficacy against *S. enterica* planktonic suspension dried on stainless steel. Formulation A was decanted into a trigger spray bottle and applied to each DSB coupon using two sprays from a 20 cm distance at a 45° angle – the volume delivered covered the entire surface of the coupons. Material-2 was immersed in Formulation A for five minutes before wiping followed with a 1 min contact time post-wiping. *S. enterica* serovar Typhimurium SL1344 test suspension was prepared and resuspended in TSC supplemented with 0.3 g/L BSA as described above. 20 µL of the bacterial suspension (1–5 × 10^8 cfu/mL) was dispensed onto sterile stainless-steel coupons (10 mm, grade 2B finish), which were then placed to dry in an incubator at 37°C for 30 minutes. When visibly dry, formulation A was applied and left in contact with the coupon for 1 minute before wiping. Coupons were wiped using a Wiperator device (based on ASTM 2967:2015) for 5 seconds with a 300 g weight. Wipe products A and B were left in contact with the coupon for 1 minute after wiping. All coupons were placed into 10 mL neutralizer containing 3 g glass beads and vortexed for 3 minutes. Viable bacteria were enumerated using the drop count method. Log_{10} reduction in viable bacteria was calculated relative to untreated control samples. The performance of formulation A was compared to a water-treated control with each appropriate material (Table 3).

Product efficacy against DSBs. Formulation A was prepared and applied to DSBs as described above. Material-2 was prepared with formulation A as described above. After a 1-min contact time, DSB coupons were wiped using a Wiperator for 5 seconds with a 300 g weight. Formulation A was used in combination with six wipe materials (cut to 4 × 4 cm) (Table 3). Brown paper was folded in half to ensure the material did not tear during wiping. All wipe materials were pre-sterilized by autoclaving at 121°C for 20 minutes.

Wipe products A and B were cut to 4 × 4 cm. After wiping using a Wiperator for 5 seconds with a 300 g weight, the wiped coupons were left for a further 1-minute contact time before neutralization. Treated coupons and control DSBs were transferred to tubes containing 10 mL of a neutralizing solution and glass beads (3 g). Following vortexing for 3 minutes, suspensions were serially diluted in TSC, and viable bacteria were enumerated using the drop count method. Log_{10} reduction in viable bacteria was calculated relative to untreated control samples. The performance of formulation A was compared to a water-treated control with each appropriate material.

Bacterial transfer post-treatment. Bacterial transfer from DSBs was evaluated following wiping. Transfer was determined by 36 successive adpressions of the wiped coupons (using a 100 g weight) across the surface of Dey-Engley (DE) neutralizing agar plates (Oxoid, UK; 120 × 120 mm) (Ledwoch et al., 2021b). The plates were then incubated at 37°C for 24 hours, and positive growth was recorded. Transfer was evaluated after wiping with either one wipe or three successive fresh wipe materials. In the case of three successive wiping events, the formulation contact time increased to 3 minutes in total due to the time taken to change the wipe material between wipes.

Statistical analysis. Three biological replicates were evaluated for each test. One-way ANOVA was performed for the DDAC equivalent concentration test. Two-way ANOVA with multiple comparisons was performed for log_{10} reduction tests and transfer tests. All treatments were compared to a water-treated control. All statistical analyses were performed using GraphPad Prism® version 9.4.0 (GraphPad Software Inc.).

Results and discussion

Salmonella spp. and *L. monocytogenes* can persist in dry environments (Iibuchi et al., 2010; Guerrero et al., 2022). *Salmonella* spp. have been shown to survive for more than 200 days on dry surfaces at ambient temperature, posing a risk of cross-contamination of foods and foodborne outbreaks (Iibuchi et al., 2010). Bacteria in biofilms poses an additional challenge for disinfection (Maillard & Centeleghe, 2023). The decreased efficacy of disinfection against bacteria embedded in hydrated biofilms compared to bacteria dried on surfaces, has been well established (Wong et al., 2010). *S. enterica* Typhimurium has been shown to form a DSB (Duggan et al., 2024) and Chaggar and colleagues (2024) reported the formation of *L. monocytogenes* DSB *in vitro*. However, their DSB formation protocol was based on the formation of a hydrated biofilm which was subsequently dried. This protocol differed significantly from the DSB protocol described in this study, which relied on a 48h sequential alternation of dry and hydrated phases over a 12-days period. DSB formation using sequential dry and hydrated phases have been well reported in the literature using a sedimentation biofilm approach (Ledwoch et al., 2018; 2021a) or the CDC reactor (Almatroudi et al., 2015). Following our DSB formation protocol, the average concentration of bacteria recovered from DSBs was as follows (log_{10} CFU/coupon): 7.43 ± 0.28 for *S. enterica* SL1344, 7.28 ± 0.58 for

S. enterica CMCC 3750, 7.13 ± 0.71 for *S. enterica* CMCC 3579, and 5.79 ± 0.22 for *L. monocytogenes*. DSBs pose an additional challenge for disinfection compared to hydrated biofilms (Maillard & Centeleghe, 2023). Combining disinfection with mechanical removal has been shown to be essential for eliminating DSBs from stainless steel surfaces (Ledwoch et al., 2021b; Duggan et al., 2024).

In the present study, the elimination of dried *S. enterica* SL1344 from a stainless steel surface was easier to achieve than that of a DSB using a combination of formulation A and most materials ($p \leq 0.0040$), except for material-6 ($p = 0.9730$) (Figure 1). Our results also suggest that material-6, a melamine sponge, is effective at reducing *S. enterica* DSBs on stainless steel compared to the other materials tested or the two products evulated (Figure 1). However, when combined with the materials, formulation A did not perform better than water in reducing bacterial concentration following surface wiping (*S. enterica* dried on surfaces: $p = 0.1379$) or *Salmonella* DSB: $p = 0.0667$), excluding material-6 (Figure 1). Previous studies have shown that the combination of a QAC-based disinfectant with wiping enabled a significant reduction of target microorganisms on surfaces and performed better than the use of water alone (Robertson et al., 2019; Ledwoch et al., 2021b). Many factors influence disinfectant efficacy (Maillard & Pascoe, 2024). The factors most relevant to this study relate to the type of material and the concentration of active ingredient released from the material. We used the DBAS assay to determine the (estimated) amount of DDAC released from material-1 and the products tested. Formulation A and products A and B are registered with different DDAC concentration (Table 2); formulation A: between 150-400 ppm, whilst product A: 200-400 ppm and product B, a ready to use product: 380 ppm.

DDAC concentrations ranging from 150 to 400 ppm were extracted from the two products and material-1 treated with formulation A. The DDAC concentration released differed significantly between substrates ($p = 0.0004$) (Figure 2). Material composition can affect the release of QACs which in turn influences their availability on substrata and, consequently, their efficacy (Wesgate et al., 2019; Pascoe et al., 2022). In this study, the amount of DDAC released from material-1 and both products exceeded 150 ppm, which did not appear to be sufficient to produce a significant difference in bacterial reduction from materials compared with water following wiping.

The two pre-formulated wipes (Product A and B), generally performed significantly worse against *S. enterica* isolates than the combination of Formulation A and the materials tested (Figure 3). Although we did not measure the QAC concentration on stainless steel after spraying Formulation A, it is conceivable that more QAC is available on the surface after spraying and before wiping with the different materials. Nevertheless, >150 ppm QAC was released from both products (Figure 2). As observed for Products A and B, Material-2 (which was pre-soaked in formulation A) did not perform as well as the combination of sprayed Formulation A and the other materials against *Salmonella* DSBs (Figure 3). Based on our results, differences in QAC chain length (Formulation A and product B: C_8-$C_{18,}C_{22}$, and Product A: C_8-$C_{18,}$ C_{12}-C_{14}; Table 2) did not impact on efficacy against DSBs. In this study, the effect of material composition on compatibility with the formulation was not comprehensively investigated. However, it is recognized that cellulosic wiping materials, such as those that are viscose-based, exhibit extensive adsorption of QACs, whereas polypropylene materials do not (Bloss et al., 2010; Sattar & Maillard, 2013; Hinchliffe et al., 2018)

When the elimination of DSBs is considered, mechanical removal has been found to be essential (Ledwoch et al., 2021b). However, initial observations found that when formulation A was combined to material-1, the addition of wiping (5 sec; 300 g weight) did not significantly impact bacterial reduction from DSBs ($p = 0.244$; Figure 4). Nevertheless, all efficacy testing was performed with wiping to better reflect product usage in practice.

When considering the different *Salmonella* isolates tested, formulations/materials or the products performed similarly regardless of the isolates (Figure 5). The two *Salmonella* food isolates (CMCC3750 and CMCC3759) are described as tolerant to QAC and alcohol-based disinfectants (Table 1); however, the extent of the *tolerance* is not clear and did not seem to impact efficacy. The melamine sponge (Material-6) removed significantly more bacteria when used with Formulation A (SL1344: $p < 0.0001$; CMCC3750: $p = 0.0013$; CMCC3759: $p < 0.0001$) than any other materials (Figure 5 a, b, c). The efficacy of Material-5 against isolate CMCC3750 was significantly increased ($p = 0.0008$) with the addition of Formulation A (Figure 5b).

Not all paper towels perform equally against *S. enterica* according to our test results (Figure 5), with Material-5 performing better than material-3 and -4 ($p = 0.0009$). Paper towels (Material -3 to -5) generally achieved better results ($p = 0.0002$) than wiping cloths (Material-1, -2 and product A, B). The parameters that impact of the microbicidal efficacy of wipes have been described (Sattar & Maillard, 2013). Product-related factors, including the type and thickness of material would impact efficacy. Material-4 was the only material that was folded upon usage, yet its overall thickness was less than the other materials tested (data not shown).

We tested only one reference strain of *L. monocytogenes* (Table 1). This strain (NCTC11944) formed a DSB containing less bacteria (5.79 ± 0.22 $\log_{10}$/coupon) that *Salmonella* DSBs. To date, DSB formation has been mostly confirmed using scanning electron microscopy (SEM), which shows bacterial aggregates forming a thin layer (~30 µm in depth) on surfaces, with the presence of extra polymeric substances (Almatroudi et al., 2015; Ledwoch et al., 2109; Duggan et al., 2024). *L. monocytogenes* (NCTC11944) DSB was significantly less susceptible ($p < 0.0001$) than *Salmonella* DSBs to both products and the combination of Formulation A and different materials (Figures 4,5). No significant differences ($p > 0.05$) in efficacy were observed between water and formulation A when *L. monocytogenes* DSBs were tested. However, Material-1 (100% viscose) with Formulation A demonstrated the highest reduction in *L. monocytogenes* DSB (2.26 Log_{10} reduction) (Figure 5d). We are not aware of studies comparing the susceptibility of Gram-positive and gram-negative DSBs. Scientific studies on DSBs typically report product efficacy against either Gram-positive or Gram-negative bacteria, making it difficult to determine whether the lower susceptibility of *L. monocytogenes* DSBs is related to its Gram-positive nature. SEM observations tend to show Gram-negative bacteria in a DSBs exhibiting greater structural stress (Centeleghe et al., 2023; Duggan et al., 2024) compared to Gram-positive ones (Ledwoch et al., 2019). Such stress is likely linked to desiccation (Maillard & Centeleghe, 2023), which explain why *Ps. aeruginosa* DSBs are produced from a hydrated biofilm that have subsequently been dried (Chaggar et al., 2024). In real-world settings, DSBs are composed of multiple species predominantly Gram positive bacteria (Hu et al., 2015; Ledwoch et al., 2018).

To confirm the decreased susceptibility of *L. monocytogenes* DSBs compared to *Salmonella* spp. DSBs a broader range of isolates should be tested. Furthermore, to provide information relevant to the food industry, the persistence of *L. monocytogenes* and *Salmonella* DSBs under environmentally relevant conditions requires further investigation. This study showed that the spray-and-wipe performed better than pre-formulated products. Previous studies have highlighted both the resilience of DSBs to disinfection and the importance of the formulation–material combination in eliminating pathogens from surfaces (Siani et al., 2013; Almatroudi et al., 2015; Guerrero et al., 2022). When an appropriate combination is identified, the efficacy of a wipe product can be better than that of a disinfectant spray followed by wiping (Panousi et al., 2009). However, under the standardized wiping conditions used in this study (5 s; 300 g applied weight) and contact times reflecting product use, formulation A combined with most materials (with the exception of material-6) did not outperform water alone. Notably, material-6 combined with the QAC-based formulation

performed better than any other materials. Without detailed information on material composition, it is difficult to determine the mechanisms underlying this enhanced efficacy. The QAC-based formulation primarily reduced bacterial transfer following wiping, although multiple wipes were required. If the objective of no-rinse food contact surface sanitizers (NR-FCS) is the removal of bacteria dried on surfaces, this study shows that water combined with mechanical action using various materials can achieve a 99.99% reduction on stainless steel surfaces. If the objective is the elimination of bacteria within DSBs, the combination of an NR-FCS with a melamine sponge provided the most effective outcome.

When assessing the efficacy of disinfectant-type products against DSBs, both the reduction in bacteria on surfaces and the transfer of bacteria from the surface post-treatment should be assessed (Ledwoch et al., 2021b). Evidence indicates that bacteria within DSBs can be easily transferred when the biofilm is disturbed (Tahir et al., 2019; Ledwoch et al., 2021a; Duggan et al., 2024). In this study, the combination of water alone and materials resulted in a high transfer of bacteria post-wiping than when formulation A was used, regardless of the isolate tested (Figure 6). The use of water alone has been shown not to be effective in controlling the transfer of bacteria from DSBs post-wiping, even though the combination of water with materials removed a high concentration of bacteria from surfaces (Robertson et al., 2019).

The number of bacteria transferred following one wiping event significantly depended on the bacterial isolate ($p < 0.0001$), with *S. enterica* SL1344 and *L. monocytogenes* being the most transferred (Figure 6).

Increasing the number of wiping events (from one to three) with different materials reduced the number of *S. enterica* SL1344 on stainless steel ($p < 0.0001$), but did not reduce the transfer of *S. enterica* CMCC3750 (Figure 6). When the two products were considered, the use of one wipe or three wipes did not impact the transfer of bacteria, which remained high ($p = 0.4893$) regardless of the species (Figure 6). Of note, product A, Material-2, and Material-6 are stated by the manufacturers as “reusable.” in healthcare settings it is generally recommended that wiping products be used once, in a single direction, and then disposed of (Williams et al., 2009; Edwards et al., 2020). This principle is supported by the findings of the present study.

Conclusion

Salmonella spp. and *L. monocytogenes* can exist as DSBs on stainless steel surfaces. *Salmonella* DSBs were significantly more difficult to eradicate than dried planktonic inocula alone. In addition, the *L. monocytogenes* reference strain used in this study was less susceptible, as a DSB, to QAC-based product or QAC-based formulation/materials combinations than *Salmonella* DSBs.

Overall, these findings emphasize the importance of informed selection of wiping materials and highlight the need for further investigation into the interactions between no-rinse food contact surface sanitizers, substrates, wiping materials, and target organisms.

Declaration of competing interest

Rebecca Wesgate and Jean-Yves Maillard have no competing interests to report.

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Declaration of generative AI in scientific writing

AI has not been used at any stage of the writing up of this manuscript.

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Table 1 Bacterial isolate name and provenance

Isolate	Provenance	Source
Salmonella enterica serovar Typhimurium SL1344	Originally isolated from calves	Veterinary Laboratories Agency Culture Collection (Weybridge, Surrey, UK)
Salmonella enterica serovar Havana CMCC3759	Isolated from a contaminated food source showing an increased tolerance to QAC and alcohol based disinfectant*	Safety and Environmental Assurance Center, Unilever R&D, Colworth, Bedfordshire, UK
Salmonella enterica serovar Agona CMCC3750	Isolated from a contaminated dried vegetable, showing increased tolerance to QAC and an alcohol based disinfectant*	
Listeria monocytogenes NCTC11994	Reference strain - *Listeria* serovar 4b – isolated from food	UK Health Security Agency

* information about the strains‘ tolerance is not available. These strains were used here because they are food isolates

Table 2 Active ingredients of formulation and pre-impregnated wipe products

Food contact surface sanitizer	Disclosed ingredients*	Material Texture	Material preparation	Material Usage
Formulation A#	~ 400 ppm (registered range for use is 150 – 400 ppm) DDAC#/ADBAC at 1.5/1 ratio (C_8-$C_{18,}C_{22}$)†	N/A	Dilute according to manufacturer's directions	Spray liquid
Product A	~400 ppm ADBAC/ADEBAC at 1/1 ratio (C_8-$C_{18,}C_{12}$-C_{14}) †		Immersed in water, wrung 10 times and left for 5 minutes to equilibrate	Reusable, soaking towel
Product B	380 ppm DDAC#/ADBAC at 1.5/1 ratio (C_8-$C_{18,}C_{22}$) †		N/A	Premoistened wipe, single use, disposable

*Complete formulations constitute proprietary information

Formulation A: DDAC is a blend of octyl, decyl, dioctyl, and didecyl ammonium chloride; For Product B: DDAC is didecyl dimethyl ammonium chloride.

† alkyl chain lengths in the QAC mixtures

ADEBAC – alkyl dimethyl ethyl benzyl ammonium chloride; ADBAC - alkyl dimethyl benzyl ammonium chloride

Table 3 Wipe materials used with Formulation A

Material	Composition	Material texture	Material usage
1	100% Viscose		Reusable cloth towel
2	Synthetic polymeric fiber blend towel, compatible with QACs		Reusable cloth towel
3	4-Ply, nylon reinforced cellulose fibers, Sustainable Forestry Initiative certified		Single use, disposable paper towel
4	1-Ply, kraft paper, Green Seal certified, 50% minimum recycled content		Single use, disposable paper towel
5	2-Ply, wood pulp & water, improved spacing between plies		Single use, disposable paper towel
6	Melamine sponge	 *	Reusable sponge, (150+ uses)

* magnified

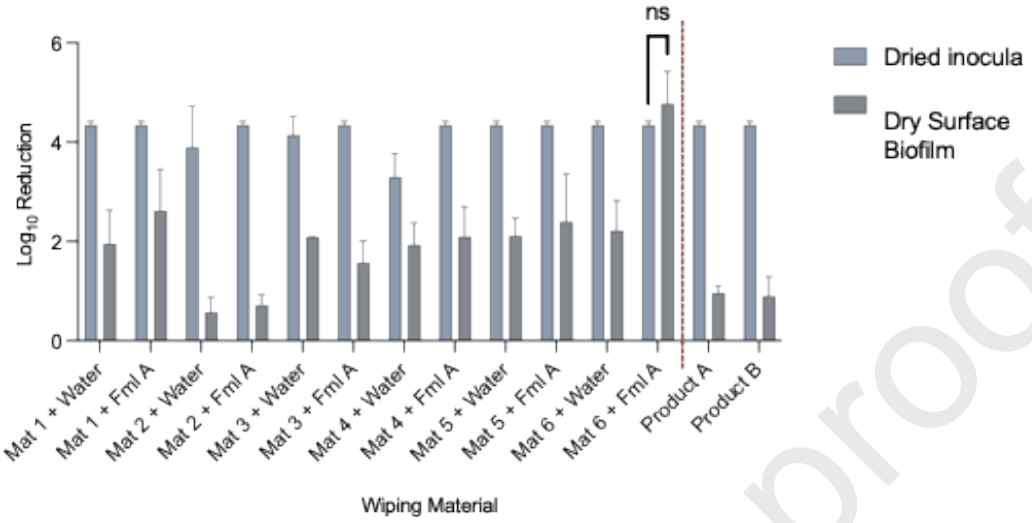


Figure 1 Reduction in *S. enterica* serovar Typhimurium SL1344 DSBs and planktonic bacteria dried on stainless steel surface. (n=3). Material-1, -3 to -6 were wiped (5 sec; 300 g weight) after formulation A was sprayed onto a stainless-steel disc and left for a 1 minute contact time. Material-2 was immersed in Formulation A for five minutes before wiping (5 sec; 300 g weight) followed with a 1 min contact time post-wiping. Product A is a pre-formulated material (~400 ppm ADBAC/ADEBAC) which is immersed in water, wrung 10 times and left for 5 minutes, before wiping (5 sec; 300 g weight) and 1 min contact time post-wiping. Product B is a pre-formulated wiping material (380 ppm DDAC/ADBAC) which needs no preparation before use (wiping 5 sec; 300 g weight) and 1 min contact time post-wiping.

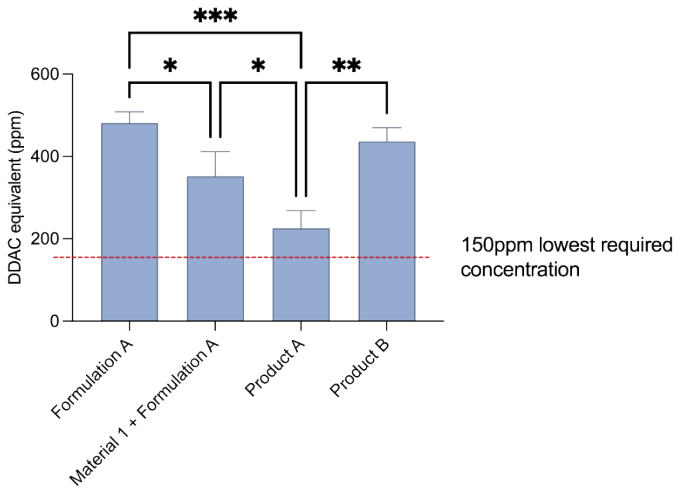
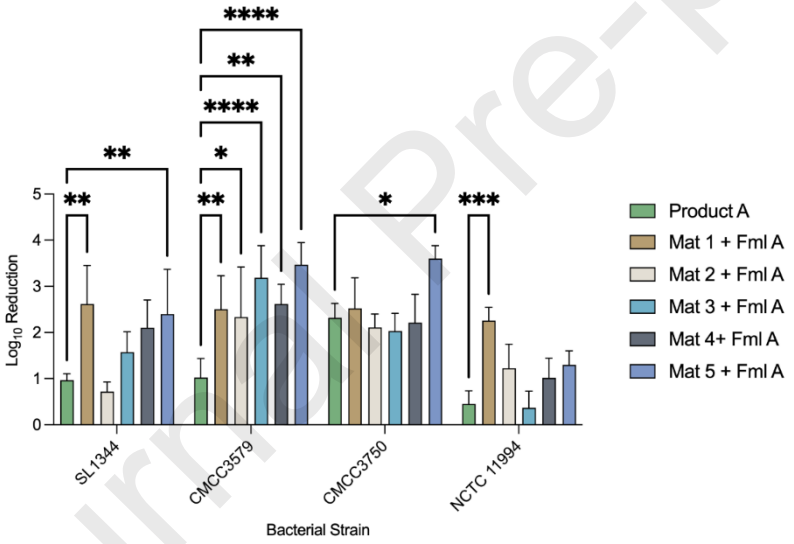


Figure 2 DDAC equivalent concentrations of formulation A and liquid extracted from products A and B using the DBAS assay. (n=3) Analyzed by ONE-WAY ANOVA Tukey's multiple comparison test; * p <0.05, ** p <0.01, *** p = <0.0001

A



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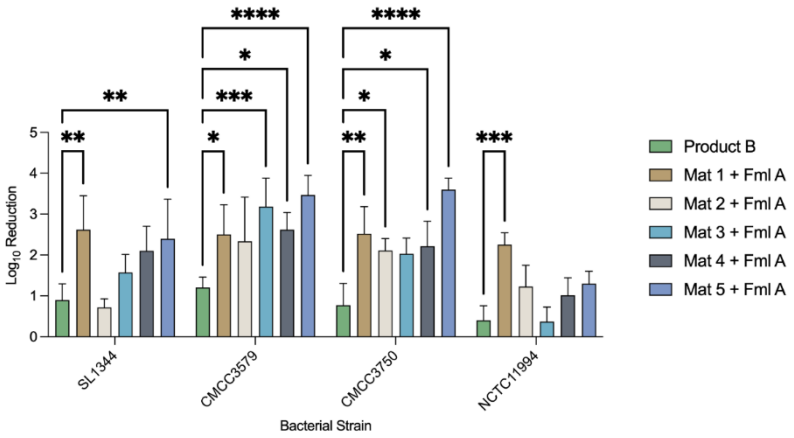


Figure 3 Efficacy of pre-formulated (A) Product A and (B) Product B against DSBs compared to Formulation A (Fml A) with different materials. Figure indicates the Log_{10} reduction of *S. enterica (*SL1344, CMCC3750, CMCC3759*)* and *L. monocytogenes* (NCTC11994) after wiping. (n=3) Material-1, -3 to -6 were wiped (5 sec; 300 g weight) after formulation A was sprayed onto a stainless-steel disc and left for a 1 minute contact time. Material-2 was immersed in Formulation A for five minutes before wiping (5 sec; 300 g weight) followed with a 1 min contact time post-wiping. Product A is a pre-formulated material (~400 ppm ADBAC/ADEBAC) which is immersed in water, wrung 10 times and left for 5 minutes, before wiping (5 sec; 300 g weight) and 1 min contact time post-wiping). Product B is a pre-formulated wiping material (380 ppm DDAC/ADBAC) which needs no preparation before use (wiping 5 sec; 300 g weight(and 1 min contact time post-wiping). Analysed by TWO-WAY ANOVA Dunnet's multiple comparison test; * p <0.05, ** p <0.01, *** p = <0.001, **** p =<0.0001

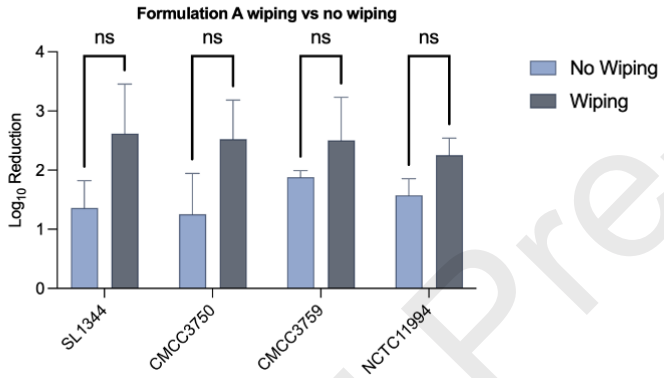
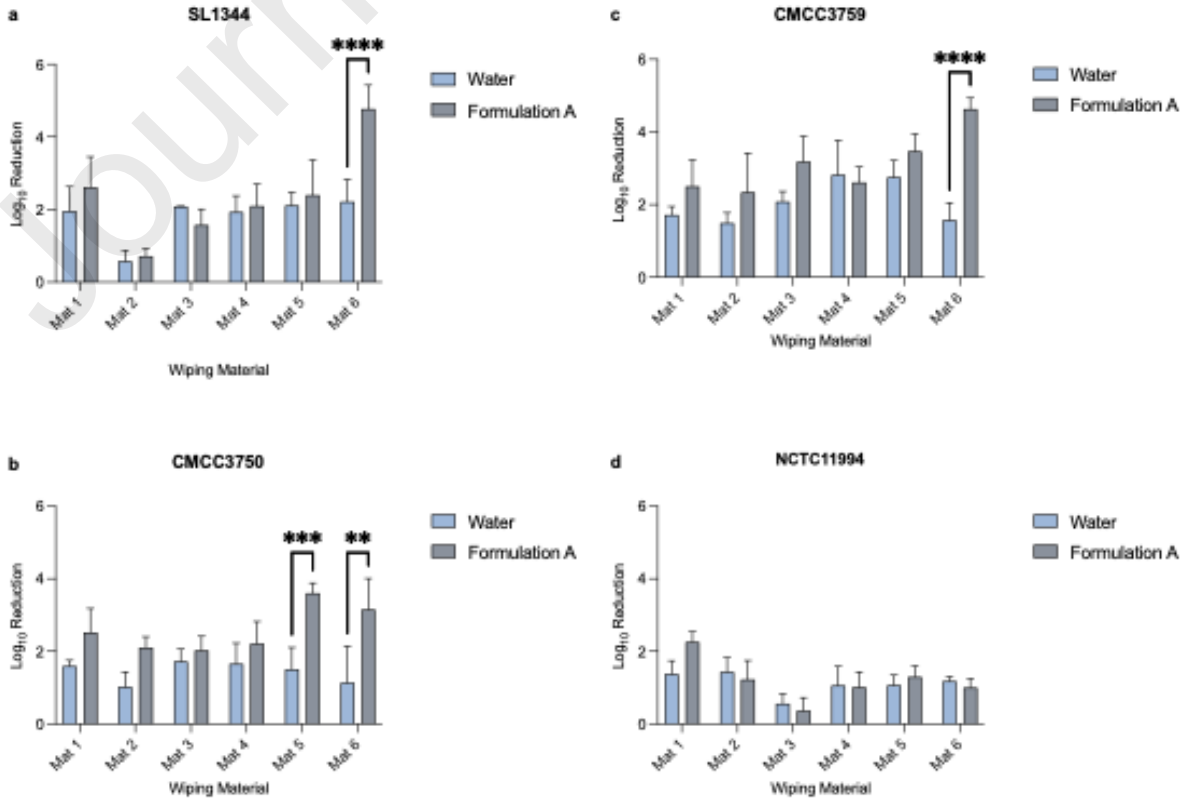


Figure 4 Log_{10} reduction of *S. enterica* DSBs (SL1344; CMCC3750; CMCC3759) and *L. monocytogenes* (NCTC11994) DSB treated with formulation A after wiping (5 sec; 300 g weight) with material-1 or no wiping (n=3)



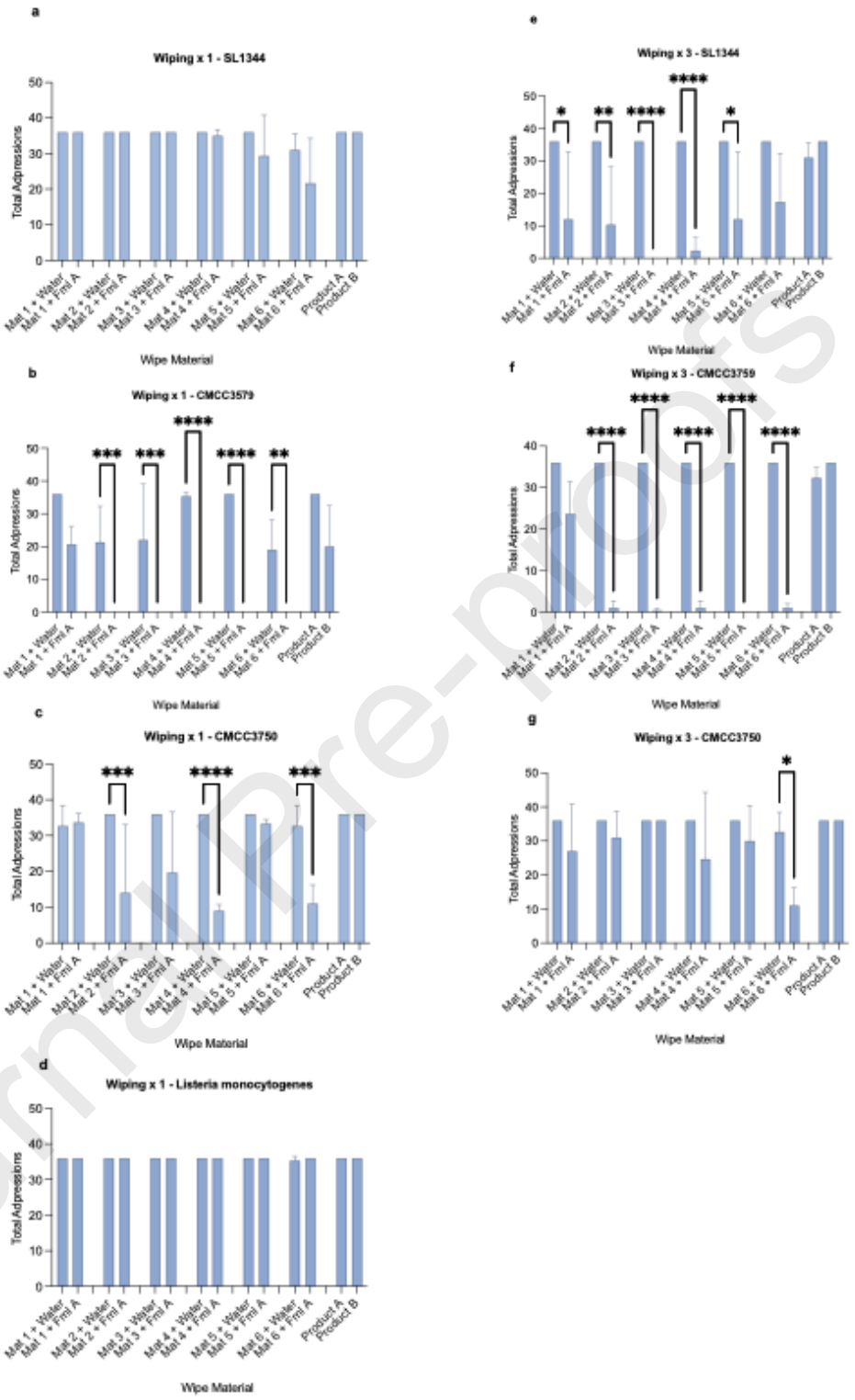


Figure 6 Successive transfer events of *Salmonella enterica* (SL1344, CMCC3759, CMCC3750) and *L. monocytogenes* (NCTC11994) DSB following wiping with formulation A/ materials combination or products A and B. Means of three replicates plotted with error bars representing SD. Two-way ANOVA was performed comparing treatments to a water treated control, p <0.05, ** p <0.01, *** p = <0.001, **** p =<0.0001

Impact of wiping materials on the elimination from surfaces of dry surface biofilm of bacteria of food safety concern

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The authors declare they have no competing interests

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