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1 **Treatment with disinfectants may induce an increase in viable but non culturable**  
2 **populations of *Listeria monocytogenes* in biofilms formed in smoked salmon**  
3 **processing environments**

4

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21

22 **Abstract**

23 The objectives of this study were 1) to evaluate the impact of two industrial disinfectants on  
24 the viability of *Listeria monocytogenes* populations in biofilm and 2) to investigate the viability  
25 state of *L. monocytogenes* cells present on contact surfaces in the smoked salmon  
26 processing environment. In the first step, we cultured mono species and mixed species  
27 biofilms containing *L. monocytogenes* on stainless steel or polyvinyl chloride (PVC) at 8°C for  
28 48h. The biofilms were then exposed to quaternary ammonium- and hydrogen peroxide-  
29 based disinfectants. Residual total populations of *L. monocytogenes* were measured by  
30 qPCR, and viable culturable (VC) cell populations were quantified using standard  
31 microbiological culture-based techniques and by a quantitative PCR (qPCR) assay coupled  
32 with a propidium monoazide treatment. Decreases in VC populations and the appearance of  
33 viable but non culturable (VBNC) populations were observed in response to treatment with  
34 the disinfectants. An 8 month sampling campaign in 4 smoked salmon processing plants  
35 was also carried out to detect *L. monocytogenes* in environmental samples. VBNC cells were  
36 detected mainly after the cleaning and disinfection operations. This study showed that  
37 industrial disinfectants did not inactivate all *L. monocytogenes* cells on inert surfaces. The  
38 presence of VBNC populations of *L. monocytogenes* in the smoked salmon processing  
39 environment is a public health concern.

40

## 41 1. Introduction

42 *Listeria monocytogenes* (*L. monocytogenes*) is a Gram-positive, facultative anaerobic and  
43 intracellular bacterial pathogen which is responsible for human cases of listeriosis. The  
44 specie can cause severe illness in at-risk populations (Swaminathan and Gerner-Smidt,  
45 2007). An increase of the number of outbreaks was observed in the European Union  
46 between 2009-2013 and confirmed in 2017 (EFSA, 2018a). Several listeriosis outbreaks  
47 were reported around the world were linked to diverse food products: ready-to-eat sausages  
48 (South Africa, 2017-2018 : 1.060 cases, 216 deaths) (Olanya et al., 2019), cold-smoked fish  
49 products (Europe, 2014 – 2019 : 22 cases, 5 deaths) (EFSA, 2018b), frozen vegetables  
50 (Europe, 2015-2018 : 47 cases, 9 deaths) (Authority et al., 2018) and dairy products  
51 (France, 2019 : 7 cases). Outbreaks caused by *L. monocytogenes* pose a major threat to  
52 consumer health and result in important economic losses. For example, the cost valuations  
53 of listeriosis cases from a South African outbreak were over US\$ 260 million (234 M€),  
54 including US\$ 10.4 million (9.4 M€) in hospitalization cots and losses amounting to US\$ 15  
55 million (13.5 M€) (Olanya et al., 2019). Inadequate cleaning and disinfection procedures  
56 resulting in cross contamination between the surfaces and food are suspected to have  
57 caused many outbreaks. Jami et al. (2014) and Rodríguez-López et al. (2019) examined  
58 sources of *L. monocytogenes* in the fish processing environment and often observed cross-  
59 contamination between surfaces.

60  
61 *L. monocytogenes* can persist during several months or years on surfaces in food processing  
62 environments. For example, *L. monocytogenes* were detected at a prevalence of 16% in  
63 environmental samples collected over a period of six-years (2003-2008) in Italy (Di Ciccio et  
64 al., 2012); and with a prevalence of 3.7% in environmental and food samples from different  
65 sites over a period of 1.5-years in Thailand (Vongkamjan et al., 2017). Hoffman (2003)  
66 reported a prevalence of 43.8% and 1.2 % in two plants, over two 8-week periods. The  
67 persistence of *L. monocytogenes* could be due to its capacity to form biofilms even under  
68 stressful conditions, such as those encountered in food environments (low temperature,

69 desiccation) (Ferreira et al., 2014). The complex, multicellular structure characteristic of  
70 biofilms could offer bacterial cells protection during cleaning and disinfection (C&D)  
71 procedures. Furthermore, several researchers have reported that biofilms promote the viable  
72 but non culturable (VBNC) state, which would also contribute to resistance under stressful  
73 conditions (Flemming et al., 2016; Stewart and Franklin, 2008). Bacteria in the VBNC state  
74 have very low metabolic activity and do not divide. Consequently, VBNC cells do not grow on  
75 standard microbiological media but retain the ability to recover and become culturable under  
76 favourable conditions (ie. resuscitation). Different environmental stresses can induce the  
77 VBNC state during food processing, such as starvation, oxidative stress, temperature  
78 changes and disinfection (Zhao et al., 2017). We recently showed that VBNC populations  
79 increased in *L. monocytogenes* biofilms following treatment with a NaOH solution (Brauge et  
80 al., 2018).

81 The objective of this work was to study the impact of industrial disinfectants on the  
82 prevalence and viability of *L. monocytogenes* present on food contact surfaces in the  
83 smoked salmon processing environment. The effects of two such disinfectants (quaternary  
84 ammonium compound (QA) and hydrogen peroxide (HP) based products) on the viability of  
85 *L. monocytogenes* and the development of VBNC populations were examined in single  
86 species and mixed biofilms grown on stainless steel and PVC in the laboratory. Extensive  
87 sampling of food contact surfaces before and after cleaning and disinfection in four  
88 commercial smoked salmon processing plants was also carried out over an 8 month period  
89 to examine the prevalence of culturable and VBNC populations of *L. monocytogenes*.

90

## 91 **2. Material and methods**

### 92 **Bacterial strains and culture conditions**

93 Three bacterial strains were used in the study: *Listeria monocytogenes* Lm1 (serogroup 1/2a-  
94 3a), *Carnobacterium maltoromaticum* P1AV5 and *Carnobacterium divergens* P9AV5. All  
95 were isolated from contact surfaces in seafood processing plants. One hundred ml of fresh

96 culture were spun at 5,000 g for 10 min at 4°C, and the pellets were washed with 100 ml of a  
97 sterile saline solution (8.5 g/ml NaCl). The procedure was repeated twice and the pellets  
98 were resuspended in 2.5 ml sterile saline. Concentrations of each inoculum were adjusted to  
99 obtain a final concentration at  $1 \cdot 10^8$  CFU/ml. Final inoculation solutions were prepared by  
100 mixing equal volumes of each bacterial suspension.

#### 101 **Preparation of smoked salmon filtrates**

102 Fifty grams of smoked salmon were homogenized in sterile water (1:5 ratio) for 1 min in a  
103 Stomacher. Supernatant was collected after 1 h of decantation at room temperature and  
104 filtered through a 0.22 µm pore size Stericup® filter (Millipore, Saint Quentin en Yvelines,  
105 France). The filtrate was stored at -18°C and thawed at 4°C for one day before prior to  
106 experimentation.

#### 107 **Biofilm formation**

108 Coupons of AISI 316 L 2B stainless steel (Sapim Inox, Loison-sous-Lens, France) or  
109 polyvinyl chloride (PVC) (Ammeraal Beltech, Noyelles-lès-Seclin, France) measuring 40 mm  
110 x 20 mm were washed with a 2% (v/v) solution of RBST105 (RBS France, Vallerois-le-Bois,  
111 France) and disinfected with a 0.2% (v/v) Oxygal (France-ANTI-GERM, Vaas, France)  
112 solution for 5 min. The coupons were placed in 60-mm diameter Petri dishes (Nunc,  
113 Roskilde, Denmark). Each 60-mm diameter petri dish was placed in a 140-mm diameter Petri  
114 dish (Grosseron, Saint Herblain, France) containing paper towel soaked with 25 ml sterile  
115 distilled water to prevent dehydration during incubation. Ten ml of smoked salmon filtrate  
116 were deposited on each coupon and the plates were incubated at 8°C for 1 h. The smoked  
117 salmon filtrate was then removed with a serological pipette and replaced with 10 ml bacterial  
118 inoculum. The inoculated coupons were subsequently incubated at 8°C for 48 h.

119 **Disinfectant treatments**

120 After incubation, non-adhering bacteria were removed by immersing the coupons twice in  
121 12.5 ml sterile saline for 1 min. The coupons were then treated for 20 min with 12.5 ml of  
122 hydrogen peroxide (HP) or quaternary ammonium (QA, active ingredients: quaternary  
123 ammonium and glutaraldehyde) solutions at 2% (v/v), as recommended by the supplier  
124 (Ecolab, Arceuil, France). The disinfectant solutions were then removed, the coupons were  
125 immersed in 12.5 ml of universal neutralizing broth (AEB610753, Biomérieux) for 30 min  
126 and rinsed twice with 12.5 ml of sterile saline for 1 min. All experiments were carried out at  
127 room temperature. The same protocols were used for controls, using sterile water instead of  
128 disinfectant solutions. Stick-mounted sponges supplied in sterile bags with 10 ml of  
129 neutralizing buffer (Sponge-Stick, 3M, Minnesota, USA) were used as recommended by  
130 Brauge et al. (2020) to sample bacterial populations on the surfaces. The sponges were  
131 homogenized with a Stomacher for 1 min prior to further analysis.

132 **Microscopy**

133 Coupons were stained with a 5 µg/mL solution of 4',6 diamidino-2-phenyl-indole (DAPI,  
134 Merck, Darmstadt, Germany). After 15 min incubation in the dark, the coupons were rinsed  
135 and immediately examined with an epifluorescence microscope (Imager.Z1, Zeiss, France)  
136 connected to a CCD camera (Axiocam-MRm, Zeiss) and a digital image acquisition system.  
137 Four fields of view were recorded for each sample.

138 **Quantification of total, viable and culturable bacteria**

139 Viable culturable bacteria were enumerated on ALOA® agar (BioMérieux) incubated for 24 h  
140 at 37°C for *L. monocytogenes*, and on plate count agar (PCA, Oxoid, Basingstoke, United  
141 Kingdom) supplemented with Novobiocin (Sigma Aldrich, Missouri, USA) at 1900 µg/mL  
142 incubated for 24 h at 30°C for *Carnobacterium*.

143 Total DNA and DNA from viable cells were quantified by qPCR and PMA-qPCR,  
144 respectively. A portion of each bacterial supernatant was spun at 5,000 g in a centrifuge for 5  
145 min at room temperature. The resulting pellet was re-suspended in 990  $\mu\text{L}$  of sterile water. A  
146 495  $\mu\text{L}$  aliquot was used for DNA extraction for standard qPCR. The remaining 495  $\mu\text{L}$  was  
147 treated with PMA (Propidium monoazide, Biotium, Hayward, United States) to a final  
148 concentration of 50  $\mu\text{M}$ . Samples with PMA were incubated for 5 min at room temperature in  
149 the dark and then subjected to light exposure at 80% for 10 min in an Eppendorf tube using a  
150 PhAST Blue lamp (GenIUL, Barcelone, Espagne). Cells were then collected by centrifugation  
151 at 5,000 g for 10 min at room temperature. For both qPCR and PMA-qPCR, DNA was  
152 extracted with DNeasy® Blood & Tissue kits for the “Purification of Total DNA from Animal  
153 Tissues” (Qiagen, Hilden, Germany), using the ATL lysis buffer. The elution step was carried  
154 out with 100  $\mu\text{L}$  of AE buffer. Quantification of total and viable cells by qPCR and PMA-qPCR  
155 was performed as described in (Brauge et al., 2018). Standard curves were obtained using  
156 DNA covering the range from  $10^1$  to  $10^9$  genome equivalents (GE) per ml. The GE was  
157 calculated on the basis of a *L. monocytogenes* serotype 1/2a genome of  $2.94 \cdot 10^6$  bp (Allnutt  
158 et al., 2016). The quantification limit was 3.22 log GE/ ml in an initial concentration of the  
159 sample which corresponds to 2.32 log GE/  $\text{cm}^2$  for a 8  $\text{cm}^2$  coupon.

## 160 **Environmental sampling in smoked salmon plants**

161 Samples were taken from surfaces at the end of the production day before cleaning and  
162 disinfection (C&D) operations, and the following morning before the start of a new production  
163 cycle. Two critical points (areas with a high risk of contamination) were identified in each of  
164 the four smoked salmon processing plants and a sampling campaign was conducted for  
165 eight months at a frequency of four samples (2 samples before and 2 samples after C&D  
166 procedure) per month and per plant (n= 128). The identified surfaces (100  $\text{cm}^2$ ) were wiped  
167 with a sterile sponge, using a zigzag motion in two perpendicular directions. Five ml of  
168 peptone water were added to the bag containing the sponge and the contents were  
169 homogenized for 1 min with a stomacher. The quantification of dead and viable populations



170 of *L. monocytogenes* was carried out by qPCR and PMA-qPCR as described previously.  
171 Enumeration of VC populations was carried out on ALOA agar incubated at 37°C for 48 h.  
172 For the detection of *L. monocytogenes*, 5 ml of the suspension were diluted with half-Fraser  
173 broth and were incubated at 30°C for 24 h. Fluids from the enrichment culture were applied  
174 to ALOA agar and the plates were incubated at 37°C for 24h.

## 175 **Statistical analysis**

176 All biofilm experiments were replicated six times (two coupons per experiment for three  
177 independent experiments). Data was analyzed using general linear model procedures in  
178 Statgraphics centurion V18 software (Sigmaplus, Neuilly-sur-seine, France). For each  
179 biofilm, variance analyses (ANOVA) were performed to determine the impact of substrate  
180 and disinfectant treatment on counts of i) culturable cells, ii) total viable cells (both culturable  
181 and not), and iii) total populations of *L. monocytogenes*. Differences were considered  
182 statistically significant at  $P < 0.05$ .

## 183 **3. Results**

### 184 **Impact of disinfectant treatments on viability of *L. monocytogenes* in a mixed biofilm**

185 Bacterial populations in the biofilm were enumerated by plate counting (viable and culturable  
186 cells), PMA-qPCR (viable cells) and qPCR (total cells) after treatment with water (control) or  
187 with disinfectant solutions (Figure 1). The difference between quantification by qPCR assay  
188 and PMA-qPCR assay was indicative of a dead population. The difference between the  
189 quantification by PMA-qPCR assay and the enumeration on the agar plate indicated the  
190 presence of VBNC populations.

191 Total and viable *L. monocytogenes* populations quantified by qPCR and PMA-qPCR on  
192 control stainless steel coupons were 6.80 Log (GE/cm<sup>2</sup>) and 5.93 Log (GE/cm<sup>2</sup>), respectively  
193 (Figure 1A). Measurement of VC populations on selective media yielded similar results, or  
194 5.88 and 6.42 Log (CFU/cm<sup>2</sup>), respectively. No significant difference was observed between

195 culturable, viable and total populations ( $P > 0.05$ ), demonstrating that most *Listeria* cells  
196 remained viable and culturable in the biofilm under grown under control conditions.

197 Microscopic observations of biofilms showed a heterogeneous structure, with the presence of  
198 thick clusters surrounded by diffuse material (white arrows) separated by lightly colonized  
199 areas, where only small clusters and single cells were observed (Figure 2). Biofilms  
200 cultivated on stainless steel were also treated with hydrogen peroxide (HP) and quaternary  
201 ammonium compounds (QA), two disinfectants frequently used in the seafood industry.

202 Significant decrease in total *L. monocytogenes* populations was observed after QA treatment  
203 (reduction of 1.06 Log (GE/cm<sup>2</sup>), and a comparatively lower decrease after HP treatment  
204 (reduction of 0.60 Log (GE/cm<sup>2</sup>)) (Figure 1A). These results were confirmed by microscopic  
205 observations (Figure 2) which showed that disinfection treatments caused the appearance of  
206 larger aggregates on stainless steel, with the presence of some thick clusters surrounded by  
207 diffuse material (white arrows) and areas where only single cells were seen.

208 Significant reductions ( $P < 0.05$ ) in viable populations of *L. monocytogenes* were measured  
209 on stainless steel surfaces (1.79 and 1.88 log (GE/cm<sup>2</sup>) for HP and QA treatments,  
210 respectively). The difference between qPCR and PMA-qPCR assay measurements was  
211 indicative of the presence of dead cells after treatment of the biofilms (2.06 log GE/cm<sup>2</sup> after  
212 HP treatment and of 1.70 log GE/cm<sup>2</sup> after QA treatment). Significant ( $P < 0.05$ ) decreases in  
213 *L. monocytogenes* VC populations were observed (5.22 Log (CFU/cm<sup>2</sup>) after HP treatment  
214 and 4.49 Log (CFU/cm<sup>2</sup>) after QA treatment). This was indicative of VBNC populations in the  
215 biofilms treated with HP (difference of 2.95 log GE/cm<sup>2</sup>) and QA (difference of 2.12 log  
216 GE/cm<sup>2</sup>). In summary, VBNC and VC populations of *L. monocytogenes* were detected in  
217 biofilms after treatment with both HP and QA disinfectants. VC populations of  
218 *Carnobacterium* were also affected by exposure to the disinfectants, which resulted in  
219 reductions of 4.25 Log (CFU/cm<sup>2</sup>) and 3.88 Log (CFU/cm<sup>2</sup>) after HP and QA treatment,  
220 respectively.

221

222 Similar results were found with *L. monocytogenes* biofilms cultivated on PVC (Figure 1B).  
223 The control biofilm was mainly composed of *Carnobacterium* and *L. monocytogenes* VC cells  
224 (approximately 5.5 Log (CFU/cm<sup>2</sup>) and large populations of non culturable or dead cells were  
225 found after treatment with the disinfectants. Microscopic observations confirmed the absence  
226 of bacterial detachment from the PVC surface (Figure 2).

227

#### 228 **Quantification of *L. monocytogenes* populations (viable culturable, viable and total) in** 229 **environmental samples collected in smoked salmon processing plants**

230 Before C&D operation, the prevalence of VC *L. monocytogenes* in environmental samples in  
231 4 industries was 3.17% for VC *L. monocytogenes*, 10.32% for viable population and 15.87%  
232 for total population. After C&D operation, the prevalence of VC *L. monocytogenes* in  
233 environmental samples in 4 industries was 1.59% for VC *L. monocytogenes*, 9.52% for viable  
234 population and 14.29% for total population. Prevalence was low in three of the four plants,  
235 where contamination with *L. monocytogenes* was sporadic (Figure 3 A, C, D). In plant (B),  
236 the VC *L. monocytogenes* was detected before C&D operations in two critical areas,  
237 including 50% of samples collected on slicer blades and 25 % on a slicing tray, but was not  
238 detected after C&D operations (Figure 3 B). For the viable population we also showed a  
239 decrease for the slicer blade after C&D operations and a stability for the slicing tray. This  
240 stability were also observed for the total populations after C&D operations for both critical  
241 points. Moreover, the prevalence of VBNC populations increased after C&D operations on  
242 slicer blades and was stable on the slicing tray after C&D operations. Populations of dead *L.*  
243 *monocytogenes* also increased at both locations after C&D procedures.

#### 244 **4. Discussion**

245 We evaluated the impact of two disinfectants frequently used in the seafood processing  
246 industry on the viability of *L. monocytogenes*, including a disinfectant composed of  
247 quaternary ammonium compounds (QA) and a disinfectant containing hydrogen peroxide

248 (HP). Biofilms grown for 48 h were treated with both disinfectants at concentrations  
249 recommended by the supplier. Culturable bacteria were enumerated on agar media and total  
250 and viable cells were quantified by qPCR and PMA-qPCR, respectively. We showed that the  
251 HP and QA products did not inactivate all *L. monocytogenes* cells in biofilms grown for 48 h  
252 on PVC or stainless steel. Instead, *L. monocytogenes* cells became unculturable but  
253 remained viable after the treatments. These results are in agreement with previous research  
254 which showed that populations of VBNC *L. monocytogenes* increased in biofilms exposed to  
255 0.5% NaOH (Brauge et al., 2018). Similarly, Overney et al. (2017) showed induction of the  
256 VBNC state in *L. monocytogenes* biofilms formed by strain LO28 (serotype 1/2c) on stainless  
257 steel following treatment with chlorinated alkaline cleaner used at a concentration of 3%  
258 (vol/vol) (Topax 66, Ecolab, Arcueil, France) or a quaternary ammonium-based disinfectant  
259 at a recommended concentration of 2% (vol/vol) (Triquart MS, Ecolab). Other researchers  
260 have reported induction of the VBNC state in *L. monocytogenes* exposed to commercially  
261 available household cleaners in combination with inorganic salts. *L. monocytogenes* cells  
262 exposed to combinations of surfactants and salts for 5 min up to 1 h lost their culturability on  
263 standard growth media while retaining ATP production, fermentation of sugars and  
264 membrane integrity, which are suggestive of active metabolism (Robben et al., 2018). The  
265 mechanisms responsible for the inactivation of microorganisms by disinfectants are often  
266 unclear. Depending on their molecular structure and physico-chemical properties, different  
267 disinfectants may interfere with the bacterial cell membrane, molecules responsible for  
268 energy metabolism or nucleic acids (Maris, 1995). The VBNC state may confer enhanced  
269 resistance of *L. monocytogenes* to disinfectants. Using an ATP-based approach, Robben et  
270 al. (2019) showed that *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa* and  
271 *Listeria monocytogenes* in the VBNC state were resistant to several antibiotics (ampicillin,  
272 imipenem, ciprofloxacin, and gentamicin) and were insensitive to selected disinfectants  
273 (benzalkonium chloride and trioctylmethylammonium chloride) and preservatives (bronopol  
274 and sodium azide). Nowakowska and Oliver (2013) have also shown that *Vibrio vulnificus* in  
275 the VBNC state is more resistant to environmental stresses.

276

277 We carried out repeated samplings in four smoked salmon processing plants that use the  
278 disinfectants HP and / or QA to verify the presence of viable *L. monocytogenes* populations  
279 in food processing plants. We confirmed the presence of *L. monocytogenes* VC populations  
280 with a prevalence of 3.17% and 1.59% before and after C&D operations. This is in  
281 agreement with several studies which have confirmed that VC *L. monocytogenes* populations  
282 may become established in food-processing environments (Thimothe et al., 2004; Vaz-Velho  
283 et al., 2001). Leong et al. (2015) found *L. monocytogenes* in 2.5% of 508 samples, including  
284 433 environmental swabs and 75 food samples. Others studies have indicated higher  
285 prevalence rates (Gambarin et al., 2012; Ricci et al., 2018; Schjørring et al., 2017). However,  
286 there have been few attempts to determine the prevalence of dead or non-culturable  
287 populations of *L. monocytogenes* in food processing environments. In the present study, we  
288 have shown that both were present in the smoked salmon processing environment, and that  
289 VBNC populations likely arise in response to stresses induced by industrial disinfectants  
290 used in cleaning and disinfection operations.

## 291 **5. Conclusion**

292 The present work showed that exposure of *L. monocytogenes* biofilms grown in the  
293 laboratory to HP and / or QA disinfectants reduced VC populations and induced the VBNC  
294 state in the species. Sampling of contact surfaces in smoked salmon processing plants  
295 suggested that use of these disinfectants in routine cleaning and sanitation operations leads  
296 to the development of persistent VBNC populations on such surfaces. Alternative strategies  
297 are urgently needed to mitigate the risks associated with *L. monocytogenes* in the seafood  
298 processing environment.

299

300

## 301 **6. Acknowledgements**

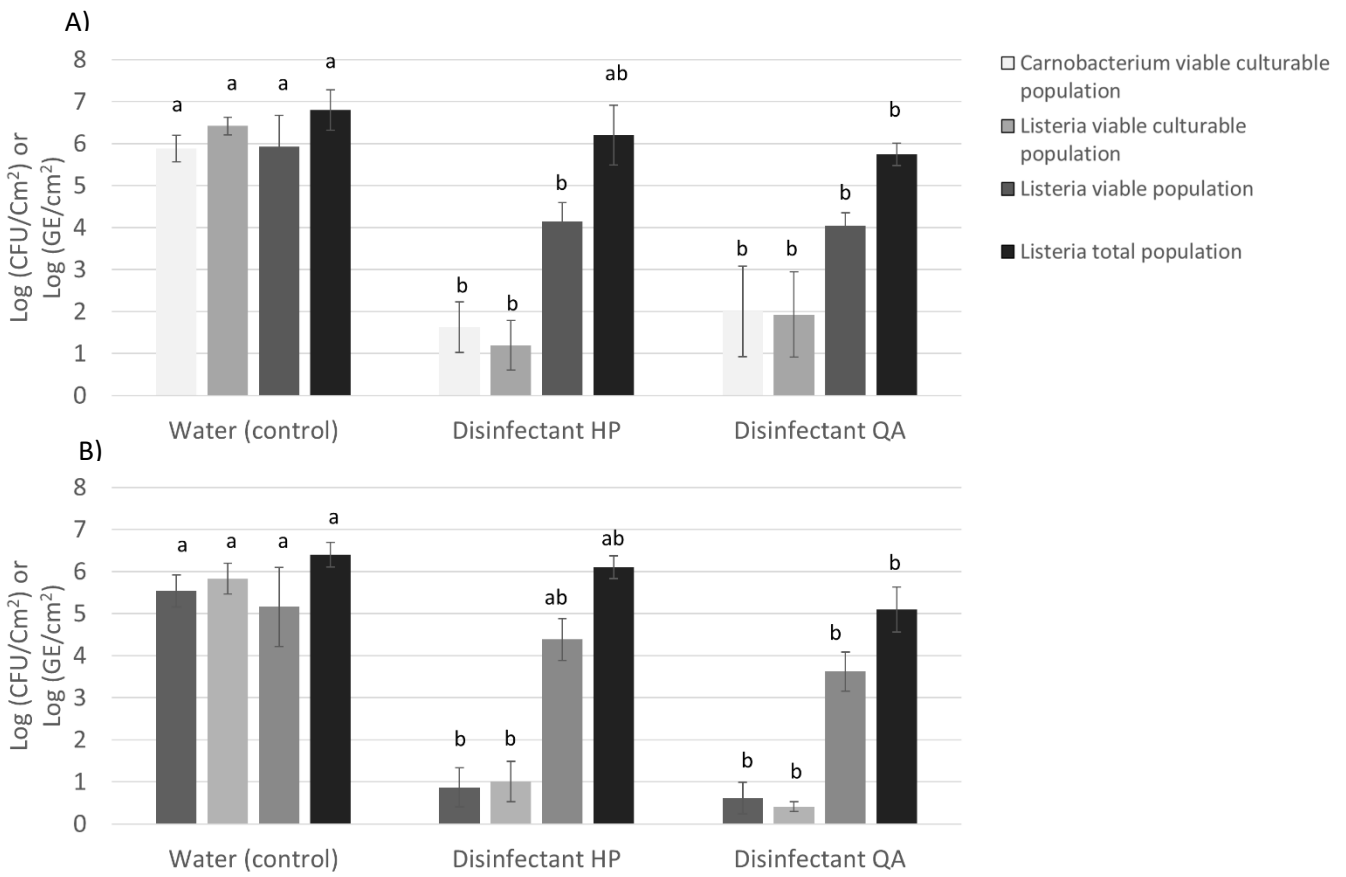
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305

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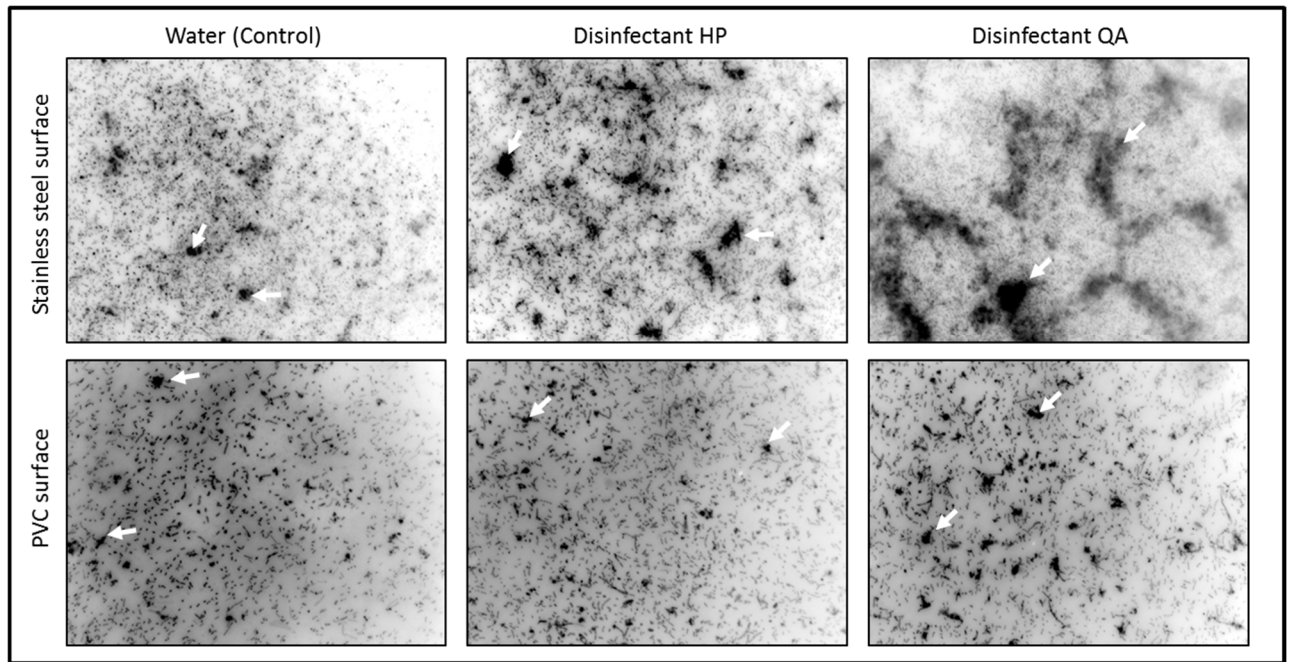
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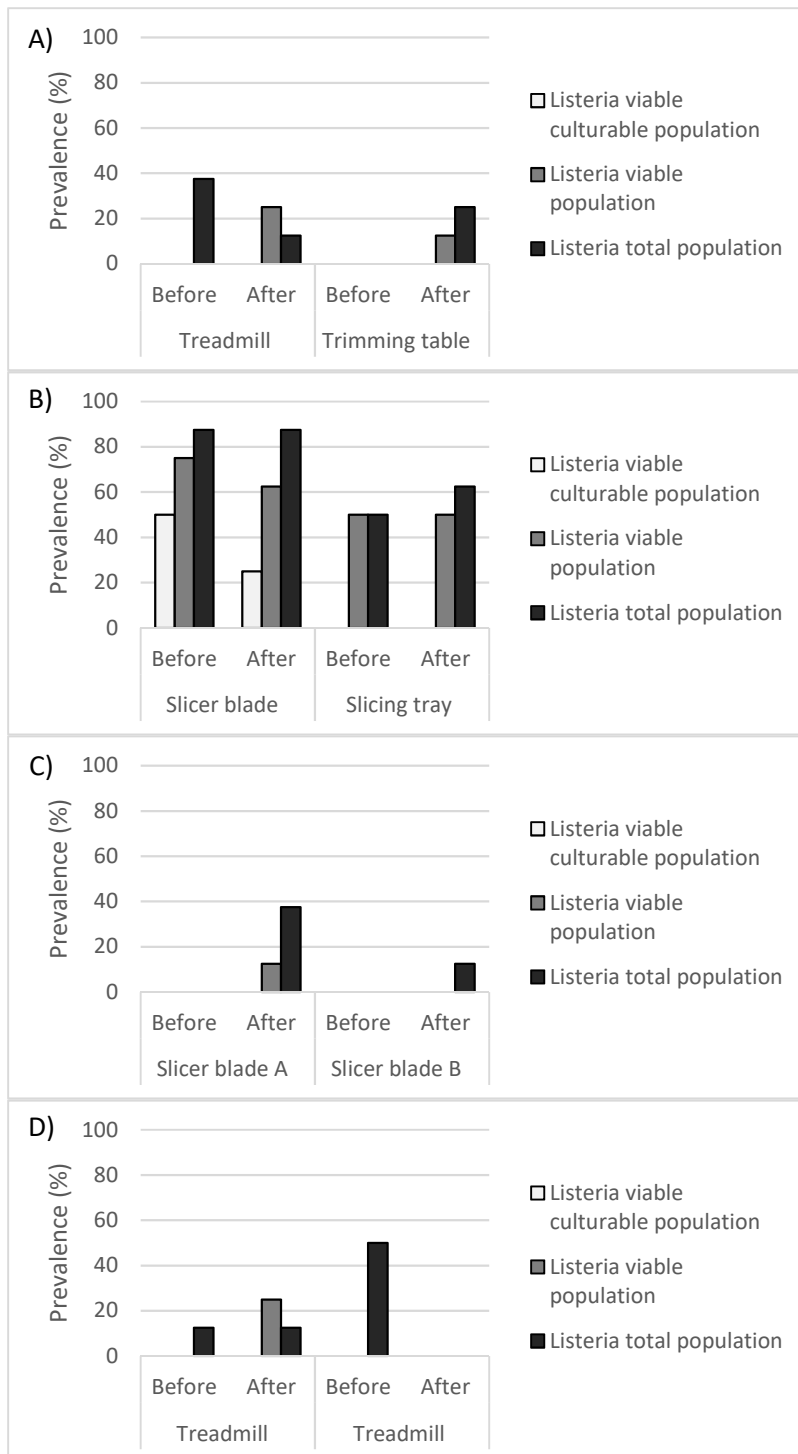


**Figure 1: Quantification of viable culturable (Log (CFU/cm<sup>2</sup>)), viable (Log (GE/cm<sup>2</sup>)) and total (Log (GE/cm<sup>2</sup>)) populations of *L. monocytogenes* and viable culturable of *Carnobacterium* in mixed biofilm treated with water (control), Hydrogen Peroxyde (HP) or Quaternary Ammonium (QA) disinfectants. Biofilms were grown for 48 h at 8°C on (A) stainless steel and (B) PVC conditioned with smoked salmon filtrate. The error bars represented the standard deviation (n = 6). Statistical significant differences were evaluated at  $P < 0.05$  and represented by letters a, b, ab.**





**Figure 2: Observations by epifluorescence microscopy of *L. monocytogenes* and *Carnobacterium* in mixed biofilms stained with DAPI.** Biofilms were grown on stainless steel or PVC conditioned with smoked salmon filtrate and incubated at 8°C for 48H. After, biofilms were treated with water (control), Hydrogen Peroxyde (HP) or Quaternary Ammonium (QA) disinfectants. Magnification x400. White arrows indicate the some thick clusters surrounded by diffuse material.



**Figure 3: Prevalence of viable culturable (Log (CFU/cm<sup>2</sup>)), viable (Log (GE/cm<sup>2</sup>)) and total (Log (GE/cm<sup>2</sup>)) *L. monocytogenes* populations from smoked salmon plant of the (A) company A, (B) company B, (C) company C, (D) company D before (n=32) and after (n=32) cleaning and disinfection procedure.**