Table of contents

Title page

Listeria monocytogenes in food businesses: from persistence strategies to intervention/prevention strategies, a review

Tessa Tuytschaever[1], Katleen Raes[1], Imca Sampers[1,#]

Tessa Tuytschaever: tessa.tuytschaever@ugent.be

Katleen Raes: katleen.raes@ugent.be

Imca Sampers: imca.sampers@ugent.be

[1]Research Unit VEG-i-TEC, Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Campus Kortrijk, Sint-Martens-Latemlaan 2B, 8500 Kortrijk, Belgium.

[#]Corresponding author, e-mail: imca.sampers@ugent.be, address: Sint-Martens-Latemlaan 2B, 8500 Kortrijk, phone: +32 56 32 21 98.

Short version of title (running head)

Listeria monocytogenes persistence

Choice of journal/topic

Comprehensive Reviews in Food Science and Food Safety

ABSTRACT

In 2023, *Listeria monocytogenes* persistence remains a problem in the food business. A profound understanding of how this pathogen persists may lead to better-aimed intervention/prevention strategies. The lack of a uniform definition of persistence makes the comparison between studies complex. Harbourage sites offer protection against adverse environmental conditions and form the ideal habitat for the formation of biofilms, one of the major persistence strategies. A retarded growth rate, disinfectant resistance/tolerance, desiccation resistance/tolerance and protozoan protection complete the list of persistence strategies for *Listeria monocytogenes* and can occur on themselves or in combination with biofilms. Based on the discussed persistence strategies, intervention strategies are proposed. By enhancing the focus on four precaution principles (cleaning and disinfection, infrastructure/hygienic design, technical maintenance, and work methodology) as mentioned in Regulation (EC) No. 852/2004, the risk of persistence can be decreased. All of the intervention strategies result in obtaining and maintaining a good general hygiene status throughout the establishment at all levels ranging from separate equipment to the entire building.

1. Introduction

Listeria monocytogenes is a Gram-positive, facultatively anaerobic, non-spore-forming rod, and has at 20-25°C tumbling motility. The foodborne pathogen is ubiquitous in the environment due to its broad temperature (-2 to +45°C) and pH (4.2 to 9.5) growth range, its ability to withstand and grow in an a_w-environment up to 0.92 and salt concentrations up to 16% (all when other conditions are optimal). The bacteria can be found in a variety of environmental sources, e.g. soil, plants, water, silage, and animal sources e.g. cattle, sheep and poultry), which give rise to the initial entrance into food businesses (FAO & WHO, 2004).

Listeriosis, the disease caused by *L. monocytogenes*, has a whole array of possible symptoms ranging from nausea to meningitis (EFSA, 2014). Newborns, the elderly, pregnant women and people with a compromised immune system are most vulnerable to the disease. The importance of *L. monocytogenes* is not reflected in its incidence, which is low (0.42 per 100 000 in 2020) compared to other foodborne pathogens (e.g. 13.7 per 100 000 for *Salmonella* spp. in 2020), but rather in its high mortality rate (13.0% in 2020) compared to other foodborne pathogens (e.g. 0.19% for *Salmonella* spp. in 2020) (EFSA, 2014, 2021).

L. monocytogenes is psychrotrophic and thus able to grow in refrigerated temperatures (< 7°C), which makes the pathogen of upmost importance in ready-to-eat foods, smoked salmon, raw vegetables and dairy products (EFSA, 2014; FAO & WHO, 2004). This is reflected in the number of alerts in the Rapid Alert System for Foods and Feeds (RASFF) related to these categories. Looking over 27 years (1996-2022) in the RASFF system, an increase is observed, not only in the number of alerts but also in the number of food products in which the pathogen is found (Figure 1).

It is impossible to eliminate *L. monocytogenes* from food businesses as problems still occur despite the efforts done by authorities, e.g. EFSA, to estimate the consumer's risk (EFSA BIOHAZ panel, 2018, 2020) and to aid in sampling and testing strategies (EFSA, 2018b). Contamination can, on the one hand, be attributed to the continuous entrance of *L. monocytogenes* via the raw material, characterized by different molecular types found over time. On the other hand, equal molecular clones, indicate that a particular strain might be persistent and thus the likely cause for the reoccurring contamination. *L.*

monocytogenes can persist for several years in meat, fish, and dairy processing companies with the equipment being the source of contamination rather than the raw material (Møretrø & Langsrud, 2004). This review focuses on the persistence strategies relevant to *L. monocytogenes*. Five persistence strategies i.e. biofilm protection, retarded growth rate, disinfectant resistance/tolerance, desiccation resistance/tolerance and protection by protozoa will be discussed. Additionally, the interaction seen between biofilm formation and each one of the other four persistence strategies is studied. Before discussing the persistence strategies themselves, the relevance of the lack of a uniform definition of the term persistence and the relevance of harbourage sites is explained. Afterwards, different intervention strategies will be discussed that aid in the prevention of persistence.

2. The problem with defining persistence

Persistence is, although seemingly simple, a complex phenomenon. It was first described by Bigger (1944) who mentioned a group of surviving cells towards a penicillin treatment and called them "persisters". The term "resisters" was avoided on purpose as it was unsure if this phenomenon was attributed to "special" qualities. It is an ongoing debate whether persistent cells carry special qualities, and abilities or not. This will pop up in several of the persistence strategies in this review. One of the problems to solve this question lies in the lack of a uniform definition. In different studies, definitions go from rather simple e.g. in Djordjevic et al. (2002) to more detailed e.g. in Rodríguez-Campos et al., (2019). Carpentier & Cerf (2011) analyzed different definitions given in studies and tried to distinguish some key items that would enhance comparability. According to them, persistence should be defined as the repeated isolation of a certain organism of a specific molecular subtype from the same location, which can range from a whole area to a particular device, despite the frequent and correct application of cleaning and disinfection (C&D). Accompanied by this definition, a specific (e.g. at least 2 times over 2 months with 6 sampling occasions) or imprecise (e.g. reoccurrence over 2 months) time range mentioning the period and threshold of positive samples should be given to set the border between persistence and non-persistence. This improved definition, provided that details on the location, the number of samples taken within a defined period and the number of samples taken/being positive are given, enhances the comparability between studies (Borucki et al., 2003). Following this definition,

molecular subtyping seems crucial to determine persistence. In the field of epidemiology, molecular subtyping is on the rise to improve multi-country outbreak studies and surveillances and speed-up cluster detection (CDC, 2023; ECDC, 2019, 2020; Walle et al., 2018). To evaluate national public health reference laboratories on their ability to determine serotypes and detect molecular clusters, the ECDC established the external quality assessment (EQA) scheme reports on the typing of *L. monocytogenes* on a regular basis (eight reports in the period 2014-2022)(ECDC, 2022). The enhancement of molecular methods in epidemiology could result in a more rapid development and lower cost of these techniques making them easier accessible for food businesses to imply them. Additionally, studying these reports could also aid food producers to choose the appropriate methodology when executing persistence studies or to evaluate the applied methodology against the ones used in national public health reference laboratories.

Another problem that causes difficulties in comparing persistence studies is the group of non-persistent cells. These may be persistent at other locations inside the production facility or become persistent over time if the strain is only recently introduced. Therefore, it is better to call these strains presumed non-persistent (Carpentier & Cerf, 2011; Larsen et al., 2014; Pan et al., 2006). This complexifies comparison as the group of strains defined as presumed non-persistent may contain persistent strains which are new or from other locations (Borucki et al., 2003).

3. Harbourage sites

Persistence may arise simply by the location where microorganisms end up during processing (Carpentier & Cerf, 2011). Harbourage sites such as poorly designed equipment (dead spaces, welds…), worn materials (cracks, crevices, frayed conveyor belts…), irregularities left by the degassing of freshly deposited resins, and difficult-to-reach places inside the building are suggested to hold appropriate conditions for microorganisms to grow, adhere and adapt (Carpentier, 2005, 2009; Carpentier & Cerf, 2011; Fagerlund et al., 2017; Lundén et al., 2002). EFSA noted that *L. monocytogenes* could be found in many of these harbourage sites (EFSA, 2018b). How bacteria reach these harbourage sites (after C&D), is still unsure with several possibilities, the first one being ‘accidental’. This can be due to an abnormally high number of cells entering the harbourage site and/or that the previous C&D did not

142 eliminate all the bacteria and thus allows these bacteria to grow (Carpentier, 2009). Another possibility
143 is the permanent entrance via the raw food ingredient, here the raw ingredient is the cause rather than
144 the factory environment and cannot be called real persistence as it will not be the same clone every time
145 (Carpentier, 2009; Gibson et al., 1999; Pan et al., 2006). Entrance/transmission to different areas inside
146 the processing environment can be via aerosols, which consist of dispersed solid and/or liquid particles
147 in the air, as they can go through every existing opening such as doorways, hatches, drains and occupy
148 nooks, crevices and other difficult-to-each areas (Burfoot, 2005; EFSA, 2018b). They may arise from
149 various sources including raw materials, people, packaging, transfer of equipment, C&D (an important
150 source of aerosols) (Holah, 1995; Reij & den Aantrekker, 2004).

151 Harbourage sites are ideal for biofilm development and some zones like wetting fronts contain biofilms
152 that are more tolerant towards mechanical stress and sanitisers. Poor hygienic design creates many of
153 these zones (Colagiorgi et al., 2017; EFSA BIOHAZ panel, 2020). The lower cleanability causes the
154 entrapment of food particles and the formation of a food-conditioning film which may boost attachment,
155 growth and biofilm formation (Chmielewski & Frank, 2003; Verghese et al., 2011). Floor drains and
156 conveyor belts are an example of poorly cleaned areas where biofilm formation occurs (Dzieciol et al.,
157 2016; Fagerlund et al., 2017). Conveyor belts may know surface irregularities which increase when
158 being used for some time e.g. edges that start to fringe out causing new harbourage sites to appear
159 (Fagerlund et al., 2017). The presence of food particles also enhances the effect of other persistence
160 strategies e.g. disinfectant resistance/tolerance and desiccation resistance/tolerance. The protection
161 bacteria get from the harbourage sites gives them a head start in persisting. When discussing persistence
162 strategies, harbourage sites are often mentioned.

163 # 4. How do bacteria become persistent in food processing facilities?

164 ## 4.1 Biofilm protection

165 The first persistence strategy are biofilms, an assemblage of viable and non-viable microbial cells
166 irreversibly associated with an (a)biotic surface, embedded in a matrix of extracellular polymeric
167 substances (EPS) (Carpentier & Cerf, 2011; Chmielewski & Frank, 2003; Costerton et al., 1987; Donlan,
168 2002; EFSA, 2018b; Vasudevan, 2014). The formation of a biofilm (Figure 2) starts with

reversible/irreversible attachment to the surface, followed by the growth of microcolonies and EPS production. Subsequently, the biofilm matures which is characterized by the presence of an EPS matrix and the downregulation of flagella synthesis (de Kievit, 2011). How long this process takes depends on the microorganisms and environmental factors. When biofilms are mature, they can release cells back into the environment (Chmielewski & Frank, 2003; Costerton et al., 1987; Donlan, 2002; Vasudevan, 2014; Wimpenny et al., 2000). Biofilms offer protection from various unfavourable environments e.g. toxic substances, nutrient depletion, oxygen/pH fluctuations, and lead, besides food safety problems and early spoilage, to economic losses caused by, among other things mechanical blockage, the impedance of heat transfer, corrosion to metal surfaces, hampered filtration (Chmielewski & Frank, 2003; Møretrø & Langsrud, 2017; Xu et al., 2011).

L. monocytogenes can form biofilms on a large variety of surfaces such as stainless steel, polystyrene, polypropylene and glass. However, these are often thin biofilms with colony counts varying between 10^4-10^7 log CFU/cm^2 in contrast to thick multilayer biofilms of other bacteria where colony counts can reach 10^9-10^{12} CFU/cm^2 (EFSA, 2018b). The ability of *L. monocytogenes* to form monospecies biofilms, which is most often studied, is not a key factor in its persistence, more important is the presence of *L. monocytogenes* in multispecies biofilms as most species live together in multispecies communities and interact with each other (Annous et al., 2009; Fagerlund et al., 2021). Especially, in hard-to-reach places where C&D is less efficient, interactions between bacteria in multi-species biofilms are important for their survival (Møretrø & Langsrud, 2017).

The relationship between *L. monocytogenes* and other microorganisms inside one biofilm may be synergistic (=cooperative) (Puga et al., 2014; Xu et al., 2017), antagonistic (=competitive) (da Silva Fernandes et al., 2015; Giaouris et al., 2015; Hossain et al., 2020; Yamakawa et al., 2018; Zhao et al., 2004) or neutral (de Grandi et al., 2018). *L. monocytogenes* possesses an antagonistic relationship with several lactic acid bacteria as studied by Hossain et al. (2020) where, on stainless steel at 30°C after 24h, maximal inhibition of 2.2 log CFU/cm^2 was caused by *Latilactobacillus curvatus.* Several hypotheses exist on the antagonistic action of lactic acid bacteria against *L. monocytogenes* e.g. the production of bacteriocins, organic acids or hydrogen peroxide which hamper the growth of the pathogens and thus

196 inhibits biofilm formation (Fagerlund et al., 2021; Gao et al., 2019; Hossain et al., 2020). A synergistic
197 effect was seen by Puga et al. (2014) where *L. monocytogenes* reached at least 1 log CFU/cm^2 higher
198 cell counts, after 24h on glass at 4 or 20°C, in combination with *Pseudomonas fluorescens*. The higher
199 cell counts would be attributed to the EPS production of *Pseudomonas* spp., which protects and embeds
200 the *L. monocytogenes* cells (Puga et al., 2014). The type of EPS produced in multispecies compared to
201 monospecies biofilms might differ and play a role in an enhanced cell count as mentioned by Xu et al.
202 (2017) where shorter EPS fragments were found connecting neighbouring cells in a duo-species biofilm
203 between *Ralstonia insidiosa* and *L. monocytogenes*. de Grandi et al. (2018) looked into a neutral
204 relationship of *L. monocytogenes* where cell counts didn't change with the presence of *E. coli*. The
205 interaction between *L. monocytogenes* and the surrounding microbiota in multispecies biofilms is
206 affected by various environmental factors such as the food residue, nutrient availability, temperature,
207 humidity, pH, surface material/characteristics and C&D (Fagerlund et al., 2021). An example of this is
208 the influence of temperature on the growth of dual-species biofilms of *L. monocytogenes* and
209 *Enterococcus faecalis* and *Enterococcus faecium* where at 25°C higher cell counts of *L. monocytogenes*
210 were seen and on 39°C lower (da Silva Fernandes et al., 2015). Several hypotheses exist to explain the
211 effect of temperature i.a. *L. monocytogenes* flagella not being present at 39°C, nutrient competition, the
212 potential production of bacteriocins, and differences in growth rate depending on the applied
213 temperature.

214 The persistence of *L. monocytogenes* is thus likely, or at least partially, due to biofilm formation.
215 Consequently, questions arise: Do biofilms found in the food industry contain persistent strains or not?
216 And if so, does that mean that persistent strains form "better" biofilms? The latter question is difficult
217 to answer, as what is seen as better? First, the lack of an appropriate uniform definition of persistence
218 makes it difficult to make comparisons. "Better" might refer to a higher adherence rate and thus faster
219 biofilm formation. Lundén et al. (2000) noticed that the biomass of persistent strains was higher after 1
220 and 2h incubation compared to presumed non-persistent strains, while this effect disappeared when
221 looking at 72h-old biofilms. Thus, the adherence rate may be shorter for persistent strains, which gives
222 them a head start compared to presumed non-persistent strains. An explanation for this faster adherence

might be the on average shorter lag time of persistent strains under certain stress conditions such as exposure to 2.5%, 4% or 8% NaCl concentrations or a pH of 5 as noticed by Magalhães et al. (2016). This shorter lag time was subjected to strain variability and some presumed non-persistent strains showed shorter lag times compared to some persistent strains.

"Better biofilm formers" can also refer to higher biomass production. Some studies (Borucki et al., 2003; Norwood & Gilmour, 2001) measured, on average, higher biomass production in persistent strains compared to presumed non-persistent strains. The study of Norwood & Gilmour, (2001) only tested one persistent and one presumed non-persistent strain. In the study of Borucki et al. (2003) strain variability was noticed and some persistent strains showed lower biomass compared to some presumed non-persistent strains. Other studies (Costa et al., 2016; Harvey & Keenan, 2007; Magalhães et al., 2017; Nilsson et al., 2011; Ochiai et al., 2014) attribute differences, if found, between persistent and presumed non-persistent strains to strain variability and observed no difference in biomass production. The enhanced biomass production is thus more likely only an adaptational difference rather than an inheritable aspect of persistent strains. Additionally, the differences between persistent and presumed non-persistent strains, if found, can also be material (Magalhães et al., 2017) or temperature (Ochiai et al., 2014) dependent which might be again prone to strain variability.

At last, "better biofilm formers" can also be seen as more resistant, meaning able to withstand chemical/mechanical/environmental stresses better compared to presumed non-persistent strains. For some persistence strategies e.g. disinfectant resistance or tolerance, persistent strains are compared to presumed non-persistent strains. These parts shed a light on "better biofilm formers" in the context of higher resistance.

4.2 Retarded growth rate

The following mechanism is not investigated for *L. monocytogenes,* yet worth discussing due to its ostensible simplicity. As a result, in this part other microorganisms e.g. *E. coli*, *Salmonella* spp., *Staphylococcus aureus* than *L. monocytogenes* are used as examples to explain this mechanism. Bacterial cells are most vulnerable in their exponential phase compared to the stationary phase (Salcedo-Sora & Kell, 2020), with molecules like e.g. penicillin killing bacterial cells during cell division (Bigger,

1944). Retarded or ceased growth rate, i.e. an inhibition in core cellular processes which cease growth and bring the cell to a dormant state, seems thus a simple explanation for persistence, yet it proves to be complex (Pontes & Groisman, 2019). Various explanations were found regarding the growth rate of bacteria and persistence. Four theories of slow-growing bacteria are distinguished. The first theory is the natural heterogeneity of bacterial populations. Within a bacterial species, small variations exist between bacterial cells, despite being the same isolate. In bacterial populations, two states, both related to the growth rate, can be found that withstand adverse conditions better than normal fast-growing cells. The first of these "retarded growth" states, is growth arrest, and the second one is a slow growth rate compared to the majority of the cells. Bacterial cells are able to switch between a normal fast-growing rate and the two "retarded growth" states.

Retarded growth states cause a small fraction of the population to survive. After the adverse conditions (e.g. antibiotic removal), the cells can switch back to the fast-growing state and form new populations that are as vulnerable as before (Balaban et al., 2004). The switch between the states can be achieved by various stochastic molecular cycles, an example of this is the buildup and energy-consuming repurposing cycle of protein aggregation through the growth cycles which has been shown to result in different kinetics among individual cells (Salcedo-Sora & Kell, 2020).

In a second theory, a reduced growth rate does not depend on specific genes, or molecules, but is rather due to random errors and damage in the interior part of genetically identical cells that causes failure to replicate. Each time when a cell is damaged and undergoes growth arrest is unique, the same counts for the mechanisms to repair and resuscitate the cell (Kaldalu & Tenson, 2019).

A third theory relies on the existence of specific mechanisms, to decline the growth rate (Goormaghtigh et al., 2018; Harms et al., 2017, 2018; Kaldalu & Tenson, 2019; Pontes & Groisman, 2019; Ramisetty et al., 2016; Salcedo-Sora & Kell, 2020). Toxin-antitoxin (TA) modules were believed to play a key role in bacterial persistence in healthcare. The antitoxin prevents the toxin from inhibiting core cellular processes. In stressful conditions e.g. nutrient starvation, or exposure to sublethal antibiotic concentrations (Harms et al., 2018), the antitoxin is degraded by a Lon protease activated by guanosine (penta-)tetraphosphate and the toxin is set free. By inhibiting some core cellular processes in a subpart

of the population, hence slowing down growth, this subpart of the population is protected from antibiotic treatments (Goormaghtigh et al., 2018; Harms et al., 2017, 2018; Kaldalu & Tenson, 2019; Pontes & Groisman, 2019; Ramisetty et al., 2016). This hypothesis for the medical persistence of pathogens is currently debatable and studies no longer report a link between persistence and TA modules in certain microorganisms e.g. *E. coli* and *S. aureus* (Goormaghtigh et al., 2018; Harms et al., 2017, 2018; Kaldalu & Tenson, 2019; Pontes & Groisman, 2019; Ramisetty et al., 2016).

The fourth theory states that a slow growth rate is a consequence rather than the main goal. This is, for example, seen in a study by Kim et al. (2018) where *Pseudomonas aeruginosa* could withstand high concentrations (1200-1600 mg/L) of benzalkonium chloride (a well-known disinfectant). A whole range of mechanisms was found that caused the increased MIC (minimal inhibitory concentration), among which reduced growth (Kim et al., 2018).

Slow growth, caused in biofilms by i.a. nutrient gradients, aid in the enhanced resistance of cells of biofilms compared to planktonic cultures (Mah & O'Toole, 2001). According to Sutherland (2001) it is also expected that the slow growth of bacteria could potentially lead to enhanced EPS production as it is part of the stress response and is promoted by nutrient-limiting conditions.

4.3 Disinfectant resistance or tolerance

Terminology is key when trying to understand disinfectant resistance/tolerance as persistence strategy. Codex Alimentations, ISO and FAO defined many terms frequently used when discussing disinfectant tolerance (Table 1). Inadequate C&D of equipment can lead to a *L. monocytogenes* foodborne outbreak as was the case in 2019 in the Netherlands (VMT-Food, 2019a, 2019b). Similar, Di Ciccio et al. (2012) found that *L. monocytogenes* contamination of smoked salmon happened mostly via the production environment even when the raw material was contaminated, highlighting the importance of an adequate C&D procedure.

An adequate C&D procedure needs to remove at least the number of cells that grow/attach during the production period on the surface (Carpentier & Cerf, 2011; Møretrø & Langsrud, 2017). If the removal rate is lower than the growth rate, they reside on the surface (Carpentier & Cerf, 2011). Complete removal of bacteria from the surface is impossible (Gibson et al., 1999), bacteria that remain after an

adequate C&D are seen as residential bacteria (Møretrø & Langsrud, 2017). During environmental monitoring, swabbing is often used to detach bacteria. However, this mainly removes bacteria with a lower attachment strength which is only a fraction of the total bacterial load. Cells may also turn into a viable-but-not-culturable (VBNC) state which makes them undetectable with normal culture-based methods (Cerf et al., 2010; Noll et al., 2020; Overney et al., 2017).

A selection of studies, discussing disinfectant resistance/tolerance is given in Table 2. Studies were included if the results were compared against the MRC (minimal recommended concentration), and an answer was given to at least one of the following parameters for biofilms and/or planktonic cultures: increased tolerance determination and its stability, the effect of the presence of organic matter or variability among strains (including differences between persistent and presumed non-persistent strains). The parameters only concern *L. monocytogenes*, not the other microorganisms (if others are used in the studies). Occasionally, entire C&D cycles are evaluated on lab scale e.g. Overney et al. (2017) and Pan et al. (2006). For example, Fagerlund, Heir, et al. (2020) evaluated different C&D *L. monocytogenes* biofilm control strategies towards conventional C&D procedures using alkaline, acidic, or enzymatic cleaning agents. These studies resemble reality more and could be used as a starting point for validation studies for new C&D protocols.

4.3.1 Inadequate cleaning as cause of C&D failure

If the cleaning procedure fails, organic matter remains on the surface with inhibitory substances like proteins, lipids, and carbohydrates interfering with the disinfectant and consequently decreasing the active disinfectant concentration to sublethal levels (Cerf et al., 2010; Martínez-Suárez et al., 2016). Besides the removal of organic matter, cleaning is also used to lower the microbial load on the surface and is for this at least as important as the disinfection step. For example, Luyckx et al. (2014) found a 100-fold reduction in the total aerobic count for the cleaning procedure inside a broiler stable, whereas the disinfection led to a 31-fold decrease in the total aerobic count. Table 2 provides several examples, e.g. Aarnisalo et al. (2007), Pang et al. (2019) and Stoller et al. (2019), of how the presence of organic matter decreases disinfectant efficiency.

4.3.2 Inadequate disinfection as cause of C&D failure

A failed disinfection results in a higher number of surviving cells than wanted/expected. Not considering interference of organic matter due to insufficient cleaning, four major pathways could explain disinfection failure. The first one is related to the disinfectant itself e.g. wrong dosage, wrong product, wrong contact time or wrong temperature. Applying the wrong dosage might result in an insufficient reduction/increased tolerance. Aarnisalo et al. (2007) studied the effectivity of eight disinfectants for 5 minutes on *L. monocytogenes* in suspension tests (both soiled and clean conditions) (Table 2). For Three out of eight disinfectants, slightly increasing the concentration/contact time (still well below the upper limit of the MRC, close to the lower limit of the MRC) was needed to achieve 5-6 log reduction. For one product, a sodium hypochlorite/sodium hydroxide-based product, sufficient reductions could not be achieved near the lower limit of the MRC. Besides the wrong dosage, one might simply use the wrong product. A large offer of cleaning agents and disinfectants is available on the market, so it is important to select products considering the food matrix, the targeted microorganisms and other surrounding conditions (e.g. processing temperature, processing time) and to at least handle it conform to the manufacturer's instructions (González-Fandos et al., 2005). The recommended exposure time can also be provided by manufacturers (Boucher et al., 2021). The contact time is an important parameter in C&D, the longer the contact time, the more bacteria might be eliminated (Griffith, 2016). The contact time needs to be long enough as disinfectants do not work instantaneously and require some time before coming into effect. Insufficient contact time, might be the reason of failing to achieve sufficient reductions (González-Fandos et al., 2012) (Table 2). The temperature might also influence the efficacy of disinfectants. Boucher et al. (2011) noticed a significant influence of temperature mainly present on the efficacy of benzalkonium chloride and a citric-acid based sanitizer. Lower temperatures (4°C) resulted in lower efficacies with an optimum working efficacy at 23°C. Concentration, time and temperature are also three out of four aspects mentioned in the Sinner's circle (5.2 PRP Cleaning and disinfection procedure).

The second reason for disinfection failure when applying the correct dosage is the dilution of the disinfectant e.g. the presence of stagnant water in drains, pipes or cracks/crevices in flooring materials/other parts of the equipment or any other harbourage site (Cerf et al., 2010). When rinsing the equipment with water during the cleaning phase, water might reside in some areas. This causes dilution of the disinfectant and thus the application of insufficient concentrations in these areas, enhancing disinfectant tolerance of bacteria.

A third reason why disinfectant procedures fail is related to the food business itself e.g. execution of the C&D procedures (work methodology). Both cleaning and disinfection might fail due to "time is money". Stopping the production for C&D means losing money due to not producing sellable products. Consequently, the frequency of the C&D might be reduced and the time taken to perform the C&D decreased to minimize downtime. The fact that C&D is often performed during the least appealing work hours (Bland et al., 2021) i.e. night shifts makes that a well-established procedure might fail. Accompanied by this, companies might invoke a third-party company to perform the C&D completely or partially, causing a higher risk for deviations in the execution of the C&D protocol. This deviation might be attributed to the change in staff executing the C&D or the variability in the number of staff members performing the C&D. Further, inconsistent execution might be due to the lack of training for that particular production environment as each company uses different products, protocols, and dosages.

Studies often discuss if *L. monocytogenes*, but also bacteria in general, may become resistant or tolerant towards disinfectants, this is a fourth possibility of disinfection failure. Resistance and tolerance are terms that are submitted to various misinterpretations, especially when talking about disinfectant resistance which causes difficulties in comparing studies (Cerf et al., 2010; Martínez-Suárez et al., 2016). Bland et al. (2021) highlights this difference by comparing various interpretations of "resistance" and "tolerance" found in studies. According to Bland et al. (2021), tolerance should be defined as a decreased susceptibility towards a substance compared to sensitive isolates but with inhibition or inactivation at the MRC. The term resistance is not that straightforward as multiple definitions can be found in the literature (Table 3). Making it even more complicated, two types of resistance can be distinguished, intrinsic and acquired resistance. Intrinsic resistance is comparing the susceptibility of

two species to a substance (Cerf et al., 2010; Meyer, 2006; Meyer & Cookson, 2010). This is often the case with disinfectants where some species/groups are less susceptible to certain concentrations than other species (Iñiguez-Moreno et al., 2017). Acquired resistance means that within one species some strains show a significantly higher tolerance towards a substance at the bactericidal level (=increased MBC) (Cerf et al., 2010; Meyer, 2006; Meyer & Cookson, 2010). For the present review, real disinfectant resistance is defined as bacteria capable to survive, despite the application of the recommended in-use disinfectant concentrations and exposure time provided by the manufacturer which is tested in-house in a surrounding where there is no dilution/inhibition by organic matter in the planktonic state, which fits with the interpretation of the term resistance given by Bland et al. (2021).

Disinfectant resistance/tolerance of *L. monocytogenes* is often tested by analyzing an increased MIC or MBC (Table 2), rather than using recommended in-use concentrations to detect disinfectant resistance as defined here (Carpentier & Cerf, 2011; Meyer, 2006). Contrary to the in-use concentration for antibiotics which is situated near the MIC, the in-use concentration of disinfectants is much higher than the MIC and must at least be equal to the MBC value, preferably higher (Figure 3) (Carpentier & Cerf, 2011; Holah et al., 2002; Kastbjerg & Gram, 2009; Meyer, 2006; Meyer & Cookson, 2010). A (stable) increase in the MIC/MBC might happen due to sublethal exposure to disinfectants caused by e.g. high organic matter content, a hiding place in the harbourage site, dilution, biofilm formation, wrong dosage/use (Table 2) (Aase et al., 2000; Kastjerg & Gram, 2012; Lundén et al., 2003; Riazi & Mathews, 2011).

Overall, no report has been made for foodborne pathogens, among which *L. monocytogenes*, claiming real disinfectant resistance as defined here (Bland et al., 2021). If resistance is claimed then this is attributed to the presence of organic matter, biofilm formation, dilution, wrong dosage/contact time or using the term MIC for defining resistance (González-Fandos et al., 2005; Iñiguez-Moreno et al., 2017, 2018; Stoller et al., 2019).

Considering that no foodborne pathogens, among which *L. monocytogenes*, were found to possess disinfectant resistance as defined here, one may ask if persistent *L. monocytogenes* strains possess a higher tolerance to disinfectants in comparison to presumed non-persistent strains which enables them

to reside easier in food premises. Aase et al. (2000) found that persistent strains possessed more often benzalkonium resistance genes compared to presumed non-persistent strains enabling them to withstand higher concentrations of benzalkonium chloride. In some cases, the initial MIC of persistent strains is higher compared to presumed non-persistent strains. However, when submitting both persistent and presumed non-persistent strains to sublethal disinfectant concentrations, the final obtained MIC value will approximately be the same (Table 2) (Kastbjerg & Gram, 2009; Lundén et al., 2003). The initially higher MIC of persistent strains is most likely due to the already repeated exposure (= preadaptation) to sublethal disinfectant concentrations in the food processing companies (Holah et al., 2002; Kastbjerg & Gram, 2009; Magalhães et al., 2016). Stressful environments (e.g. growth before exposure in a lower pH environment) can also cause enhanced tolerance towards disinfectants (Dhowlaghar et al., 2019). Preadaptation, by benzalkonium chloride, has been shown to increase the survival of cells towards benzalkonium chloride accompanied by an increase in viable-but-non-culturable cells (Noll et al., 2020). Regardless, strains/species show variability in tolerance towards disinfectants (Table 2).

4.3.3 Cross-adaptation in disinfectant tolerance

Disinfectant tolerance studies could be combined with testing the cross-adaptation towards other disinfectants or even antibiotics. This phenomenon is proven to be possible by some (Kampf, 2019; Lundén et al., 2003) but refute by others (Maertens et al., 2019; Riazi & Matthews, 2011; Roedel et al., 2019) indicating that cross-adaptation may depend on for example species/strain, substances tested or even the experimental setup e.g. media, temperature (Aarnisalo et al., 2007; Lundén et al., 2003; Noll et al., 2020; Riazi & Matthews, 2011; Roedel et al., 2019; Yu et al., 2018).

4.3.4 Biofilms and disinfectant tolerance

Biofilms are known for their high tolerance towards mechanical and chemical cleaning (i.e. cleaners and disinfectants) (Cunault et al., 2019). The enhanced resistance to chemical cleaning (=disinfectants) in biofilms is most likely multifactorial. For example, the diffusion coefficient present inside biofilm causes exposure to sublethal disinfectant levels in the deeper layers of the biofilm, hence protecting bacteria (Bridier et al, 2011). Additionally, the presence of the EPS matrix (existing out of organic matter) might decrease the efficacy of disinfectants (Bridier et al, 2011).

Exposure to sublethal disinfectant concentrations might, besides survival, also stimulate biofilm formation. Rodríguez-Melcón et al. (2019) looked at the biofilm formation of *L. monocytogenes* during exposure to benzalkonium chloride concentrations near the MIC (0.5xMIC, 1xMIC, 1.5xMIC) and found enhanced biomass for some strains after exposure to 0.5xMIC benzalkonium chloride, for benzalkonium tolerant strains (Table 2). Rodríguez-Campos (2019) confirmed the stimulated biofilm formation in sublethal levels of benzalkonium chloride, for both persistent strains and sporadic strains (Table 2). However, it is not possible to continuously have this concentration present in the production process when producing.

The efficacy of disinfectants on biofilms is subjected to the same parameters as biofilm formation. Surface material dependency is seen but with contradictory results indicating that the type of disinfectant determines if differences in removal efficiency between surface materials occur. (Aarnisalo et al., 2007; Krysinski et al., 1992; Overney et al., 2017; Pan et al., 2006; Poimenidou et al., 2016). Further, strain variability can be spotted (Table 2) (Luque-Sastre et al., 2018; Poimenidou et al., 2016). Some authors state no difference between persistent and presumed non-persistent strains in disinfectant tolerance in biofilms (Fagerlund et al., 2017; Overney et al., 2017). Rodríguez-Campos et al. (2019) found biofilms formed by persistent strains being more susceptible towards sodium hypochlorite compared to biofilms formed by sporadic strains. Despite this, persistent strains managed to form more biomass before the disinfection treatment, thus even with the reductions from sodium hypochlorite, the OD_{580} value (indicating biomass) was higher for persistent strains (Table 2).

Skowron et al. (2019) noticed a higher tolerance towards disinfectants in biofilms formed under nutrient-limiting conditions compared to nutrient-rich conditions, contrary to Kyoui et al. (2016), who noticed a decreased resistance of biofilms formed with 0.1% glucose compared to 1 or 2% glucose. In the latter study, an enhanced EPS matrix was found with higher glucose concentrations which contributed to the higher resistance. It is thus possible that the presence of certain components in the growth media, such as glucose and sodium chloride, might cause an enhanced or decreased tolerance towards disinfectants which can be partially attributed to changes in the EPS matrix, and biofilm structure. Further, many tests are performed under static conditions, which is less realistic as many water flows exist in food

businesses. The temperature at which the biofilms are formed might also influence their resistance with enhanced tolerances towards disinfectants being found at lower temperatures. This is an important finding as many food business environments have zones that are kept at lower temperatures (Puga et al., 2016a). Puga et al. (2016a) noticed that *L. monocytogenes* biofilms formed at 4°C showed higher tolerance towards chitosan compared to biofilms formed at 20°C. Similarly, Lourenço et al. (2011) noticed higher tolerance towards four different sanitisers (Topax 99, Topax 66, Basochlor DD, Basotop) used in the dairy industry in biofilms formed at 12°C compared to 37°C. Boucher et al. (2021) noticed differences in the MBC for a citric acid-based sanitiser between 4 and 23°C, with lower MBC values at 23°C but no significant influence of temperature for a conventional quaternary ammonium compound (Table 2). On the contrast, Belessi et al. (2011) formed biofilms at 20°C and showed higher tolerance towards 2% peracetic acid compared to biofilms formed at 5°C. Additionally, the influence of temperature can be biofilm age-dependent (Pang et al., 2019). Pang et al. (2019) saw no influence of temperature (4 vs 15°C) in 7-14-day-old biofilms treated with a commercial disinfectant (Whisper™ V, quaternary ammonium compound 200ppm as the active ingredient) but noticed a higher sensitivity in one to four-day-old biofilms at 4°C compared to 15°C (Table 2). These studies indicate that the effect of temperature depends on other factors such as type of disinfectant, age of the biofilm, media, and contact time among other factors. Thus, considering the environment when establishing in-house testing is necessary to assure the most realistic/useful outcome of the verification/validation tests.

Although it is shown that the age of the biofilm might also have a significant influence on its resistance towards disinfection, many studies use immature/young biofilms (max. 72h) to investigate the disinfectant resistance/tolerance (Fagerlund, Heir, et al., 2020; Haubert et al., 2019; Kocot & Olszewska, 2019; Pang et al., 2019; Pan et al., 2006). Ibusquiza et al. (2011) noticed that 11-day-old *L. monocytogenes* biofilms had an enhanced biofilm disinfectant resistance in comparison to 4-day-old biofilms and planktonic cells. A 4-day-old biofilm is already mature, indicating that even when the EPS matrix is fully developed, longer residence times can still attribute to enhanced resistance. Kocot & Olszewska (2019) confirmed the enhanced resistance in more mature biofilms (72h biofilms) compared to more immature/younger biofilms (24h biofilms) but this is not always the case (Ibusquiza et al., 2011;

Pang et al., 2019; Somers & Wong, 2004). The influence of age could depend on additional factors, as in the study of Pang et al. (2019) only an influence of age on the disinfectant tolerance was visible in diluted TSB and not when using salmon broth (Table 2). The overall higher tolerance in mature biofilms might be attributed to the spatial arrangement, formation of a 3-D structure with a thick EPS-matrix and the shielding of dead cells protecting the underlying cells from adverse environmental conditions (Ibusquiza et al., 2011; Kocot & Olszewska, 2019). Due to the possibility that mature biofilms are less susceptible towards disinfectants and the fact that biofilms have sufficient time to reach maturation in between C&D cycles when establishing the C&D procedure, in-house testing must take mature biofilms into account. Factors influencing the effect of the age, e.g. media (Pang et al. (2019), on disinfectant tolerance could be explained by possible differences in biofilm structure.

The presence of other (non-pathogenic) microorganisms in multispecies biofilms might increase the resistance towards disinfectants even further compared to monospecies biofilms (Bridier et al., 2011; Fagerlund et al., 2017; Giaouris et al., 2015; Lourenço et al., 2011; Olszewska & Diez-Gonzalez, 2021; Overney et al., 2017; Pang et al., 2019; Puga et al., 2016b; van der Veen & Abee, 2011). The complexity of multispecies makes it difficult to find an unambiguous explanation. On the one hand, the spatial arrangement, i.e. shielding of *L. monocytogenes* by the other species, offers protection towards the disinfectant concentration. On the other hand, a possible increased EPS matrix, and/or an increased EPS-matrix viscosity hinders the diffusion of the disinfectant (Olszewska & Diez-Gonzalez, 2021). For bacteria in general, an increase in the number/types of enzymes to degrade toxic compounds from the environment might also attribute to the increased tolerance (Bridier et al., 2011; Fagerlund et al., 2017; Olszewska & Diez-Gonzalez, 2021). However, increased protection towards *L. monocytogenes* is not always the case and studies can be found where no difference (Overney et al., 2017) or even a decrease (Pang et al., 2019) in the survival rate of *L. monocytogenes* possible in multispecies biofilms compared to monospecies biofilms when subjected to C&D, using concentrations of disinfectants recommended by the manufacturer (Table 2).

4.3.5 *Efficacy tests in scientific studies and authorities*

Testing the efficacy of disinfectants is in the US regulated by the United States Environmental Protection Agency (EPA), manufacturers must test the efficacy in standardized tests against selected target organisms. In Europe, biocide, among which disinfectants, approval is covered by ECHA (European Chemical Agency) and several tests according to standards (ISO, CEN) need to be performed together with the application for approval form. Modifications to these tests are often done in scientific studies making studies between themselves more difficult to compare (Bland et al., 2021; Martínez-Suárez et al., 2016). A modification is for instance the use of *L. monocytogenes*, a less vulnerable species, in pure culture for testing disinfectant efficacy. This is in contrast with official efficacy testing studies where both vulnerable and less vulnerable microorganisms are used. If a study is being done only with a monoculture (planktonic or in biofilm) of a less vulnerable species, it might be possible that the lowest recommended concentration is insufficient (Table 2), however, this does not conclude that the manufacturer provided an insufficient range/product. Similar, higher inoculum concentrations of pathogens are used than what one finds in real situations. Factors like bacterial overgrowth, production of bacteriocins by accompanying microflora, nutrient depletion, space depletion, and production of organic acids are likely to occur within multispecies biofilms with lower pathogen inoculums. The effect of these factors is thus often missed when using high, unrealistic inoculum concentrations (Giaouris et al., 2015). Further, studies testing disinfectant tolerance, often only test the active compound itself. This might lead to wrong conclusions as it is the combination of multiple chemical compounds that form the efficacy of commercial products rather than one compound on its own (Bland et al., 2021).

Official tests e.g. standards (ISO, CEN) do not incorporate biofilms, despite ECHA (ECHA, 2018) and EPA (EPA, 2022) suggesting biofilms should be incorporated in tests, especially when products claim antibiofilm activity. Biofilms need a higher lower limit of MRC compared to planktonic cells to achieve wanted reductions (Table 2) (Barroso et al., 2019; Cruz & Fletcher, 2012; Fagerlund, Langsrud, et al., 2020; Krysinski et al., 1992; Pang et al., 2019; Poimenidou et al., 2016). Studies done to test disinfectant efficacy on biofilms are thus adaptations with the fundaments laying in official tests (Bland et al., 2021).

In the review of Bland et al. (2021), the lack of standardization is further investigated with considerations for designing disinfectant efficacy studies and pitfalls when comparing studies.

It is important to consider that sanitation/disinfection is not equal to sterilization and that the criteria of sanitiser/disinfectant efficacy are set on a certain reduction that must be achieved, and not a certain maximum cell count that is allowed to remain (Bland et al., 2021). According to the US EPA, disinfectants need to reach a 6-log reduction, for both food-contact surfaces and non-food-contact surfaces. For sanitisers, different criteria hold and a 5-log reduction must be achieved on food contact surfaces and a 3-log reduction within 5 minutes on non-food contact surfaces (Bland et al., 2021). On the European level, ECHA has passed criteria for the approval of disinfectants stating the quantity of the reduction. For bacteria in general in the food production area, this is 4/5 log CFU depending on the contact time (ECHA, 2018). These are, however, the criteria for planktonic cells. Theoretically, this should be the same for biofilms, as biofilms also exist out of cells which can potentially harm the product. Only limited studies testing *L. monocytogenes* biofilm disinfectant resistance reach 5 log CFU/cm^2 reduction and/or see this as a boundary for effectivity (Aryal & Muriana, 2019; Boucher et al., 2021; Fagerlund, Heir, et al., 2020; Kocot & Olszewska, 2019; Overney et al., 2017). This is mainly due to the lack of reaching sufficient cell counts to start with, which must be high enough (at least 5 log CFU/cm^2 + LOD). More often studies testing *L. monocytogenes* biofilm disinfectant resistance reach a maximum of 4 log CFU/cm^2 reduction and see this as effective (Aarnisalo et al., 2007; da Silva Fernandes et al., 2015; Olszewska & Diez-Gonzalez, 2021; Poimenidou et al., 2016; van der Veen & Abee, 2011).

4.4 Desiccation resistance/tolerance

Water is essential for bacteria, in general, to survive, and low water availability affects all biological functions negatively. The minimal amount of water needed for bacteria to grow is reflected in the water activity (a_w). At the minimal a_w required for growth, bacteria do not immediately die, they can survive for prolonged periods and proliferate after rehydration including in food premises (Esbelin et al., 2017). Looking at some well-known examples, linked with dry environments, such as *Cronobacter sakazakii*

569 in powdered infant formula and *Salmonella* spp. in chocolate (Beuchat et al., 2011), it is not unlikely
570 that *L. monocytogenes* might possess the ability to withstand/persist in dry environments (Table 4).

571 The removal of water can be done in two different environments. In osmotic systems, water is removed
572 from cells by the surrounding which contains high amounts of solutes (= low a_w). In matric systems,
573 water is removed by exposure to the atmosphere (= air drying). Desiccation is caused by matric stress
574 and is defined as the removal of a substantial fraction of the bulk water from cells through drying stress
575 which can be done slowly or rapidly (Burgess et al., 2016). Air drying causes an array of damages to
576 cells (e.g. free radicals attacking phospholipids, and DNA) and cells might die immediately when air-
577 dried or remain vital for years (Potts, 1994). Bacteria are tolerant to desiccation when they can undergo
578 nearly absolute dehydration through the air without being killed (Billi & Potts, 2002). To survive a
579 period of desiccation, bacteria need to overcome three phases: an initial dehydration period, a period
580 with low moisture conditions and a rehydration period (Streufert et al., 2021).

581 In the context of persistence, desiccation occurs in periods of ceased production e.g. weekends, holidays,
582 and maintenance, causing a drop in relative humidity. Further, desiccation occurs due to the periodic
583 use of pipes, open surfaces, cutting boards, and partially filled tanks… during production itself or just
584 before C&D (Faille et al., 2018).

585 How well bacteria can adapt to desiccation determines their ability to persist (Esbelin et al., 2017) with
586 the adaptations coming hand in hand with altered gene expression (Burgess et al., 2016). Desiccation
587 tolerance knows many different mechanisms, with some being strain/species-specific such as the
588 formation of spores (Checinska et al., 2015) and others not such as biofilm formation, import/synthesis
589 of osmolytes and extracellular polysaccharides (Burgess et al., 2016; Esbelin et al., 2017; Mensink et
590 al., 2017; Potts, 1994). Comprehensive reviews discussing desiccation tolerance mechanisms in general
591 (Esbelin et al., 2017; Potts, 1994), focusing on one desiccation mechanism specifically (Laskowska &
592 Kuczyńska-Wiśnik, 2020; Mensink et al., 2017), or focusing on desiccation mechanisms specific for
593 foodborne pathogens (Burgess et al., 2016) are available. From these reviews, it can be concluded that
594 not one single mechanism or macromolecule on its own is enough to protect bacteria against desiccation.
595 It is rather the synergistic effect of multiple mechanisms that leads to protection and survival (Esbelin

et al., 2017). The exact mechanisms of desiccation tolerance are not fully understood and further research is necessary for each separate species of interest, the combination of multiple species (Burgess et al., 2016) and each desiccation mechanism separately (Laskowska & Kuczyńska-Wiśnik, 2020; Mensink et al., 2017). Even less clear are the mechanisms determining the rehydration efficiency as rehydration also causes stress to microorganisms which can have detrimental consequences (Potts, 1994).

The desiccation survival of planktonic *L. monocytogenes* is tested by several studies of which an overview is given in Table 3: Variability of definitions given to the term resistance in studies.

DEFINITION RESISTANCE	SOURCE
An isolate is defined as resistant when it is not inhibited by a concentration that would inhibit most of the strains of that particular species.	Martinez-Suárez et al. (2016)
Resistance is used to indicate that the extent of the killing of a microorganism is less than expected at bactericidal concentrations.	Carpentier & cerf (2011)
In the context of disinfectants, the word "resistance" is used for comparative purposes. A strain of a species is said to be more resistant than another one if the time needed to achieve a given number of decimal reductions of suspended cells at a given disinfectant concentration is significantly longer, or if the concentration needed to achieve a given number of decimal reductions for a given exposure time is significantly higher.	Cerf et al. (2010)
Resistant microorganisms could be described as a species or strain that was more able to survive repeated C&D programs such that that species or strain dominated the environmental microbial flora, albeit in low numbers.	Holah et al. (2002)
Bacterial resistance is the capacity of bacteria to withstand the effects of antibiotics or biocides that are intended to kill or control them.	Glossary: Bacterial Resistance (n.d.)

605 Table 2. For this table, only articles were used in which the relative humidity was given. Further, articles
606 needed at least, one of the following parameters investigated: the influence of temperature, the influence
607 of relative humidity, strain variability or the influence of additional microorganisms. The use of different
608 media, temperatures, and surfaces in desiccation tolerance studies indicates a lack of standardized tests,
609 making the comparison of results very complex. Some studies test desiccation tolerance without
610 indicating the relative humidity used in the study which causes difficulties in interpreting and comparing
611 the obtained conclusions (Kuda et al., 2015; Streufert et al., 2021; Takahashi et al., 2011).

612 The presence of food residues can result in higher survival rates of *L. monocytogenes* against desiccation
613 (Hingston et al., 2013; Lim et al., 2020; Takahashi et al., 2011; Vogel et al., 2010) which might be due
614 to the decrease in drying rate due to the presence of solutes in the food matrices or to preadaptation in
615 the salt solutions preceding desiccation (Hingston et al., 2013). The effect of food residues was seen for
616 both biofilm cells and non-biofilm cells. Among others, Lim et al. (2020) and Kuda et al. (2015) noticed
617 a protective effect of small quantities of food residues, both protein-rich as carbohydrate-rich ones, on
618 the survival of several pathogens, among which *L. monocytogenes*, on a stainless steel surface. Vogel et
619 al. (2010) added that food residues might also negatively impact the desiccation tolerance of *L.*
620 *monocytogenes*, e.g. when the food residue contains toxic compounds which are up-concentrated during
621 desiccation (Table 4). Additionally, differences in the impact of food residues on desiccation survival
622 can be influenced by the number of protective compounds available in the food matrix (Vogel et al.,
623 2010).

624 Overall, reduction rates caused by desiccation for *L. monocytogenes* vary between 0 and 2 log CFU/cm^2
625 (Piercey et al., 2017; Takahashi et al., 2011). The tolerance towards desiccation of *L. monocytogenes* is
626 strain, serotype dependent with no differences between persistent and presumed non-persistent strains
627 (Hansen et al., 2011; Vogel et al., 2010; Zoz et al., 2017).

628 In food production environments, temperature differences occur which influence the survival of *L.*
629 *monocytogenes*. Temperature can influence the tolerance but is limited studied for *L. monocytogenes*
630 (Bremer et al., 2001; Jones & Gibson, 2022; Redfern & Verran, 2017). The influence of temperature

631 can be humidity depended, on both biofilm and non-biofilm (drop inoculated) cells (Bremer et al., 2001;
632 Redfern & Verran, 2017). Redfern & Verran (2017) noticed that at lower temperatures, 4/10°C, a higher
633 percentage of attached *L. monocytogenes* cells could be recovered compared to 21°C for different
634 relative humidities (11, 50, 85% RH).

635 The applied relative humidity also affects the survival of desiccated *L. monocytogenes* cells; however,
636 results must be treated carefully when recovery percentages are used instead of log reductions. As an
637 example, Redfern & Verran (2017) studied the effect of the different relative humidities (11, 50, 85%)
638 on the survival of attached *L. monocytogenes*. At a relative humidity of 11%, no cells could be recovered
639 after 1h for all temperatures. At a relative humidity of 50%, a higher percentage of cells could be
640 recovered and even growth was observed at 4°C. At the highest relative humidity, 85%, recovery was
641 significantly less compared to the relative humidity of 50%. However, percentages are not an ideal way
642 to look at reductions and absolute numbers give more information. Indeed at 85% RH, the recovery
643 percentage was lower compared to 50% RH, however, the number of cells at time 0 differed and was
644 higher at 85% RH compared to 50% RH, resulting in higher absolute numbers at 85% RH for time 0, 1,
645 5 and 7 hours at 4 °C and 10°C. Further, as long as recovery percentages could be measured, reductions
646 stayed below 2 logs CFU/cm^2. Only at 11% RH (>1h, all temperatures) and 86% RH (24h at 4°C),
647 recovery was not possible. Despite this, survival depending on the applied relative humidity is a well-
648 proven fact (Alavi & Truelstrup Hansen, 2013; Jones & Gibson, 2022; Zoz et al., 2016) and often the
649 lowest reductions are found at intermediate relative humidities e.g. 43/68% RH and the highest at low
650 relative humidities e.g. 2/11% RH. Another trend that can be seen is the reduction rate, a faster decline
651 is often seen at the lowest relative humidity (Alavi & Truelstrup Hansen, 2013; Zoz et al., 2016).

652 Different temperatures and humidities might occur in production areas indicating that this might
653 influence the desiccation survival very locally. For enclosed spaces such as closed production
654 equipment, Vogel et al. (2010) noticed a difference in desiccation survival of *L. monocytogenes*
655 depending on the location inside a drying oven, this difference was mostly levelled out after prolonged
656 desiccation (e.g. 91 days in the study of Vogel et al. (2010), Table 4). Locations where temperature and

relative humidity are less hostile, e.g. niches, and hard-to-reach places, might thus form an ideal habitat against desiccation tolerance.

To the best of our knowledge, desiccation experiments with *L. monocytogenes* were carried out for a maximum of 91 days (15°C, 2 or 43% RH) (Vogel et al., 2010). Longer survival for *L. monocytogenes* might be possible but is not studied so far. The decrease in viable cell counts depends on the studied time-interval (Alavi & Truelstrup Hansen, 2013; Bremer et al., 2001; Hansen et al., 2011; Hingston et al., 2013; Kim et al., 2019; Kuda et al., 2015; Pang & Yuk, 2019; Piercey et al., 2017; Takahashi et al., 2011; Vogel et al., 2010; Zoz et al., 2016) as dehydration is concomitant with cell death (Zoz et al., 2016) and longer storage periods result in a lower viable cell count. The majority of the cell death is at the start of the desiccation period where the majority of the water evaporation takes place (Alavi & Truelstrup Hansen, 2013; Hansen et al., 2011). At the start, differences in desiccation rate are visible depending on e.g. strain, relative humidity, and temperature (Hansen et al., 2011; Hingston et al., 2013). After this strong decline, the cell count remains fairly stable (Hingston et al., 2013; Vogel et al., 2010) which indicates that if cells survive the start of the desiccation period, survival chances enhance (Carpentier & Cerf, 2011). A Weibull fitting is often used to model the effect of desiccation (Kuda et al., 2015; Vogel et al., 2010).

It was noticed that cells in the exponential, early stationary phase were most prone to desiccation (Kuda et al., 2015). This corresponds to the enhanced resistance seen with retarded growth rate where also a subpart of the population showed enhanced tolerance.

Applying a certain relative humidity might decrease the survival of bacteria and might thus serve as a potential elimination/prevention strategy as Zoz et al. (2016) noticed with consecutive dehydration/ rehydration at 68% RH resulting in >4.5 log CFU/ml decrease of *L. monocytogenes* with several strains tested (Table 4). Similarly, at 43% RH, also higher reductions (± 1 log CFU/ml more) could be achieved with two consecutive dehydration/rehydration cycles compared to one. Accompanied by this, many governments suggest control of relative air humidity as a microbiological control strategy, keeping the execution for the food processing companies themselves as each company is unique (Redfern & Verran,

683 2017). However, maintaining a fixed relative humidity (and/or deliberately wetting and drying the
684 surface) seems not economically nor practically feasible considering small local variations. This aside,
685 air dehumidification processes are often applied after routine C&D procedures (Esbelin et al., 2017;
686 Overney et al., 2017). Applying an additional drying step (3h, 75% RH, 25°C) after daily C&D may
687 reduce the amount of culturable, surviving bacteria from a *L. monocytogenes* biofilm (Overney et al.,
688 2017).

689 The rehydration process itself can also have detrimental effects for *L. monocytogenes*. Zoz et al. (2016)
690 tested the survival of instantaneous rehydration (immersion in phosphate buffer solution) compared to
691 progressive rehydration (99% RH) for *L. monocytogenes* and found higher survival rates with the latter,
692 especially after a drying period of 180 min at 43% RH. No influence of the applied rehydration method
693 was seen after a drying period of 180 min at 68% RH for all strains tested. The exact mechanisms behind
694 rehydration remain unclear.

695 Biofilms are one of the main strategies bacteria use to cope with desiccation. Biofilms enhance the
696 tolerance against desiccation (Hansen et al., 2011; Hingston et al., 2013). The EPS matrix is water-rich
697 and protects the bacteria inside the biofilm against desiccation (Bremer et al., 2001; Burgess et al., 2016;
698 Esbelin et al., 2017). Besides the EPS-matrix, other factors like altered cell metabolism and cell envelope
699 composition could also explain the higher survival rates (Hansen et al., 2011). The desiccation resistance
700 of *L. monocytogenes* in biofilms might also be influenced by the species inhabiting the biofilm. Similar
701 to disinfectant resistance, some species enhance (Alavi & Truelstrup Hansen, 2013; Bremer et al., 2001;
702 Pang & Yuk, 2019) while others decrease the desiccation tolerance (Kim et al., 2019) (Table 4). For
703 example, in the study of Alavi & Truelstrup Hansen (2013) the desiccation tolerance of *L.*
704 *monocytogenes* in duo-species biofilms depends on the desiccation tolerance of the accompanying
705 species where species with a similar desiccation tolerance as *L. monocytogenes* cause lower survival
706 compared to duo-species biofilms composed of *L. monocytogenes* and species with lower desiccation
707 tolerance compared to *L. monocytogenes*. Besides the presence of other species, the order in which the
708 different species colonize the surface can influence the desiccation tolerance as Pang & Yuk (2019)

showed (Table 4). In their study, duo-species biofilms were formed with *L. monocytogenes* and *P. fluorescens*, which were subjected to 43% RH, at 15°C for up to 15 days on stainless steel. The biofilms used were grown for 48h, at 15°C with the second culture added to a preformed monospecies biofilm (48h, 15°C, Tryptic soy broth). The final obtained cell count did not depend on the colonization sequence, however the highest desiccation tolerance for *L. monocytogenes* was seen with *P. fluorescens* as the first colonizer, this enhanced the survival of *L. monocytogenes* with 0.8 logs CFU/cm^2. This colonization sequence also corresponded with a higher EPS production. Besides the higher EPS production, the spatial arrangement further enhanced the survival of *L. monocytogenes*. The pathogen proved to be able to invade pre-existing biofilms of *P. fluorescens* finding shelter and thus more protection, in the deeper layers of the biofilm (Esbelin et al., 2017; Puga et al., 2018).

The EPS production is paired with the maturity of the biofilms, with immature biofilms having rudimentary EPS matrices and mature biofilms with 3D structures existing out of EPS. Hingston et al. (2013) noticed that biofilms need sufficient time at the maximum cell count to develop EPS to show optimal desiccation tolerance. Without EPS, the immature cells showed a similar desiccation tolerance as non-biofilm cells. Once some EPS is formed, biofilm cells were more tolerant towards desiccation and the influence of age ceased.

4.5 Protection by protozoa

Protozoa are ubiquitous present in the soil, air, water, and sediments and tend to be more tolerant towards stressful conditions in comparison with free-living bacteria (Lambrecht et al., 2017; Vaerewijck et al., 2014). Many protozoa have two life cycles: trophozoites and dormant cysts. Trophozoites are the active multiplying, preying form. In adverse environmental conditions, e.g. food shortage, temperature, disinfectants, and desiccation, the trophozoites convert to dormant cysts which offer higher protection against these adverse conditions, when conditions are right again the cysts convert back to trophozoites (Lambrecht et al., 2015). Cysts pose an immense tolerance towards procedures commonly encountered in food processing environments such as heat and chlorine with interstrain, interspecies and experimental setup variability (Gabriel & Panaligan, 2020). An example of this is given by Gabriel & Panaligan (2020) who isolated an *Acanthamoeba* sp. from the soil surrounding a food pilot plant and found it has a D-value of 25.06 min in 200ppm chlorine which is much more resistant compared to *L. monocytogenes* which shows a D-value of 12 seconds in 100ppm hypochlorite (Erkmen, 2004). *Acanthamoeba* sp. showed a D-value of 1.81 min at 91°C which shows its higher heat resistance compared to *L. monocytogenes* where 2 min at 70°C results in a 6 D-reduction (Debevere et al., 2021).

Little research has been done to establish the number and species of non-pathogenic protozoan in food processing facilities. Only limited studies have tried to determine whether typical in-house protozoa exist in food production facilities and if so, what the persistence strategies of these organisms are. Vaerewijck et al. (2008) studied the occurrence of free-living protozoa in meat-cutting plants and found that free-living protozoa are widely spread in the production environment. One possible reason for this is that they seem harmless which makes screening and detection in the context of food safety useless (Vaerewijck et al., 2014). Despite the harmless attitude of the protozoan itself, the locations where Vaerewijck et al. (2008) spotted these organisms were locations such as holes in plastic, the undersides of cutting boards and conveyor belts, upper sides of rails that were inadequately cleaned and disinfected due to ignorance or inaccessibility. This gives the pathogens, ingested by these protozoa, double

750 protection against C&D, one by the protozoa itself and one by being in a harbourage site where C&D is
751 less efficient (Vaerewijck et al., 2008).

752 Various pathogens like *Salmonella* spp., *L. monocytogenes* (Fieseler et al., 2014; Lambrecht et al., 2017;
753 Minh et al., 1990a, 1990b; Zhou et al., 2007), *Legionella* spp., *Yersinia enterolitica* (Lambrecht et al.,
754 2013), pathogenic *E. coli,* and *Campylobacter jejuni* can be protected and/or obtain an increased
755 tolerance by protozoa like *Acanthamoeba castellanii*, *Acanthamoeba polyphaga,* and/or *Tetrahymena*
756 *pyriformis* towards various treatments like hydrogen peroxide, benzalkonium chloride, sodium
757 hypochlorite, NaCl (Lambrecht et al., 2015). More pathogen-protozoa relationships are given in Amaro
758 & Martín-González (2021). Depending on which protozoan species, different survival mechanisms are
759 used by bacteria (Raghu Nadhanan & Thomas, 2014). Studied *L. monocytogenes*-protozoa relationships
760 are given in Table 3. Concerning the *L. monocytogenes – Acanthamoeba* relationship, contradictory
761 results can be found. Older (Minh et al., 1990a, 1990b) studies mention survival after internalization for
762 up to 34 days in *Acanthamoeba* sp. Zhou et al. (2007) suggested possible intracellular survival for at
763 least 72h (Table 5). More recently, Lambrecht et al. (2015, 2017) noticed survival of *L. monocytogenes*
764 in cysts, but not intra-amboebal in trophozoites with the cysts being tolerant towards several disinfectant
765 treatments (70% EtOH, 2.5% sodium hypochlorite, 0.3% H_2O_2 and 10mg/l benzalkonium chloride).
766 Further, bacterial cells grew in the presence of the trophozoites and even possessed an increased
767 tolerance after this cystic passage towards these stressors (Lambrecht et al., 2017) (Table 5). Doyscher
768 et al. (2013) noticed that *L. monocytogenes* forms large aggregates at the uroid (=terminal region of
769 protozoa) of *A. castellanii* or *A. polyphaga* for 10-30 min after which they were lysed and digested. The
770 aggregates were used as transient storage for bacterial cells. Internalization in trophozoites/cysts is not
771 the only way pathogens can be protected. The presence of trophozoites can enhance the tolerance of
772 bacteria against disinfectants without the need for physical contact (Table 5). Metabolic waste and dead
773 protozoa may become nutrients for foodborne pathogens and in this way enhance their survival
774 (Vaerewijck et al., 2014).

775 Similarly, the *L. monocytogenes* - *Tetrahymena* relationship is also known for some contradictions. The
776 review of Schuppler (2014) mentions survival in several studies, in contrast, in the study of
777 Meinersmann et al. (2020) *T. pyriformis* is suggested as a possible prevention/elimination strategy for
778 *L. monocytogenes* by predation. This study lasted only 24h, which is a fairly short period when
779 comparing all protozoa-*L. monocytogenes* studies (Table 5). Further, Meinersmann et al. (2020) showed
780 that attached *L. monocytogenes* were less prone to predation from *T. pyriformis* (10-fold) compared to
781 planktonic bacteria (Table 5).

782 Many studies (Akya et al., 2010; Doyscher et al., 2013; Lambrecht et al., 2015, 2017; Minh et al., 1990a)
783 studying *Acanthamoeba* spp./ *Tetrahymena* spp. – *L. monocytogenes* relationships see a decline of
784 culturable *L. monocytogenes* at the start of the incubation period, but still, end up with viable cells when
785 studying over a longer period which lasts between 96h and 34 days (Table 5). Overall, differences in
786 survival characteristics are seen between studies (Table 5). This can be due to different methods used or
787 due to the possible influence of various parameters such as media, pathogen strain, protozoa strain, time,
788 temperature (Akya et al., 2010), ratio bacteria/protozoa, cysts versus trophozoites (Lambrecht et al.,
789 2015), planktonic or attached bacteria and even motility (Fieseler et al., 2014) (Table 5).

790 *Acanthamoeba* spp. and *Tetrahymena* spp. are the protozoa of choice because they are easy to handle,
791 culturable, cheap, and well-characterized (Vaerewijck et al., 2014). Amongst many others, these
792 protozoans are also often found on vegetables and leafy greens (Gourabathini et al., 2008; Vaerewijck
793 et al., 2011) similar to *L. monocytogenes* (EFSA BIOHAZ panel, 2020). Besides these protozoa, other
794 non-pathogenic species also can be found on/inhabit products/processing environments. Some of these
795 might harbour *L. monocytogenes* and consequently might stimulate survival/offer protection against
796 adverse conditions (Lyashchuk et al., 2021).

797 Besides *Acantamoeba* spp. and *Tetrahymena* spp. one article could be found studying the *Colpoda* sp.
798 (also found in food processing environments) – *L. monocytogenes* relationship. *Colpoda* sp. secreted
799 viable *L. monocytogenes* cells in faecal pellets indicating that *Colpoda* sp. can serve as a protection

800 organism for the pathogen. Further research is necessary to confirm this (Raghu Nadhanan & Thomas,
801 2014).

802 Finally, the studies referred to were all conducted with free-living protozoa, however, protozoa might
803 also occur in natural biofilms giving additional protection for pathogens (Arndt et al., 2003). Studies
804 concerning protozoa and *L. monocytogenes* cohabitating biofilms are not available yet.

5. Intervention strategies

Prevention of *L. monocytogenes* persistence implicates preventing the opportunity of *L. monocytogenes* to reside on/in processing equipment for extended periods. This can be achieved by the cooperation of multiple intervention/prevention strategies already (partially) implemented in food production facilities through European law. The proposed intervention strategies are not restricted to *L. monocytogenes* but can be used to enhance overall hygiene (EFSA BIOHAZ panel, 2020; PROFEL, 2020).

5.1 Intervention strategies embedded in the food hygiene regulation

Food hygiene is defined in the European law Regulation (EC) No. 852/2004 as "the measures and conditions necessary to control hazards and to ensure fitness for human consumption of a foodstuff taking into account its intended use" and covers the whole food chain, from production and processing to distribution and placing on the market (European Commission, 2020).

Food hygiene knows many regulations and directions with Regulation (EC) No. 852/2004, 853/2004, and 2017/625 being the main ones. Based on these regulations several other regulations were constructed to aid in the assurance of food hygiene and safety such as Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. These regulations tend to harmonize, simplify, and merge hygiene requirements to obtain a single, transparent hygiene policy. Across the intervention strategies, some of the corresponding legal frameworks will be mentioned to highlight the already established incorporation of these strategies in European law.

Food safety management systems (FSMS) are the result of the obligation to comply with the general hygiene rules mentioned in Article 4 of Regulation (EC) No. 852/2004, the requirement to establish a permanent procedure based on the hazard analysis critical control point principles (HACCP-principles) mentioned in Article 5 of Regulation (EC) No. 852/2004 and general aspects such as the precaution principle and traceability mentioned in the general food law (Regulation (EC) No. 178/2002. Before HACCP principles can be implemented, the general hygiene needs to be on point. To do this prerequisite programs (PRP) which are basic rules necessary to operate hygienically are established. The number and type of PRPs depend on the sector but overall many of the 12 proposed PRPs in the Commission

notice (2016/C 278/01) are applied. This list with possible PRPs was extended from 12 to 13 by the BIOHAZ panel of EFSA and often traceability is added as a 14th PRP (Figure 4). The PRPs provide safe and hygienic surroundings for the production process and form the minimum level of hygiene required and apply for the entire production facility thus not restricted to one hazard. Out of the 13 PRPs suggested by the BIOHAZ panel of EFSA, cleaning and disinfection, infrastructure (hygienic design), technical maintenance and work methodology are considered crucial to prevent *L. monocytogenes* contamination in frozen vegetables (EFSA BIOHAZ panel, 2020). These four PRPs on their own and in interaction with one another should result in a decrease of *L. monocytogenes* persistence if implemented correctly (Figure 4).

Besides legal requirements, standards provide additional requirements. Complying with certain standards (e.g. BRC, IFS) can be seen as a quality indicator of the company and can be obligatory for some customers. Further, technical standards such as the ISO standards describe criteria to perform a task and include definitions, procedures, and references towards work instructions. These international standards are often translated towards European standards (by the European committee for standardisation –CEN) or American standards (by the American national standards institute -ANSI). European standards can then again be translated to national scale by the normalisation institutes of each country e.g. NBN for Belgium, NEN for the Netherlands, AFNOR for France.

5.2 Guidance on hygiene, control measures and surveillance strategies of *L. monocytogenes* in the food industry

The ongoing problems with *L. monocytogenes* resulted in the establishment of many guides by official agencies and sector organisations. These can be for food products in general such as the guide of the codex Alimentarius on the applications of the general principle of food hygiene to control *L. monocytogenes* in foods (CAC/GL 61-2007). More often, guides are developed for a specific food category, making them straightforward for implementation. Examples of official agencies are the FSIS (Food Safety and Inspection Service, United States) for retail delicatessens (FSIS, 2015), FAO and WHO on RTE-foods (FAO & WHO, 2022) and EFSA for frozen vegetables (EFSA, 2018b). The guides provided by sector organisations are also food category-specific with for example PROFEL (the

858 European Association of Fruit and Vegetable Processors) for quick-frozen vegetables (PROFEL, 2020)
859 and the Innovation Center for US Dairy for dairy products (Innovation Center for US Dairy, 2018).

860 The FAO & WHO mention a summary of the surveillance programs related to *L. monocytogenes* in
861 Australia and New Zealand, Canada, China, Egypt, Latin America (with details for each country),
862 Europe, South Africa and the United States themselves in the meeting report concerning *L.*
863 *monocytogenes* in RTE-foods (FAO & WHO, 2022). Guides on a national scale could thus be an
864 addition to international guides and could include country-specific aspects. The Federal Agency for the
865 Safety of the Food Chain of Belgium (FASFC) gives the option to incorporate the GM4 module in all
866 the (unique for Belgium) self-checking guides available for the food industry. The module is one of four
867 additional modules that can be incorporated into the many approved self-checking guides and is
868 developed by FEVIA (federation of the Belgian food industry) and Ghent University (Belgium). The
869 GM4 module concerns the control of environmental pathogens (no food product category specified),
870 among which *L. monocytogenes*, in the food industry (FASFC, 2019,2023; FEVIA, 2023). Even
871 companies develop guides to control *L. monocytogenes* (Christeyns, 2019). Independently if the guides
872 are on a national or international scale, cover food products in general or a specific food category, the
873 practical implementation of pre-requisite programs and/or sampling strategies to monitor hygiene is
874 included in the scope.

875 ## 5.3 PRP Cleaning and disinfection procedure

876 When the C&D procedure removes the pathogens of interest, persistence is unlikely. In the persistence
877 strategy disinfectant resistance/tolerance, an elaborate part is attributed to the consequences of a failed
878 C&D procedure. The intervention strategy PRP Cleaning and disinfection focuses on the legal context
879 of C&D, how a C&D program should be established, and the necessity of validation/verification.

880 Cleaning and disinfection must comply with some legal requirements. Besides the definitions for C&D,
881 the Codex Alimentarius (CAC/RCP 1-1969) mentions how to store cleaning chemicals, what a cleaning
882 procedure needs to involve and that the cleaning equipment, utensils, and surfaces also need to be
883 cleaned and disinfected where necessary. Rules surrounding the products, more specifically the
884 disinfectants, used in C&D of the food and feed industry are laid down in the biocide regulation (EU)

528/2012. Regulation (EU) No. 528/2012 states that disinfectants and their active components have to undergo an authorization procedure before they can be placed on the market and used. The European chemicals agency (ECHA) handles these authorization procedures (ECHA, n.d.). A whole document on the procedure and testing norms are provided by ECHA to authorize a disinfectant, this procedure contains different phases starting from simple suspension tests to field trials done as specified in international standards (ISO, CEN) (ECHA, 2018). Meyer et al. (2010) confirmed that one of these EN-norms (EN13697:2001, now replaced by EN13697:2015 + A12019) was a realistic worst-case scenario of the food business industry both for the microbial load and for total organic carbon. Not always provide efficacy tests a realistic scenario e.g. biofilms (part 4.3.5).

EHEDG (European Hygienic Engineering & Design Group) established various guidelines and books concerning hygienic design (EHEDG, n.d.). Special rules concerning the use of cleaning and disinfection agents in production and storage areas apply for organic products where the commission is authorised to make a restrictive list on which substances can be used (Regulation (EC) No. 2018/848).

Developing a C&D protocol takes time and information needs to be gathered to come up with an appropriate C&D draft preceding validation (Griffith, 2016; Holah, 2013). When anything changes in the process, equipment or products used, revalidation must be done (EHEDG, 2016). During validation, extra information, e.g. the location of difficult-to-clean places, the influence of the food matrix, and the influence of the environment (e.g. humidity, water hardness) can be noticed and used to optimise the C&D regime (Bland et al., 2021; C. Griffith, 2016).

Designing a C&D procedure is complex and only when all the practical and potential problems are identified, a draft C&D procedure can be made (Griffith, 2016; Holah, 2013). The C&D depends on the characteristics of the site, so a site survey must be done and used to determine an effective C&D regime. With this site survey, aspects like construction, production flow, shift patterns, temperature/humidity patterns and types of food residue are determined (Griffith, 2016; Holah, 2013; Perni et al., 2007).

Further, many parameters influence the applied dosing of the disinfectant and might result in the need to apply higher concentrations than recommended by the manufacturer of the cleaning/disinfection

product (part 4.3.2). Overall, higher processing temperatures and longer running periods before cleaning increase the difficulty of removing the soil (Holah, 2013). Disinfectants may contain several active compounds to obtain the most effective solution for food establishments (Debevere et al., 2021). Once this combination is optimized and passes in-house tests, the disinfectant used can remain the same for decades (Holah et al., 2002). As will be discussed in the intervention strategy infrastructure (part 5.3), the material of the surfaces and the design of the equipment influence the C&D regime strongly (Holah, 2013; Perni et al., 2007).

The removal kinetics consist of different actions each having its contribution depending on the circumstances (Bénézech & Faille, 2017). A C&D procedure is a combination of mechanical energy (= physical removal of soils), chemical energy (= breaking down soils), thermal energy and time (= Sinner's circle). Each piece of equipment (or even equipment parts) may require its specific combination and thus C&D procedure (Bénézech & Faille, 2017; Faille et al., 2018; Holah, 2013). In general, chemical and mechanical energy is increased when applying higher temperatures and/or longer contact times (Faille et al., 2018; Holah, 2013). Mechanical energy can be physical abrasion e.g. brushes and scraping but also fluid abrasion (Faille et al., 2018; Holah, 2013). Fluid abrasion uses water to create shear stress on the surface and can be done by a continuous pressure jet stream or by a pulsating stream which tends to give better lower residual contamination due to higher shear wall stresses (Bénézech & Faille, 2017; Blel et al., 2013; Bode et al., 2007; Gillham et al., 2000). Chemical and mechanical action may play an important role in the removal of biofilms (Cunault et al., 2019). The main goal to remove biofilms is the breakdown of the EPS matrix, which will be mostly achieved by mechanical action (Bridier et al., 2011). Disrupting the EPS matrix, removes the diffusion of the products inside the biofilm and thus enhances the exposure concentration of the cells to the products (Bridier et al., 2011). Bénézech & Faille (2017) investigated the role of the mechanical and chemical action on the CIP removal of *P. fluorescens* biofilms (48h, 20°C). The mechanical action (shear stress 0 to 20 Pa) was responsible for the removal of biofilm chunks and single cells, and the chemical action (0.1 – 0.5 w/w% NaOH) mainly disrupted, and destabilized biofilm clusters. The synergistic effect of both actions gave the highest removal rates. The importance of the chemical action was confirmed by Faille et al. (2013) on *Bacillus*

spores attached to a stainless steel surface and was strain-dependent. Biofilms are considered highly resistant and harsh C&D procedures are needed to remove them sufficiently. Fagerlund, Heir, et al. (2020) looked at the effect of commercial cleaners and enzymes removing *L. monocytogenes* biofilms (seven-day-old biofilms) and noticed that only elaborative C&D protocols with higher cleaning product concentrations were capable of reaching >5.5 logs CFU/cm^2 reductions.

Besides the end-production C&D procedure, several other types of C&D procedures can be done such as in-production C&D, inter-production C&D and site decontamination. The C&D procedure itself is more complex than just applying the product and rinsing. Food residues need to be removed as much as possible and equipment needs to be prepared (e.g. disassembly if needed) before the C&D procedure. The removal of soil and a pre-rinse is followed by a visual control to see if the surface is clean enough to apply the cleaning agent. After the application of the cleaning agent and subsequent rinsing another control is conducted to see if the surface is clean enough to apply the disinfectant. This can be done both visually and with biochemical tests. Drying can be applied after the cleaning phase to avoid dilution of the disinfectant. The disinfectant is rinsed off and another test is done to verify if the surface is adequately cleaned and disinfected (de Reu et al., 2022). Drying the surface after the C&D procedure might not only enhance the effectiveness of the procedure as proven by Overney et al. (2017) (part 4.3.2), but it may also aid in the prevention of cross-contamination as wet surfaces may enhance cross-contamination in comparison to dry surfaces by offering circumstances for microbial growth (Griffith, 2016). The C&D procedure should end with the cleaning and disinfection of the cleaning utensils and loose equipment.

When finishing the C&D program for the equipment, it is important to realize that the environment and equipment inside a certain environment form a whole and thus need to be cleaned at the same time to avoid recontamination after C&D and cross-contamination during production (Holah, 2013). Cleaning utensils, which are a major source of cross-contamination, are rarely considered in food safety. As an example, a UK government-funded study (Holah, 1999) found that 47% of the cleaning utensils were contaminated with *L. monocytogenes* (Smith, 2019). Further, Schäfer et al. (2017) noticed that 87% of the equipment and processing utensils used for thighs in a poultry processing plant were contaminated

with *L. monocytogenes* and that after C&D, this was still 67%. To overcome this, GFSI-approved (Global Food Safety Initiative) standards like BRC (British Retail Consortium) and IFS (International Food Standard) incorporated the cleaning of cleaning utensils in their standards so that food business operators pay attention to them. Cleaning utensils need to be cleaned and disinfected at least once a day, or more if required. When using "sanitiser baths", frequently replacing the fluid is necessary to avoid a decrease in efficiency due to the presence of organic matter (Smith, 2019). To determine the cleaning level, a decision tree like the one suggested by Smith (2019) can be used.

A good C&D involves monitoring (Holah, 2013). Bad cleaning might result in a failed C&D. Monitoring the production area after the cleaning phase is thus crucial. Failure or absence of this might endanger food safety. The same counts for disinfection, after the complete C&D procedure, monitoring based on sampling needs to be conducted to verify the efficiency of the C&D procedure. Visual control, disinfectant temperatures, chemical concentrations… are examples of monitoring measurements (Holah, 2013).

Target (the value that is supposed to be achieved with "best practices") and tolerance (the value that is the maximum value which is reasonably achieved with "best practices", exceeding this value indicates a problem) values need to be established before monitoring and verification can take place, which is often done during validation (Uyttendaele et al., 2018). For microbiology, this means determining the acceptable numbers of microorganisms remaining on surfaces and this depends on the food product, risk area and sanitation procedure undertaken (Holah, 2013). A zero tolerance on microorganisms on the surface after C&D is not realistic, and so is a tolerance value of 0 CFU/cm^2. With disinfection, the aim is to achieve a surface, where the number of microorganisms does not hamper food safety and quality for the desired shelf life. Food producers themselves must determine what they consider appropriate, this must be risk-based with the goal of guaranteed safety and acceptable shelf life (Møretrø & Langsrud, 2017). Despite that no legal standards other than visual cleanliness exist, guidelines may suggest some values but the reasoning behind these values is often unclear. Starting from in-house data, target and threshold values can be set up based on what is achievable (Griffith, 2016; Møretrø & Langsrud, 2017). Studies showed that a mean level of <2.5 CFU/cm^2 after routine C&D is attainable (Bagge-Ravn et al.,

2003; Griffith, 2005, 2016; Griffith et al., 2000; Moore & Griffith, 2007). Higher numbers could indicate an inadequate C&D program of execution thereof.

Both microbiological and non-microbiological tests can be used for validation, verification and monitoring. An example of a non-microbiological test is visual control which is a widely used method to assess cleanliness and can be done with the unaided eye of the microscope (Griffith, 2016). Visual controls are often used to verify if the cleaning procedure is sufficient. This is however a subjective method and the absence of visual soil does not imply a surface free from organic matter (Griffith, 2016). Other non-microbiological tests are ATP bioluminescence readings which pose an objective alternative to test quickly if the surface is adequality cleaned and thus can be disinfected (Holah, 2014; Uyttendaele et al., 2018). By using a non-microbiological test to check if the surface is adequately clean, corrective actions like recleaning the surface can be done which is not possible with microbiological tests due to the incubation period (Griffith, 2016).

5.4 PRP Infrastructure (hygienic design)

Paying attention to hygienic design e.g. when constructing the site, renovating the site and the purchase of (new) equipment, aids in the prevention of persistent bacteria as it may give rise to possible hazards concerning hygiene (Beetz et al., 2017; EHEDG, n.d.; Holah, 2008). Special attention must be paid to second-hand equipment as food particles may harbour pathogenic organisms. Lundén et al. (2002) found the same persistent *L. monocytogenes* strain contaminating three food businesses due to the resale of a dicing machine meaning that contamination was spread from buying second-hand equipment. Poor hygienic design leads to the existence of harbourage sites which may cause food safety/quality problems e.g. dripping condensate from pipes and cooling systems (3M and Cornell University, 2019; EFSA BIOHAZ panel, 2018; FDA - CFSAN, 2017). Hygienic design and engineering are defined according to EHEDG as “the design and engineering of equipment and premises assuring the food is safe and suitable for human consumption” (EHEDG, n.d.). This includes the physical aspects of the equipment but also the design of the building and the surrounding itself, the flow of the raw materials, products, and the people inside the building (Holah, 2008), the design and installation of services such as air, water, steam, the maintenance of the equipment (EHEDG, n.d.). Considering hygienic design before the

actual construction phase of an equipment/processing plant and considering the actual usage may give rise to possible hazards concerning hygiene which can then be immediately solved/prevented (Beetz et al., 2017; EHEDG, n.d.; Holah, 2008).

Construction-wise, zonation can be incorporated to avoid the spreading of contamination from one part of the production area to another. If no zonation is applied, contamination from low-risk areas to high-risk areas can easily be spread by the hands/shoes of the staff, and transport trolleys (Griffith, 2016). Zonation is mentioned in different standards like BRC and GFSI and is required when handling products that are prone to recontamination and require higher hygiene standards and sometimes restricted entrance (BRC, 2018; Holah, 2008).

The hygienic design of equipment may avoid the existence of many harbourage sites, a major contributing factor to persistence (part 3). Equipment must be designed in a way that cleaning or maintenance does not introduce food safety hazards. All parts and components should be easily accessible for actions like inspection, maintenance and troubleshooting. For this, pipes, pumps, and large-volume heating should be located in a technical corridor to avoid disruption of the cleanliness in the production area itself. Maintenance, lubrication and repair should be done according to the principles of hygienic design (Moerman et al., 2013).

Paying attention to a good hygienic design is mandatory nowadays. Codex Alimentarius (CAC/RCP 1-1969) mentions requirements to which equipment and facilities need to comply regarding the design of equipment and facility layout. For the United States, the code of federal regulations (CRF-title 21-part 110) describes some general aspects of food premises. This is on European level Regulation (EC) No 852/2004 with further, legal requirements, like directive 2006/42/EC, to describe several general aspects for the design of the equipment keeping in mind hygiene, cleanability, and safety.

Based on legal requirements, guidelines and standards are being developed to help equipment manufacturers (Beetz et al., 2017). Institutes, like EHEDG, provide a practical interpretation of the standards/laws and provide interaction/cooperation between equipment manufacturers, users and legislators concerning hygienic design (EHEDG, n.d.). The EHEDG is linked to several other

1045 international standardization organizations like 3-A sanitary design (USA), NSF International (National
1046 Sanitation Foundation), ISO, DIN (Deutsches Institut für Normung, Germany) … to make it more
1047 practical and efficient to incorporate hygienic design in the industry (EHEDG, n.d.).

1048 Focusing more on the hygienic design itself, the geometry inside the equipment is an important factor
1049 as it determines the flow characteristics (Faille et al., 2018). Angles, corners, and sudden, or gradual
1050 expansion or contraction may alter the flow pattern severely and as a consequence also affect bacterial
1051 removal (Blel et al., 2007). Several examples of geometric requirements are welds, angels, fasteners,
1052 crevices, and slopes (EHEDG, n.d.) A well-known example of a hygienic design guideline concerning
1053 geometric aspects is avoiding sharp 90-degree angles in the inner edges and rounding them off (Beetz
1054 et al., 2017).

1055 Hygienic design is not only keeping in mind the geometry but also the materials used as this may affect
1056 the adherence of bacteria and the removal of them (Faille et al., 2018). Materials may influence the
1057 adherence of bacteria through their physicochemical properties and topography (Faille et al., 2018).
1058 Textured surfaces may harbour more contamination than smooth surfaces (Chasseignaux et al., 2002)
1059 and decrease the efficiency of C&D as bacteria hide in the valleys (Fagerlund et al., 2017). Solely using
1060 the Ra (average roughness) as a topographic parameter is insufficient to estimate the hygienic status of
1061 a surface and other parameters about the shape of the topographic features need to be included to get a
1062 more accurate estimation (Faille et al., 2018). Besides the microbiological reason, the chosen material
1063 also needs to be resistant to cracking, chipping, flaking and abrasion, be inert to the food product and
1064 disinfectants, corrosion-resistant, non-toxic, non-tainting, and mechanically stable (EHEDG, n.d.).
1065 Adhesives, sealants, lubricants, signal transfer liquids, and thermal insulation materials are also
1066 subjected to (legal) requirements, standards, and norms (EHEDG, n.d.; Moerman et al., 2013). For
1067 Europe, for all food-contact materials, Regulation (EC) No. 1935/2004 is applicable, more specific
1068 regulations for some materials are also available like Directives 78/142/EEC for vinyl chloride
1069 monomers, 84/500/EEC for ceramics, 2004/13/EC for certain epoxy, 93/10/EEC for regenerated
1070 cellulose film and Regulation (EU) No 10/2011 regarding plastics.

Applying hygienic design is not only mandatory, but it also holds benefits other than the reduced risk of contamination. Softer and shorter C&D methods can be applied which means that there is a reduction in the use of (aggressive) chemicals and cleaning and decontamination cycles. As a result, more time comes free for actual production, equipment may have an enhanced lifespan, enhanced sustainability, lower operating cost, and less effluent is being produced (EHEDG, n.d.; Holah, 2013).

5.5 PRP Technical maintenance

Equipment does not have an endless lifespan and parts are susceptible to wear and break thus making preventive maintenance regularly necessary/obliged to keep the equipment running properly and avoid a breakdown during production (CAC/GL 61 – 2007). Preventive maintenance or pre-operational maintenance, in case of infrequently used equipment, is also desired as maintenance during production is discouraged (PROFEL, 2020). During maintenance, the equipment must be checked on physical and microbiological risk factors e.g. created due to wear. Areas where food and moisture can accumulate e.g. parts that are cracked or worn, gaskets or belts, porous welds, product contact surfaces, and worn or frayed hoses pose microbiological risk factors (CAC/GL 61 – 2007). These may have various origins e.g. repeated heating and cooling cycles that may decrease the elasticity of seals (Moerman et al., 2013), junction separation, corrosion and water encroachment in freezing tunnels (EFSA BIOHAZ panel, 2020) and corrosion which increases the surface roughness (Mai et al., 2006). Parts must likely be replaced because they form a microbiological contamination risk despite still functioning and not forming a physical risk (Moerman et al., 2013).

The actions performed during preventive maintenance like dismantling, servicing, reassembling equipment and the personal hygiene of the operative can form a microbiological contamination risk as well e.g. disruption of biofilms (Moerman et al., 2013). Before installing new parts/equipment, the equipment must be physically fine, no parts are damaged or missing and must also be cleaned and disinfected before entering the food production area and after installation before production (Moerman et al., 2013)(CAC/GL 61 – 2007).

Maintenance operators must follow the same hygiene measures as the other staff working in that area when conducting maintenance and may thus vary according to the zone they work in (FDA - CFSAN,

2017; Moerman et al., 2013)(CAC/GL/ 61). Awareness must be created that technical maintenance staff moves from zone to zone and thus poses a higher food safety risk than operators staying in the same area (Moerman et al., 2013). Tools and even uniforms and footwear should be provided for each zone separately or cleaned thoroughly before use in another area (PROFEL, 2020).

5.6 PRP Work methodology

Even when the C&D protocol is effective, it is not guaranteed that it is executed correctly. Griffith (2016) mentions work culture, training of the staff and missing documentation as reasons for failing C&D. Documentation helps to maintain consistency and transparency associated with processes, it is also a requirement of certification standards such as BRC and IFS (Griffith, 2016). Many different types of documents exist such as work instructions, standard operating procedures (SOP), and registration forms. SOPs are needed for every procedure, for the C&D itself but also the monitoring and sampling. SOP documents are needed to make sure everybody knows how a certain process e.g. C&D needs to be done, accompanied by these work instructions (WI) can be carried out. Registration forms offer documented evidence that the approved C&D procedure is applied and will provide clean equipment intended for its use (EHEDG, 2016).

Guidelines may specify certain aspects that must be present in SOPs. For instance, the guideline Cleaning Validation in the Food Industry - General Principles, Part 1 of the EHEDG describes aspects that should be present in the SOPs of C&D procedures (EHEDG, 2016).

Contamination can easily spread throughout one facility for example by moving equipment from one room to another, by products that get contaminated in a particular processing step (Hoelzer et al., 2012), through movable fomites e.g. trolley wheels (Lindsay et al., 2019) or personnel e.g. through contaminated shoes (Lindsay et al., 2019; Morita et al., 2006), contaminated hands due to handling of contaminated product (Chen et al., 2001), gloves (Lindsay et al., 2019; Wu & Ponder, 2018).

Not only written documents to assure good workflow, but the operative personnel must also have had adequate training concerning their task (EHEDG, 2016) and be aware of the consequences when not executing the job well. Protocols on their own will not be successful or correctly implemented without

an appropriate compliance culture (Griffith, 2016). Food safety culture must be present and encouraged in the facilities to obtain the best result (De Boeck, 2018). As an example, neatness is mentioned by PROFEL as important to avoid *L. monocytogenes* in the quick-frozen vegetable industry. Paying attention to neatness can be seen as part of the food safety culture, indicating that the staff knows the importance of a neat working space.

6. Conclusion

Much research has been done concerning the persistence of *L. monocytogenes*, however, information is still lacking. First, defining persistence in each study (what the authors define as persistence) would enhance comparability between studies. Second, a retarded growth rate is here proposed as a possible persistence strategy, however, no data is available yet to support this for *L. monocytogenes*. Likewise, the presence of other protozoa than *Acanthamoeba* spp. and *Tetrahymena* spp. in food production areas and their relationship with *L. monocytogenes* needs to be investigated as many more protozoa could provide shelter. Further, standard tests should be developed for the approval of disinfectants towards biofilms as this is lacking. Additionally, studies with realistic scenarios (including biofilms with in-house flora, using the MRC) are lacking and could provide essential information.

The proposed intervention strategies had all one thing in common, improving the hygiene status of the surroundings as the presence of organic matter proved key e.g. biofilm formation, enhanced tolerance towards desiccation, and disinfectants. Limited interventions could prove big differences. By optimizing the C&D procedure, and the execution thereof in both work methodology and hygienic design, the remaining organic matter decreases. Adding to that, a visual check-up of the infrastructure during technical maintenance to prevent additional harbourage sites and optimizing work instructions should result in the prevention of the persistence of *L. monocytogenes*.

Acknowledgments

This work was supported by the European Regional Development Fund under the Interreg VEG-I-TEC project (Interreg France-Wallonia-Flanders, GoToS3).

Conflicts of Interest

None

References

3M and Cornell University. (2019). *Environmental Monitoring Handbook*.

Aarnisalo, K., Lundén, J., Korkeala, H., & Wirtanen, G. (2007). Susceptibility of *Listeria monocytogenes* strains to disinfectants and chlorinated alkaline cleaners at cold temperatures. *LWT - Food Science and Technology*, *40*(6), 1041–1048. https://doi.org/10.1016/J.LWT.2006.07.009

Aase, B., Sundheim, G., Langsrud, S., & Rørvik, L. M. (2000). Occurrence of and a possible mechanism for resistance to a quaternary ammonium compound in *Listeria monocytogenes*. *International Journal of Food Microbiology*, *62*(1–2), 57–63. https://doi.org/10.1016/S0168-1605(00)00357-3

Akya, A., Pointon, A., & Thomas, C. (2010). Listeria monocytogenes does not survive ingestion by Acanthamoeba polyphaga. *Microbiology*, *156*(3), 809–818. https://doi.org/10.1099/mic.0.031146-0

Alavi, H. E. D., & Truelstrup Hansen, L. (2013). Kinetics of biofilm formation and desiccation survival of *Listeria monocytogenes* in single and dual species biofilms with *Pseudomonas fluorescens*, *Serratia proteamaculans* or *Shewanella baltica* on food-grade stainless steel surfaces. *Biofouling*, *29*(10), 1253–1268. https://doi.org/10.1080/08927014.2013.835805

Amaro, F., & Martín-González, A. (2021). Microbial warfare in the wild-the impact of protists on the evolution and virulence of bacterial pathogens. *International Microbiology : The Official Journal of the Spanish Society for Microbiology*, *24*(4), 559–571. https://doi.org/10.1007/S10123-021-00192-Y

Annous, B. A., Fratamico, P. M., & Smith, J. L. (2009). Quorum sensing in biofilms: Why bacteria behave the way they do. *Journal of Food Science*, *74*(1), 24–37. https://doi.org/10.1111/j.1750-3841.2008.01022.x

1191 Arndt, H., Schmidt-Denter, K., Auer, B., & Weitere, M. (2003). Protozoans and biofilms. *Fossil and*
1192 *Recent Biofilms*, 161–179. https://doi.org/10.1007/978-94-017-0193-8_10

1193 Aryal, M., & Muriana, P. M. (2019). Efficacy of commercial sanitizers used in food processing
1194 facilities for inactivation of *Listeria monocytogenes, E. coli* O157:H7, and *Salmonella* biofilms.
1195 *Foods*, *8*(12), 639. https://doi.org/10.3390/FOODS8120639

1196 Bagge-Ravn, D., Ng, Y., Hjelm, M., Christiansen, J. N., Johansen, C., & Gram, L. (2003). The
1197 microbial ecology of processing equipment in different fish industries - Analysis of the
1198 microflora during processing and following cleaning and disinfection. *International Journal of*
1199 *Food Microbiology*, *87*(3), 239–250. https://doi.org/10.1016/S0168-1605(03)00067-9

1200 Balaban, N. Q., Merrin, J., Chait, R., Kowalik, L., & Leibler, S. (2004). Bacterial persistence as a
1201 phenotypic switch. *Science*, *305*(5690), 1622–1625. https://doi.org/10.1126/SCIENCE.1099390

1202 Barroso, I., Maia, V., Cabrita, P., Martínez-Suárez, J. v., & Brito, L. (2019). The benzalkonium
1203 chloride resistant or sensitive phenotype of *Listeria monocytogenes* planktonic cells did not
1204 dictate the susceptibility of its biofilm counterparts. *Food Research International*, *123*, 373–
1205 382. https://doi.org/10.1016/J.FOODRES.2019.05.008

1206 Beetz, J.-P., Kloberdanz, H., & Kirchner, E. (2017). New ways of hygienic design – A methodical
1207 approach. *21ST INTERNATIONAL CONFERENCE ON ENGINEERING DESIGN, ICED17*.

1208 Belessi, C. E. A., Gounadaki, A. S., Psomas, A. N., & Skandamis, P. N. (2011). Efficiency of different
1209 sanitation methods on *Listeria monocytogenes* biofilms formed under various environmental
1210 conditions. *International Journal of Food Microbiology*, *145*(SUPPL. 1), S46-52.
1211 https://doi.org/10.1016/J.IJFOODMICRO.2010.10.020

1212 Bénézech, T., & Faille, C.-. (2017). Two-phase kinetics of biofilm removal during CIP. Respective
1213 roles of mechanical and chemical effects on the detachment of single cells vs cell clusters from
1214 a. *Journal of Food Engineering*, *219*(February 2018), 121–128.

Beuchat, L., Komitopoulou, E., Betts, R., Beckers, H., Bourdichon, F., Joosten, H., Fanning, S., & B., ter K. (2011). Persistence and survival of pathogens in dry foods and dry food processing environments. *ILSI Europe Report Series. ILSI Europe, Brussels, Belgium*, 1–48. https://www.narcis.nl/publication/RecordID/oai:dare.uva.nl:publications%2F66faf8c0-607b-40ab-a12f-d77019c6a016

Bigger, J. W. (1944). Treatment of Staphylococcal infections with penicillin intermittent sterilisation. *The Lancet*, *244*(6320), 497–500. https://doi.org/10.1016/S0140-6736(00)74210-3

Billi, D., & Potts, M. (2002). Life and death of dried prokaryotes. *Research in Microbiology*, *153*(1), 7–12. www.elsevier.com/locate/resmicMini-review

Bland, R., Brown, S. R. B., Waite-Cusic, J., & Kovacevic, J. (2021). Probing antimicrobial resistance and sanitizer tolerance themes and their implications for the food industry through the *Listeria monocytogenes* lens. *Comprehensive Reviews in Food Science and Food Safety*, *21*(2), 1777–1802. https://doi.org/10.1111/1541-4337.12910

Blel, W., Bénézech, T., Legentilhomme, P., Legrand, J., & Le Gentil-Lelièvre, C. (2007). Effect of flow arrangement on the removal of *Bacillus* spores from stainless steel equipment surfaces during a Cleaning In Place procedure. *Chemical Engineering Science*, *62*(14), 3798–3808. https://doi.org/10.1016/j.ces.2007.04.011

Blel, W., Legentilhomme, P., Bénézech, T., & Fayolle, F. (2013). Cleanability study of a Scraped Surface Heat Exchanger. *Food and Bioproducts Processing*, *91*(2), 95–102. https://doi.org/10.1016/j.fbp.2012.10.002

Bode, K., Hooper, R. J., Paterson, W. R., Wilson, D. I., Augustin, W., & Scholl, S. (2007). Pulsed flow cleaning of whey protein fouling layers. *Heat Transfer Engineering*, *28*(3), 202–209. https://doi.org/10.1080/01457630601064611

1238 Borucki, M. K., Peppin, J. D., White, D., Loge, F., & Call, D. R. (2003). Variation in biofilm
1239 formation among strains of *Listeria monocytogenes*. *Applied and Environmental Microbiology*,
1240 *69*(12), 7336–7342. https://doi.org/10.1128/AEM.69.12.7336-7342.2003

1241 Boucher, C., Waite-Cusic, J., Stone, D., & Kovacevic, J. (2021). Relative performance of commercial
1242 citric acid and quaternary ammonium sanitizers against *Listeria monocytogenes* under
1243 conditions relevant to food industry. *Food Microbiology*, *97*, 103752.
1244 https://doi.org/10.1016/J.FM.2021.103752

1245 BRC. (2018). *BRC Global Standard Food Safety Issue 8*.

1246 Bremer, P. J., Monk, I., & Osborne, C. M. (2001). Survival of *Listeria monocytogenes* attached to
1247 stainless steel surfaces in the presence or absence of *Flavobacterium* spp. *Journal of Food*
1248 *Protection*, *64*(9), 1369–1376.

1249 Bridier, A., Briandet, R., Thomas, V., & Dubois-Brissonnet, F. (2011). Resistance of bacterial
1250 biofilms to disinfectants: A review. *Biofouling*, *27*(9), 1017–1032.
1251 https://doi.org/10.1080/08927014.2011.626899

1252 Burfoot, D. (2005). Aerosols as a contamination risk. In *Handbook of Hygiene Control in the Food*
1253 *Industry* (pp. 93–102). Elsevier Inc. https://doi.org/10.1533/9781845690533.1.93

1254 Burgess, C. M., Gianotti, A., Gruzdev, N., Holah, J., Knøchel, S., Lehner, A., Margas, E., Esser, S. S.,
1255 Sela Saldinger, S., & Tresse, O. (2016). The response of foodborne pathogens to osmotic and
1256 desiccation stresses in the food chain. *International Journal of Food Microbiology*, *221*, 37–53.
1257 https://doi.org/10.1016/j.ijfoodmicro.2015.12.014

1258 Carpentier, B. (2005). Improving the design of floors. In *Handbook of Hygiene Control in the Food*
1259 *Industry* (pp. 168–184). Elsevier Inc. https://doi.org/10.1533/9781845690533.2.168

1260 Carpentier, B. (2009). Biofilms in red meat processing. In *Biofilms in the Food and Beverage*
1261 *Industries* (pp. 375–395). Elsevier Inc. https://doi.org/10.1533/9781845697167.4.375

1262 Carpentier, B., & Cerf, O. (2011). Review - Persistence of *Listeria monocytogenes* in food industry
1263 equipment and premises. *International Journal of Food Microbiology*, *145*(1), 1–8.
1264 https://doi.org/10.1016/j.ijfoodmicro.2011.01.005

1265 Cerf, O., Carpentier, B., & Sanders, P. (2010). Tests for determining in-use concentrations of
1266 antibiotics and disinfectants are based on entirely different concepts: "Resistance" has different
1267 meanings. *International Journal of Food Microbiology*, *136*(3), 247–254.
1268 https://doi.org/10.1016/j.ijfoodmicro.2009.10.002

1269 Chasseignaux, E., Gérault, P., … M. T.-F. M., & 2002, U. (2002). Ecology of *Listeria monocytogenes*
1270 in the environment of raw poultry meat and raw pork meat processing plants. *FEMS*
1271 *Microbiology Letters*, *210*, 271–275.

1272 Checinska, A., Paszczynski, A., & Burbank, M. (2015). *Bacillus* and other spore-forming genera:
1273 variations in responses and mechanisms for survival. *Annual Review of Food Science and*
1274 *Technology*, *6*, 351–369. https://doi.org/10.1146/ANNUREV-FOOD-030713-092332

1275 Chen, Y., Jackson, K. M., Chea, F. P., & Schaffner, D. W. (2001). Quantification and Variability
1276 Analysis of Bacterial Cross-Contamination Rates in Common Food Service Tasks. In *Journal of*
1277 *Food Protection* (Vol. 64, Issue 1).

1278 Chmielewski, R. A. N., & Frank, J. F. (2003). Biofilm formation and control in food processing
1279 facilities. *Comprehensive Reviews in Food Science and Food Safety*, *2*(1), 22–32.
1280 https://doi.org/10.1111/j.1541-4337.2003.tb00012.x

1281 Christeyns. (2019). *Listeria monocytogenes in the meat industry* (1st ed.). BETELGEUX, S.L.,
1282 CHRISTEYNS FOOD HYGIENE.

1283 Colagiorgi, A., Bruini, I., di Ciccio, P. A., Zanardi, E., Ghidini, S., & Ianieri, A. (2017). *Listeria*
1284 *monocytogenes* Biofilms in the wonderland of food industry. *Pathogens*, *6*(3), 41.
1285 https://doi.org/10.3390/pathogens6030041

Costa, A., Bertolotti, L., Brito, L., & Civera, T. (2016). Biofilm formation and disinfectant susceptibility of persistent and Non-persistent *Listeria monocytogenes* isolates from gorgonzola cheese processing plants. *Foodborne Pathogens and Disease*, *13*(11), 602–609. https://doi.org/10.1089/fpd.2016.2154

Costerton, W., Cheng, K. -l., Geesey, G. G., Ladd, T. l., Nickel, C., Dasgupta, M., & Marrie, T. I. (1987). Bacterial biofilms in nature and disease. *Annual. Review of Microbiology*, *41*, 435–464. https://doi.org/10.1146/annurev.mi.41.100187.002251

Cruz, C. D., & Fletcher, G. C. (2012). Assessing manufacturers' recommended concentrations of commercial sanitizers on inactivation of *Listeria monocytogenes*. *Food Control*, *26*(1), 194–199. https://doi.org/10.1016/J.FOODCONT.2012.01.041

Cunault, C., Faille, C., Calabozo-Delgado, A., & Benezech, T. (2019). Structure and resistance to mechanical stress and enzymatic cleaning of *Pseudomonas fluorescens* biofilms formed in fresh-cut ready to eat washing tanks. *Journal of Food Engineering*, *262*, 154–161. https://doi.org/10.1016/j.jfoodeng.2019.06.006

da Silva Fernandes, M., Kabuki, D. Y., & Kuaye, A. Y. (2015). Behavior of *Listeria monocytogenes* in a multi-species biofilm with *Enterococcus faecalis* and *Enterococcus faecium* and control through sanitation procedures. *International Journal of Food Microbiology*, *200*, 5–12. https://doi.org/10.1016/j.ijfoodmicro.2015.01.003

Debevere, J., Devlieghere, F., & Jacxsens, L. (2021). *Levensmiddelenmicrobiologie en -conservering : Frank Devlieghere, Johan Debevere, Liesbeth Jacxsens, Andreja Rajkovic, Mieke Uyttendaele, An Vermeulen*. Brugge : Die Keure, 2021. https://lib.ugent.be/nl/catalog/rug01:003005101?i=3&q=Levensmiddelenmicrobiologie+en+-conservering

De Boeck, E. (2018). Food safety culture and climate, exploring the human factor in food safety management. *Ghent University. Faculty of Bioscience Engineering, Ghent, Belgium*.

de Grandi, A. Z., Pinto, U. M., & Destro, M. T. (2018). Dual-species biofilm of *Listeria monocytogenes* and *Escherichia coli* on stainless steel surface. *World Journal of Microbiology and Biotechnology*, *34*(3), 61. https://doi.org/10.1007/s11274-018-2445-4

de Kievit, T. (2011). Biofilms. In M.-Y. Murray (Ed.), *Comprehensive Biotechnology, Second Edition* (2nd ed., Vol. 1, pp. 547–558). Academic Press. https://doi.org/10.1016/B978-0-08-088504-9.00064-7

de Reu, K., Rasschaert, G., & Lambrecht, E. (2022). Hygiene monitoring and verification of hygiene interventions in food industry. *Twenty-Sixth Conference on Food Microbiology, Pag. 30, October 13-14 , 2022 Brussels, Belgium.*

Dhowlaghar, N., Shen, Q., Nannapaneni, R., Schilling, W., & Samala, A. (2019). Survival of acid stress adapted cells of *Listeria monocytogenes* serotypes 1/2a and 4b in commonly used disinfectants in broth and water models. *LWT*, *109*, 201–206. https://doi.org/10.1016/J.LWT.2019.04.007

Di Ciccio, P., Meloni, D., Festino, A. R., Conter, M., Zanardi, E., Ghidini, S., Vergara, A., Mazzette, R., & Ianieri, A. (2012). Longitudinal study on the sources of *Listeria monocytogenes* contamination in cold-smoked salmon and its processing environment in Italy. *International Journal of Food Microbiology*, *158*(1), 79–84. https://doi.org/10.1016/j.ijfoodmicro.2012.06.016

Djordjevic, D., Wiedmann, M., & Mclandsborough, L. A. (2002). Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. *Applied and Environmental Microbiology*, *68*(6), 2950–2958. https://doi.org/10.1128/AEM.68.6.2950-2958.2002

Donlan, R. M. (2002). Biofilms: Microbial Life on Surfaces. *Emerging Infectious Diseases*, *8*(9), 881–890. http://www.microbelibrary.org/

Doyscher, D., Fieseler, L., Dons, L., Loessner, M. J., & Schuppler, M. (2013). *Acanthamoeba* feature a unique backpacking strategy to trap and feed on *Listeria monocytogenes* and other motile

1336 bacteria. *Environmental Microbiology*, *15*(2), 433–446. https://doi.org/10.1111/j.1462-
1337 2920.2012.02858.x

1338 Dzieciol, M., Schornsteiner, E., Muhterem-Uyar, M., Stessl, B., Wagner, M., & Schmitz-Esser, S.
1339 (2016). Bacterial diversity of floor drain biofilms and drain waters in a *Listeria monocytogenes*
1340 contaminated food processing environment. *International Journal of Food Microbiology*, *223*,
1341 33–40. https://doi.org/10.1016/j.ijfoodmicro.2016.02.004

1342 ECHA. (n.d.). *Authorisation of biocidal products - ECHA*. Retrieved July 10, 2020, from
1343 https://echa.europa.eu/regulations/biocidal-products-regulation/authorisation-of-biocidal-
1344 products

1345 ECHA. (2018). *Guidance on the Biocidal Products Regulation Volume II Efficacy-Assessment and*
1346 *Evaluation (Parts B+C)*. https://doi.org/10.2823/49865

1347 EFSA. (2014). *Listeria EFSA explains zoonotic diseases*. https://doi.org/10.2805/52269

1348 EFSA. (2018a). *Listeria infections increase in vulnerable groups*.
1349 https://www.efsa.europa.eu/en/press/news/180124

1350 EFSA. (2018b). Urgent scientific and technical assistance to provide recommendations for sampling
1351 and testing in the processing plants of frozen vegetables aiming at detecting *Listeria*
1352 *monocytogenes*. *EFSA Supporting Publications*, *15*(7). https://doi.org/10.2903/sp.efsa.2018.en-
1353 1445

1354 EFSA. (2021). The European Union One Health 2020 Zoonoses Report. *EFSA Journal*, *19*(12).
1355 https://doi.org/10.2903/J.EFSA.2021.6971

1356 EFSA BIOHAZ panel. (2017). Hazard analysis approaches for certain small retail establishments in
1357 view of the application of their food safety management systems. *EFSA Panel on Biological*
1358 *Hazards*, *15*(3). https://doi.org/10.2903/j.efsa.2017.4697

1359 EFSA BIOHAZ panel. (2018). SCIENTIFIC OPINION *Listeria monocytogenes* contamination of
1360 ready-to-eat foods and the risk for human health. *EFSA Journal*, *16*(1), 5134.
1361 https://doi.org/10.2903/j.efsa.2018.5134

1362 EFSA BIOHAZ panel. (2020). The public health risk posed by *Listeria monocytogenes* in frozen fruit
1363 and vegetables including herbs, blanched during processing EFSA Panel on Biological Hazards
1364 (Panel members. *EFSA Journal*, *18*(4), 6092. https://doi.org/10.2903/j.efsa.2020.6092

1365 EHEDG. (n.d.). *EHEDG-European Hygienic Engineering and Design Group*. Retrieved September 8,
1366 2020, from https://www.ehedg.org/guidelines/

1367 EHEDG. (2016). *Cleaning Validation in the Food Industry - General Principles, Part 1*.
1368 https://www.ehedg.org/guidelines/

1369 EPA. (n.d.). *What are Antimicrobial Pesticides?* Retrieved May 5, 2022, from
1370 https://www.epa.gov/pesticide-registration/what-are-antimicrobial-pesticides

1371 EPA. (2022, September). *Efficacy Test Methods, Test Criteria, and Labeling Guidance for*
1372 *Antimicrobial Products with Claims Against Biofilm on Hard, Non-Porous Surfaces | US EPA*.
1373 https://www.epa.gov/pesticide-analytical-methods/efficacy-test-methods-test-criteria-and-
1374 labeling-guidance#criteria

1375 Erkmen, O. (2004). Hypochlorite inactivation kinetics of *Listeria monocytogenes* in phosphate buffer.
1376 *Microbiological Research*, *159*(2), 167–171. https://doi.org/10.1016/J.MICRES.2004.03.002

1377 Esbelin, J., Santos, T., & Ebraud, M. H. (2017). Desiccation: An environmental and food industry
1378 stress that bacteria commonly face. *Food Microbiology*, *69*, 82–88.
1379 https://doi.org/10.1016/j.fm.2017.07.017

1380 EU Commission. (2023). *RASFF Portal*. https://webgate.ec.europa.eu/rasff-
1381 window/portal/?event=SearchForm&cleanSearch=1#

1382 European commission. (2020). *Legislation | Food Safety*.
1383 https://ec.europa.eu/food/safety/biosafety/food_hygiene/legislation_en

European Centre for Disease Prevention and Control. ECDC strategic framework for the integration of molecular and genomic typing into European surveillance and multi-country outbreak investigations – 2019–2021. Stockholm: ECDC; 2019.

European Centre for Disease Prevention and Control, ECDC (2020). *European Food- and Waterborne Diseases and Zoonoses Network (FWD-Net)*. https://www.ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/fwd-net

European Centre for Disease Prevention and Control. Eighth external quality assessment scheme for *Listeria monocytogenes* typing. Stockholm: ECDC; 2022.

Fagerlund, A., Heir, E., Møretrø, T., & Langsrud, S. (2020). *Listeria monocytogenes* biofilm removal using different commercial cleaning agents. *Molecules (Basel, Switzerland)*, *25*(4), 792. https://doi.org/10.3390/MOLECULES25040792

Fagerlund, A., Langsrud, S., & Møretrø, T. (2020). In-depth longitudinal study of *Listeria monocytogenes* ST9 isolates from the meat processing industry: Resolving diversity and transmission patterns using whole-genome sequencing. *Applied and Environmental Microbiology*, *86*(14). https://doi.org/10.1128/AEM.00579-20/SUPPL_FILE/AEM.00579-20-S0001.PDF

Fagerlund, A., Langsrud, S., & Møretrø, T. (2021). Microbial diversity and ecology of biofilms in food industry environments associated with *Listeria monocytogenes* persistence. *Current Opinion in Food Science*, *37*, 171–178. https://doi.org/10.1016/J.COFS.2020.10.015

Fagerlund, A., Møretrø, T., Heir, E., Briandet, R., & Langsruda, S. (2017). Cleaning and disinfection of biofilms composed of *Listeria monocytogenes* and background microbiota from meat processing surfaces. *Applied and Environmental Microbiology*, *83*(17). https://doi.org/10.1128/AEM.01046-17

Faille, C., Bénézech, T., Blel, W., Ronse, A., Ronse, G., Clarisse, M., & Slomianny, C. (2013). Role of mechanical vs. chemical action in the removal of adherent Bacillus spores during CIP procedures. *Food Microbiology*, *33*(2), 149–157. https://doi.org/10.1016/j.fm.2012.09.010

Faille, C., Cunault, C., Dubois, T., & Bénézech, T. (2018). Hygienic design of food processing lines to mitigate the risk of bacterial food contamination with respect to environmental concerns. *Innovative Food Science and Emerging Technologies*, *46*, 65–73. https://doi.org/10.1016/j.ifset.2017.10.002

FAO, & WHO. (2004). *Risk assessment of Listeria monocytogenes in ready-to-eat foods*. http://www.fao.org/es/esn

FAO, & WHO. (2019). *Joint FAO/WHO expert meeting in collaboration with OIE on foodborne antimicrobial resistance: role of the environment, crops and biocides: meeting report*. https://www.who.int/publications/i/item/9789241516907

FAO, & WHO. (2022). *Listeria monocytogenes in ready-to-eat (RTE) food: attribution, characterization and monitoring: meeting report*. https://www.who.int/publications/i/item/9789240034969

FASFC. (2019). *Self-Checking system*. https://www.fasfc.be/control-system/self-checking-system

FASFC. (2023). *Autocontrole - Autocontrôle*. https://www.favv-afsca.be/comites-nl/raadgevend/verslagenvergaderingen/_documents/B3_NL-FR_Presentatie.pdf

FDA - CFSAN. (2017). *Control of Listeria monocytogenes in Ready-To-Eat Foods: Guidance for Industry Draft Guidance*.

FEVIA, & Ghent university. (2023). *Module GM4 Beheersing van omgevingspathogenen in voedingsindustrie-Aanvullende module bij de autocontrolegidsen-Fevia-UGent 2023*.

Fieseler, L., Doyscher, D., Loessner, M. J., & Schuppler, M. (2014). *Acanthamoeba* release compounds which promote growth of *Listeria monocytogenes* and other bacteria. *Applied*

Microbiology and Biotechnology, *98*(7), 3091–3097. https://doi.org/10.1007/S00253-014-5534-9

FSIS. (2015). *Best Practices Guidance for Controlling Listeria monocytogenes (Lm) in Retail Delicatessens*.

Gabriel, A. A., & Panaligan, D. C. (2020). Heat and chlorine resistance of a soil *Acanthamoeba sp.* cysts in water. *Journal of Applied Microbiology*, *129*(2), 453–464. https://doi.org/10.1111/JAM.14600

Gao, Z., Daliri, E. B. M., Wang, J. U. N., Liu, D., Chen, S., Ye, X., & Ding, T. (2019). Inhibitory Effect of Lactic Acid Bacteria on Foodborne Pathogens: A Review. *Journal of Food Protection*, *82*(3), 441–453. https://doi.org/10.4315/0362-028X.JFP-18-303

Giaouris, E., Heir, E., Desvaux, M., Hébraud, M., Møretrø, T., Langsrud, S., Doulgeraki, A., Nychas, G. J., Kacániová, M., Czaczyk, K., Ölmez, H., & Simões, M. (2015). Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. In *Frontiers in Microbiology* (Vol. 6, Issue JUL, p. ART 841). Frontiers Research Foundation. https://doi.org/10.3389/fmicb.2015.00841

Gibson, H., Taylor, J. H., Hall, K. E., & Holah, J. T. (1999). Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. *Journal of Applied Microbiology*, *87*(1), 41–48. https://doi.org/10.1046/j.1365-2672.1999.00790.x

Gillham, C. R., Fryer, P. J., Hasting, A. P. M., & Wilson, D. I. (2000). Enhanced cleaning of whey protein soils using pulsed flows. *Journal of Food Engineering*, *46*(3), 199–209. https://doi.org/10.1016/S0260-8774(00)00083-2

Glossary: Bacterial resistance. (n.d.). Retrieved November 12, 2020, from https://ec.europa.eu/health/scientific_committees/opinions_layman/en/biocides-antibiotic-resistance/glossary/abc/bacterial-resistance.htm

González-Fandos, E., Sanz, J., García-Fernández, M. C., & García-Arias, M. T. (2005). Effectiveness of disinfectants used in the food industry on microorganisms of sanitary interest. *Acta Alimentaria*, *34*(3), 253–258. https://doi.org/10.1556/AALIM.34.2005.3.7

Goormaghtigh, F., Fraikin, N., Putrinš, M., Hallaert, T., Hauryliuk, V., Garcia-Pino, A., Sjödin, A., Kasvandik, S., Udekwu, K., Tenson, T., Kaldalu, N., & van Melderen, L. (2018). Reassessing the role of type II toxin-antitoxin systems in formation of *Escherichia coli* type II persister cells. *MBio*, *9*(3), 1–14. https://doi.org/10.1128/mBio.00640-18

Gourabathini, P., Brandl, M. T., Redding, K. S., Gunderson, J. H., & Berk, S. G. (2008). Interactions between food-borne pathogens and protozoa isolated from lettuce and spinach. *American Society for Microbioly*, *74*(8), 2518–2525. https://doi.org/10.1128/AEM.02709-07

Griffith, C. (2005). Improving surface sampling and detection of contamination. In *Handbook of Hygiene Control in the Food Industry* (pp. 588–618). Elsevier Inc. https://doi.org/10.1533/9781845690533.3.588

Griffith, C. (2016). Surface Sampling and the Detection of Contamination. In *Handbook of Hygiene Control in the Food Industry: Second Edition* (pp. 673–696). Elsevier Inc. https://doi.org/10.1016/B978-0-08-100155-4.00044-3

Griffith, C. J., Cooper, R. A., Gilmore, J., Davies, C., & Lewis, M. (2000). An evaluation of hospital cleaning regimes and standards. *Journal of Hospital Infection*, *45*(1), 19–28. https://doi.org/10.1053/jhin.1999.0717

Hansen, T., Vogel, L., & Fonnesbech, B. (2011). Desiccation of adhering and biofilm *Listeria monocytogenes* on stainless steel: Survival and transfer to salmon products. *International Journal of Food Microbiology*, *146*(1), 88–93. https://doi.org/10.1016/j.ijfoodmicro.2011.01.032

1478 Harms, A., Brodersen, D. E., Mitarai, N., & Gerdes, K. (2018). Toxins, Targets, and Triggers: An
1479 Overview of Toxin-Antitoxin Biology. *Molecular Cell*, *70*(5), 768–784.
1480 https://doi.org/10.1016/J.MOLCEL.2018.01.003

1481 Harms, A., Fino, C., Sørensen, M. A., Semsey, S., & Gerdes, K. (2017). Prophages and growth
1482 dynamics confound experimental results with antibiotic-tolerant persister cells. *MBio*, *8*(6), 1–
1483 18. https://doi.org/10.1128/mBio.01964-17

1484 Harvey, J., & Keenan, K. (2007). Assessing biofilm formation by *Listeria monocytogenes* strains.
1485 *Food Microbiology*, *24*(4), 380–392.
1486 https://www.sciencedirect.com/science/article/pii/S0740002006001365

1487 Haubert, L., Zehetmeyr, M. L., & da Silva, W. P. (2019). Resistance to benzalkonium chloride and
1488 cadmium chloride in *Listeria monocytogenes* isolates from food and food-processing
1489 environments in southern Brazil. *Canadian Journal of Microbiology*, *65*(6), 429–435.
1490 https://doi.org/10.1139/CJM-2018-0618

1491 Hingston, P. A., Stea, E. C., Knøchel, S., & Hansen, T. (2013). Role of initial contamination levels,
1492 biofilm maturity and presence of salt and fat on desiccation survival of *Listeria monocytogenes*
1493 on stainless steel surfaces. *Food Microbiology*, *36*(1), 46–56.
1494 https://doi.org/10.1016/J.FM.2013.04.011

1495 Hoelzer, K., Pouillot, R., Gallagher, D., Silverman, M. B., Kause, J., & Dennis, S. (2012). Estimation
1496 of *Listeria monocytogenes* transfer coefficients and efficacy of bacterial removal through
1497 cleaning and sanitation. *International Journal of Food Microbiology*, *157*(2), 267–277.
1498 https://doi.org/10.1016/j.ijfoodmicro.2012.05.019

1499 Holah, J. (1995). *Airborne microorganism levels in food processing environments*.

1500 Holah, J. (1999). *Effective microbiological sampling of food processing environments*.

Holah, J. T. (2008). The hygienic design of chilled food plants and equipment. In *Chilled Foods: A Comprehensive Guide: Third Edition* (pp. 262–303). Elsevier Ltd. https://doi.org/10.1533/9781845694883.2.262

Holah, J. T. (2013). Cleaning and disinfection practices in food processing. In *Hygiene in Food Processing: Principles and Practice: Second Edition* (pp. 259–304). Elsevier Inc. https://doi.org/10.1533/9780857098634.3.259

Holah, J. T. (2014). Microbiological environmental sampling, records and record interpretation. In *Hygiene in Food Processing: Principles and Practice: Second Edition* (pp. 539–576). Elsevier Inc. https://doi.org/10.1533/9780857098634.3.539

Holah, J. T., Taylor, J. H., Dawson, D. J., & Hall, K. E. (2002). Biocide use in the food industry and the disinfectant resistance of persistent strains of *Listeria monocytogenes* and *Escherichia coli*. *Journal of Applied Microbiology*, *92*(1), 111–120. https://doi.org/10.1046/j.1365-2672.92.5s1.18.x

Hossain, M. I., Mizan, M. F. R., Ashrafudoulla, M., Nahar, S., Joo, H. J., Jahid, I. K., Park, S. H., Kim, K. S., & Ha, S. do. (2020). Inhibitory effects of probiotic potential lactic acid bacteria isolated from kimchi against *Listeria monocytogenes* biofilm on lettuce, stainless-steel surfaces, and MBEC™ biofilm device. *LWT*, *118*, 108864. https://doi.org/10.1016/J.LWT.2019.108864

Ibusquiza, P. S., Herrera, J. J. R., & Cabo, M. L. (2011). Resistance to benzalkonium chloride, peracetic acid and nisin during formation of mature biofilms by *Listeria monocytogenes*. *Food Microbiology*, *28*(3), 418–425. https://doi.org/10.1016/j.fm.2010.09.014

Iñiguez-Moreno, M., Avila-Novoa, M. G., & Gutiérrez-Lomelí, M. (2018). Resistance of pathogenic and spoilage microorganisms to disinfectants in the presence of organic matter and their residual effect on stainless steel and polypropylene. *Journal of Global Antimicrobial Resistance*, *14*, 197–201. https://doi.org/10.1016/J.JGAR.2018.04.010

Iñiguez-Moreno, M., Avila-Novoa, M. G., Iñiguez-Moreno, E., Guerrero-Medina, P. J., & Gutiérrez-Lomelí, M. (2017). Antimicrobial activity of disinfectants commonly used in the food industry in Mexico. *Journal of Global Antimicrobial Resistance*, *10*, 143–147. https://doi.org/10.1016/J.JGAR.2017.05.013

Innovation center for US Dairy. (2018). *Control of Listeria monocytogenes - Guidance for the U.S. Dairy Industry*. https://www.usdairy.com/getmedia/aee7f5c2-b462-4f4f-a99d-870f53cb2ddc/control%20of%20listeria%20monocytogenes%20guidance%20for%20the%20us%20dairy%20industry.pdf.pdf

ISO. (2018). *Sterilization of health care products — Vocabulary of terms used in sterilization and related equipment and process standards (ISO 11139:2018(en))*. https://www.iso.org/obp/ui#iso:std:iso:11139:ed-1:v1:en

Jones, S. L., & Gibson, K. (2022). Temperature, Time, and Type, Oh My! Key Environmental Factors Impacting the Recovery of *Salmonella Typhimurium*, *Listeria monocytogenes*, and Tulane Virus from Surfaces. *Journal of Food Protection*, *85*(8). https://doi.org/10.4315/JFP-22-057

Kaldalu, N., & Tenson, T. (2019). Slow growth causes bacterial persistence. *Science Signaling*, *12*(592), 1167. https://doi.org/10.1126/scisignal.aay1167

Kampf, G. (2019). Antibiotic resistance can be enhanced in Gram-positive species by some biocidal agents used for disinfection. *Antibiotics*, *8*(13), 1–15. https://doi.org/10.3390/antibiotics8010013

Kastbjerg, V. G., & Gram, L. (2009). Model systems allowing quantification of sensitivity to disinfectants and comparison of disinfectant susceptibility of persistent and presumed nonpersistent *Listeria monocytogenes*. *Journal of Applied Microbiology*, *106*(5), 1667–1681. https://doi.org/10.1111/j.1365-2672.2008.04134.x

Kastbjerg, V. G., & Gram, L. (2012). Industrial disinfectants do not select for resistance in *Listeria monocytogenes* following long term exposure. *International Journal of Food Microbiology*, *160*(1), 11–15. https://doi.org/10.1016/j.ijfoodmicro.2012.09.009

1550 Kim, K. Y., & Frank, J. F. (1994). Effect of growth nutrients on attachment of *Listeria monocytogenes*
1551 to stainless steel. *Journal of Food Protection*, *57*(8), 720–724. https://doi.org/10.4315/0362-
1552 028x-57.8.720

1553 Kim, M., Hatt, J. K., Weigand, M. R., Krishnan, R., Pavlostathis, S. G., & Konstantinidis, K. T.
1554 (2018). Genomic and transcriptomic insights into how bacteria withstand high concentrations of
1555 benzalkonium chloride biocides. *Applied and Environmental Microbiology*, *84*(12), 1–15.
1556 https://doi.org/10.1128/AEM.00197-18

1557 Kim, Y., Kim, H., Beuchat, L. R., & Ryu, J. H. (2019). Inhibition of *Listeria monocytogenes* using
1558 biofilms of non-pathogenic soil bacteria (*Streptomyces* spp.) on stainless steel under desiccated
1559 condition. *Food Microbiology*, *79*, 61–65. https://doi.org/10.1016/J.FM.2018.11.007

1560 Kocot, A. M., & Olszewska, M. A. (2019). Interaction and inactivation of *Listeria* and *Lactobacillus*
1561 cells in single and mixed species biofilms exposed to different disinfectants. *Journal of Food*
1562 *Safety*, *39*(6). https://doi.org/10.1111/jfs.12713

1563 Krysinski, E. P., Brown, L. J., & Marchisello, T. J. (1992). Effect of cleaners and sanitizers on *Listeria*
1564 *monocytogenes* attached to product contact surfaces. *Journal of Food Protection*, *55*(4), 246–
1565 251. http://meridian.allenpress.com/jfp/article-pdf/55/4/246/2321831/0362-028x-55_4_246.pdf

1566 Kuda, T., Shibata, G., Takahashi, H., & Kimura, B. (2015). Effect of quantity of food residues on
1567 resistance to desiccation of food-related pathogens adhered to a stainless steel surface. *Food*
1568 *Microbiology*, *46*, 234–238. https://doi.org/10.1016/j.fm.2014.08.014

1569 Kyoui, D., Hirokawa, E., Takahashi, H., Kuda, T., & Kimura, B. (2016). Effect of glucose on *Listeria*
1570 *monocytogenes* biofilm formation, and assessment of the biofilm's sanitation tolerance.
1571 *Http://Dx.Doi.Org/10.1080/08927014.2016.1198953*, *32*(7), 815–826.
1572 https://doi.org/10.1080/08927014.2016.1198953

1573 Lambrecht, E., Baré, J., Chavatte, N., Bert, W., Sabbe, K., & Houf, K. (2015). Protozoan cysts act as a
1574 survival niche and protective shelter for foodborne pathogenic bacteria. *Applied and*
1575 *Environmental Microbiology*, *81*(16), 5604–5612. https://doi.org/10.1128/AEM.01031-15

1576 Lambrecht, E., Baré, J., Sabbe, K., & Houf, K. (2017). Impact of *Acanthamoeba* Cysts on Stress
1577 Resistance of *Salmonella enterica* Serovar Typhimurium, *Yersinia enterocolitica* 4/O:3, *Listeria*
1578 *monocytogenes* 1/2a, and *Escherichia coli* O:26. *Applied Environmental Microbiology*, *83*(14).
1579 https://doi.org/10.1128/AEM.00754-17

1580 Lambrecht, E., Baré, J., Van Damme, I., Bert, W., Sabbe, K., & Houf, K. (2013). Behavior of *Yersinia*
1581 *enterocolitica* in the Presence of the Bacterivorous *Acanthamoeba castellanii*. *Applied and*
1582 *Environmental Microbiology*, *79*(20), 6407–6413. https://doi.org/10.1128/AEM.01915-13

1583 Larsen, M., Dalmasso, M., Ingmer, H., Control, S. L.-F., & 2014, U. (2014). Persistence of foodborne
1584 pathogens and their control in primary and secondary food production chains. *Food Control*, *44*,
1585 92–109. https://www.sciencedirect.com/science/article/pii/S0956713514001649

1586 Laskowska, E., & Kuczyńska-Wiśnik, D. (2020). New insight into the mechanisms protecting bacteria
1587 during desiccation. *Current Genetics*, *66*(2), 313–318. https://doi.org/10.1007/S00294-019-
1588 01036-Z

1589 Lee, B. J., Gibson, O. R., Thake, C. D., Tipton, M., Hawley, J. A., & Cotter, J. D. (2019). Cross
1590 adaptation and cross tolerance in human health and disease. *Frontiers in Physiology*, *10*, 1827.
1591 https://doi.org/10.3389/fphys.2018.01827

1592 Lim, S. M., Lim, E. S., Kim, J. S., Paik, H. D., & Koo, O. K. (2020). Survival of foodborne pathogens
1593 on stainless steel soiled with different food residues. *Food Science and Biotechnology*, *29*(5),
1594 729–737. https://doi.org/10.1007/s10068-019-00705-6

1595 Lindsay, D., Laing, S., Fouhy, K. I., Souhoka, L., Beaven, A. K., Soboleva, T. K., & Malakar, P. K.
1596 (2019). Quantifying the uncertainty of transfer of *Cronobacter* spp. between fomites and floors

and touch points in dairy processing plants. *Food Microbiology*, *84*(December). https://doi.org/10.1016/j.fm.2019.103256

Lourenço, A., Machado, H., & Brito, L. (2011). Biofilms of *Listeria monocytogenes* produced at 12 °C either in pure culture or in co-culture with *Pseudomonas aeruginosa* showed reduced susceptibility to sanitizers. *Journal of Food Science*, *76*(2), M143–M148. https://doi.org/10.1111/J.1750-3841.2010.02011.X

Lundén, J., Autio, T., Markkula, A., Hellström, S., & Korkeala, H. (2003). Adaptive and cross-adaptive responses of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants. *International Journal of Food Microbiology*, *82*(3), 265–272. https://doi.org/10.1016/S0168-1605(02)00312-4

Lundén, J. M., Autio, T. J., & Korkeala, H. J. (2002). Transfer of persistent *Listeria monocytogenes* contamination between food-processing plants associated with a dicing machine. *Journal of Food Protection*, *65*(7), 1129–1133. https://doi.org/10.4315/0362-028X-65.7.1129

Lundén, J. M., Miettinen, M. K., Autio, T. J., & Korkeala, H. J. (2000). Persistent *Listeria monocytogenes* strains show enhanced adherence to food contact surface after short contact times. *Journal of Food Protection*, *63*(9), 1204–1207. https://doi.org/10.4315/0362-028X-63.9.1204

Luque-Sastre, L., Fox, E. M., Jordan, K., & Fanning, S. (2018). A comparative study of the susceptibility of *Listeria* species to sanitizer treatments when grown under planktonic and biofilm conditions. *Journal of Food Protection*, *81*(9), 1481–1490. https://doi.org/10.4315/0362-028X.JFP-17-466

Luyckx, K., Dewulf, J., Van Weyenberg, S., Herman, L., Zoons, J., Vervaet, E., Heyndrickx, M., & De Reu, K. (2014). Comparison of sampling procedures and microbiological and non-microbiological parameters to evaluate cleaning and disinfection in broiler houses. *Poultry Science*, *94*(4), 740–749. https://doi.org/10.3382/ps/pev019

1622 Lyashchuk, Y. O., Novak, A. I., Kostrova, Y. B., Shibarshina, O. Y., Evdokimova, O. v., & Kanina, I.
1623 v. (2021). The study of persistence of microorganisms and parasites in food products. In *IOP*
1624 *Conference Series: Earth and Environmental Science* (Vol. 640, Issue 6, p. 062002). IOP
1625 Publishing. https://doi.org/10.1088/1755-1315/640/6/062002

1626 Maertens, H., De Reu, K., Meyer, E., Van Coillie, E., & Dewulf, J. (2019). Limited association
1627 between disinfectant use and either antibiotic or disinfectant susceptibility of *Escherichia coli* in
1628 both poultry and pig husbandry. *BMC Veterinary Research*, *15*(1), 310.
1629 https://doi.org/10.1186/s12917-019-2044-0

1630 Magalhães, R., Ferreira, V., Biscottini, G., Brandão, T. R. S., Almeida, G., & Teixeira, P. (2017).
1631 Biofilm formation by persistent and non-persistent *Listeria monocytogenes* strains on abiotic
1632 surfaces. *Acta Alimentaria*, *46*(1), 43–50. https://doi.org/10.1556/066.2017.46.1.6

1633 Magalhães, R., Ferreira, V., Brandão, T. R. S., Palencia, R. C., Almeida, G., & Teixeira, P. (2016).
1634 Persistent and non-persistent strains of *Listeria monocytogenes*: A focus on growth kinetics
1635 under different temperature, salt, and pH conditions and their sensitivity to sanitizers. *Food*
1636 *Microbiology*, *57*, 103–108. https://doi.org/10.1016/j.fm.2016.02.005

1637 Mah, T. F. C., & O'Toole, G. A. (2001). Mechanisms of biofilm resistance to antimicrobial agents.
1638 *Trends in Microbiology*, *9*(1), 34–39. https://doi.org/10.1016/S0966-842X(00)01913-2

1639 Mai, T. L., Sofyan, N. I., Fergus, J. W., Gale, W. F., & Conner, D. E. (2006). Attachment of *Listeria*
1640 *monocytogenes* to an Austenitic Stainless Steel after Welding and Accelerated Corrosion
1641 Treatments. In *Journal of Food Protection* (Vol. 69, Issue 7).

1642 Martínez-Suárez, J. v., Ortiz, S., & López-Alonso, V. (2016). Potential impact of the resistance to
1643 quaternary ammonium disinfectants on the persistence of *Listeria monocytogenes* in food
1644 processing environments. *Frontiers in Microbiology*, *7*, 638.
1645 https://doi.org/10.3389/fmicb.2016.00638

1646 Meinersmann, R. J., Berrang, M. E., & Rigsby, L. L. (2020). Recoverability of *Listeria*
1647 *monocytogenes* after coculture with *Tetrahymena pyriformis*. *Journal of Food Safety*, *40*(3).
1648 https://doi.org/10.1111/JFS.12778

1649 Mensink, M. A., Frijlink, H. W., van der Voort Maarschalk, K., & Hinrichs, W. L. J. (2017). How
1650 sugars protect proteins in the solid state and during drying (review): Mechanisms of
1651 stabilization in relation to stress conditions. In *European Journal of Pharmaceutics and*
1652 *Biopharmaceutics* (Vol. 114, pp. 288–295). Elsevier B.V.
1653 https://doi.org/10.1016/j.ejpb.2017.01.024

1654 Meyer, B. (2006). Does microbial resistance to biocides create a hazard to food hygiene? *International*
1655 *Journal of Food Microbiology*, *112*(3), 275–279.
1656 https://doi.org/10.1016/j.ijfoodmicro.2006.04.012

1657 Meyer, B., & Cookson, B. (2010). Does microbial resistance or adaptation to biocides create a hazard
1658 in infection prevention and control? In *Journal of Hospital Infection* (Vol. 76, Issue 3, pp. 200–
1659 205). J Hosp Infect. https://doi.org/10.1016/j.jhin.2010.05.020

1660 Meyer, B., Morin, V. N., Rödger, H.-J., Holah, J., & Bird, C. (2010). Do European Standard
1661 Disinfectant tests truly simulate in-use microbial and organic soiling conditions on food
1662 preparation surfaces? *Journal of Applied Microbiology*, *108*(4), 1344–1351.
1663 https://doi.org/10.1111/j.1365-2672.2009.04530.x

1664 Minh, T., Ly, C., & Muller, H. E. (1990a). Ingested *Listeria monocytogenes* survive and multiply in
1665 protozoa. *Journal of Medical Microbiology*, *33*(1), 51–54. https://doi.org/10.00

1666 Minh, T., Ly, C., & Muller, H. E. (1990b). Interactions of *Listeria monocytogenes*, *Listeria seelegri*,
1667 and *Listeria innocua* with protozoans. *Journal of General and Applied Microbiology*, *36*(3),
1668 143–150.

1669 Moerman, F., Holah, J. T., & Steenaard, P. (2013). Hygienic practices for equipment maintenance. In
1670 *Hygiene in Food Processing: Principles and Practice: Second Edition* (pp. 384–407). Elsevier
1671 Inc. https://doi.org/10.1533/9780857098634.3.384

1672 Moore, G., & Griffith, C. (2007). Problems associated with traditional hygiene swabbing: The need for
1673 in-house standardization. *Journal of Applied Microbiology*, *103*(4), 1090–1103.
1674 https://doi.org/10.1111/j.1365-2672.2007.03330.x

1675 Møretrø, T., & Langsrud, S. (2004). *Listeria monocytogenes* : biofilm formation and persistence in
1676 food-processing environments. *Biofilms*, *1*(2), 107–121.
1677 https://doi.org/10.1017/s1479050504001322

1678 Møretrø, T., & Langsrud, S. (2017). Residential bacteria on surfaces in the food industry and their
1679 implications for food safety and quality. *Comprehensive Reviews in Food Science and Food*
1680 *Safety*, *16*(5), 1022–1041. https://doi.org/10.1111/1541-4337.12283

1681 Morita, T., Kitazawa, H., Iida, T., & Kamata, S. (2006). Prevention of *Salmonella* cross-contamination
1682 in an oilmeal manufacturing plant. *Journal of Applied Microbiology*, *101*(2), 464–473.
1683 https://doi.org/10.1111/j.1365-2672.2006.02972.x

1684 Nilsson, R. E., Ross, T., & Bowman, J. P. (2011). Variability in biofilm production by *Listeria*
1685 *monocytogenes* correlated to strain origin and growth conditions. *International Journal of Food*
1686 *Microbiology*, *150*(1), 14–24. https://doi.org/10.1016/j.ijfoodmicro.2011.07.012

1687 Noll, M., Trunzer, K., Vondran, A., Vincze, S., Dieckmann, R., al Dahouk, S., & Gold, C. (2020).
1688 Benzalkonium chloride induces a VBNC state in *Listeria monocytogenes*. *Microorganisms*,
1689 *8*(2), 184. https://doi.org/10.3390/MICROORGANISMS8020184

1690 Norwood, D. E., & Gilmour, A. (2001). The differential adherence capabilities of two *Listeria*
1691 *monocytogenes* strains in monoculture and multispecies biofilms as a function of temperature.
1692 *Letters in Applied Microbiology*, *33*(4), 320–324. https://doi.org/10.1046/j.1472-
1693 765X.2001.01004.x

Ochiai, Y., Yamada, F., Mochizuki, M., Takano, T., Hondo, R., & Ueda, F. (2014). Biofilm formation under different temperature conditions by a single genotype of persistent *Listeria monocytogenes* strains. *Journal of Food Protection*, *77*(1), 133–140. https://doi.org/10.4315/0362-028X.JFP-13-074

Olszewska, M. A., & Diez-Gonzalez, F. (2021). Characterization of binary biofilms of *Listeria monocytogenes* and *Lactobacillus* and their response to chlorine treatment. *Frontiers in Microbiology*, *12*, 638933. https://doi.org/10.3389/FMICB.2021.638933

Overney, A., Jacques-André-Coquin, J., Ng, P., Carpentier, B., Guillier, L., & Firmesse, O. (2017). Impact of environmental factors on the culturability and viability of *Listeria monocytogenes* under conditions encountered in food processing plants. *International Journal of Food Microbiology*, *244*, 74–81. https://doi.org/10.1016/j.ijfoodmicro.2016.12.012

Pang, X., Wong, C., Chung, H. J., & Yuk, H. G. (2019). Biofilm formation of *Listeria monocytogenes* and its resistance to quaternary ammonium compounds in a simulated salmon processing environment. *Food Control*, *98*, 200–208. https://doi.org/10.1016/j.foodcont.2018.11.029

Pang, X., & Yuk, H. G. (2019). Effects of the colonization sequence of *Listeria monocytogenes* and *Pseudomonas fluorescens* on survival of biofilm cells under food-related stresses and transfer to salmon. *Food Microbiology*, *82*, 142–150. https://doi.org/10.1016/j.fm.2019.02.002

Pan, Y., Breidt, F., & Kathariou, S. (2006). Resistance of *Listeria monocytogenes* biofilms to sanitizing agents in a simulated food processing environment. *Applied and Environmental Microbiology*, *72*(12), 7711–7717. https://doi.org/10.1128/AEM.01065-06

Perni, S., Aldsworth, T. G., Jordan, S. J., Fernandes, I., Barbosa, M., Sol, M., Tenreiro, R. P., Chambel, L., Zilhão, I., Barata, B., Adrião, A., Leonor Faleiro, M., Andrew, P. W., & Shama, G. (2007). The resistance to detachment of dairy strains of *Listeria monocytogenes* from stainless steel by shear stress is related to the fluid dynamic characteristics of the location of isolation. *International Journal of Food Microbiology*, *116*(3), 384–390. https://doi.org/10.1016/j.ijfoodmicro.2007.03.002

Piercey, M. J., Ells, T. C., Macintosh, A. J., & Truelstrup Hansen, L. (2017). Variations in biofilm formation, desiccation resistance and Benzalkonium chloride susceptibility among *Listeria monocytogenes* strains isolated in Canada. *International Journal of Food Microbiology*, *257*, 254–261. https://doi.org/10.1016/J.IJFOODMICRO.2017.06.025

Poimenidou, S. v., Chrysadakou, M., Tzakoniati, A., Bikouli, V. C., Nychas, G. J., & Skandamis, P. N. (2016). Variability of *Listeria monocytogenes* strains in biofilm formation on stainless steel and polystyrene materials and resistance to peracetic acid and quaternary ammonium compounds. *International Journal of Food Microbiology*, *237*, 164–171. https://doi.org/10.1016/j.ijfoodmicro.2016.08.029

Pontes, M. H., & Groisman, E. A. (2019). Slow growth determines nonheritable antibiotic resistance in *Salmonella enterica*. *Science Signaling*, *12*(592), eaax3938–eaax3938. https://doi.org/10.1126/scisignal.aax3938

Potts, M. (1994). Desiccation tolerance of prokaryotes. *Microbiological Reviews*, *58*(4), 755–805. https://doi.org/10.1128/MR.58.4.755-805.1994

PROFEL. (2020). *Hygiene guidelines for the control of Listeria monocytogenes in the production of quick-frozen vegetables*.

Puga, C., Dahdouh, E., SanJose, C., & Orgaz, B. (2018). *Listeria monocytogenes* colonizes *Pseudomonas fluorescens* biofilms and induces matrix over-production. *Frontiers in Microbiology*, *9*, 1706. https://doi.org/10.3389/FMICB.2018.01706/BIBTEX

Puga, CH., Orgaz, B., & SanJose, C. (2016). *Listeria monocytogenes* impact on mature or old *Pseudomonas fluorescens* biofilms during growth at 4 and 20°C. *Frontiers in Microbiology*, *7*, 134. https://doi.org/10.3389/fmicb.2016.00134

Puga, C., SanJose, C., & Orgaz, B. (2014). Spatial distribution of *Listeria monocytogenes* and *Pseudomonas fluorescens* in mixed biofilms. In E. C. Hambrick (Ed.), *Listeria monocytogenes: Food sources, prevalence and management strategies* (pp. 115–131). Nova Science Publishers.

https://www.researchgate.net/profile/Carmen_Sanjose/publication/293072072_Spatial_distribution_of_listeria_monocytogenes_and_pseudomonas_fluorescens_in_mixed_biofilms/links/5b0da4e6a6fdcc80995969eb/Spatial-distribution-of-listeria-monocytogenes-and-pseudo

Puga, C., SanJose, C., & Orgaz, B. (2016). Biofilm development at low temperatures enhances *Listeria monocytogenes* resistance to chitosan. *Food Control*, *65*, 143–151. https://doi.org/10.1016/j.foodcont.2016.01.012

Raghu Nadhanan, R., & Thomas, C. J. (2014). Colpoda secrete viable *Listeria monocytogenes* within faecal pellets. *Environmental Microbiology*, *16*(2), 396–404. https://doi.org/10.1111/1462-2920.12230

Ramisetty, B. C. M., Ghosh, D., Chowdhury, M. R., & Santhosh, R. S. (2016). What is the link between stringent response, endoribonuclease encoding type II toxin-antitoxin systems and persistence? *Frontiers in Microbiology*, *7*, 1882. https://doi.org/10.3389/fmicb.2016.01882

Redfern, J., & Verran, J. (2017). Effect of humidity and temperature on the survival of *Listeria monocytogenes* on surfaces. *Letters in Applied Microbiology*, *64*(4), 276–282. https://doi.org/10.1111/LAM.12714

Reij, M. W., & den Aantrekker, E. D. (2004). Recontamination as a source of pathogens in processed foods. In *International Journal of Food Microbiology* (Vol. 91, Issue 1, pp. 1–11). Elsevier. https://doi.org/10.1016/S0168-1605(03)00295-2

Riazi, S., & Matthews, K. R. (2011). Failure of foodborne pathogens to develop resistance to sanitizers following repeated exposure to common sanitizers. *International Biodeterioration and Biodegradation*, *65*(2), 374–378. https://doi.org/10.1016/j.ibiod.2010.12.001

Rodríguez-Campos, D., Rodríguez-Melcón, C., Alonso-Calleja, C., & Capita, R. (2019). Persistent *Listeria monocytogenes* isolates from a poultry-processing facility form more biofilm but do not have a greater resistance to disinfectants than sporadic strains. *Pathogens*, *8*(4). https://doi.org/10.3390/pathogens8040250

1770 Rodríguez-Melcón, C., Capita, R., Rodríguez-Jerez, J. J., Martínez-Suárez, J. v., & Alonso-Calleja, C.
1771 (2019). Effect of low doses of disinfectants on the biofilm-forming ability of *Listeria*
1772 *monocytogenes*. *Foodborne Pathogens and Disease*, *16*(4), 262–268.
1773 https://doi.org/10.1089/fpd.2018.2472

1774 Roedel, A., Dieckmann, R., Brendebach, H., Hammerl, J. A., Kleta, S., Noll, M., al Dahouk, S., &
1775 Vincze, S. (2019). Biocide-tolerant *Listeria monocytogenes* isolates from German food
1776 production plants do not show cross-resistance to clinically relevant antibiotics. *Applied and*
1777 *Environmental Microbiology*, *85*(20), 1–15. https://doi.org/10.1128/AEM.01253-19

1778 Salcedo-Sora, J. E., & Kell, D. B. (2020). A quantitative survey of bacterial persistence in the presence
1779 of antibiotics: towards antipersister antimicrobial discovery. *Antibiotics*, *9*(8), 1–36.
1780 https://doi.org/10.3390/ANTIBIOTICS9080508

1781 Schäfer, D. F., Steffens, J., Barbosa, J., Zeni, J., Paroul, N., Valduga, E., Junges, A., Backes, G. T., &
1782 Cansian, R. L. (2017). Monitoring of contamination sources of *Listeria monocytogenes* in a
1783 poultry slaughterhouse. *LWT*, *86*, 393–398. https://doi.org/10.1016/j.lwt.2017.08.024

1784 Skowron, K., Wałecka-Zacharska, E., Grudlewska, K., Gajewski, P., Wiktorczyk, N., Wietlicka-
1785 Piszcz, M., Dudek, A., Skowron, K. J., & Gospodarek-Komkowska, E. (2019). Disinfectant
1786 susceptibility of biofilm formed by *Listeria monocytogenes* under selected environmental
1787 conditions. *Microorganisms 2019, Vol. 7, Page 280*, *7*(9), 280.
1788 https://doi.org/10.3390/MICROORGANISMS7090280

1789 Smith, D. L. (2019). Global Food Safety Initiative scheme audit requirements regarding cleaning tool
1790 and utensil selection and maintenance – a review. *Quality Assurance and Safety of Crops and*
1791 *Foods*, *11*(7), 603–611. https://doi.org/10.3920/QAS2018.1409

1792 Somers, E. B., & Wong, A. C. L. (2004). Efficacy of two cleaning and sanitizing combinations on
1793 *Listeria monocytogenes* biofilms formed at low temperature on a variety of materials in the
1794 presence of ready-to-eat meat residue. *Journal of Food Protection*, *67*(10), 2218–2229.
1795 http://meridian.allenpress.com/jfp/article-pdf/67/10/2218/1676080/0362-028x-67_10_2218.pdf

1796 Stoller, A., Stevens, M., Stephan, R., & Guldimann, C. (2019). Characteristics of *Listeria*
1797 *monocytogenes* strains persisting in a meat processing facility over a 4-year period. *Pathogens*,
1798 *8*(1), 32. https://doi.org/10.3390/pathogens8010032

1799 Streufert, R. K., Keller, S. E., & Salazar, J. K. (2021). Relationship of growth conditions to desiccation
1800 tolerance of *Salmonella enterica, Escherichia coli*, and *Listeria monocytogenes*. *Journal of*
1801 *Food Protection*, *84*(8), 1380–1384. https://doi.org/10.4315/JFP-21-077

1802 Sutherland, I. W. (2001). Biofilm exopolysaccharides : a strong and sticky framework. *Microbiology*,
1803 *147*, 3–9.

1804 Takahashi, H., Kuramoto, S., Miya, S., & Kimura, B. (2011). Desiccation survival of *Listeria*
1805 *monocytogenes* and other potential foodborne pathogens on stainless steel surfaces is affected
1806 by different food soils. *Food Control*, *22*(3–4), 633–637.
1807 https://doi.org/10.1016/j.foodcont.2010.09.003

1808 Uyttendaele, M., Vermeulen, A., Jacxsens, L., Debevere, J., Devlieghere, F., & de Loy-Hendrickx, A.
1809 (2018). *Microbiological Guidelines*.

1810 Vaerewijck, M. J. M., Baré, J., Lambrecht, E., Sabbe, K., & Houf, K. (2014). Interactions of
1811 foodborne pathogens with free-living protozoa: potential consequences for food safety.
1812 *Comprehensive Reviews in Food Science and Food Safety*, *13*(5), 924–944.
1813 https://doi.org/10.1111/1541-4337.12100

1814 Vaerewijck, M. J. M., Sabbe, K., Baré, J., & Houf, K. (2008). Microscopic and molecular studies of
1815 the diversity of free-living protozoa in meat-cutting plants. *Applied and Environmental*
1816 *Microbiology*, *74*(18), 5741–5749. https://doi.org/10.1128/AEM.00980-08

1817 Vaerewijck, M. J. M., Sabbe, K., Baré, J., & Houf, K. (2011). Occurrence and diversity of free-living
1818 protozoa on butterhead lettuce. *International Journal of Food Microbiology*, *147*(2), 105–111.
1819 https://doi.org/10.1016/J.IJFOODMICRO.2011.03.015

1820 Van Der Linden, I., Cottyn, B., Uyttendaele, M., Vlaemynck, G., Maes, M., & Heyndrickx, M. (2013).
1821 Long-term survival of *Escherichia coli* O157:H7 and *Salmonella enterica* on butterhead lettuce
1822 seeds, and their subsequent survival and growth on the seedlings. *International Journal of Food*
1823 *Microbiology*, *161*(3), 214–219. https://doi.org/10.1016/j.ijfoodmicro.2012.12.015

1824 van der Veen, S., & Abee, T. (2011). Mixed species biofilms of *Listeria monocytogenes* and
1825 *Lactobacillus plantarum* show enhanced resistance to benzalkonium chloride and peracetic acid.
1826 *International Journal of Food Microbiology*, *144*(3), 421–431.
1827 https://doi.org/10.1016/j.ijfoodmicro.2010.10.029

1828 Vasudevan, R. (2014). Biofilms: Microbial Cities of Scientific Significance. *Journal of Microbiology*
1829 *and Experimentation*, *1*(3), 84–98. https://doi.org/10.15406/jmen.2014.01.00014

1830 Verghese, B., Lok, M., Wen, J., Alessandria, V., Chen, Y., Kathariou, S., & Knabel, S. (2011). *comK*
1831 prophage junction fragments as markers for *Listeria monocytogenes* genotypes unique to
1832 individual meat and poultry processing plants and a model for rapid niche-specific adaptation,
1833 biofilm formation, and persistence. *Applied and Environmental Microbiology*, *77*(10), 3279–
1834 3292. https://doi.org/10.1128/AEM.00546-11

1835 VMT-Food. (2019a). *Offerman negeerde advies schoonmakers CSU over schoonmaken fabriek.*

1836 VMT-Food. (2019b). *Vleesfabriek Offerman reinigde machines niet correct*.

1837 Vogel, B. F., Hansen, L. T., Mordhorst, H., & Gram, L. (2010). The survival of *Listeria*
1838 *monocytogenes* during long term desiccation is facilitated by sodium chloride and organic
1839 material. *International Journal of Food Microbiology*, *140*(2–3), 192–200.
1840 https://doi.org/10.1016/j.ijfoodmicro.2010.03.035

1841 Walle, I. van, Björkman, J. T., Cormican, M., Dallman, T., Mossong, J., Moura, A., Pietzka, A.,
1842 Ruppitsch, W., Takkinen, J., Mattheus, W., Christova, I., Maikanti-Charalampous, P.,
1843 Karpíšková, R., Halbedel, S., Nielsen, E. M., Koolmeister, M., Mandilara, G., Torreblanca, R.
1844 A., Salmenlinna, S., … Grant, K. (2018). Retrospective validation of whole genome sequencing

1845 enhanced surveillance of listeriosis in Europe, 2010 to 2015. *Eurosurveillance*, *23*(33), 1–11.
1846 https://doi.org/10.2807/1560-7917.ES.2018.23.33.1700798

1847 Wimpenny, J., Manz, W., & Szewzyk, U. (2000). Heterogeneity in biofilms. *FEMS Microbiology*
1848 *Reviews*, *24*(5), 661–671.

1849 Wu, J., & Ponder, M. A. (2018). Evaluation of transfer rates of *Salmonella* from single-use gloves and
1850 sleeves to dehydrated pork jerky. *Food Control*, *84*, 17–22.
1851 https://doi.org/10.1016/j.foodcont.2017.07.019

1852 Xu, H., Lee, H. Y., & Ahn, J. (2011). Characteristics of biofilm formation by selected foodborne
1853 pathogens. *Journal of Food Safety*, *31*(1), 91–97. https://doi.org/10.1111/j.1745-
1854 4565.2010.00271.x

1855 Xu, Y., Nagy, A., Bauchan, G. R., Xia, X., & Nou, X. (2017). Enhanced biofilm formation in dual-
1856 species culture of *Listeria monocytogenes* and *Ralstonia insidiosa*. *AIMS Microbiology*, *3*(4),
1857 774–783. https://doi.org/10.3934/microbiol.2017.4.774

1858 Yamakawa, T., Tomita, K., & Sawai, J. (2018). Characteristics of Biofilms Formed by Co-Culture of
1859 *Listeria monocytogenes* with *Pseudomonas aeruginosa* at Low Temperatures and Their
1860 Sensitivity to Antibacterial Substances. In *Biocontrol Science* (Vol. 23, Issue 3, p. 107119).

1861 Yu, T., Jiang, X., Zhang, Y., Ji, S., Gao, W., & Shi, L. (2018). Effect of benzalkonium chloride
1862 adaptation on sensitivity to antimicrobial agents and tolerance to environmental stresses in
1863 *Listeria monocytogenes*. *Frontiers in Microbiology*, *9*, 2906.
1864 https://doi.org/10.3389/FMICB.2018.02906/BIBTEX

1865 Zhao, T., Doyle, M. P., & Zhao, P. (2004). Control of *Listeria monocytogenes* in a Biofilm by
1866 Competitive-Exclusion Microorganisms. *APPLIED AND ENVIRONMENTAL*
1867 *MICROBIOLOGY*, *70*(7), 3996–4003. https://doi.org/10.1128/AEM.70.7.3996-4003.2004

Zhou, X., Elmose, J., & Call, D. R. (2007). Interactions between the environmental pathogen *Listeria monocytogenes* and a free-living protozoan (*Acanthamoeba castellanii*). *Environmental Microbiology*, *9*(4), 913–922. https://doi.org/10.1111/j.1462-2920.2006.01213.x

Zoz, F., Grandvalet, C., Lang, E., Iaconelli, C., Gervais, P., Firmesse, O., Guyot, S., & Beney, L. (2017). *Listeria monocytogenes* ability to survive desiccation: Influence of serotype, origin, virulence, and genotype. *International Journal of Food Microbiology*, *248*, 82–89. https://doi.org/10.1016/j.ijfoodmicro.2017.02.010

Zoz, F., Iaconelli, C., Lang, E., Iddir, H., Guyot, S., Grandvalet, C., Gervais, P., & Beney, L. (2016). Control of relative air humidity as a potential means to improve hygiene on surfaces: a preliminary approach with *Listeria monocytogenes*. *PLOS ONE*, *11*(2), e0148418. https://doi.org/10.1371/journal.pone.0148418

Tables

Table 1: Definitions of some terms commonly used when talking about disinfection resistance.

TERM	DEFINITION	SOURCE
Antimicrobial resistance	The ability of a microorganism to multiply or persist in the presence of an increased level of an antimicrobial agent relative to the susceptible counterpart of the same species.	CAC/GL 77- 2011
Disinfection	The reduction, through chemical agents and/or physical methods, of the number of microorganisms in the environment, to a level that does not compromise food safety or suitability.	CAC/RCP 1-1969
Cleaning	The removal of soil, food residue, dirt, grease or other objectionable matter.	CAC/RCP 1-1969
Cross-resistance	The ability of a microorganism to multiply or persist in the presence of other members of a particular class of antimicrobial agents or across different classes due to a shared mechanism of resistance.	CAC/GL 77- 2011
Cross-adaptation	Cross-adaptation occurs when exposure to one stressor induces physiological and behavioural protection to a novel stressor, without prior exposure to the novel stressor.	Lee et al. (2019); Riazi & Matthews (2011)
Co-resistance	The ability of a microorganism to multiply or persist in the presence of different classes of antimicrobial agents due to the possession of various resistance mechanisms.	CAC/GL 77- 2011
MIC	The Minimal Inhibitory Concentration is the lowest concentration of antimicrobial at which cell growth is visibly inhibited.	FAO & WHO (2019)
MSC	Minimum Selective Concentration is the lowest concentration of antimicrobial at which resistance is positively selected.	FAO & WHO (2019)
MEC	Minimum Effective Concentration is the lowest concentration of a chemical or product, used in a specified process, that achieves a claimed activity. The concentration where 99.9% or more of the initial inoculum is killed.	ISO 11139:2018 Bland et al. (2021)
MBC	The Minimal Bactericidal Concentration is the concentration at which bacteria are killed off (an increased MBC can be seen as acquired resistance). The concentration where 99.9% or more of the initial inoculum is killed.	Meyer (2009); Meyer & Cookson (2010) Bland et al. (2021)
MRC	The minimal recommended concentration is the lowest concentration of a chemical or product specified for use in a process.	ISO 11139:2018
Sterilization	The validated process is used to render the product free from viable microorganisms.	ISO 11139:2018
Sanitation	Used to reduce, but not necessarily eliminate, microorganisms from the inanimate environment to levels considered safe as determined by public health codes or regulations. Process of reducing microbiological contamination on an effectively cleaned surface using a bactericidal treatment such as heat or chemicals, to a level that is acceptable to local health regulations. For effectiveness, this must be preceded by cleaning (a mix of detergent and disinfectant or a disinfectant).	EPA (n.d.) GFSI

1883 Table 2: Selection of articles investigating disinfectant resistance/tolerance of planktonic/attached *Listeria monocytogenes*. To be included in the table the studies
1884 had to compare results against the MRC (=minimal recommended concentration), and give an answer to at least two of the following parameters for biofilms
1885 and/or planktonic cultures separately: increased tolerance determination and its stability, the effectiveness of the MRC, the effect of the presence of organic
1886 matter, variability among strains (includes differences between persistent and presumed non-persistent strains) and, cross adaptation. The parameters only
1887 concern *Listeria monocytogenes*, not the other microorganisms (if others are used in the studies).

	PLANKTONIC/ ATTACHED/ BIOFILM CELLS USED	DISINFECTANT/CLEANERS USED + TEST PERFORMED/ C&D SIMULATED	INCREASED TOLERANCE DETECTED AND ITS STABILITY	MRC SUFFICIENT?	EFFECT OF ORGANIC MATTER	VARIABILITY AMONG STRAINS?
Kastbjerg & Gram (2012)	Planktonic,	**Prod. 1:** Disinfect CL (hypochlorite) **Prod. 2:** Incimaxx DES (peracetic acid and hydrogen peroxide) **Prod. 3:** Triquart SUPER (QAC) MRC: 1-5%. **Test 1:** MIC was determined before and after adaptation in BHI. **Test 2:** For Triquart SUPER (QAC), killing assay with 0.0031%; 0.0063% and 0.0125%.	Yes, 4-8-fold only for prod. 3, this increase was stable for 6 transfers.	Yes	/	/
Lundén et al. (2003)	Planktonic	**Prod. 1:** 2 QAC (alkyl-benzyl-dimethyl ammonium chloride and n-alkyl dimethyl ethyl benzyl ammonium chloride); MIC= 0.63-5mg/l **Prod. 2**: Tertiary alkylamine (1,3-propane diamine-N-(3-aminopropyl) N-dodecyl); MIC=0.63-2.5mg/l **Prod. 3**: Sodium hypochlorite; MIC=2500mg/l **Prod. 4:** Potassium persulphate; MIC=2500mg/l **Test 1**: MIC tests (all products), serial 2-fold dilutions were made. **Test 2:** Submit the strains to 1xMIC, 2xMIC and 3xMIC.	Yes, max. 3-fold increase in the MIC, for QAC and tertiary alkylamine this effect was stable for up to 28 days.	Yes	/	Initial MIC differed between persistent and presumed non-persistent strains, but after adaptation, all strains reached similar MIC values.
Roedel et al. (2019)	Planktonic	**Prod. 1:** BAC (>95%); test range: 10 to 0.08 mg/l **Prod. 2:** Glutaraldehyde (50%); test range: 5,650 to 44 mg/l **Prod. 3:** Isopropanol (>99.9%); test range: 249,600 to 3,900 mg/l **Prod. 4:** Sodium hypochlorite (12%); test range: 8,000 to 62.5 mg/l **Prod. 5:** Peracetic acid (36-40%); test range: 2,875 to 22 mg/l **Prod. 6**: Biocidal product containing bis(3-aminopropyl) dodecylamine (7.5wt/wt%); test range: 48 to 0.7 mg/l **Test:** Determine MIC/MBC values for each product according to the given test range.	/	Yes	/	Yes, but the majority of the strains possessed similar MIC/MBC values.
Pang et al. (2019)	Biofilm	**Test:** Whisper™ (3.00% alkyl dimethyl benzyl ammonium chloride, 2.25% octyl decyl dimethyl ammonium chloride, 1.35% dodecyl dimethyl ammonium chloride, 0.90% dioctyl dimethyl ammonium chloride) MRC: 200ppm for 1 minute. Clean (diluted TSB) and soiled (salmon broth) conditions were tested. Besides mono-species *Listeria monocytogenes* biofilms also multi-species (cultivatable indigenous microorganisms of salmon) biofilms were used.	/	No, 4 log CFU/cm^2 reductions were not always achieved.	Yes, a higher resistance in salmon broth compared to diluted TSB.	Older biofilms showed higher resistance. Mixed species showed varying effects on resistance (decreased/ no difference)

1888 Table 2 continued

	PLANKTONIC/ ATTACHED/ BIOFILM CELLS USED	DISINFECTANT/CLEANERS USED + TEST PERFORMED/ C&D SIMULATED	INCREASED TOLERANCE DETECTED AND ITS STABILITY	MRC SUFFICIENT?	EFFECT OF ORGANIC MATTER	VARIABILITY AMONG STRAINS?
Boucher et al. (2021)	Planktonic	**Prod. 1:** Citric acid; MRC: 0.660% (w/v); adaptation in 0.04% of citric acid)) **Prod. 2:** Conventional quaternary ammonium compound; MRC: 200ppm; adaptation in 1.6ppm **Prod. 3:** Benzalkonium chloride; MRC: 200ppm		Yes, this at 4, 15, 23, and 30 °C (temperature did influence efficacy)		Yes, variability was seen mainly for prod. 1
	Biofilm	**Test 1:** MBC tests (all products), serial 2-fold dilutions were made **Test 2:** Determination of growth rates in the presence of sublethal concentrations of prod. 1 or 2. **Test 3**: Use of prod. 1 and 2 in MRC concentrations on biofilms on stainless steel and high-density polyethylene.	/	No, prod. 1 had an efficieny at 4°C of 87%, prod. 2 of 50%	/	Yes, mainly on stainless steel
Stoller et al. (2019)	Planktonic	**Prod. 1:** Benzalkonium chloride; MRC: 500–1000 µg/mL **Prod. 2:** Peracetic acid; MRC: 0.5-1% **Test 1**: MIC determination: growth BHI agar plates supplemented with prod. 1 **Test 2:** Determine MIC/MBC values for each product according to the given test range of prod. 2 in BHI and tap water.	No	Yes	Yes, the MBC was above the MRC in BHI, but well below the MRC in tap water.	Yes
González-Fandos et al. (2012)	Planktonic	**Prod. 1:** Tertiary alkylamine (+ sequestrant, antifoaming); MRC: 0.25-1% **Prod. 2:** QAC (+non-ionic surfactants, polyphosphates, potassium hydroxide, alkaline salts); MRC: 1-8% **Prod. 3:** Formaldehyde + glutaraldehyde + glyoxal + dodecyl dimethyl ammonium chloride (QAC) (+ionic surfactants); MRC: 0.4-1% **Prod. 4:** Chlorhexidine (+glycerine); MRC: 7-10% **Prod. 5:** Peracetic acid + hydrogen peroxide; MRC: 0.05-0.5% **Test:** Test effectiveness using the highest and the lowest recommended concentration (5 or 10 minutes exposure time), this with or without the presence of 2.5% organic matter.	/	Yes, but the lowest recommended concentrations were not always capable of reaching sufficient reduction. The same counts for 5 minutes exposure time.	Yes, effectiveness could be affected by the presence of organic matter and no 5 log/ml reduction could be achieved for some products.	/
Iñiguez-Moreno et al. (2018)	Planktonic	**Prod. 1:** SANICIP Q5 (QAC); MRC: 400mg/ **Prod. 2:** SANICIP PLUS (iodine-based); MRC: 25mg/l **Prod. 3:** SANICIP PAA (peracetic acid + hydrogen peroxide + acetic acid); MRC: 200mg/l **Prod. 4:** SANICIP OSA (disinfectant detergent acid anionic grease, Mix of anionic surfactant and phosphoric, succinic and octanoic acids); MRC: 200mg/l **Test**: Test the effectiveness of the products against the MRC concentrations in the presence of meat extract, egg yolk and whole milk (30s contact time).	/	Yes, without organic matter always 5 log/ml reduction.	Yes, organic matter decreased efficiency (effect varied depending on product and type of organic matter).	Between species (only 1 *Listeria monocytogenes* strain was used)

1889 Table 2 continued

	PLANKTONIC/ ATTACHED/ BIOFILM CELLS USED	DISINFECTANT/CLEANERS USED + TEST PERFORMED/ C&D SIMULATED	INCREASED TOLERANCE DETECTED AND ITS STABILITY	MRC SUFFICIENT?	EFFECT OF ORGANIC MATTER	VARIABILITY AMONG STRAINS?
Aarnisalo et al. (2007)	Planktonic	**Prod. 1:** Peracetic acid (<5%), hydrogen peroxide (15-30%), acetic acid (5-15%), phosphonic acid (<1%); MRC: 0.05-3% **Prod. 2:** Peracetic acid (<5%), hydrogen peroxide (15-30%), acetic acid (5-15%), phosphonic acid (<1%), non-ionic tensides (<5%); MRC: 1-3% **Prod. 3:** Peracetic acid (<5%), hydrogen peroxide (15-30%), acetic acid (5-15%), phosphonic acid (<1%), anionic tensides (<5%); MRC: 0.05-1% **Prod. 4:** Ethanol (<70%); MRC: 100% **Prod. 5:** Sodium hypochlorite (>60%, active chlorine 13%), sodium hydroxide (<5%); MRC: 0.05-2% **Prod. 6:** Alkyl dimethyl benzyl ammonium chloride (>30%), synthetic tensides; MRC: 0.1-0.5% **Prod. 7:** Isopropanol (15-30%), 1-propanol (>30%); MRC: 100% **Prod. 8:** Peracetic acid (<5%), hydrogen peroxide (15-30%), acetic acid (5-15%); MRC: 0.1-4%	/	Maybe, in both clean and soiled conditions some products (prod. 1, 3, 8) needed higher concentrations than the lowest MRC boundary or a longer incubation time. For prod. 5 this still was not enough.	Yes, soiled conditions showed lower reductions compared to clean conditions.	Yes, MIC values differed.
	Biofilm	**Prod. 9:** Sodium hydroxide (5-15%), sodium hypochlorite (<35%, active chlorine 13%); MRC: 1-5% **Prod. 10:** Potassium hydroxide (5-15%), sodium hypochlorite (<38%, active chlorine 13%), non-ionic tensides (>5%), phosphonates (<5%); MRC: 1-5% **Prod. 11:** Sodium hydroxide (5-15%), sodium hypochlorite (<5%), non-ionic tensides (<5%), phosphonates (<5%); MRC: 2-10% **Prod. 12:** Potassium hydroxide (5-15%), sodium hypochlorite (<5%), anionic tensides (<5%); MRC: 2-5% Planktonic: **Test 1:** Suspension test (product 1-8), lowest recommended concentration was used to start and increased if proved ineffective. Different concentration of bovine albumin indicated clean (1.5%) or soiled(10%) conditions. **Test 2:** MIC tests (all products), serial 2-fold dilutions were made. Biofilm: **Test 1:** Suspension test with the lowest recommended concentration.	/	Maybe, complete ineffectiveness cannot be determined as insufficient reductions were achieved for prod. 6 and 7 and no concentrations near the upper bound of the MRC were tested.	Yes, soiled conditions occasionally give less reduction.	Yes, not all strains could be sufficiently reduced by prod. 6 and 7 at the initial tested concentration.

1890

1891 Table 2 continued

	PLANKTONIC/ ATTACHED/ BIOFILM CELLS USED	DISINFECTANT/CLEANERS USED + TEST PERFORMED/ C&D SIMULATED	INCREASED TOLERANCE DETECTED AND ITS STABILITY	MRC SUFFICIENT?	EFFECT OF ORGANIC MATTER	VARIABILITY AMONG STRAINS?
Poimenidou et al. (2016)	Planktonic	**Disinfectant solutions:** **Prod. 1**: P3-oxysan (peracetic acid with a MRC of 800-2500ppm at 4-20°C for 5-30 min) **Prod. 2**: P3-triquart (quaternary ammonium compound with a MRC of 2000-5000ppm at ambient temperature for 20-120 min)	/	Yes, but the lower limit of the MRC was not always sufficient.	TSB used	Yes
	Biofilm	**Test 1:** MIC determination of planktonic strains in TSB. **Test 2:** Biofilm eradication concentration (BEC) only on polystyrene (contact of 5 min at 20°C). concentration at which no growth of the biofilm is observed. **Test 3:** Comparative resistance to disinfectants: Exposing 72h old biofilms to disinfectants (2000ppm prod. 1 and 500ppm prod. 2) and enumerated afterwards. Both polystyrene and stainless steel.		Test 2: Yes, BEC values did not exceed MRC Test 3: No, some strains failed to reach 4 log CFU/cm^2 reduction.		Test 2: Yes
Overney et al. (2017)	Biofilm:	**Prod. 1:** Topax 66 (chlorinated alkaline cleaner, MRC: 3v%) **Prod 2:** Triquart MS (quaternary ammonium compound, MRC: 2 v%). Products were diluted to model what happens in the **retention zones** which are difficult to reach for C&D **Test 1:** Daily C&D (4 days): prod. 1 at 0.3 v% for 15 min at room temperature, rinsing step with water of 55°C for 2 min, prod. 2 at 0.2v% for 30 min at room temperature, soiling with salmon juice or meat exudate and re-incubation. Before enumeration, neutralization was applied after C&D. **Test 2:** Drying step (at 25 °C with high relative humidity (75%)): a daily drying step without C&D is tested, a daily C&D with only the last day a drying step is tested and daily C&D with daily drying is tested. Mono-and dual species (with *Pseudomonas fluorescens)* were used.	/	Yes	No, at the MRC both soiling conditions resulted in <LOD results.	No difference between mono-and dual species
Luque-Sastre et al. (2018)	Planktonic	**Prod. 1**: triclosan (MRC: 0.3% (V/V)) **Prod. 2:** Triquart MS (10-20% (V/V) EDTA, 7-10% BC, 1-3% alcohol ethoxylates, 1-3% didecyldimethylammonium chloride) MRC; 1% **Prod. 3:** 70% (V/V) ethanol **Prod. 4:** Undiluted P3- alcodes (ethanol 50-100%) **Prod. 5**: Undiluted P3-manodes (ethanol 30-50%; propan-2-ol, 25-30%) **Prod. 6:** Benzalkonium chloride (MRC: 10 v/v%)	/	Yes	BHI used	Yes , no higher values for persistent strains
	Biofilm	**Test 1:** MIC/MBC determination in BHI. **Test 2:** Biofilm susceptibility tested at the recommended in-use concentration (18h exposure at 37°C).		No, most products did not reduce biomass, an increase was sometimes even seen.		

1892

1893 Table 2 continued

	PLANKTONIC/ ATTACHED/ BIOFILM CELLS USED	DISINFECTANT/CLEANERS USED + TEST PERFORMED/ C&D SIMULATED	INCREASED TOLERANCE DETECTED AND ITS STABILITY	MRC SUFFICIENT?	EFFECT OF ORGANIC MATTER	VARIABILITY AMONG STRAINS?
Baroso et al. (2019)	Planktonic	**Prod. 1**: Benzalkonium chloride (MCR: 2000-5000mg/l for 20-120 min) **Prod. 2:** Peracetic acid (MCR: 800-2500mg/l, 4-20°C, 5-30min)	/	Yes	/	Yes, especially for prod. 1.
	Biofilm	**Test 1:** MIC determination. **Test 2:** Biofilm susceptibility tested on 2-days old biofilms (grown in low (diluted TSB-YE) or high (TSB-YE) nutrient concentration).		Yes, but the concentrations needed are higher than the lower boundary of the MRC.	Yes, biofilms grown in soiled conditions required occasionally higher concentrations.	Yes
Kocot & Olszewska (2019)	Biofilm	**Prod. 1:** QAC based sanitizer Pursept-AF (12.5 g didecyl-dimethylammonium chloride, 1.5 g N-(3-aminopropyl)-N-dodecylopropane-1,3-diamine); MCR: 2% **Prod. 2:** Tertiary alkyl amine-based sanitizer-Barren (<5% anionic surfactants, <5% EDTA + salts); MCR: 2% **Prod. 3: C**hlorine based-sanitizer-Medicarine (99% sodium dichloroisocyanurate); MCR: 0.18% (1000ppm) **Test 1:** Biofilm susceptibility tested on 1- and 3-days old biofilms.	/	Yes, not always could >4 logs CFU/cm^2 reductions be achieved, 72h biofilms showed higher resistance compared to 24h biofilms.	/	Yes
(Rodríguez-Melcón et al. (2019)	Biofilm	**Prod. 1:** Sodium hypochlorite (10% active chlorine) **Prod. 2:** benzalkonium chloride **Test 1:** MIC determination in TSB **Test 2:** Biofilm formation in presence of disinfectant (0.5xMIC, 1xMIC, 1.5x MIC)	Adapted strains to prod. 2 showed in the presence of 0.5xMIC higher biofilm formation	Yes, biofilm formation was inhibited when cells were exposed to 1xMIC or 1.5x MIC	/	/

1894

1895 Table 2 continued

	PLANKTONIC/ ATTACHED/ BIOFILM CELLS USED	DISINFECTANT/CLEANERS USED + TEST PERFORMED/ C&D SIMULATED	INCREASED TOLERANCE DETECTED AND ITS STABILITY	MRC SUFFICIENT?	EFFECT OF ORGANIC MATTER	VARIABILITY AMONG STRAINS?
Pan et al. (2006)	Biofilm	Products used in recommended in-use concentrations. **Prod. 1:** Matrixx (6.9% hydrogen peroxide, 4.4% peroxyacetic acid, 3.4% octanoic acid) **Prod. 2:** Multi-Quat (3.0% dimethyl benzyl ammonium chloride, 2.3% octyl decyl dimethyl ammonium chloride, 1.1% dodecyl dimethyl ammonium chloride, 1.1% di octyl dimethyl ammonium chloride) **Prod. 3**: Chlorine solution (4-6% NaOCl) **Test 1:** 24h-cycle applied for 3 weeks: - Sanitation by Matrixx (100ppm) for 60 seconds - Storage without liquid medium for 15h at 22.5°C (only 0.5ml water to maintain relative humidity) - Incubation in TSB-YE/10 for 8h at 22.5°C **Test 2:** Susceptibility of biofilms towards sanitizers: biofilms on different days before the sanitation procedure were removed and exposed to Matrixx (100ppm) or Multi-Quat (150ppm) or chlorine solution (200ppm) for 60s. **Test 3:** Susceptibility of detached cells towards sanitizers: cells swabbed from biofilms were exposed to: Matrixx (50ppm, 60s) or chlorine solution (0.2 ppm of Free Available Chlorine, 60s).	/	No, the obtained reduction was followed by an increase, probably due to adaptation to the sublethal concentrations	/	No difference in susceptibility was seen between detached cells and fresh planktonic cells

1896

1897 Table 3: Variability of definitions given to the term resistance in studies.

DEFINITION RESISTANCE	SOURCE
An isolate is defined as resistant when it is not inhibited by a concentration that would inhibit most of the strains of that particular species.	Martinez-Suárez et al. (2016)
Resistance is used to indicate that the extent of the killing of a microorganism is less than expected at bactericidal concentrations.	Carpentier & cerf (2011)
In the context of disinfectants, the word "resistance" is used for comparative purposes. A strain of a species is said to be more resistant than another one if the time needed to achieve a given number of decimal reductions of suspended cells at a given disinfectant concentration is significantly longer, or if the concentration needed to achieve a given number of decimal reductions for a given exposure time is significantly higher.	Cerf et al. (2010)
Resistant microorganisms could be described as a species or strain that was more able to survive repeated C&D programs such that that species or strain dominated the environmental microbial flora, albeit in low numbers.	Holah et al. (2002)
Bacterial resistance is the capacity of bacteria to withstand the effects of antibiotics or biocides that are intended to kill or control them.	Glossary: Bacterial Resistance (n.d.)

1898

1899 Table 2: Examples of desiccation studies carried out on *Listeria monocytogenes*.

	BIOFILM/DROP INOCULATION	DESICCATION TREATMENT	INFLUENCE OF TEMPERATURE	INFLUENCE OF RH	INFLUENCE OF STRAIN	INFLUENCE OF ADDITIONAL MICROORGANISM
Zoz et al. (2016)	Drop inoculation	- Humidity: 11, 43, 68, 73% RH - Time 30-180 min - Temperature: 25°C Rehydration: - Progressively: 99% RH for 60 min, only tested for 43/68% RH - Instantaneously: a drop of phosphate buffer saline on dried cells **Test:** Testing dehydration/rehydration cycles with dehydration periods of 180 min.	/	Faster decrease in viability at lower RH.	Mostly pronounced at 68% RH.	/
Pang & Yuk (2019)	Biofilm duo-species	- Humidity: 43% RH - Time 0 to 21 days - Temperature: 15°C **Test:** Different colonization sequences tested with *Pseudomonas fluorescens*.	/	/	Similar reductions reached (>3 log CFU/cm^2) for monospecies.	Lower reductions in duo-species, lowest reduction when *P. fluorescens* was the first colonizer.
Kim et al. (2019)	Biofilm: Monospecies or inoculating *Streptomyces* biofilm	- Humidity: 43% RH - Time 0-120h - Temperature 25°C	/	/	A 5-strain cocktail of *Listeria monocytogenes* was used.	Higher reductions with *Streptomyces* (to below the detection limit).
Piercey et al. (2017)	Biofilm	- Humidity: 23% RH - Temperature: 15°C - Time: 7 days	/	/	Strains showed no reduction, intermediate reduction or high reductions.	/
Bremer et al. (2001)	Biofilm mono and duo-species with *Flavobacterium spp.*	- Temperature 4/15 °C - Humidity: 75% RH - Time: 10 days	Faster decline at 15°C compared to 4°C in monospecies, opposite for duo-species.	An increasing number of injured cells at 75% RH.	/	Higher resistance in duo-species compared to monospecies.
Vogel et al. (2010)	Drop inoculation	- Humidity: 2, 43, 75% RH - Temperature: 15°C - Time 21 or 91 days **Test**: Influence organic material + salt: physiological salt solution/ TSB + glucose with 0.5/5% NaCl Influence of salmon juice from fresh (0.5% NaCl) or cold-smoked (5% NaCl) salmon.	/	Slower reductions at higher RH compared to 2% RH. The effect of a high salt concentration gets lower as RH increases.	No difference between persistent and presumed non-persistent strains after 91 days of desiccation.	/

1900

1901 Table 3:Examples of protozoa-bacteria relationships.

	PROTOZOA	BACTERIA TESTED	ENCYSTMENT SURVIVAL	TROPHOZOITE INGESTION SURVIVAL	OTHER ASPECTS
Minh et al. (1990a)	***Acanthamoeba,***	*Listeria monocytogenes* (1 strain)	No	Yes, lysis of the protozoa happened after 8 days.	/
	Tetrahymena pyriformis		*Not applicable*		
Doyscher et al. (2013)	***Acanthamoeba castellanii,***	*Listeria monocytogenes* (4 wild-type strains)	/	No	*Acanthamoeba* carries *Listeria monocytogenes* at the uriod in densely packed aggregates in phagolysosomes and were rapidly lysed and digested.
	Acanthamoeba polyphaga				
Lambrecht et al. (2017)	***Acanthamoeba castellanii***	*Salmonella enterica Serovar Typhimurium, Yersinia enterocolitica, Listeria monocytogenes,* and *E. coli O:26* (1 strain of each)	Yes, up to 14 days.	Yes, in co-culture after excystment.	Increased tolerance towards several disinfectants and other chemical stressors. After excystment, *Listeria monocytogenes* showed lower reductions towards sodium hypochlorite (2.5%), H_2O_2 (0.3%) and 70% EtOH. Some treatments were cysticidal, indicating that sheltering alone enhances *Listeria monocytogenes* survival.
Zhou et al. (2007	***Acanthamoeba castellanii***	*Listeria monocytogenes* (12 different strains)	/	Yes, up to 96h	Variability among strains.
Akya et al. (2010)	***Acanthamoeba polyphaga***	*Listeria monocytogenes* (3 strains) *Salmonella enterica serovar Typhimurium*	/	Yes at 15°C still countable results after 96h, no survival after 3h at 22 or 37°C.	/
Lambrecht et al. (2015)	***Acanthamoeba castellanii***	*Listeria monocytogenes, Salmonella enterica, E. coli, Yersinia enterocolitica, Campylobacter jejuni* (2 strains of each)	Yes, up to 14 days survival in cysts with growth after excystment.	/	The invasion time is longer than 30 min.
Meinersmann et al. (2020)	***Tetrahymena pyriformis***	*Listeria monocytogenes* (20 different strains)	/	No, barely survival after 24h	Attached *Listeria monocytogenes* was a 10-fold less prone to predation.
Fieseler et al. (2014)	***Acanthamoeba castellanii***	*Listeria monocytogenes, Listeria grayii, Listeria innocua, Listeria seeligeri, Listeria ivanovii, Listeria welshimeri, Bacillus subtilis, Brevibacterium epidermis, Brochothrix thermopshacta,, Corynebacterium ammoniagenes, Cronobacter sakazakii, E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus carnosus, Staphylococcus epidermidis*	Cyst formation not observed.	Only co-culture is possible, not ingestion.	The presence of the *Acanthamoeba* alone proved to enhance *Listeria monocytogenes* growth. The absence of motility protects *Listeria monocytogenes* from predation/ aggregate formation at the uroid.

1902

Figures

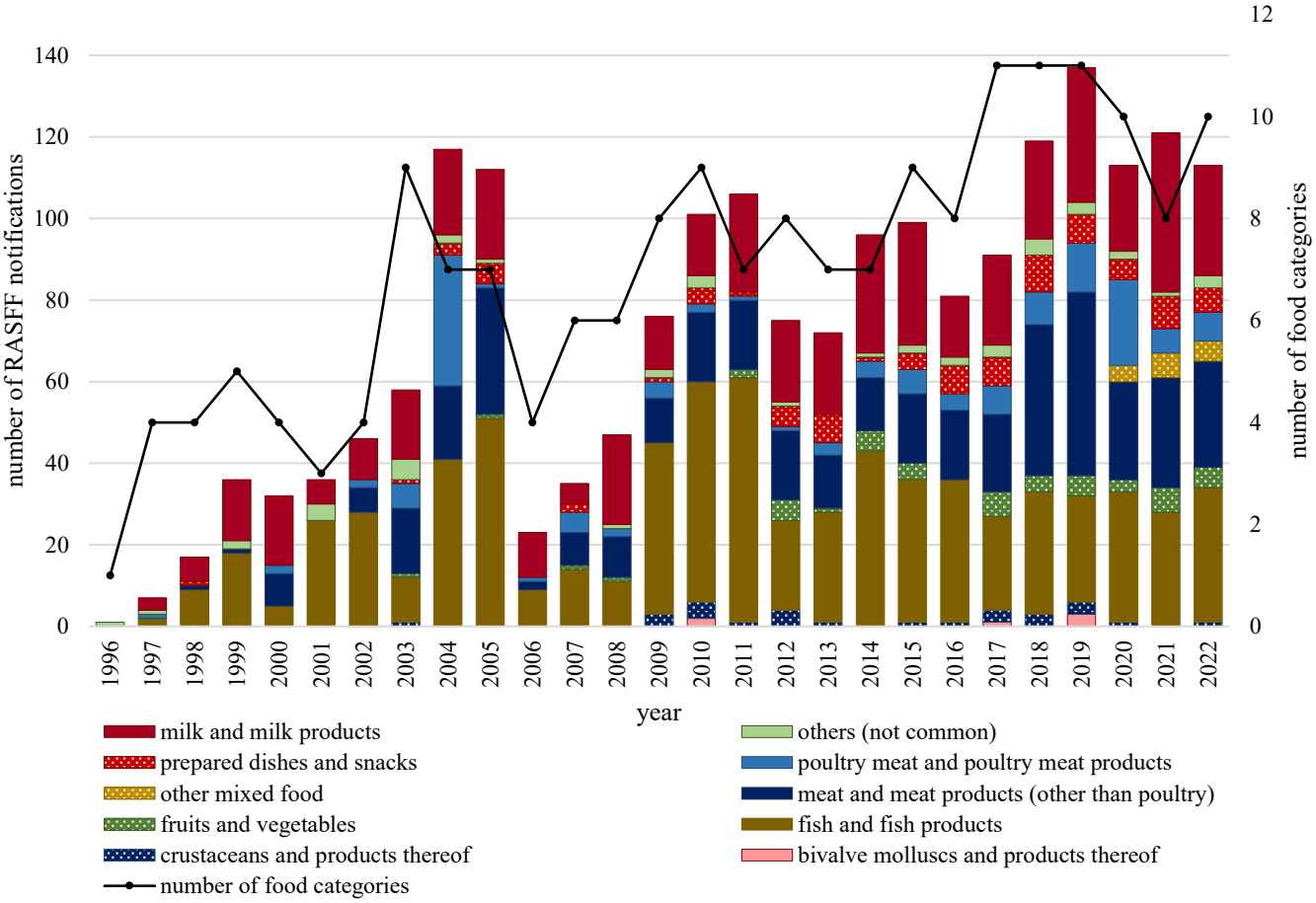

Figure 1:Number of RASFF (Rapid Alert System for Food and Feed) alerts in the period 1996-2022. Left y-axis: The bars represent the number of notifications of the (possible) presence of *Listeria monocytogenes* from 1996-2022. The contribution of different food categories is represented by the different colors/patterns. Food products that contained less than or equal to 5 notifications in total for the whole period collected in the food category others (not common). Right y-axis; the line represents the number of different food categories for each year from 1996-2022 with the food categories belonging to "others (not common)" counted separately. X-axis is the year ranging from 1996-2022 (EU Commission, 2023).

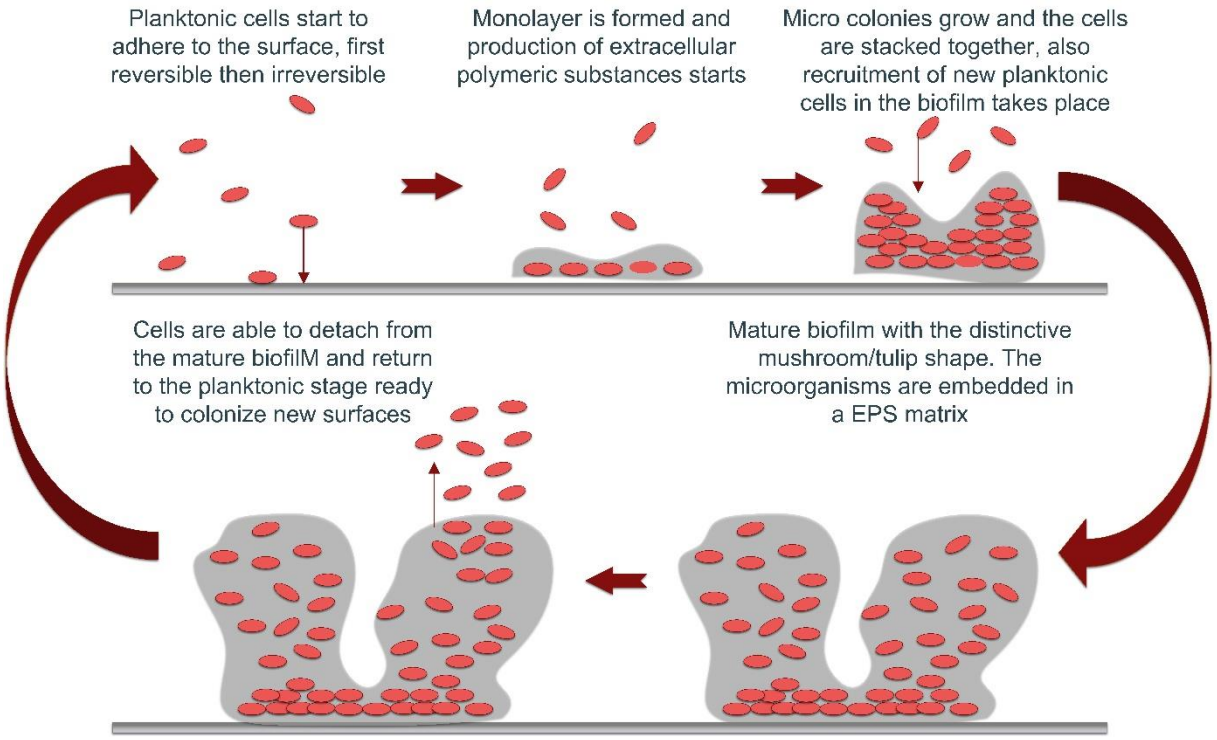

Figure 2: Development stages of biofilms based on Chmielewski & Frank, 2003; Costerton et al., 1987; Donlan, 2002; Kim & Frank, 1994; Vasudevan, 2014; Wimpenny et al., 2000

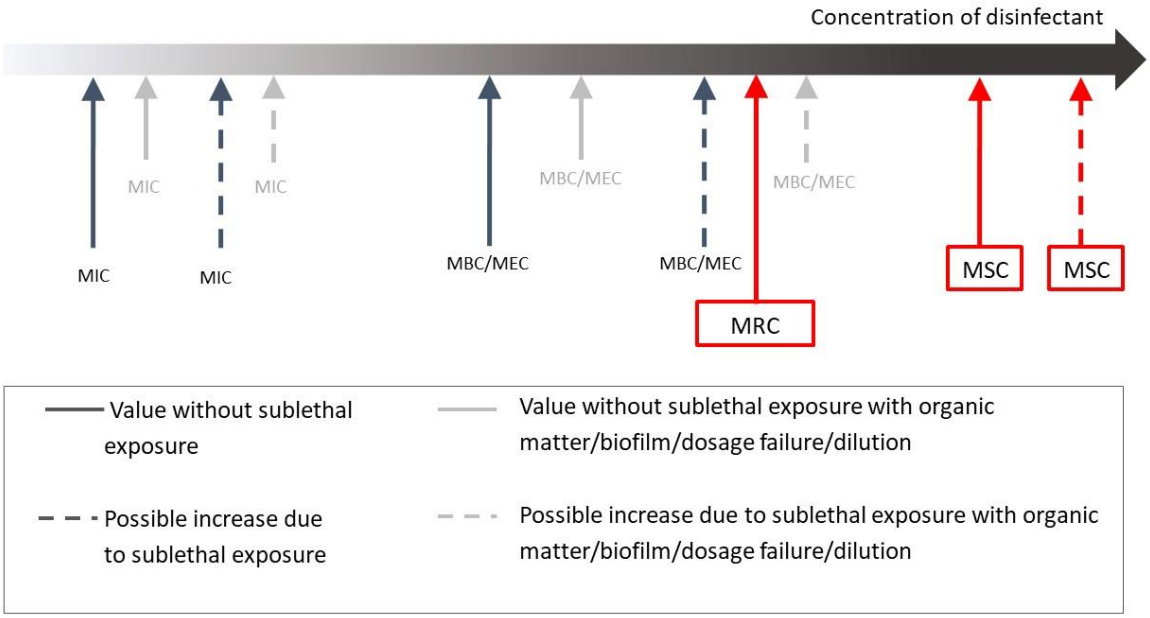

Figure 3: Overview of the diverse terminologies (minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), minimum effective concentration (MEC), minimal recommended concentration (MRC), minimum selective concentration (MSC)) used in disinfectant efficacy testing. Original values are given with the possible increase due to sublethal exposure. The effect of disinfectant failure is included as well (Bland et al., 2021; FAO & WHO, 2019; ISO, 2018).

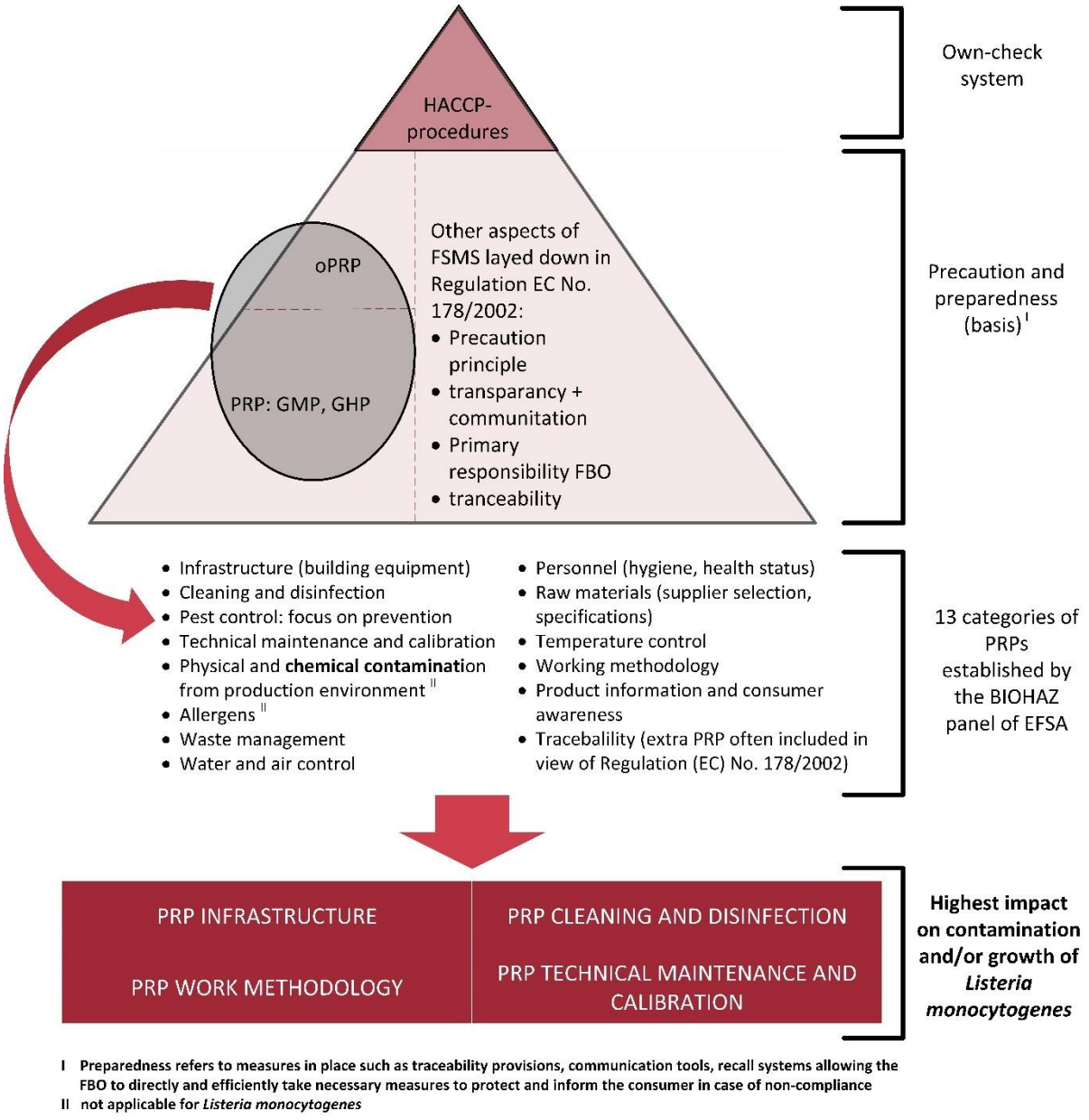

Figure 4: Situation of the proposed solutions for persistent microorganisms within the food safety management system based on Figure 1 of the Commission Notice (EC) No 2016/C 278/01, (EFSA BIOHAZ panel, 2017, 2020)