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SCIENTIFIC OPINION

Persistence of microbiological hazards in food and feed production and processing environments

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Abstract

Listeria monocytogenes (in the meat, fish and seafood, dairy and fruit and vegetable sectors), *Salmonella enterica* (in the feed, meat, egg and low moisture food sectors) and *Cronobacter sakazakii* (in the low moisture food sector) were identified as the bacterial food safety hazards most relevant to public health that are associated with persistence in the food and feed processing environment (FFPE). There is a wide range of subtypes of these hazards involved in persistence in the FFPE. While some specific subtypes are more commonly reported as persistent, it is currently not possible to identify universal markers (i.e. genetic determinants) for this trait. Common risk factors for persistence in the FFPE are inadequate zoning and hygiene barriers; lack of hygienic design of equipment and machines; and inadequate cleaning and disinfection. A well-designed environmental sampling and testing programme is the most effective strategy to identify contamination sources and detect potentially persistent hazards. The establishment of hygienic barriers and measures within the food safety management system, during implementation of hazard analysis and critical control points, is key to prevent and/or control bacterial persistence in the FFPE. Once persistence is suspected in a plant, a 'seek-and-destroy' approach is frequently recommended, including intensified monitoring, the introduction of control measures and the continuation of the intensified monitoring. Successful actions triggered by persistence of *L. monocytogenes* are described, as well as interventions with direct bactericidal activity. These interventions could be efficient if properly validated, correctly applied and verified under industrial conditions. Perspectives are provided for performing a risk assessment for relevant combinations of hazard and food sector to assess the relative public health risk that can be associated with persistence, based on bottom-up and top-down approaches. Knowledge gaps related to bacterial food safety hazards associated with persistence in the FFPE and priorities for future research are provided.

KEYWORDS

cleaning and disinfection, *Cronobacter sakazakii*, food processing, interventions, *Listeria monocytogenes*, Persistence, risk factors, *Salmonella enterica*, subtypes

CONTENTS

SUMMARY

The European Food Safety authority (EFSA) asked the Panel on Biological Hazards (BIOHAZ) to deliver a scientific opinion on the persistence of microbiological hazards in food and feed production and processing environments (FFPE), excluding primary production. In the scope of this mandate, microbial 'persistence' was defined as the ability of a given organism to be established in niches (or harbourage sites) within the FFPE for a long term, despite the frequent application of cleaning and disinfection (C&D). It requires prolonged existence (spanning months or years) usually with multiplication of the microorganism in the specific FFPE. Feed-producing environments (FePE) were restricted to the environments of facilities producing and processing feed for food-producing animals. Food-producing environments (FoPE) cover environments where food of animal or non-animal origin is industrially produced or processed at post-harvest level. The sectors considered were: (i) feed for food animal production, (ii) meat (incl. slaughterhouses and processing plants), (iii) fish and seafood, (iv) dairy, (v) egg and egg products, (vi) fruit and vegetable (including herbs) and (vii) low moisture food (LMF).

In Term of Reference 1 (ToR1), EFSA was requested to identify the most relevant microbiological food safety hazards associated with persistence in the FFPE of these sectors in the EU/EEA. Based on the definition of persistence, viruses and parasitic protozoa were excluded because, due to their inability to multiply on abiotic surfaces, they cannot become established for a long term or constitute a contamination reservoir in the FFPE. Likewise, microbial toxins and other hazardous microbial metabolites were excluded. The most relevant bacterial food safety hazards were identified as: *Salmonella enterica* in the feed for food animal production sector; *Listeria monocytogenes* and *S. enterica* in the meat processing sector; *L. monocytogenes* in the fish and seafood processing sector; *L. monocytogenes* in the dairy sector; *S. enterica* in the eggs and egg processing sector; *L. monocytogenes* in the fruit and vegetables processing sector; and *S. enterica* and *Cronobacter sakazakii* in the LMF sector. Other bacterial hazards were either not of highest public health (PH) relevance in the specific sector or were not considered as most relevant food safety hazards associated with persistence in the FFPE in the specified sector based on the available information.

In ToR2, EFSA was requested to identify the main (sub)types of the most relevant hazards involved in persistence and the main features responsible for their persistence in the FFPE. It was concluded that there is a wide range of subtypes reported to be involved in persistence in the FFPE for the three most relevant hazards listed above. Some specific subtypes are more commonly reported as persistent: for *L. monocytogenes*, especially CC 121, CC8, CC9 from lineage II and CC 5, CC6, CC2 from lineage I; for *S. enterica*, *S.* Typhimurium and *S.* Agona; and also *S.* Derby, *S.* Anatum, *S.* Infantis, *S.* Heidelberg, *S.* Mbandaka and *S.* Senftenberg; and for *C. sakazakii*, CC64, CC1, CC83 and CC4. For *L. monocytogenes*, some markers have been identified as possibly associated with persistence: stress survival islets SSI-1 and SSI-2, genomic islands LGI-1 and LGI-2, heavy metal (cadmium and arsenic) and biocide (*bcrABC, qacC, qacH, emrE* and *emrC*) resistance determinants, often located on mobile genetic elements (mainly plasmids) and bacteriophage regions (*comK*), globally linked to increased environmental robustness, tolerance to disinfection and/or biofilm formation. The set of phenotypic and genomic features that have been investigated for *Salmonella* and *C. sakazakii* in relation to persistence in the FFPE is incomplete. As such, it is difficult to deduce certain features, that are either indispensable for or may markedly contribute to, persistence, alone or in combination with other key genotypic and phenotypic elements. For *Salmonella*, most studies focused on features inherent to most infectious foodborne hazards, and reported resistance of some strains to one or more antimicrobials, carriage of plasmid-mediated virulence factors, biofilm formation ability or reduced susceptibility to alkaline disinfectants. Several features have been associated with the ability of *C. sakazakii* to survive for long time periods and persist in the dry conditions of the LMF FoPE, including the ability to form biofilms on a variety of abiotic surfaces; a high heat tolerance and desiccation resistance; the production of a capsule that aids attachment and provides resistance to biocides and desiccation; and the production of a yellow carotenoid pigment which stabilises cell membranes and protects against stress. However, none of these features seem to be specifically linked to particular subtypes frequently associated with persistence. Overall, no universal markers or features, responsible for persistence have been identified. Although the carriage of different combinations of genetic determinants linked to increased environmental robustness possibly confers the ability to persist on particular subtypes, persistence is a multifactorial process that also depends on specific environmental conditions and risk factors.

In ToR3, EFSA was requested to identify the risk factors i.e. those factors at facility level that increase the likelihood of persistence of the food safety hazards in the FFPE. The main risk factor of the three bacterial hazards listed above in the FFPE is poor hygienic design of equipment and machines. This leads to niches (or harbourage sites) which are difficult to clean and disinfect and where food debris and moisture can accumulate, and the hazards can survive and persist. Other important factors are: (i) inadequate zoning and hygiene barriers, that enables the spread of contamination from contaminated to clean areas; (ii) inadequate C&D of the facilities; (iii) introduction of the hazards through raw materials, which may lead to the colonisation and spread of persistent clones in the processing environment; and (iv) humidity, which favour persistence. Specifically for hazards of relevance in dry (LMF/feed) processing environments, additional risk factors are airborne transmission through dust, the limited use of disinfectants due to dry cleaning operations or the presence of water in the FoPE, whether from wet cleaning, condensation generated through temperature gradients within the facility or within equipment, or other sources.

In ToR4, EFSA was requested to assess available and enhanced measures or interventions for monitoring, preventing and/or controlling the persistence of the most relevant microbiological food safety hazards in the FFPE. It was concluded that a well-designed environmental sampling and testing programme, following a risk-based approach, is the most effective strategy to identify contamination sources and detect potentially persistent hazards. The establishment of hygienic

barriers and measures within the food safety management systems (FSMS), during implementation of hazard analysis and critical control point (HACCP), is key to prevent and/or control bacterial persistence in the FFPE through avoiding the entry of the hazard(s) to the processing plant and/or their spread across the facility. The following prerequisites are of particular importance: infrastructure (building, equipment), C&D, technical maintenance and calibration, water and air control, personnel (hygiene, health status), working methodology and food safety culture. The confirmation of the presence of a persistent strain and identification of its niche within the facility requires the detailed characterisation of isolates of the specific hazard(s) recovered from positive samples using subtyping methods with enough resolution, preferably whole genome sequencing (WGS). Once persistence is suspected in a plant, a 'seek-and-destroy' approach has been frequently recommended, which includes: (i) intensified monitoring; (ii) the introduction of measures to control the event; and (iii) the continuation of the intensified monitoring programme to confirm the efficacy of the measures taken or to identify the requirement for additional measures. Alternatively, systematic 'root cause analyses' can be applied to identify the most probable factors/sites within the facilities contributing to the problem and define the most appropriate interventions to eliminate the pathogen from the premises. Successful actions triggered by *L. monocytogenes* persistence in the FoPE were identified, for example, the introduction of new or specialised (deep) C&D, the implementation of workflows, the installation of a new drainage system; the implementation of structural changes and renovations; the control of the contamination of raw ingredients and the improvement of the compartmentalisation, or the simultaneous implementation of various corrective actions. In addition, some options of interventions to eliminate the persistent hazard(s) with direct bactericidal activity and of different nature (i.e. as chemical (e.g. biocides), physical (e.g. heat or novel non-thermal technologies) or biological (e.g. competitive exclusion, phage)) are described but in some cases these are not yet commercially available and/or their efficacy is not yet fully validated under industrial conditions.

In ToR5, EFSA was requested to identify knowledge gaps and priorities for future research and develop the perspectives (or future opportunities) of integrating the information gathered in the previous ToRs in risk assessment. Perspectives are provided for the use of risk assessment for relevant combinations of hazard and food product to assess the relative PH risks that can be associated with persistence, based on bottom-up (or forward) and top-down (or backward) approaches. A basic model for persistence, proposed to be used in bottom-up food chain QMRA, can be used to study the role of persistence in the PH risk for a specific food production process. The dynamics of persistence and its role in PH risk will however be very food process specific, and the model proposed may be too simple to capture important biological processes, such as biofilm formation. With the currently available data, top-down risk assessment cannot be used to assess the relative PH risk that can be attributed to persistence. Risk assessment cannot fully exploit the data gathered to support answering the previous AQs, and the data needs for risk assessment are not well covered. Application of these data would require better translation of genotypic information of strains into phenotypic characteristics that can be converted into parameters of risk assessment models, as well as extensive quantitative data to describe the dynamics of transfer, survival and growth of bacteria in the FoPE.

Nine specific knowledge gaps have been identified and translated into recommendations for research. Most recommendations would involve activities at industry settings, but some of the research activities could be performed using industrial-like model systems of certain niches, where different strains, environmental conditions and potential interventions can be tested. These research activities would enable to establish the contribution of specific genetic markers and their link to phenotypes associated with persistence, and to monitor the impact of particular interventions in reducing or preventing persistence. They would also allow the generation of quantitative data to describe the dynamics of transfer, survival and growth of bacterial hazards and to define strain- or subtype-specific parameters for QMRA.

It is recommended to apply clear definitions of persistence in all involved research areas (observational, experimental, epidemiological, etc.) aiming at the same unambiguous definition for all of them. The environmental sampling and testing programme should be robust and carefully planned by the food business operators and ensure an adequate surveillance of higher risk niches for target bacterial hazards, and, during outbreak investigation, the sampling strategy should be optimised and data reporting of official and industrial sampling improved, in order to strengthen the link between FFPE and the outbreak cases. It is also recommended to promote the use of interoperable standards to collect and report metadata associated to WGS data to ensure auditability, to streamline data sharing and to reduce uncertainty. Finally, it is recommended to promote the open access to both WGS data and complete and unambiguous associated metadata related to the strain isolation, respecting data confidentiality and the interests of different partners in the food chain for investigating persistence in the FFPE.

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

In slaughterhouses and facilities where food and feed are produced and/or processed, persistence of microbiological hazards in the production environment occurs commonly and often involves repeated occurrence of the same strain for months or even years at the same premises or equipment (Davies & Wray, 1997; Unnerstad et al., 1996). This represents a great concern for public health, and food and feed business operators, since the persistent microbiological hazards in the food and feed processing environment (FFPE) can lead to contamination of processed products, with important associated health risks for consumers and economic losses for producers.

The ability of some microbiological hazards to persist in food processing environments (FoPE) is well documented, with *Listeria monocytogenes* persistence being a major focus of attention (Fagerlund et al., 2021; Townsend et al., 2021). Indeed, the persistence of *L. monocytogenes* strains has been described in cheese factories (Fox et al., 2011; Lomonaco et al., 2009), salmon or crab meat production plants (Elson et al., 2019; Wulff et al., 2006), meat and meat products processing facilities (Nesbakken et al., 1996; Ojeniyi et al., 2000), and produce packing houses and fresh-cut facilities (Estrada et al., 2020; Sullivan & Wiedmann, 2020). Nevertheless, other biological hazards also have the ability to persist in FoPE. Larsen et al. (2014) reviewed persistence of *Campylobacter* spp. in food processing facilities and of *Cronobacter* spp. in processing facilities for powdered foodstuffs, as examples to highlight factors involved in the persistence of microbiological hazards at FoPE. Of note is that persistence of *Campylobacter* spp. has been demonstrated to occur for longer periods than expected, given the supposed 'fragility' of the organism (Garcia-Sanchez et al., 2017). Persistence of *Salmonella* in low-moisture and high lipid matrices (such as chocolate, sesame-based halva, tahini or peanut butter) and their production environments also represents a challenge due to the pathogen's ability for long-term survival in low-moisture products and dry production environments (Finn et al., 2013). *Salmonella* persistence has also been described in pig and poultry slaughterhouses (Hald et al., 2003; Zeng et al., 2021). Moreover, apart from bacteria, some pathogenic viruses can persist in FoPE,[1] as reviewed by Kotwal and Cannon (2014) for enteric viruses.

In recent years several high-profile foodborne outbreaks (FBO) have been associated with strains persistently colonising FoPE or equipment, even for several years, and some processing facilities have been recurrently linked to FBOs and cases of infection caused by closely-related genotypes of some microbiological hazards. As an example, a nationwide outbreak of human listeriosis in Switzerland was traced to persisting environmental contamination of a cheese processing plant with *L. monocytogenes* serotype 4b, multi-locus sequence type clonal complex 6 (CC6) (Nüesch-Inderbinen et al., 2021). Luth et al. (2020) described one of the largest listeriosis outbreaks in Germany, with 83 cases of invasive listeriosis between 2013 and 2018, linked to persistence of the pathogen in a single producer of ready-to-eat (RTE) meat products. The finding of *L. monocytogenes* 4b, ST6, matching a multi-country FBO strain in frozen corn and other frozen vegetables produced during the 2016–2018 production seasons at a freezing plant, led to the suggestion that the outbreak strain could have been persisting in the FoPE of the plant after standard cleaning and disinfection (C&D)[2] procedures carried out, in conjunction with periods of inactivity (EFSA and ECDC, 2018b). Likewise, outbreaks of salmonellosis have been traced back to persistent contamination of pig slaughterhouse equipment (Bertrand et al., 2010; Kuhn et al., 2013; Schroeder et al., 2016), and a large proportion of the *Salmonella* strains associated with food animals are associated with persistence in feed processing plants and compound feed mills. These examples highlight the challenge of preventing environmental persistence of microbiological hazards in the FFPE.

The factors influencing microbial persistence in the FFPE, and the causes and genetic determinants involved in this, are a matter of intense debate. The existence of harbourage sites that are difficult to clean and disinfect adequately, or the special abilities of certain microbial strains to withstand conditions of environmental stress, desiccation, or disinfection, or to form biofilms in industrial environments, have been mentioned among the most relevant determinants of persistence. Some review articles have highlighted factors involved in the persistence of microbiological hazards, including *L. monocytogenes*, *Cronobacter* spp., and *Campylobacter* spp. in selected food chains (Larsen et al., 2014); they identified, for *Listeria*, locations where it is commonly found to persist (i.e., floors, drains, conveyor belts, slicers, and tables), the most common risk factors at processing facility level (equipment cleanability and lack of hygienic zoning), and interventions for the elimination of persistent strains with variable results (Belias et al., 2022).

Remarkably, evidence from recent years suggests that, within the most relevant microbiological hazards, particular lineages or genotypes more frequently colonise and persist in processing environments. For example, some *L. monocytogenes* sequence types (e.g., ST121, ST9) have been recurrently identified as persistent in FoPE (Alvarez-Molina et al., 2021; Ferreira et al., 2014; Schmitz-Esser et al., 2015). However, the molecular mechanisms underlying these frequent associations are not yet fully elucidated. In 2018, the EFSA BIOHAZ Panel described that some hypovirulent molecular subtypes of *L. monocytogenes*, such as ST121, seem to encompass multiple isolates with a proven capability to persist. Whether their persistence is a result of improper hygiene conditions, or the involvement of strains equipped with an arsenal of specific genetic determinants is under investigation. A high adaptive capacity against physical–chemical factors as well as biofilm-forming capacity could partly explain the persistence phenomenon (EFSA BIOHAZ Panel, 2018). Recent advances in next generation sequencing technologies have promoted a more detailed characterisation of persistent isolates through whole genome sequencing (WGS) and transcriptomics, and offer opportunities for the study of persistence episodes at

[1]This review article deals with transfer and environmental survival of foodborne and waterborne viruses outside the human host. It does not contain evidence on the long-term establishment of viral hazards in FoPE.

[2]Disinfection is used as a synonym of sanitation. The terms have been explained in the glossary.

processing plant level through the characterisation of the FFPE microbiome through metagenomics, which will provide information on the role of complex microbial communities and the cellular processes and microbial interactions driving microbial persistence in the FFPE (Fenner et al., 2021; Hu et al., 2022).

The strategies that can be employed to combat persistence by some microbiological hazards have been also reviewed (for example by Larsen et al. (2014)). Those at the processing environment included hygiene measures; cleaning routines of facilities and design of equipment; cleaning, disinfection and biofilm removal; and sampling. The EFSA BIOHAZ Panel provided possible control options that may be implemented by food business operators during the production process of frozen fruit and vegetables including herbs, blanched during processing. Additional control measures (technologies and antimicrobial solutions) were identified to reduce or eliminate *L. monocytogenes* in the environment, mainly on surfaces, and on the product (EFSA BIOHAZ Panel, 2020). In addition, measures for enhanced environmental monitoring can be implemented, for example through agent-based in silico modelling to simulate the dynamics of foodborne pathogens in the built environment (surfaces and equipment) of processing facilities, and to reduce the time and cost usually linked to classical environmental monitoring activities (Sullivan et al., 2021).

EFSA is asked to deliver a scientific opinion on the persistence of microbiological hazards in food and feed production and processing environments, excluding primary production.

More specifically, EFSA is requested to address the following terms of reference (ToRs):

ToR1. To identify the most relevant microbiological food safety hazards associated with persistence in the FFPE

ToR2. To identify the main (sub)types of the most relevant hazards involved in persistence and the main features responsible for their persistence in the FFPE

ToR3. To identify the risk factors at facility level responsible for the persistence of the most relevant hazards in the FFPE

ToR4. To assess available and enhanced measures or interventions for monitoring, preventing and/or controlling the persistence of the most relevant microbiological food safety hazards in the FFPE

ToR5. To identify knowledge gaps and priorities for future research and develop the perspectives of integrating the information gathered in the previous ToRs in risk assessment

1.2 | Interpretation of the Terms of Reference

In the scope of this mandate, **microbial 'persistence'** was defined as the ability of a given organism to be established in niches (or harbourage sites) within the FFPE for a long term, despite the frequent application of C&D. It requires prolonged existence usually with multiplication of the microorganism in the specific FFPE. It is a phenomenon which may lead to recurrent food contamination events and is normally detected through the repeated isolation from the same premises or equipment on different dates (spanning months or years) of strains that are subsequently identified as highly related subtypes (as determined by phenotypic or genotypic methods). Persistence does not include continuous reintroduction in the facility of the same organism, although in practice it is often not possible to distinguish between both phenomena. Strains identified as sporadically (not repeatedly) contaminating the FFPE of a processing plant are frequently referred to as 'presumed non-persistent', as a more intensified or a longer sampling campaign could result in their repeated isolation from the FFPE. This terminology is also used in this assessment.

The **'microbiological food safety hazards'** include all microorganisms which may adversely affect human health through food consumption, including bacteria, viruses and parasitic protozoa, and any hazardous substance they may produce. However, based on the definition of persistence (see above), it was agreed that viruses and parasitic protozoa are excluded from the assessment because, due to their inability to multiply on abiotic surfaces, they cannot become established for a long term and then constitute a contamination reservoir in the FFPE. Indeed, their occurrence is mainly linked to contamination at the primary production level or through human manipulation of food matrices that are not processed afterwards or are minimally processed, such as raw milk, fresh meat, oysters or berries. Likewise, microbial toxins and other hazardous microbial metabolites (e.g. histamine) are excluded. Antimicrobial resistance (AMR) determinants are considered as possible features related to persistence under ToR2, but they are not considered to define the most relevant hazards in ToR1.

The **'food and feed production and processing environments'** cover slaughterhouses and facilities where food and feed are produced and/or processed. It excludes primary production. Feed-producing environments are restricted to the environments of facilities producing and processing feed for food processing animals. FoPE cover all environments where food of animal or non-animal origin is industrially produced or processed, at post-harvest level (e.g. slaughterhouses, plant factories, processing facilities). As such, the retail stage is not considered. Although farm environments are not included, small scale food production facilities linked to a farm are covered (for example artisanal cheese production on a dairy farm). The focus is on EU food and feed production systems, or, where applicable, in similar production systems from other regions of the world. The following sectors are considered: (i) feed processing for food-producing animals, (ii) meat processing (including slaughterhouses and processing plants), (iii) fish and seafood processing, (iv) dairy processing, (v) egg and egg products processing, (vi) fruit and vegetable processing (including herbs), including Controlled Environment Agriculture (CEA)/production through indoor hydroponic operations and (vii) low moisture food (LMF) processing. According to ILSI (2011), a wide range of products fall in this LMF category (with a water activity below 0.85): cereals, chocolate, cocoa powder, dried fruits and vegetables, egg powder, fermented dry sausages, flour, meal and grits, dried herbs, spices and condiments,

honey, hydrolysed vegetable protein powder, meat powders, dried meat, milk powder, pasta, peanut butter, peanuts and tree nuts, powdered infant formula, rice and other grains, and seeds.

For **ToR1**, with 'relevant' it is understood those food safety hazards that have public health impact and have been found to persist in the FFPE of each of the sectors.

For **ToR2**, (sub)types refers to a grouping of bacteria within a species that share certain characteristics, usually derived by molecular typing (molecular or genotypic subtype) and/or spectroscopy/spectrometry based phenotypic methods. Different types (e.g. derived from serotyping, multi-locus sequence typing or MLST, pulsed-field gel electrophoresis or PFGE, WGS) will be considered as the methods have evolved over time. With 'features', it is considered the phenotypic characteristics (e.g. ability to persist/survive in competition, survive disinfection, form biofilms, etc.) and the genotypic characteristics (i.e. carriage of genetic determinants of resistance/persistence).

For **ToR3**, the 'risk factors' are interpreted as those factors at facility level that increase the likelihood of persistence of the food safety hazards in the FFPE.

For **ToR4**, 'measures' or 'interventions' are considered synonyms. Strategies and control options cover those that are already in place (either exceptionally or routinely used), as well as those not yet implemented. Enhanced control options are considered the latter ones and those used already exceptionally. It was clarified that only measures or interventions assessed in industry settings are to be considered. Economic or environmental impacts as well as the impact on the human exposure to the hazards resulting from these mitigation options are outside the scope. Food businesses are obliged to develop and implement food safety management systems (FSMS) including prerequisite programme (PRP) activities and hazard analysis and critical control point (HACCP) principles. For this reason, control options will be based on prerequisite programmes (PRPs; e.g. C&D), operational prerequisite programmes (oPRP) and, if possible, control points (CP) and critical control points (CCP; i.e. the steps at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level). PRP are preventive practices and conditions needed prior to and during, the implementation of HACCP and which are essential for food safety. However, some prerequisites, typically linked to a specific production process, may be identified as essential to control the likelihood of the introduction, survival and/or proliferation of food safety hazards in the product(s) or in the processing environment. These are referred to as oPRP.

For **ToR5**, 'perspectives' was understood to mean future opportunities. It is expected that limited data are available, and there are no standard methods available to integrate the information gathered in a risk assessment of the public health risk related to persistence. Therefore, it was agreed to explore future opportunities to perform such a risk assessment, if possible, illustrated by some case studies.

The ToRs have been translated into assessment questions (AQs). A conceptual map of the AQs to be addressed in the current assessment can be found in Figure 1.

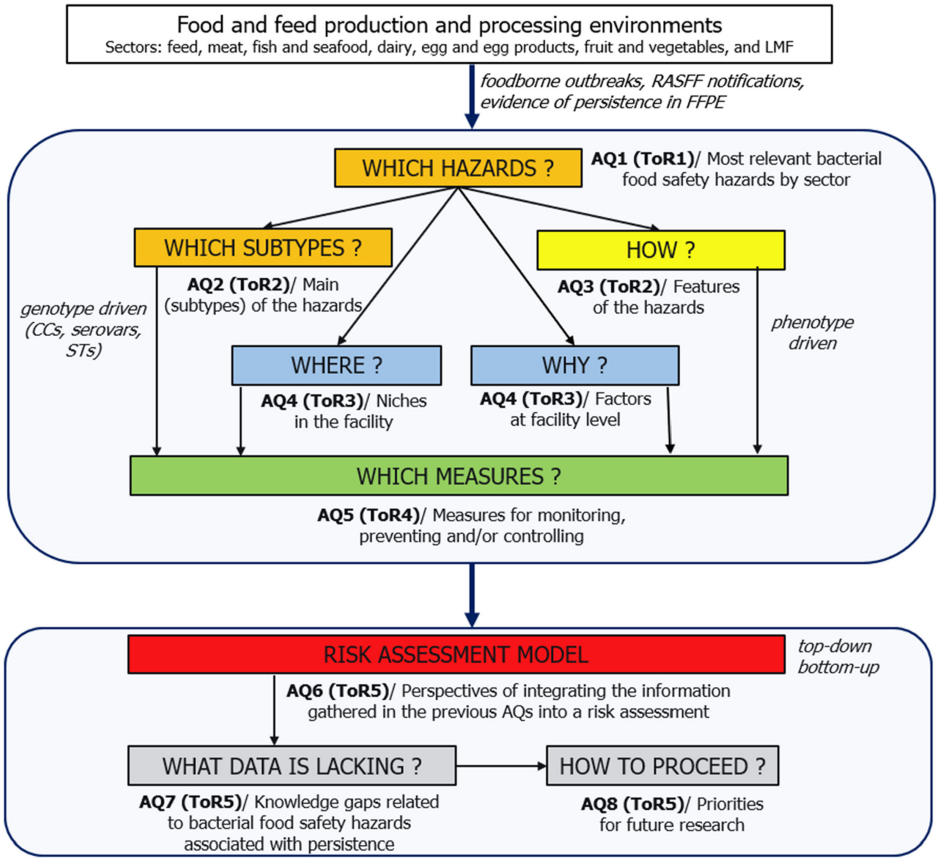

FIGURE 1 Conceptual map of the AQs to be addressed in the current assessment.

The AQs are as follows:

- **AQ1 (ToR1).** What are the most relevant bacterial food safety hazards associated with persistence in the FFPE of the various food and feed production and processing sectors in the EU/EEA?
- **AQ2 (ToR2).** Considering the most relevant bacterial hazards (from AQ1), what are the main (sub)types of these hazards involved in persistence in the specific sector?
- **AQ3 (ToR2).** What are the main bacterial features responsible for the persistence of the most relevant bacterial hazards/(sub)types (from AQ2) in the FFPE across sectors?
- **AQ4 (ToR3).** Considering the most relevant bacterial hazards (from AQ1), what are the factors at facility level that increase the likelihood of persistence in the FFPE?
- **AQ5 (ToR4).** Considering the most relevant bacterial hazards (from AQ1), what are the available and enhanced measures for monitoring, preventing and/or controlling their persistence in the FFPE?
- **AQ6 (ToR5).** What are the perspectives of integrating the information gathered in the previous AQs into a risk assessment?
- **AQ7 (ToR5).** What are the knowledge gaps related to bacterial food safety hazards associated with persistence in the FFPE?
- **AQ8 (ToR5).** What are the priorities for future research related to bacterial food safety hazards associated with persistence in the FFPE?

1.3 | Additional information

The approach to answer the ToR was defined in advance and is described in the protocol (Annex A). It covers both the problem formulation (i.e. what the assessment aims to address) and which methods will be used for addressing the problem. The problem formulation ('what') includes the clarification of the mandate (see further refined in Section 1.2) and consists of the steps (1) translation of the mandate into scientifically answerable AQs, (2) definition of the sub-questions (SQs) of each AQ, if needed, and their relationship (conceptual model) and (3) the selection of the approach for the assessment. The planning of the methods for conducting the assessment ('how') consists of (1) specifying the evidence needs and the methods for answering each AQ/SQ, including uncertainty analysis and (2) the methods for integrating evidence across AQs/SQs and addressing the remaining and overall uncertainty. Protocol development followed the draft framework for protocol development for EFSA's scientific assessments (EFSA, 2020).

2 | DATA AND METHODOLOGIES

2.1 | Most relevant bacterial food safety hazards associated with persistence in the FFPE (AQ1)

To identify the most relevant bacterial hazards associated with persistence in the FFPE, first the most relevant bacterial pathogens of public health (PH) relevance in the various sectors in the EU/EEA were listed. Then, for those most relevant pathogens, the evidence for their persistence in the FFPE of the corresponding sector was evaluated.

2.1.1 | Most relevant bacterial pathogens of public health relevance in the various food and feed production and processing sectors in the EU/EEA

2.1.1.1 | *Food production and processing sectors*

Bacterial pathogens of PH relevance in the various food sectors were identified based on FBO as derived from various sources and notifications in the Rapid Alert System for Food and Feed (RASFF) database. Some causative agents were excluded from the extracted data as defined in the clarifications to the ToR (i.e. viruses, parasitic protozoa, microbial toxins and other hazardous microbial metabolites such as histamine).

Strong evidence FBO at EU/EEA level. Data on 'strong evidence' FBO from 2010 to 2020 were extracted from the EFSA zoonoses database. The available epidemiological evidence was summarised by sector and causative agent, retrieving information on the number of outbreaks, number of cases, number of hospitalised cases and number of deaths. The food vehicle (i.e. the food or foodstuff, that is suspected of causing human cases) was categorised as follows: meat and meat products[3] (for meat processing), fish and fishery products[4] (for fish and seafood processing), fruit and vegetables and

[3]Bovine meat and products thereof; Broiler meat (*Gallus gallus*) and products thereof; Cooked cured (or seasoned) poultry meat; Meat and meat products; Meat from bovine animals – meat products; Meat from bovine animals – meat products – ready-to-eat; Meat from pig – fresh; Meat from pig – meat products – fresh raw sausages; Meat from poultry, unspecified – fresh; Meat from poultry, unspecified – meat products – non-ready-to-eat; Meat from wild boar – meat products; Meat from wild boar – meat products – fresh raw sausages; Meat, mixed meat – meat products – ready-to-eat; Other, mixed or unspecified poultry meat and products thereof; Pig meat and products thereof; Sheep meat and products thereof; Turkey meat and products thereof.

[4]Crustaceans, shellfish, molluscs and products thereof; Fish – smoked; Fish - smoked – hot-smoked; Fish and fish products; Live bivalve molluscs – oysters.

products thereof[5] (for fruit and vegetable processing), milk and milk products[6] (for dairy processing), egg and egg products[7] (for egg and egg products processing) or various other food groups such as bakery products, nuts or sweets (for LMF processing). Further information from other data fields, such as 'more food vehicle information' and 'contributory factors', was consulted, when available. More information about the reporting of FBO can be found in the technical report (EFSA, 2023).

Multi-country outbreaks in EU described in the Rapid Outbreak Assessments (ROA). Information was extracted from the ROA reports of the multi-country outbreaks (2012–2023 period) in EU related to the year and date of publication, incident, causative agent, suspected vehicle, number of cases, countries concerned and duration of the outbreak. Then they were categorised by sector based on the suspected vehicle and the causative agent was evaluated.

Multi-country outbreaks described in the joint notification summaries (JNS) as included in the annual RASSF reports. The JNS mentioned in the published annual RASSF reports between 2018 and 2020 were listed (European Commission, 2019, 2020, 2021). The outbreaks were categorised by sector and the evidence on the causative agent was evaluated.

RASFF notifications. Data were extracted from the RASFF database, considering the period 1 January 2010 to 11 July 2022, on the notification type 'food', the hazard category 'pathogenic microorganisms' and for each of the following product categories: (i) meat and meat products (other than poultry), poultry meat and poultry meat products (for meat processing), (ii) bivalve molluscs and products thereof, cephalopods and products thereof, crustaceans and products thereof, fish and products thereof (for fish and seafood processing), (iii) milk and milk products, excluding dairy powder and infant formula (for dairy processing), (iv) eggs and egg products, excluding egg powder (for egg and egg products processing), (v) fruits and vegetables and herbs and spices, excluding those dried (for fruit and vegetable processing), (vi) cereals and bakery products, cocoa and cocoa preparations, coffee and tea, confectionery, fats and oils, honey and royal jelly, nuts, nut products and seeds, prepared dishes and snacks, dried fruits and vegetables, vegetable oil/flour/powder manually selected from the fruits and vegetables group, dried herbs and spices, egg powder, dairy powder and infant formula (for LMF processing). Notifications of viruses and parasitic protozoa were excluded.

FBO published on websites from non-EU authorities and agencies and on the scientific literature. The following websites from authorities and agencies were consulted on 19 July 2022 for information related to foodborne outbreaks: Centers for Disease Control and Prevention (CDC) of the U.S. Department of Health & Human Services;[8] US Food and Drug Administration (US FDA);[9] Food Standards Australia New Zealand (FSANZ);[10] Public Health Agency of Canada.[11] In addition, the Eurosurveillance journal[12] was consulted for information related to foodborne outbreaks using the search term 'outbreak' in 'All fields'. The search included reports between 1 January 2010 and 3 October 2022. Both surveillance and outbreak reports were considered eligible article types. All outbreaks were categorised by sector and the evidence on the causative agent was evaluated.

For evidence integration, a list of bacterial pathogens that were found to be associated with FBO for each food sector was prepared, obtained from these sources of evidence and the most relevant pathogen(s) were identified based on the available data and the knowledge/expertise of the Working Group and Panel members.

2.1.1.2 | *Feed production and processing sectors*

For the feed sector, only data extracted from the RASFF database, considering the period 1 January 2010 to 11 July 2022, for the category feed materials, feed additives, feed premixtures, compound feeds (for feed for food processing animals) were considered. Also, a former scientific opinion of the BIOHAZ panel on Microbiological Risk Assessment in feeding stuffs for food-producing animals for both public health and animal health (EFSA BIOHAZ Panel, 2008) was consulted. For evidence integration, a list of bacterial pathogens that were found to be associated with feed was prepared and the most relevant pathogen(s) identified based on the available data and knowledge/expertise of the Working Group and Panel members.

2.1.2 | Persistence of bacterial pathogens of public health relevance in the FFPE of the various sectors

2.1.2.1 | *Food production and processing sectors*

The evidence for persistence of the most relevant bacterial pathogens in the FoPE of the corresponding sector was evaluated by assessing the evidence (a) of at least one outbreak linked to strains persisting in the FoPE and/or (b) that the bacterial hazard persists in the FoPE of plants from that particular sector.

[5]Fruit, berries and juices and other products thereof; Fruits – whole; Herbs and spices; Lentil sprouts; Vegetables; Vegetables – pre-cut; Vegetables and juices and other products thereof.

[6]Cheese; Cheeses made from cows' milk; Cheeses, made from unspecified milk or other animal milk – spreadable; Dairy products (other than cheeses); Milk; Milk, cows' – pasteurised milk; Milk, cows' – raw milk; Milk, goats' – raw milk; Milk, sheep's; Milk, sheep's – raw milk.

[7]Eggs; Eggs – raw material (liquid egg) for egg products; Eggs – table eggs – mixed whole; Eggs – table eggs – shell; Eggs and egg products.

[8]https://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html

[9]https://www.fda.gov/food/outbreaks-foodborne-illness/investigations-foodborne-illness-outbreaks#date-posted

[10]https://www.foodstandards.gov.au/industry/FoodIncidents/Pages/default.aspx

[11]https://www.canada.ca/en/public-health/services/public-health-notices.html

[12]https://www.eurosurveillance.org/search?value1=&option1=fulltext

For the first (point a), the FBOs in the ROA reports and JNSs and those in the websites from authorities and agencies and the Eurosurveillance journal were screened for evidence of persistence of the outbreak strain in the FoPE. Also, non-systematic searches to retrieve publications linked to relevant outbreaks were conducted and the knowledge of the Working Group and BIOHAZ Panel members was considered. Scientific publications of outbreak investigations found through the literature search (next point (b)) were also assessed.

For the second point (b), a literature search was carried out, restricted to publications post 2010. The search strategy followed is described in detail in Appendix A. Firstly, review articles including information on outbreaks directly linked to a hazard persisting in the FoPE, or on primary research studies demonstrating the persistence of hazards in processing plants, were identified and consulted. Then, experimental studies were considered relevant when these: (i) took place in a FoPE from one of the sectors considered, with description of the environment/plant;[13] (ii) included sampling of specified surfaces and microbiological testing of samples with reporting of results for each specific hazard; (iii) specifically referred to the following terms related to persistence: persistence, permanent, residence, recurrence, dispersal or other relevant terms; (iv) had matching subtypes for at least two sampling events over different time points; (v) used a molecular-based subtyping method, including either genotypic or spectroscopy/spectrometry based phenotypic methods.[14] Experimental studies were excluded when these (i) were conducted in an environment not relevant to this assessment;[15] (ii) described solely food product sampling (e.g. raw material or finished product), a predictive microbial model in a food matrix or an intervention in food products; (iii) presented laboratory studies conducted on lab-scale inoculated surfaces; (iv) did not have matching subtypes for at least two sampling events over different time points; (v) did not use one of the substantive subtyping methods referred to above. The evidence obtained was used to list the relevant bacterial pathogens identified per sector.

For selecting the most relevant pathogen(s), the following criteria were considered by expert judgement: available evidence on persistence and on outbreaks related to persistence, and on the PH relevance of the hazard (e.g. number of cases or mortality rate).

2.1.2.2 | *Feed production and processing sectors*

A specific literature search was carried out, following the search strategy described in Appendix A, to retrieve evidence that the bacterial pathogen persists in the FePE of plants from the feed sector. The most relevant pathogen(s) in the FePE were selected by expert judgement.

2.2 | Main (sub)types of the most relevant bacterial hazards involved in persistence and the main features responsible for their persistence in the FFPE (AQ2 and 3)

The literature search as described in Section 2.1.2 was used to identify the subtypes most frequently identified as involved in persistence and their main features. Reviews and primary research studies were considered. The grey literature was also consulted, for example reports from agencies. Additionally, information (when available) on the subtypes that have been found in the outbreaks linked to persistence (see Section 2.1.1) was retrieved.

Data were extracted for each of the primary research studies selected as relevant in relation to persistence of the hazards in the FFPE of processing plants on: the country where sampling took place, the sector and plant within the sector, the reason for sampling (outbreak related or not), the subtype identified, the location of persistence (i.e. non-food contact surface (NFCS) and/or food contact surface (FCS)) with further details, if available, the typing method(s) used, the features of the persistent strain, the factors influencing its persistence, the intervention(s) implemented, the number of sampling events, the sampling period and the timespan for persistence.

The evidence of relevance for replying to AQ2 and AQ3 was synthesised by listing all those subtypes identified as linked to persistence in the FFPE for each of the most relevant hazards (from AQ1) and for each sector. For *L. monocytogenes* and *C. sakazakii* (formerly *Enterobacter sakazakii*), the clonal complex (CC) of strains identified as persistent was recorded and presented, while for *S. enterica* it was the serotype of persistent strains.

For those subtypes most frequently involved in persistence, the main features possibly associated with persistence, as retrieved from the literature search, were identified and listed. Further references obtained through non-systematic specific literature searches were consulted to provide more insights into the role of some of the genetic markers identified as possibly linked to persistence.

The subtypes involved in the main clusters of related genome sequences of *L. monocytogenes*, *S. enterica* and *C. sakazakii* available in the National Center for Biotechnology Information (NCBI) Pathogen Detection database[16] were further analysed, considering their distribution by source and year of isolation. For *L. monocytogenes*[17] and *Salmonella*,[18] a minimum

[13]Small scale production facilities linked to a farm were also considered (e.g. an artisanal cheese production at a dairy farm).

[14]For example, AFLP, MLVA, MLVST, MLST, PFGE, RAPD, REA, Ribotyping, WGS-based methods, serotyping, spa typing, panC typing, Fla-typing, RFLP, FTIR or MALDI-TOF.

[15]For example, home kitchen, institutional kitchen, farm or retail.

[16]https://www.ncbi.nlm.nih.gov/pathogens/

[17]Listeria 2023-4-23; https://ftp.ncbi.nlm.nih.gov/pathogen/Results/Listeria/PDG000000001.3193/

[18]Salmonella 2023-7-30; https://ftp.ncbi.nlm.nih.gov/pathogen/Results/Salmonella/PDG000000002.2708/

threshold of at least 100 isolates per cluster was used to select clusters for further interrogation; in the case of *Cronobacter*,[19] the largest cluster contained only 67 isolates, and 24 clusters contained only up to 10 isolates. The NCBI Pathogen Detection database integrates genomes from numerous ongoing surveillance and research efforts whose sources include food, environmental sources, such as water or production facilities, and patient samples. The database designates single nucleotide polymorphism (SNP) clusters using two approaches: firstly, using a reference whole genome multi-locus sequence typing (wgMLST) scheme using a 25-allele cut-off to cluster related isolates; and secondly, using k-mer distances to first cluster related isolates, then a 50-SNP single-linkage clustering SNP analysis. For *L. monocytogenes*, further analyses on the carriage of persistence-, biofilm- and virulence-related genetic determinants were undertaken for a selection of clusters from subtypes more frequently reported as persistent in experimental studies in the literature, together with two clusters from subtypes that have not been associated with persistence yet. Genetic determinants were screened by BLAST analysis of the selected genetic markers against all available genome assemblies within the selected clusters. Clusters were extracted for analysis in June 2023.

2.3 | Factors at facility level that increase the likelihood of persistence of the most relevant bacterial hazards in the FFPE (AQ4)

The literature search as described in Section 2.1 was used to identify the site (or location) where the persistent strains were isolated and, if available, the niche (or harbourage site), as well as the risk factors at facility level that increased the likelihood of persistence of the most relevant bacterial hazards in the FFPE. Reviews, primary research studies and the grey literature were consulted. Additionally, the factors at facility level, when available, that have been found in the FBOs (from AQ1) were listed.

The evidence retrieved was synthesised, for each of the most relevant hazards (from AQ1), by listing the sites or niches of persistent strains within the plants. Next, those risk factors at facility level that increase the likelihood of persistence in the FFPE were described across production sectors and further, when relevant, specific to each of the sectors.

2.4 | Measures for monitoring, preventing and/or controlling the persistence of the most relevant bacterial hazards in the FFPE (AQ5)

The literature search as described in Section 2.1 was used to summarise the measures for monitoring, preventing and/or controlling the persistence of the most relevant bacterial hazards in the FFPE. Only experimental studies on measures or interventions assessed in industry settings were considered. Reviews and primary research studies were consulted. Grey literature was also consulted, for example reports from agencies, as well as sector-specific guidance documents. Additionally, the interventions (when that info was available) that have been used to control the problem in the FBOs (from AQ1) were listed.

In addition, relevant BIOHAZ scientific opinions were reviewed and referred to for the environmental monitoring and safety control measures in specific commodities (e.g. EFSA BIOHAZ Panel (2017, 2020)).

The evidence retrieved was synthesised by listing the measures for monitoring, preventing and/or controlling persistence in the FFPE. First, the daily measures included in the FSMS, including PRP activities, were addressed. Secondly, corrective measures triggered when a persistence event was detected were also addressed.

2.5 | Perspectives of integrating the information gathered in risk assessment (AQ6)

Potential risk assessment questions related to persistence in the FFPE were discussed between Working Group members and the relevant questions were selected, with feedback from Panel members. Approaches to answer these questions included the development of quantitative mathematical risk assessment modelling structures, based on existing risk assessment models available from the literature and/or known by the experts. A non-systematic literature review was performed to identify existing risk assessment models involving persistence in the FFPE. Both 'top-down' (or backward) and 'bottom-up' (or forward) approaches were explored, i.e. approaches where risks are assessed based on human disease data and approaches where risks are assessed based on pathogen occurrence in the food chain and the effect of food chain processes on hazards, including a dose–response (DR) model. For the 'bottom-up' approach, a 'persistence model' was proposed and the perspectives of its usage were studied.

Two case studies were defined, based on information gathered from the previous ToR and on discussion within the Working Group. For these, the bottom-up approach using the developed model structure was applied. Relevance of the hazard and of persistence in the FFPE, as well as availability of existing predictive models for growth in the food product and DR models, required to perform a risk assessment, were important considerations in the selection of the two case studies. Information gathered from ToR2 ((sub)types and features of persistent strains) was used to develop and test a strain

[19]Cronobacter 2023-7-27; https://ftp.ncbi.nlm.nih.gov/pathogen/Results/Cronobacter/PDG000000043.195/

specific risk assessment, where the potential association between for example growth potential, environmental resistance and virulence is included. Based on the case studies and the modelling structures, more generic conclusions were drawn on the data needs for risk assessment of microbiological food safety hazards associated with persistence in the FFPE.

Finally, as a last step, the collected data was compared to the data needs that were identified for risk assessment. This comparison was used to draw conclusions on the perspectives of performing risk assessments considering the role of persistence in the FFPE.

2.6 | Knowledge gaps and priorities for future research (AQ7 and 8)

Uncertainties linked to answering AQ1-6 were listed and used to formulate the knowledge gaps related to bacterial hazards associated with persistence in the FFPE (answering AQ7) based on expert knowledge (Working Group and BIOHAZ Panel members). Based on the identified knowledge gaps, research needs related to bacterial food safety hazards associated with persistence in the FFPE were identified and prioritised (answering AQ8), also based on expert knowledge.

2.7 | Uncertainty analysis

As recommended by the EFSA guidance and related principles and methods on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018a, 2018b), an uncertainty analysis was implemented. Given the nature and context of the ToRs of the mandate, the uncertainty analysis was restricted to an overview of the uncertainty sources affecting the different AQs (Appendix B). They describe the strengths and weaknesses of the collected evidence and served as a source of information for the discussion on knowledge gaps and research needs.

3 | ASSESSMENT

3.1 | Most relevant bacterial food safety hazards associated with persistence in the FFPE (AQ1)

3.1.1 | Most relevant bacterial pathogens of public health relevance in the various food and feed production and processing sectors in the EU/EEA

Appendix C includes the detailed assessment for each of the food and feed production and processing sectors on the bacterial pathogens which are of PH relevance in the EU/EEA. A summary is provided here below for each sector based on reported FBO and/or RASFF notifications and an overview of the pathogens selected as of highest relevance is presented in Table 1.

Serovars of *S. enterica* are the major hazard associated with microbial contamination of feed for food processing animals.

S. enterica subsp. *enterica* (especially serovars Enteritidis and Typhimurium), *L. monocytogenes*, human pathogenic *E. coli*, *C. jejuni* and *C. coli*, and *Clostridium perfringens* and *Clostridium botulinum* are the most relevant hazards associated with meat and meat products. *Salmonella* outbreaks were associated with meat and meat products from all animal origins. *L. monocytogenes* was mainly associated with RTE meat products, human pathogenic *E. coli* with bovine meat (products), *Campylobacter* with broiler meat (products) and *Clostridium* spp. with general meat products. *S. enterica* caused the highest number of cases and hospitalisations linked to FBOs; however, *L. monocytogenes* caused most deaths. *Clostridium* spp. and *Campylobacter* spp. were also associated with a high number of cases, but human pathogenic *E. coli* caused more hospitalisations than these two *Clostridium* species. Other biological hazards which have been involved in less frequent and/or severe reported outbreaks linked to meat and meat products are *Bacillus cereus* sensu *lato*, *Staphylococcus aureus* and *Yersinia enterocolitica*.

TABLE 1 Overview of the bacterial pathogens of the highest public health (PH) relevance in the various food and feed production and processing sectors in the EU/EEA (highlighted in grey).

	Pathogens of highest PH relevance in the sector						
Bacterial pathogen	**F**	**M**	**FS**	**D**	**E**	**FV**	**LMF**
Bacillus cereus sensu *lato*							
Campylobacter jejuni/coli							
Clostridium botulinum/perfringens							
Cronobacter sakazakii[a]							
Listeria monocytogenes							
Human pathogenic *E. coli*							

TABLE 1 (Continued)

Bacterial pathogen	Pathogens of highest PH relevance in the sector						
	F	M	FS	D	E	FV	LMF
Staphylococcus aureus							
Salmonella enterica							
Vibrio parahaemolyticus							

Abbreviations: F, feed for food animal production sector; M, meat sector, excluding low moisture food (LMF) products; FS, fish and seafood sector, excluding LMF products; D, dairy sector, excluding LMF products; E, egg sector, excluding LMF products; FV, fruit and vegetable sector, excluding LMF products; LMF, low moisture food sector.

[a]Formerly *Enterobacter sakazakii*.

S. enterica and *L. monocytogenes* are the most relevant bacterial hazards linked to fish and seafood products; these hazards caused most hospitalisations and deaths linked to FBOs in the EU/EEA since 2010, were also linked to FBOs outside the EU and were most often notified in the RASFF database. Outbreaks and notifications on *S. enterica* are associated with various fish and shellfish products and on *L. monocytogenes* mostly with cold smoked fish (salmon). *V. parahaemolyticus* has also a high PH relevance, as it has been involved in FBOs inside and outside the EU and has been the topic of various RASFF notifications linked to shellfish. Other bacterial hazards which have been involved in less frequent and/or less severe outbreaks linked to fish and seafood products are *B. cereus s. l.*, *C. botulinum* and *C. perfringens*, human pathogenic *E. coli* and *Campylobacter*.

S. enterica, *Campylobacter* spp., human pathogenic *E. coli*, *L. monocytogenes* and *S. aureus* are the most important bacterial hazards associated with milk and dairy products. *S. enterica*, *S. aureus*, pathogenic *E. coli* and *L. monocytogenes* caused most of the infections associated with cheese consumption, with the latter having the highest mortality. *Campylobacter* was the main pathogen in milk-related outbreaks, followed by *S. enterica* and human pathogenic *E. coli*. Other biological hazards associated with the contamination of raw milk, cheese and dairy products that have been linked less frequently to outbreaks are *B. cereus s. l., Brucella melitiensis, Mycobacterium bovis, C. perfringens, Yersinia pseudotuberculosis* and *Shigella flexneri*.

S. Enteritidis is the most relevant bacterial hazard linked to eggs and egg products, with other serovars of *S. enterica* being also causative agents of less frequent outbreaks. *S. enterica* caused the vast majority of infections, hospitalisations and notifications associated with eggs and egg products. *B. cereus s. l.* has been involved in less frequent and/or severe reported outbreaks linked to eggs and egg products, although detailed information on the food vehicle is in most cases not available to evaluate whether they can be linked to a LMF such as egg powder.

S. enterica, human pathogenic *E. coli* and *L. monocytogenes* are the most relevant bacterial hazards linked to whole fresh, fresh-cut or frozen fruits and vegetables, berries, juices and products thereof. This is in agreement with the most relevant hazards identified in the hazard prioritisation conducted based on EU reported outbreaks (2014–2020) in the scientific opinion on microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs) – Part 1 (EFSA BIOHAZ Panel, 2023). Despite the high number of outbreaks and associated cases due to *S. enterica*, their severity is rather low with no deaths reported, as opposed to human pathogenic *E. coli* and *L. monocytogenes* infections, which, despite the lower number of cases, caused more deaths. *B. cereus s. l.*, *Shigella sonnei*, *C. botulinum, C. perfringens*, *Campylobacter* spp., *Aeromonas hydrophila, S. aureus* and *Y. enterocolitica* have been involved in less frequent and/or severe reported outbreaks linked to fresh or frozen fruits and vegetables, berries, juices and products thereof.

S. enterica is the most relevant bacterial hazard linked to LMF, being frequently involved in outbreaks associated with confectionary products and snacks, chocolate products, nuts and nut products, cereals and grains, dried fruits and vegetables, seeds for consumption and powdered foods including infant formula. Other bacterial hazards linked to outbreaks in LMF and having also a high PH relevance are *B. cereus s. l.*, mainly in cereal-based products, especially cooked rice and pasta dishes, and spices and dried aromatic herbs; human pathogenic *E. coli*, mainly in cereal-based products (flours) and nuts and nut products; and *C. sakazakii*, in powdered infant formula. Other biological hazards which have been involved in less frequent and/or severe reported outbreaks linked to LMF are *Clostridium* (*C. perfringens and C. botulinum*), *S. aureus* and *L. monocytogenes*.

3.1.2 | Persistence of bacterial pathogens of public health relevance in the FFPE of the various sectors

3.1.2.1 | *Pathogens able to persist in the processing environment of the feed for food animal production sector*

The presence of *Salmonella* in animal feed has only rarely been directly linked to human cases of salmonellosis (Bonifait et al., 2022; Schroeder et al., 2016), possibly due to the number of steps between animal feed production and human consumption of the respective food of animal origin. Nevertheless, there is evidence of *Salmonella* strains found in feed also being found in the associated livestock consuming the feed (APHA, 2022). *Salmonella* strains have been associated with persistence in feed processing plants and compound feed mills, where some serovars can be repeatedly isolated for many years. The clones may persist on parts of the factory equipment or environment (Larsen et al., 2014; Nesse et al., 2003;

Vestby et al., 2009). There have also been indications of *Salmonella* growth in feed mill environments, such as floors, valves and cyclones (Binter et al., 2011), and in storage containers when moisture had accumulated (Binter et al., 2011).

According to a previous scientific opinion of the BIOHAZ panel (EFSA BIOHAZ Panel, 2008), the repeated and long-term isolation of certain serotypes in feed ingredients or compound feed has often been found to be the result of persistent contamination of crushing and feed-producing plants. This opinion highlighted that equipment used for cooling feed may become persistently contaminated by *Salmonella* as a result of intake of contaminated cooling air or passage of feed which has been incompletely heat treated, and that in situations when the post heat treatment feed processing equipment does become persistently contaminated by *Salmonella* it is common to find that finished products are more likely to be contaminated than the ingredients used to manufacture the feed.

3.1.2.2 | *Pathogens able to persist in the processing environment of the meat sector*

There are numerous documented outbreaks of human listeriosis linked to meat product contamination, and moreover with strong evidence of persistence of the *L. monocytogenes* outbreak strain in the associated FoPE. An outbreak in Germany lasting 5 years was linked to persistent contamination of the meat processing plant environment (Luth et al., 2020). Contaminated slicers, in a RTE meat processing plant, were the source of the strain implicated in an outbreak in Canada (Currie et al., 2015; Weatherill, 2009). Moreover, other listeriosis outbreaks linked to the meat FoPE have been possibly related with persistence but with no repeated isolation of the outbreak strain (Duranti et al., 2018; ECDC and EFSA, 2019b; Hachler et al., 2013; Lachmann et al., 2021). For example, a single RTE meat facility was linked to one of the biggest listeriosis outbreaks reported worldwide, in South Africa in 2017–2018, with the implicated strain being isolated from environmental samples collected at several facility sections (precooking and post-cooking) in a unique sampling occasion (Thomas et al., 2020). Collectively, there is strong evidence that *L. monocytogenes* can persist in the environment of meat products processing premises.

In the case of *Campylobacter* outbreaks, strong evidence is lacking regarding association with persistence in the meat FoPE. Weak evidence for persistence of an outbreak strain in a poultry slaughterhouse was found in an outbreak lasting several months (Joensen et al., 2021). Moreover, although *Campylobacter* has frequently been detected in environmental samples from pork and poultry slaughterhouses and processing plants (Quintana-Hayashi & Thakur, 2012; Torralbo et al., 2015), evidence of the same subtypes of *C. jejuni* being repeatedly isolated in poultry processing environments has been only reported by Melero et al. (2012) and Garcia-Sanchez et al. (2017).

Strong evidence of persistence of *S. enterica* in the meat processing environment linked to human salmonellosis outbreaks is lacking. Several outbreaks with weak evidence of environmental persistence have been reported, where no detailed environmental sampling or repeated isolation of the strain involved was reported (Bertrand et al., 2010; Gieraltowski et al., 2016; Hobbs et al., 2017; Kuhn et al., 2013; Wingstrand et al., 2012). Schroeder et al. (2016) reported the isolation of the outbreak strain, previously associated with an outbreak related to a feed processing plant, in the environment of a pork slaughterhouse in several sampling occasions after the C&D procedures. More generally, studies targeting the environment of poultry slaughterhouses suggest persistence of strains of *Salmonella* over time (Dantas et al., 2020) with subsequent dissemination to retail also noted (Shang et al., 2019), indicating potential routes of transmission of persistent strains through contaminated meat products to retail and the consumer.

Evidence of outbreaks related to persistent strains of human pathogenic *E. coli* is lacking. King et al. (2014) traced back an *E. coli* O157:H7 outbreak strain to a beef processing facility, however, no strong evidence of persistence was presented as no environmental sampling was reported. The literature lacks longitudinal sampling studies demonstrating the persistence of STEC in meat processing environments over prolonged timeframes.

There was no evidence of outbreaks of *C. perfringens* and *C. botulinum* linked to persistence. Some studies describe *C. perfringens* outbreaks related to episodes occurring after consumption of improperly reheated meat products in restaurants (Mellou et al., 2019; Wahl et al., 2013) and a catered lunch (Rinsky et al., 2016). There are limited published studies tracing these clostridial species in meat processing or identifying persistent contamination; although Jiang et al. (2022) demonstrated distribution of the same pulsotype across the FoPE and associated meat samples of beef slaughterhouses on single sampling occasions, persistent contamination of the FoPE over time was not identified.

3.1.2.3 | *Pathogens able to persist in the processing environment of the fish and seafood sector*

There have been several multi-year outbreaks of human listeriosis linked to cold smoked or gravad fish (mostly salmon) and two outbreaks linked to crab meat. In one multi-year outbreak in Germany linked to smoked/gravad salmon, the outbreak strain was repeatedly isolated from the processing environment over 2 years (Lachmann et al., 2022). For other outbreaks, outbreak strains have been found in the processing environments, but evidence for repeated isolation over time is lacking (ECDC and EFSA, 2019a; Lassen et al., 2016). There are multiple reports of *L. monocytogenes* persisting in fish processing environments (Fagerlund et al., 2022; Ferreira et al., 2014). As an example, investigations due to increased listeriosis cases in Finland revealed two fish production plants with persistent *L. monocytogenes* contamination (Nakari et al., 2014; Wulff et al., 2006).

No strong evidence for outbreaks of *S. enterica* linked to persistence in the processing environment of fish and seafood was found. Two outbreaks with *S.* Thompson lasting several months associated with raw and cooked seafood and smoked salmon, respectively, had a possible link to the processing environment, although repeated isolation of the outbreak strains from the FoPE was not documented[20] (Friesema et al., 2014). There is some evidence of persistence of *Salmonella* in

[20] https://www.cdc.gov/salmonella/thompson-10-21/details.html

processing plants (Wang et al., 2019). Isolation of the same pulsed-field gel electrophoresis (PFGE) types of *S*. Senftenberg over time was reported in several mussel processing plants (Martinez-Urtaza & Liebana, 2005). It is not entirely clear if this was due to reintroduction of the bacteria through contaminated salt/brine or semi-processed mussels, or due to persistence of the bacteria in the processing environment. Likewise, persistence (same PFGE types) of *S*. Stanley and *S*. Bareilly has been shown in a tilapia sashimi processing plant in Taiwan (Wang et al., 2019).

Most outbreaks of *V. parahaemolyticus* are linked to raw or undercooked seafood, usually shellfish (Haque et al., 2023). Cross-contamination from raw to RTE food is reported but no evidence for persistence in the FoPE was linked to outbreaks. Re-occurring infections or outbreaks over time with the same strain can be explained by persistence of the strain in marine habitats, not in the FoPE (Yang et al., 2022).

3.1.2.4 | *Pathogens able to persist in the processing environment of the dairy sector*

Persistent bacteria in the dairy sector are mainly described in relation to primary production and farmhouse cheesemakers. *S. aureus*, a contagious mastitis pathogen, has been frequently related to persistence in the bovine udder. Some *S. aureus* subtypes are widely spread among dairy cattle (e.g. CC151 and CC97) or have been described in dairy cattle from particular countries (e.g. CC705, CC398, CC479, CC8) (Campos et al., 2022). *S. aureus* genotype B (CC8) is highly prevalent in the alpine dairy industry and has been associated with human intoxications caused by the enterotoxins Sea, Sed and Sej (Johler et al., 2015). Methicillin resistant *S. aureus* (MRSA) are rarely reported in dairy animals. Studies indicate persistence of livestock associated ST1 and ST398 among dairy goats and cattle (Cortimiglia et al., 2016; Schnitt et al., 2020). Some *S. aureus* genotypes harbouring particular capsular polysaccharide types (e.g. cap 5) have been associated with a stronger potential for biofilm production in the udder tissue and food processing environments (Salimena et al., 2016). However, although *S. aureus* is described as a biofilm former with potential to persist on abiotic surfaces in the food and medical sectors (Abdallah et al., 2014; Miao et al., 2017), data from FCS or environmental samples in dairy industry settings are lacking.

There is strong evidence from listeriosis outbreaks linked to strains persisting in the processing environment in the dairy sector. A nationwide outbreak of human listeriosis linked to cheese processing in Switzerland was traced to persistent environmental contamination with the hypervirulent *L. monocytogenes* subgroup CC6 (Nüesch-Inderbinen et al., 2021). In Italy, Gorgonzola products were linked to human cases caused by *L. monocytogenes* epidemic hypervirulent clones from CC3 (Bergholz et al., 2016; Filipello et al., 2017). Of interest are studies that retrospectively clarify the cause of outbreaks across continents such as the study by Acciari et al. (2016), who sought the causes of *L. monocytogenes* contamination in traditional Italian cheese associated with US outbreaks in the country of origin. In 2012, a US multistate outbreak of listeriosis was linked to Ricotta Salata imported from Italy. The follow-up sampling identified the same PFGE type in the Italian cheese plant suggesting an event of persistence. *L. monocytogenes* persists preferentially in cheese production with surface ripening, being found in environmental samples and product-associated samples, such as cheese smear, brine, wash water and smear robot. This has been adequately described in retrospective studies using molecular epidemiological methods (WGS, PFGE) (Barría et al., 2020; Kaszoni-Rückerl et al., 2020; Melero, Stessl, et al., 2019; Muhterem-Uyar et al., 2018; Nüesch-Inderbinen et al., 2021; Stessl et al., 2014).

There are some studies assessing the prevalence of *Campylobacter* in milk. For example, in a Portuguese cattle farm, *C. jejuni* ST-21, ST-22, ST-206 and ST-403, all strongly associated with cattle and goat milk according to the PubMLST database,[21] were present at a low prevalence (4%; (Barata et al., 2022)). *C. jejuni* ST-883, associated with an outbreak, persisted in a milk tank for more than 7 months on a Finnish dairy farm (Jaakkonen et al., 2020). Nevertheless, overall, *Campylobacter* is most likely detected in raw milk by faecal contamination during milk collection, and persistence of *Campylobacter* in milk and cheese processing environments has not been described.

There are various studies reporting the occurrence of some *S. enterica* serotypes in primary production and dairy products. *S.* Dublin, one of the most common serovars in dairy cattle (Holschbach & Peek, 2018) and raw milk cheeses, can persist in some dairy facilities, which were also associated with regional outbreaks in France between 2015 and 2017 (De Sousa et al., 2022; Ung et al., 2019). In 2020, an outbreak of *S.* Enteritidis occurred in Central Italy, transmitted by Pecorino cheese. Cheese, bulk milk, faecal and environmental samples taken at the dairies linked the outbreak to potential short-term persistence of *S.* Enteritidis in the FoPE. However, the sources of the outbreak were infected sheep and their unpasteurised milk used for cheese processing (Napoleoni et al., 2021). Overall, there is a data gap regarding the survival and persistence of *S. enterica* in the dairy processing environment.

Considering human pathogenic *E. coli*, during 2013 an *E. coli* O157:H7 outbreak occurred in five Canadian provinces transmitted by contaminated Gouda cheese originating from a cheese processing facility in British Columbia. The raw milk was the primary source of *E. coli* O157:H7, which persisted during the production and at least during 60 days of ripening. This outbreak was the third outbreak caused by *E. coli* O157:H7 attributed to Gouda cheese made from raw milk in North America within a narrow timeframe. Pathogenic *E. coli* are introduced via raw milk and are detectable at various stages of ripening during cheese processing (Dos Santos Rosario et al., 2021; Rios et al., 2020), but there is a clear lack of data on sampling of pathogenic *E. coli* in the FoPE.

[21] https://pubmlst.org/

3.1.2.5 | *Pathogens able to persist in the processing environment of the egg sector*

Large salmonellosis outbreaks extended in time linked to consumption of eggs and egg products frequently occur (ECDC and EFSA, 2020a; EFSA and ECDC, 2017b). Such outbreaks are commonly associated with *Salmonella* persistence at farm level. This persistent contamination in laying hen farms has sometimes been reflected in contamination with the same strain of contact surfaces in the corresponding egg packhouses, which test more frequently positive when working with high-prevalence farms. This supports an association between the prevalence at the laying environment and at the packhouse egg contact surfaces (Kim et al., 2015; Kingsbury et al., 2019). However, no strong evidence was found of outbreaks directly linked to persistence of *S. enterica* in the processing environment of eggs and egg products processing plants (excluding LMF).

There is evidence that *S. enterica* can persist for long periods of time in the processing environment of egg products processing plants. For example, Jakociune et al. (2014) demonstrated that the continuous contamination of lightly pasteurised egg products with *S.* Tennessee at a large European producer of industrial egg products was caused by persistent contamination of the production facility. Likewise, Kim et al. (2015) found contamination by *S.* Bareilly in liquid egg samples produced by one of the eight egg-breaking plants sampled, due to contamination of the product line. The authors concluded that the contamination could have originated from a specific farm that provided shell eggs to the plant, and that the contaminating *Salmonella* could persist at the processing plant for a long period. Indeed, the contamination was not properly controlled in the product line, even after pasteurisation, which may indicate post-process contamination from the production environment.

3.1.2.6 | *Pathogens able to persist in the processing environment of the fruit and vegetable sector*

Outbreaks linked to enteric pathogens in fresh fruits and vegetables are traced to strains present in incoming raw materials at the processing plants or accumulated in the post-harvest process water and further disseminated in the processing and packing plant (Fatica & Schneider, 2011; Jung et al., 2014; Wang, 2019). Survival of strains in the FoPE also allows a long-term presence in the processing plants (Fatica & Schneider, 2011), especially in moist environments (Williamson et al., 2018), albeit without a clear implication of persistence as the causative link to outbreaks. Listeriosis outbreaks have been commonly linked to the presence of strains in dry or wet FCS (brushes, slicers, conveyor belts, etc.) and NFCS (e.g. drains, floors, coolers) (Chen et al., 2022; Estrada et al., 2020; Truchado et al., 2022).

Considering human pathogenic *E. coli*, the available evidence on the occurrence of this hazard in the fruit and vegetable sector suggest, but do not clearly establish, a link between outbreak strains and persistence in the FoPE. Similarly, there is limited (if any) systematic link of genetically related strains (judged by subtyping) to particular niches in the processing environment of the fruit and vegetable sector, that could explain persistence and thus, re-occurrence in the products. STEC O157:H7 lineage I/II has remained the dominant lineage in England since 1980s and recently IIc has become increasingly associated with consumption of fresh produce and particularly pre-packaged salads (Dallman et al., 2021), suggesting a potential for persistence in the raw materials and the processing environment. Nevertheless, in general, the majority of single or multi-country STEC or EPEC outbreaks linked to packaged leafy greens (e.g. rocket, lettuce or mixed salad), such as those in the UK (165 cases) and Finland (237 cases), are attributed to the raw materials, rather than the packing or processing plants.

Similarly to human pathogenic *E. coli*, the available outbreak and literature evidence on *S. enterica* implies, but it does not clearly establish a link of outbreak strains with persistence in FoPE. Neither does it demonstrate a clear long-term establishment of genetically similar strains in the environment of this sector. Most *Salmonella* outbreaks during 1973–2010 caused by various serovars present in tomatoes were attributed to strains in the packing house or the farm, yet, without clear source tracing (Bennett et al., 2015). In 2008, a *Salmonella* outbreak occurred in Finland with 77 cases linked to serovar Newport and 30 cases to serovar Reading, including one case with a double infection (Lienemann et al., 2011). However, whether packing house (particularly chopping) environments were the source of contamination was not clarified. In January 2022, the FDA published the report of the first domestic investigation of a foodborne salmonellosis outbreak caused by *S.* Typhimurium associated with leafy greens grown in a CEA indoor hydroponic[22] operation. Although the investigation did not result in the identification of the specific source or route of contamination of the leafy greens, as the outbreak strain was not recovered from inside of the greenhouse, the same strain was found in a water pond located close to the farm. The final report included an overview of the various factors that potentially contributed to the introduction and spread of pathogens of PH significance into the crop, which could serve as source of strains that may subsequently be established in the processing environment of fresh-cut products (FDA, 2022).

The finding of *L. monocytogenes* 4b, CC6, matching a multi-country FBO strain in frozen corn and other frozen vegetables produced during the 2016–2018 production seasons, at a freezing plant led to the suggestion that the outbreak strain could have been persisting in the FoPE of the plant after standard C&D procedures were carried out, in conjunction with periods of inactivity (EFSA and ECDC, 2018b). WGS revealed that most fresh produce outbreaks were associated with *L. monocytogenes* contamination originating from the processing environment and equipment (Chen, Burall, et al., 2016; Garner & Kathariou, 2016; Truchado et al., 2022). This suggests its ability to establish in various niches within a processing plant, e.g. brushes, blowers, blades, dryers (Ruiz-Llacsahuanga et al., 2021). Multiple reports

[22]A cultivation technique of plants, where plants are growing in the water environment without soil or any aggregate medium.

characterising the prevalence and genotypic diversity of *L. monocytogenes* strains in the processing environments of leafy greens, fruit trees and mushrooms have demonstrated the widespread occurrence of this organism in packing houses for months to years, as the likely cause of fresh produce outbreaks (Chen et al., 2014; Chen, Burall, et al., 2016; Lake et al., 2021; Pennone et al., 2018; Simonetti et al., 2021; Sullivan & Wiedmann, 2020; Truchado et al., 2022; Viswanath et al., 2013).

3.1.2.7 | *Pathogens able to persist in the processing environment of the LMF sector*

There is strong evidence from salmonellosis outbreaks linked to *S. enterica* strains persisting in the processing environment of some LMFs. Some examples are a *S.* Bareilly outbreak (2017–2018) associated with the consumption of a powdered egg product, where a massive contamination of the equipment of the spray-drying technology, which was confirmed as the contamination source, was found (Labska et al., 2021). Another example is a *S.* Poona outbreak (2018–2019), associated with consumption of rice-based infant formula, where the outbreak isolates were linked by WGS to a 2010–2011 *S.* Poona outbreak associated with formula manufactured in the same facility, indicating a persistent contamination source. A drying tower was identified as the source of contamination (Jones et al., 2019). Also, a *S.* Mbandaka outbreak (2018), linked to sweetened puffed wheat cereal, where the thorough investigation of the manufacturing facility identified persistent environmental contamination with strains closely related genetically to the outbreak strain (Keaton et al., 2022). At last, a *S.* Agona outbreak (2008), associated with toasted oats cereal, where the outbreak isolates were linked by PFGE and WGS to a 1998 *S.* Agona outbreak associated with cereal manufactured in the same facility, indicating a persistent source of contamination (Hoffmann et al., 2020; Russo et al., 2013).

No strong evidence was found on outbreaks directly linked to *C. sakazakii* strains persisting in the LMF processing environment. However, various studies have reported the isolation of *C. sakazakii* strains of the same pulsotype or sequence type from processing equipment and finished product in powdered infant formula processing plants, with indistinguishable strains being recovered from the production environment for few months up to 4 years (Craven et al., 2010; Forsythe, 2013; Jacobs et al., 2011; Power et al., 2013). Therefore, there is sufficient evidence that *Cronobacter* spp. can persist in dry food processing and preparation environments, particularly in those of infant formula processing plants.

These observations are in agreement with the conclusions of (ILSI, 2011), where several examples of the capacity of *S. enterica* and *C. sakazakii* to survive in dry environments for long periods of time were highlighted, suggesting cross-contamination and persistence in the manufacturing areas.

Due to its capacity to form endospores *B. cereus s. l.* species may withstand harsh treatments including evaporation, drying and disinfection and survive throughout processing. In addition, *B. cereus s. l.* can produce long-lasting and hard to remove biofilms in and on equipment. Indeed, some environmental monitoring studies at LMF (mainly powdered infant formula) processing plants have reported its frequent isolation in FPPE, with high prevalence (in the range of 30%–40%) in drying and packaging areas (Liu et al., 2018; Zhuang et al., 2019). It is generally acknowledged that *B. cereus s. l.* and other spore-forming bacteria are ubiquitous and their presence in raw materials and FPPE appears to be inevitable. However, despite their capacity to colonise surfaces and equipment, no evidence was found on outbreaks directly linked to *B. cereus s. l.* strains persisting in the processing environment of LMF and very limited information was collected from studies subtyping strains recovered from FoPE and demonstrating environmental persistence of the hazard for short periods of time (Liu et al., 2018; Zhuang et al., 2019).

E. coli can be present in the FoPE, where it is commonly regarded as an important indicator of manufacturing hygiene (Xi et al., 2015). Some studies have revealed the occurrence of human pathogenic *E. coli* in the processing environment of some LMF, such as dairy powder factory environments (Duffy et al., 2009). However, no evidence was found on outbreaks directly linked to human pathogenic *E. coli* strains persisting in the processing environment of LMF and there is paucity of studies subtyping *E. coli* strains recovered along time from particular FoPE.

3.1.3 | Concluding remarks related to the most relevant bacterial food safety hazards associated with persistence

- With the available evidence, the bacterial hazards of highest PH relevance in the various food and feed production and processing sectors in the EU/EEA assessed as most relevant for persistence in the respective FFPE of these sectors can be found in Table 2.
- For example, *S. enterica* was considered among the most relevant bacterial food safety hazards associated with persistence in the FFPE of the feed for animal food production sector, the meat sector, the egg sector and the LMF sector.

TABLE 2 Overview of bacterial hazards of the highest public health (PH) relevance in the various food and feed production and processing sectors in the EU/EEA indicating which of those have been assessed as most relevant for persistence in the respective FFPE of these sectors.

	Pathogens of highest PH relevance in sector and/or persisting in the FFPE of sector						
Bacterial pathogen	**F**	**M**	**FS**	**D**	**E**	**FV**	**LMF**
Bacillus cereus sensu *lato*							
Campylobacter jejuni/coli							
Clostridium botulinum/perfringens							
Cronobacter sakasakii							
Listeria monocytogenes							
Pathogenic *E. coli*							
Staphylococcus aureus							
Salmonella enterica							
Vibrio parahaemolyticus							

Notes: Orange cells: bacterial pathogens of highest PH relevance in the specified/specific sector but not considered as most relevant bacterial food safety hazards associated with persistence in the FFPE in the specified/specific sector; Red cells: bacterial pathogens of highest PH relevance and considered as most relevant bacterial food safety hazards associated with persistence in the FFPE in the specified/specific sector; blank cells: bacterial pathogens not considered of highest PH relevance in the specified/specific sector.

Abbreviations: F, feed for food animal production sector; M, meat sector, excluding low moisture food (LMF) products; FS, fish and seafood sector, excluding LMF products; D, dairy sector, excluding LMF products; E, egg sector, excluding LMF products; FV, fruit and vegetable sector, excluding LMF products; LMF, low moisture food sector.

3.2 | Main (sub)types of the most relevant bacterial hazards involved in persistence and the main features responsible for their persistence in the FFPE (AQ2 and 3)

A wide range of subtypes of *L. monocytogenes*, *S. enterica* and/or *C. sakazakii* were identified as involved in persistence in the FFPE from the diversity of experimental studies as retrieved from the literature search. Of note is that the individual sampling approach used in each retrieved study is a source of uncertainty. Studies showed a variation in sampling methodology, period of analysis and typing methodology, and used different definitions of persistence, whereas in this assessment the harmonised criterion used for persistence evidence were matching subtypes of the specific zoonotic pathogen for at least two sampling events of the FFPE at different time points. Thus, for example, if multiple sources, including raw materials, are not included in the longitudinal samplings, the attribution of certain subtypes to persistence may be uncertain, since some widely distributed subtypes can be repeatedly introduced by raw materials.

Some of the experimental studies reported persistence-related features of the specific hazards or assessed particular genotypic or phenotypic characteristics of persistent isolates recovered from the FFPE. While the information on features available for *L. monocytogenes* was quite vast, more limited evidence was found for *S. enterica* and *C. sakazakii*.

Many studies compared a subset of persistent and presumed non-persistent strains in relation to some phenotypes sometimes with contradictory conclusions. This could be due to the fact that they use strains from different subtypes, that a presumed non-persistent strain could in some circumstances also have the capability to become persistent, or even to the different experimental conditions used in different studies.

The following subsections summarise the evidence obtained from the retrieved studies on the main subtypes and features linked to persistence of the most relevant bacterial hazards identified in AQ1 (i.e. *L. monocytogenes*, *S. enterica*, *C. sakazakii*). Further studies were only consulted to provide more insights into the role of some of the genetic markers identified as possibly linked to persistence (identified only in *L. monocytogenes*).

3.2.1 | *L. monocytogenes* subtypes and features

3.2.1.1 | *Subtypes linked to persistence*

L. monocytogenes forms a structured population consisting of four divergent lineages (I– IV). The genetic lineages have distinct, although at times overlapping, genetic, phenotypic and epidemiological characteristics, with the majority of human illness being caused by strains in lineages I and II (Painset et al., 2019).

To detect genetic similarities between potentially persistent *L. monocytogenes* isolates, the subtyping method must be of high resolution, such as the former gold standard PFGE and currently WGS. The use of different typing methods makes it difficult to compare persisting subtypes between different studies. An exception is the use of sequence-based typing methods, where the reporting of persistent CC and/or ST allows to get an overview of the subtypes involved in persistence. Currently, there are three approaches to compare *L. monocytogenes* in a food facility using WGS-based subtyping methods. The first two methods are based on the determination of core genome (cg) and/or whole genome (wg) MLST, where scoring is based on allelic similarity. Strains with less than 10 different alleles in the cgMLST analysis are counted as one genetic complex and are assigned to a clonal type (CT) (Nüesch-Inderbinen et al., 2021; Stoller et al., 2019). The third approach is based on reference genome comparison and SNP scoring. For example, isolates with than 25 SNP differences are assigned to persistent clones in an epidemiologic case (Pasquali et al., 2018).

The literature search confirmed a high number of subtypes being involved in persistence, with a total of 36 persisting CC types (of the total of 262 CC present in the Institute Pasteur MLST database[23]), belonging to two lineages. A wide range of CC types were found persisting in each of the sectors, ranging from 24 CC types in the meat sector to 10 CC types in the dairy sector and 2 CC types in the LMF sector. Overall, for all sectors, at the lineage level, persistence was mostly reported for lineage II (129 cases of persistence retrieved in the literature search), followed by lineage I (56 cases) (Figure 2A,B). The most reported persistent CC types were CC5 (17 cases), CC2 and CC6 (8 cases), of genetic lineage I, and CC121 (22 cases), CC8 (19 cases) and CC9 (18 cases), of lineage II. Twelve individual CC types were found associated with persistence in a single processing plant.

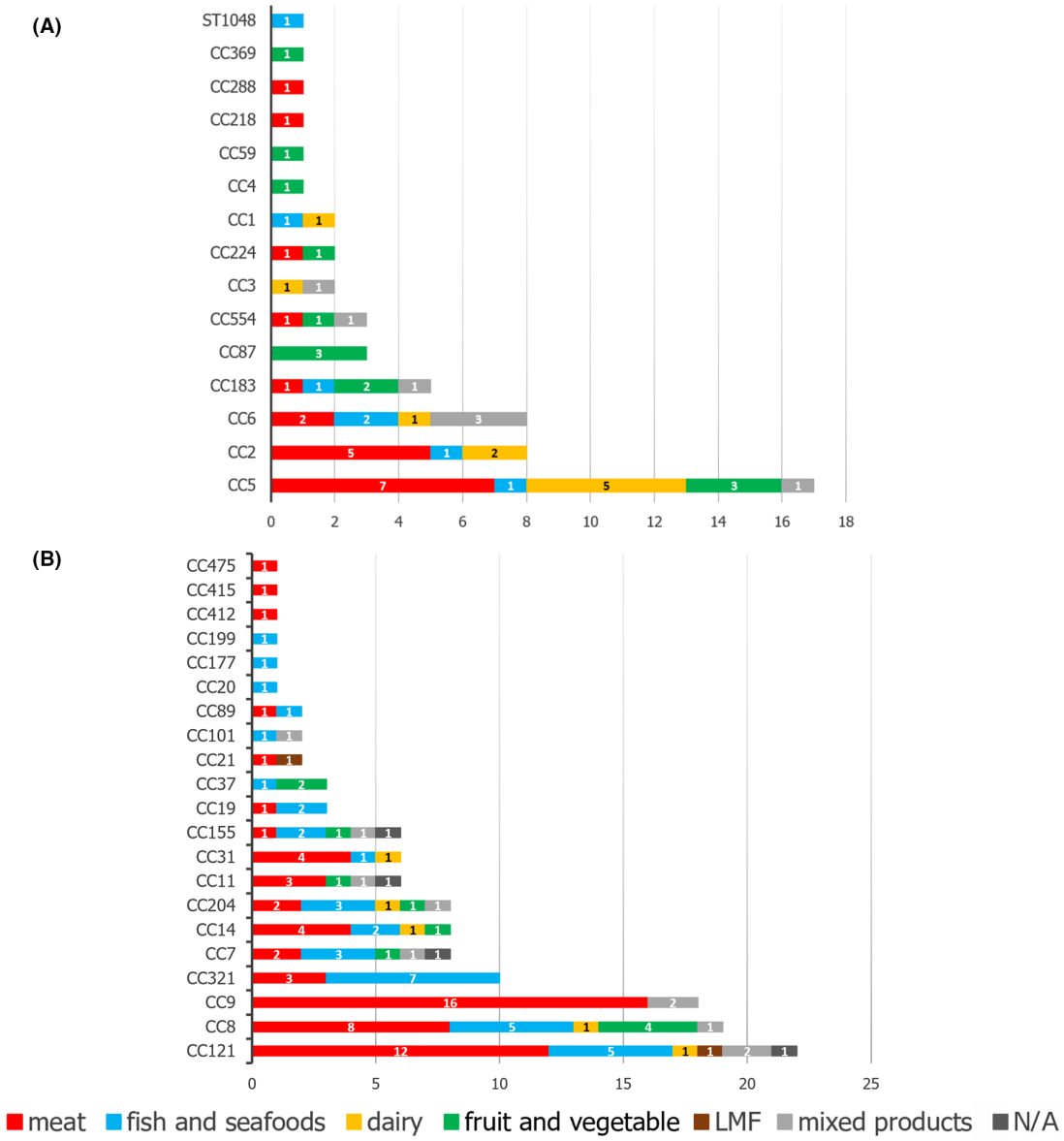

FIGURE 2 Overview of the different *L. monocytogenes* subtypes (clonal complexes; CC) persisting in the FoPE of each sector. (A) Genetic lineage I CC associated with environmental persistence; (B) Genetic lineage II CC associated with environmental persistence. *Note*: Based on (Brown et al., 2021; Burnett et al., 2022; Centorotola et al., 2021; Chase et al., 2017; Chen et al., 2017; Chen et al., 2022; Chen, Burall, et al., 2016; Cherifi et al., 2018; Chiara et al., 2014; Conficoni et al., 2016; Corcoran et al., 2013; Daeschel et al., 2022; De Cesare et al., 2017; Demaitre et al., 2021; Demaître et al., 2021; Elson et al., 2019; Fagerlund et al., 2016; Fagerlund et al., 2020; Fei et al., 2015; Gan et al., 2021; Gelbicova et al., 2018; Gelbícová et al., 2019; Guidi et al., 2021; Guidi et al., 2022; Harrand et al., 2020; Holch et al., 2013; Hurley et al., 2019; Jensen et al., 2016; Kaszoni-Rückerl et al., 2020; Knudsen et al., 2017; Kovacevic et al., 2016; Lachmann et al., 2021; Lachmann et al., 2022; Lake et al., 2021; Lassen et al., 2016; Lee et al., 2019; Liu et al., 2022; Louha et al., 2020; Lu et al., 2019; Luth et al., 2020; Madden et al., 2018; Maggio et al., 2021; Maurella et al., 2018; McLauchlin et al., 2020; Melero, Manso, et al., 2019; Melero, Stessl, et al., 2019; Mohan et al., 2021; Moretro et al., 2017; Muhterem-Uyar et al., 2018; Negrete et al., 2022; Nilsson et al., 2012; Nowak et al., 2017; Nowak et al., 2021; Nüesch-Inderbinen et al., 2021; Ortiz et al., 2016; Oswaldi et al., 2022; Oxaran et al., 2017; Palaiodimou et al., 2021; Palma et al., 2017; Palma et al., 2020; Pasquali et al., 2018; Pei et al., 2019; Pérez-Baltar et al., 2021; Ruckerl et al., 2014; Self et al., 2019; Shedleur-Bourguignon et al., 2021; Smith et al., 2019; Stessl et al., 2020; Stoller et al., 2019; Sun et al., 2021; Thomassen et al., 2021; Tirloni et al., 2020; Truchado et al., 2022; Veghova et al., 2017; Yan et al., 2015; Yang et al., 2020; Zhang et al., 2021; Zuber et al., 2019). The numbers indicate the cases of persistence being identified from the studies retrieved through the literature search for each CC and sector.

[23] https://bigsdb.pasteur.fr/listeria/, accessed on the 16 October 2023.

Some clonal complexes appear to be widely distributed across several sectors: e.g. CC5, CC8, CC121 and CC204 in five sectors (Figure 2A,B). CC9 was mainly attributed to the meat sector and was not found persisting in the fish and seafood sector. CC121, CC2 and CC6 were frequently found persisting in plants from at least three different sectors but were not reported in the fruit and vegetable sector.

The relative prevalence in humans versus food varies between *L. monocytogenes* subtypes. In a large European study with 1143 isolates, a higher relative prevalence of lineage II isolates, especially CC121 and CC9, was reported in foods than in humans, while the opposite distribution was found for lineage I isolates, especially CC1 and CC4 (Møller Nielsen et al., 2017; Painset et al., 2019). Similar findings have been reported by others, and it has been suggested that certain lineage I subtypes, such as CC1, CC4, CC6 are hypervirulent, because they are more common among human isolates, showing high virulence in in vitro studies, and contain certain specific virulence factors (e.g. internalin genes *inlG* and *inlL*, *Listeria* pathogenicity islands LIPI-3 and LIPI-4, *lapB* and *vip* genes), while certain lineage II subtypes, especially CC121, CC9 and CC31, are often associated with hypovirulence (truncated *inlA* gene, lacking specific virulence factors) and are more common among food isolates (Maury et al., 2019; Muchaamba et al., 2022; Schiavano et al., 2022; Vazquez-Boland et al., 2020). Nevertheless, *L. monocytogenes* infection is not solely based on the genetic prerequisites of strains (e.g. the presence of a functional InlA), but also involves interaction with the immune system. As a result, even hypovirulent strains can cause infection in immunocompromised individuals (Schiavano et al., 2022).

Despite isolates of lineage II are found most often to persist, also lineage I isolates have been shown to persist based on the literature search (129 vs. 56 reports). CC5 was the single most persisting CC of this lineage, but also subtypes frequently associated with outbreaks and human illness, such as CC1, CC4 and CC6, have been reported to persist in the food industry.

Examples of FBOs that have been proven to be linked to persistent environmental contamination are two multi-year outbreaks in England of CC1 and CC2 linked to crab meat (Elson et al., 2019) and a CC19 outbreak linked to salmon in Germany (Lachmann et al., 2022). In addition, long-lasting (multi-year) outbreaks where the outbreak strain has been found in the FoPE at a single occasion/sampling (or where it is unclear if found more than once) are also hypothesised to be likely due to persistence of *L. monocytogenes* in the FoPE. Examples of such outbreaks are a CC5 outbreak in USA linked to ice cream (Chen et al., 2017), the large CC6 outbreak associated with meat products in South Africa (Thomas et al., 2020), a CC6 outbreak in the UK linked to meat products (McLauchlin et al., 2020), a CC6 outbreak in Switzerland linked to soft cheese (Nüesch-Inderbinen et al., 2021), a CC6 outbreak related to frozen corn (McLauchlin et al., 2021; Sarno et al., 2021), two outbreaks of CC89 (ST391) and CC6 linked to smoked fish in Denmark (Lassen et al., 2016), and two CC8 outbreaks linked to turkey meat in the Czech republic (Gelbicova et al., 2018) and RTE meat products in Germany (Lachmann et al., 2021), respectively.

Among the *L. monocytogenes* subtypes identified as persistent in the retrieved literature (Figure 2), all but four have been linked to human clinical illness among isolates in the Institute Pasteur database (the exceptions being CC183, CC554, CC382 and ST1048). Interestingly, CC183 has been associated with recent outbreaks of listeriosis in the US and may appear to be a potential emerging clonal subgroup of *L. monocytogenes* of PH importance (Gorski et al., 2022; Kayode & Okoh, 2022). Previous studies have examined CCs over-represented among human clinical cases of illness, or those with a higher proportion of clinically derived isolates among the overall isolate population of a given CC group. Considering the top CCs here identified as persistent from lineage I and lineage II groups, all those from lineage I have been implicated as important in human clinical listeriosis (i.e. CC2, CC5 and CC6). In the case of the top five CC from lineage II (i.e. CC7, CC8, CC9, CC121 and CC321), only two of these were among the top 10 human clinical CCs identified in France (CC9 and CC121) (Maury et al., 2016); in addition to these, CC7 and CC8 were represented among those related to human clinical disease linked to RTE foods in the EU (EFSA BIOHAZ Panel, 2018). Collectively, this highlights how persistent contamination can have important PH implications, as these CC most frequently implicated in persistence are clearly important clinical subgroups.

3.2.1.2 | *Features associated with persistence and link to subtypes*

The persistence of *L. monocytogenes* in the FoPE is the most studied among foodborne pathogens. Several studies have focused on finding and understanding the characteristics that allow *L. monocytogenes* to survive in the FoPE for long periods of time, mostly related to the adaptability to physico-chemical conditions and to the identification of genetic markers associated with increased survival capacity (EFSA BIOHAZ Panel, 2018).

The BIOHAZ Panel concluded in 2018 that persistence of *L. monocytogenes* in FoPE is an often observed and important phenomenon for the contamination of RTE foods. It was highlighted that some hypovirulent molecular subtypes such as CC121 seem to encompass multiple isolates with a proven capability to persist too. It was also acknowledged that whether persistence is a result of improper hygiene conditions or more the effect of strains equipped with an arsenal of genetic determinants is under debate; and that a high adaptive capacity against physical–chemical factors and biofilm-forming capacity could partly explain the persistence phenomenon. The former EFSA BIOHAZ Panel opinion also identified the following genetic markers as possibly linked to persistence of *L. monocytogenes*: a transposon (Tn6188) and the *bcrABC* cassette, associated with tolerance against some disinfectants, and the hypervariable genetic hotspot *lmo0443-lmo0449*, which appears to play a role in stress response as it may harbour two independently acting stress survival islets (either SSI-1 or SSI-2).

As a general characteristic of *L. monocytogenes*, survival or even growth at low temperatures is possible due to an increase in the concentration of unsaturated fatty acids in the cell membrane, that prevents the formation of a gel-like state that could lead to leakage of cytoplasmic content (Beales, 2004). The expression of cold shock proteins (Csp), which act as molecular chaperones enabling replication, transcription, translation and protein folding at low temperatures has also been documented (Eshwar et al., 2017). Additionally, the σ^B-mediated accumulation of cryoprotectants, such as betaine and

carnitine, confers growth phase-dependent adaptation at low temperatures (Angelidis & Smith, 2003; Becker et al., 2000). However, the ability to adapt to low temperatures has not been demonstrated to be enhanced in persistent strains, as no differences in growth rate have been observed between persistent and presumed non-persistent strains at cold temperatures (4°C and 11°C) (Cabrita et al., 2015; Magalhaes et al., 2016).

L. monocytogenes is also able to respond positively to other stresses. Its response to osmotic stress is mainly mediated by the uptake of potassium cations, glutamate and osmoprotectants, such as glycine-betaine and carnitine, both of which contribute to maintaining turgidity and help in the stabilisation of protein structure and function (Matereke & Okoh, 2020). In the absence of osmoprotectans, tolerance to high osmolarity is associated with the expression of the protein Ctc (Gardan et al., 2003). Although the pathogen presents this general characteristic, its role in persistence is still controversial. (Harrand et al., 2020) showed that the target strains in a persistent cluster did not grow faster than those included in a non-persistent cluster at 6.5% of NaCl. Contrary to this, (Magalhaes et al., 2016) reported a shorter lag phase and higher growth rate in a set of persistent strains compared to presumed non-persistent strains when subjected to 8% NaCl.

Generally, the ability to overcome acidic stress and maintain homeostasis is mediated by the acid tolerance response (ATR), glutamate decarboxylase (GAD), arginine deiminase (ADI) and F_1F_0-ATPase systems (Wiktorczyk-Kapischke et al., 2021). Albeit a higher growth rate in acidic conditions of persistent strains from cheese processing plants at pH 5 (Magalhaes et al., 2016) and higher tolerance of persistent strains from meat processing plants at pH 2.4 (Lundén et al., 2008) have been reported, Harrand et al. (2020) showed a lower growth rate of persistent strains from a salmon processing facility at pH 5.5 than that of the presumed non-persistent ones. Regarding alkaline stress, *L. monocytogenes* also has the capacity to withstand high pH by the increased production of acids and induction of transporters and enzymes responsible for proton retention and cell surface modifications (Soni et al., 2011).

The ability to adapt to oxidative stress is mediated through the expression of σ^B, cold and heat shock proteins, proteases (ClpC, ClpP and GroEL), reactive oxygen species (ROS) detoxification systems, such as catalase (Cat), superoxide dismutase (Sod) and alkyl hydroperoxidase (AhpCF), and the ferritin-like protein (fri) (Bucur et al., 2018). However, conflicting results have been obtained regarding the behaviour of persistent and presumed non-persistent strains against oxidative stress. Manso et al. (2020) reported that a persistent strain (from CC9) isolated in a meat processing plant was more resistant to cumene hydroperoxide (CHP) at 37°C than a presumed non-persistent strain from the same CC isolated in a cheese processing plant, but the opposite occurred with two strains belonging to CC5. Moreover, when the same oxidising agent was applied at 10°C no differences were found between persistent and presumed non-persistent strains. In line with these results, Harrand et al. (2020) observed no differences between persistent and presumed non-persistent strains when exposed to 10 mM CHP at 37°C.

Stress survival islet 1 (SSI-1) or stress survival islet 2 (SSI-2) have been detected in persistent *L. monocytogenes* CC (Tables 3 and 4). SSI-1 (an 8.7-kbp region consisting of five genes: *lmo0444, lmo0445, pva, gadD1* and *gadT1*) has been shown to improve survival at high salt, bile and acid conditions and is widespread among genetic lineage I and II CC (Ryan et al., 2010). Nevertheless, Harrand et al. (2020) reported that a persistent CC321, carrying the SSI-1 in the genome, was slower to grow at pH 5.5 and 6.5% NaCl than presumed non-persistent strains lacking this genetic trait. The presence of SSI-2 (comprising *lin0464*, coding for a putative transcriptional regulator and *lin0465*, encoding an intracellular PfpI protease) in *L. monocytogenes* CC121 and CC31 enables them to overcome high pH and oxidative stress conditions (Harter et al., 2017).

Apart from the capacity to withstand a wide range of stresses, *L. monocytogenes* also has the ability to form biofilms on materials normally used in the FoPE, such as stainless steel, rubber, polystyrene, glass or polytetrafluorethylene (Nowak et al., 2021; Sinde & Carballo, 2000). However, depending on the test method used, different results have been observed (Osek et al., 2022). *L. monocytogenes* is a relatively weak biofilm former in comparison to many other bacterial species, and environmental conditions and the microbiota present in the biofilm have a significant impact on biofilm formation by *L. monocytogenes* (Rodríguez-López et al., 2018). Interactions with other microorganisms, such as *Pseudomonas, Acinetobacter* or *Janthinobacterium*, can have an important effect on listerial biofilm formation (Finn et al., 2023; Zwirzitz et al., 2021). The occurrence of persistent *L. monocytogenes* adapted to sublethal concentrations of biocides in the FoPE has not been well studied. However, it has been shown that *L. monocytogenes* cells exposed to biocides can transform into a persistent state with a higher frequency in mature biofilms (Byun & Kim, 2023). Virulence markers (flaA, ActA, InlA and InlB) and their regulator operon *prfA* are important for biofilm development, as shown in deletion mutants that are unable to form biofilms. In addition, the transcriptional regulator of stress response genes, SigB, is also required in the later stages of biofilm development. The role of the biofilm associated protein (BapL) is not well understood and is controversially associated with different serotypes. Truncation of *inlA* has been associated with increased biofilm production (Franciosa et al., 2009), whereas *inlL* mutants showed decreased attachment to surfaces. Myintzaw et al. (2023) identified the presence of *bapL* to be specific in CC121, CC14, CC204, CC9 and CC20, while *inlL* was exclusively present in CC155, CC26, CC37, CC18, CC204, CC20, CC412 and CC7.

The role of the *agrBDCA* operon of the signal peptide-based sensing system in biofilm development by *L. monocytogenes* has been questioned, while the LuxS system is important, with mutations in *luxS* leading to denser biofilms. The DNA repair and defence protein (RecO) and a putative cell wall binding protein (Lmo2504) were shown to be overexpressed in biofilms. In addition, prophage insertions into the *comK* gene are associated with enhanced biofilm production, as demonstrated in vitro with strains from a 12-year persistent epidemic clone (Colagiorgi et al., 2017; Finn et al., 2023; Orsi et al., 2008; Verghese et al., 2011).

In the literature, higher biofilm formation by persistent strains, as compared to presumed non-persistent ones, has been reported in polystyrene at 30°C for 48 h (Nowak et al., 2017) and at 37°C for 24 h, with persistent strains isolated in

a poultry processing plant (Rodríguez-Campos et al., 2019). However, Nilsson et al. (2011) observed no differences in biofilm formation between persistent and presumed non-persistent strains in polystyrene after 24 h at 10°C, 20°C, 25°C or 37°C. In addition, conflicting results were obtained with persistent and presumed non-persistent strains isolated from a poultry processing plant in Spain, where the persistent CC121 and CC9 were able to form biofilm in polystyrene as the presumed non-persistent CC1 and CC87, however the better biofilm former was the presumed non-persistent ST199 (Manso et al., 2020).

Resistance to heavy metals such as cadmium and arsenic are encoded by genetic markers often located on mobile genetic elements, mainly plasmids. The *cadA1*, *cadA2*, *cadA3* and *cadA4* genes have been associated with cadmium resistance, with the first two localised on transposon Tn5422 and plasmid pLM80, respectively, and associated with persistence (Hingston et al., 2019; Nelson et al., 2004; Osek et al., 2022). *Listeria* Genomic Island 2 (LGI2) harbours an arsenic resistance operon (*arsR1D2R2A2B1B2*) and Tn544 contains the arsenic resistance cassette *arsRDABC* (Kuenne et al., 2013; Nelson et al., 2004) that is correlated with cadmium resistance as it also contains the *cadA4* gene. Mixed results have been observed in the literature regarding the presence of these markers in persistent strains, and more studies are needed for a clear persistence association. Palaiodimou et al. (2021) found cadmium resistance genes both in persistent and presumed non-persistent strains, however *cadA1*, that allows to grow at concentrations of cadmium higher than 140 µg/mL, was more commonly present in persistent strains. Moreover, LGI2 was present only in two presumed non-persistent strains. Pasquali et al. (2018) observed differences in the cadmium genetic markers for the persistent ST121 and ST14 recovered from a rabbit meat processing plant. While none of the ST121 isolates studied harboured LGI2, it was present in 88.89% of ST14 isolates, and moreover, none of the ST14 isolates presented the *cadA1C* gen while it was present in 87.60% of ST121 isolates. These authors also confirmed that those isolates carrying the *cadA1C* gen had higher cadmium chloride MIC.

In *L. monocytogenes*, several benzalkonium chloride (BC) tolerance genetic determinants have been identified (*qacH*, located in Tn6188, *bcrABC* and *emrE*), localised on mobile genetic elements and found mainly in lineage II isolates (CC9, CC13, CC14, CC31 and CC121) (Table 4). There are reports of plasmids carrying *emrC*, which is particularly identified in CC6 (Table 3), *qacA* or *qacC* (Lakicevic et al., 2022). Overexpression of the chromosomal efflux pump MdrL is also capable of effluxing some antibiotics (such as macrolides or third generation cephalosporins and fluoroquinolones as ciprofloxacin) and heavy metals (Baquero et al., 2020; Douarre et al., 2022). Although, these genetic markers are well studied, discrepancies in their possible role in persistence have been found in the literature. The efflux cassette *bcrABC* and *emrC* were recently detected in both persistent and non-persistent candidates (Palaiodimou et al., 2021). These authors also found that persistent CC121 isolates were more likely to contain a truncated *inlA*, SSI-2 and the *qacH* gene (Palaiodimou et al., 2021). Recently, Cherifi et al. (2020) showed that the presence of the *bcrABC* cassette and *emrE* (present in the LGI1) conferred an enhanced tolerance to BC disinfectants. Moreover, the presence of CC harbouring the *bcrABC* cassette was significantly higher within the group of persistent strains, while two persistent CCs did not present any of these two genes, and only the persistent CC8 presented the *emrE* gene. Fox et al. (2011) showed that persistent strains were more tolerant to QAC based disinfectants than presumed non-persistent strains. However, Magalhaes et al. (2016) reported that the susceptibility to BC (50 ppm) of persistent and presumed non-persistent pulsotypes from cheese processing environments was similar. Manso et al. (2020) observed that a persistent strain from CC121, carrying *Tn*6188, did not show the highest BC MIC, that was reported by a presumed non-persistent CC1 strain. The authors also observed differences in BC resistance within the persistent CC9, lacking *qacH* and the *bcrABC* cassette, with two isolates showing the highest MIC for both BC disinfectants. Similarly, Harrand et al. (2020) showed that a presumed non-persistent strain, carrying the *bcrABC* cassette, had a higher BC MIC than a persistent strain harbouring the same genetic determinant. Although it is generally unknown whether the persistent strains characterised in all these studies directly come from settings where QACs have been used and they do show resistance to QAC concentrations typically used in the industry for disinfection, strains with molecular mechanisms of resistance to QACs can show a higher tolerance to them than wild type strains, and it is speculated that this could facilitate survival in some niches through long-term exposure to low doses of disinfectants (e.g. drains).

Some epidemiological studies have tried to identify mobile genetic elements and variable genetic hotspots on bacteriophage regions (e.g. *comK*, tRNA-ArgTCT loci) and plasmid types (e.g. pLM5578, pLM33, pLM80) (Fagerlund et al., 2016; Kuenne et al., 2010; Schmitz-Esser et al., 2015). A genome comparison of genetic lineage II CC121 strains persisting in different FoPE showed a high degree of conservation among prophages (tRNA-Arg-TCT prophage) and plasmids (pLM6179, pLM4423 and pLM3253 comparable to pLM5578), suggesting that strong selective pressure has acted on them (Schmitz-Esser et al., 2015). The same was observed in a genome comparison by Muhterem-Uyar et al. (2018) in genetic lineage I CC5 strains persisting in the processing environment of a cheese plant, which harboured conserved tRNA-Arg-TCT prophages and a pLM80 prototype plasmid with a *bcrABC* cassette and genes for heavy metal resistance. Some researchers apply plasmid typing, which is a helpful tool to identify the global dissemination of successful plasmids (Anast et al., 2022; Hingston et al., 2019; Muhterem-Uyar et al., 2018). In a study by Hingston et al. (2019), 26 strains from Canada and Switzerland, covering many different genotypes, carried an identical plasmid type (pLMG1-7), highlighting the unique conservation of *L. monocytogenes* plasmids worldwide. Schmitz-Esser et al. (2021) compared 1037 plasmids from 1921 genomes (54%) and clearly showed that plasmids were significantly more abundant in *L. monocytogenes* isolated from food and the FoPE compared to clinical strains, which appears to be a prerequisite for adaptation and dissemination to the environment and food niche (Tables 3 and 4).

L. monocytogenes has been traditionally considered to be genetically highly conserved. Genetic expansion was not present to the same extent as in *Salmonella* or *Campylobacter* (Buchrieser et al., 2003). However, in epidemiological analyses including food, environmental and animal isolates of *L. monocytogenes*, it has been recognised that genetic lineage

II is subject to greater selection pressure, with successful CCs and STs having incorporated genetic material from microorganisms in the specific niches (den Bakker et al., 2008; Orsi et al., 2008). Of note, this genetic expansion based on horizontal gene transfer, mutation or recombination is high among the CCs most frequently associated with persistence in the FoPE. Thus, according to the Institute Pasteur database (accessed on 16 October 2023), CC5 contains 81 different STs that diverge in a housekeeping gene, CC2 includes 96 different STs and CC6, which was also involved in the last major *Listeria* outbreaks, 51 different STs. The genomes of CC9 ($n = 94$) expanded most frequently in genetic lineage II, followed by CC8 ($n = 79$ ST) and CC121 ($n = 59$).

Certain CC types in genetic lineage II have more stress tolerance genes (*Tn*6188_qacH transposon, SSI-1 and SSI-2, plasmid-borne *brcABC* efflux pump), but have more often attenuated *inlA* genes due to premature stop codons (PMSC) than lineage I isolates and none of the *Listeria* pathogenicity islands 3 (LIPI-3) and 4 (LIPI-4) (Tables 3 and 4). In addition, the peptidoglycan binding gene (*bapL*) appears to be specific for genetic lineage II CCs, particularly CC121 and CC9. This has led to the suggestion that they (e.g. CC9, CC121) can better persist in the FoPE and infect only severely immunocompromised individuals (hypovirulence) (Palma et al., 2020). CC121 is the best described *L. monocytogenes* complex, the first to be described with a truncation of *inlA* (hypovirulent) and sublethal adaptation to quaternary ammonium compounds (QACs) (through genetic traits such as *qacH*) and appears to be the most prevalent genetic lineage II representative worldwide. These characteristics have been proposed to be related with persistence in the FoPE (Ortiz et al., 2016; Palaiodimou et al., 2021).

Nevertheless, despite the fact that some researchers highlight a unique or multiple, genetic markers (e.g. QAC efflux genes) that may contribute to *L. monocytogenes* persistence when comparing persistent versus presumed non-persistent strains (Martínez-Suárez et al., 2016; Mirena et al., 2023), there are findings that support the hypothesis that persistence is rather an interplay of different genetic markers and the ecological niche (Daeschel et al., 2022; Palaiodimou et al., 2021).

3.2.1.3 | *Analysis of clusters of related genome sequences in the NCBI Pathogen Detection database*

There were 51 SNP clusters identified for *L. monocytogenes* with ≥ 100 isolates, as shown in Figure 3. The greatest frequency was found for CC9, which had five separate SNP clusters, followed by CC7, CC155 and CC554, each with four individual SNP clusters containing ≥ 100 isolates. Of the top 10 CCs most frequently identified as persistent in the literature (Figure 2), 5 were also among the top 10 largest SNP clusters in the NCBI Pathogen Detection database including strains from both clinical and environmental/other sources (CC6, CC8, CC9, CC121 and CC321).

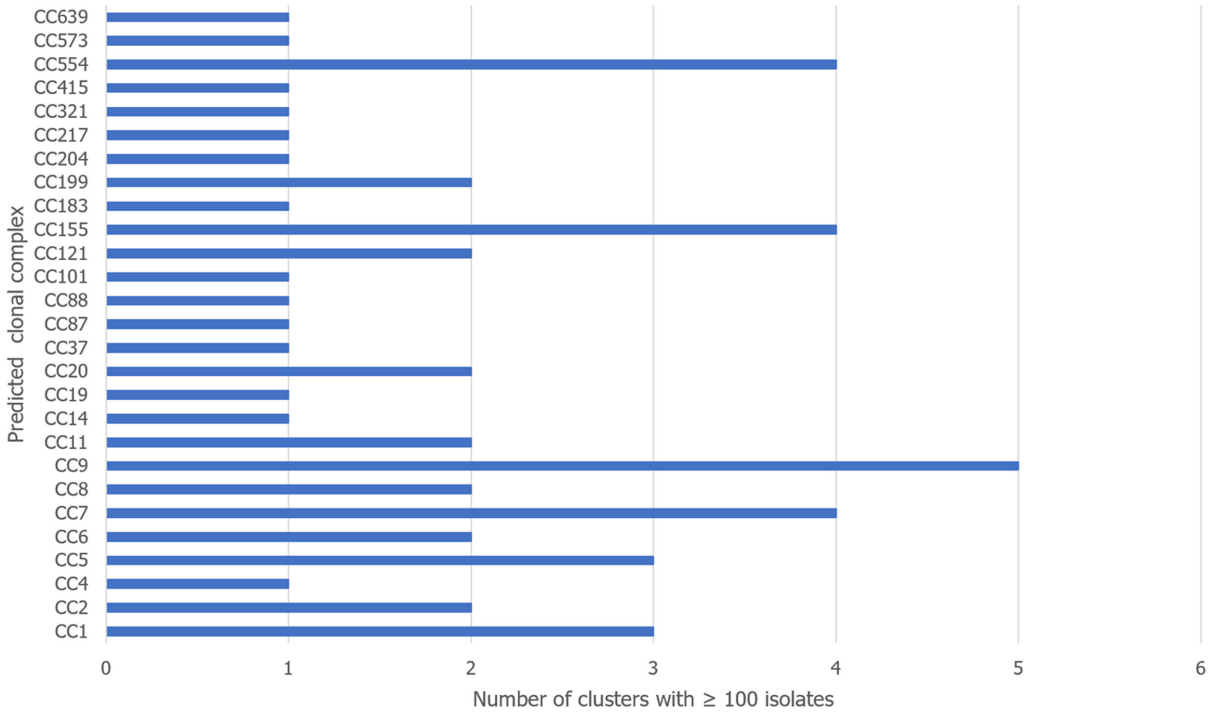

FIGURE 3 Number of SNP clusters in the NCBI Pathogen Detection database with at least 100 isolates, by predicted CC identified as persistent in the literature screening.

An overview of a selection of the largest SNP clusters from the NCBI Pathogen Detection database is shown in Figure 4, including two clusters related to CCs (CC573 and CC639) that were never reported as persistent in FoPE in any of the retrieved studies and six large clusters from CCs more frequently associated with persistence (i.e. CC5, CC6, CC7, CC8, CC9 and CC121). When comparing both groups, the two CCs not associated with persistence events lacked most of the genetic stress markers described above as possibly related to persistence in the FoPE, including disinfectant tolerance and heavy metal tolerance markers. In relation to virulence markers, while CC9 and CC121 SNP cluster isolates were associated with

production of a truncated InlA, suggesting reduced virulence, those CCs not associated with persistence (i.e. CC573 and CC639) carried full length *inlA* genes. This again suggests that important stress tolerance features of relevance to the FoPE, and truncations with loss of function of *inlA*, are more widely represented across persistent subtypes. Similar trends have been identified previously (Palaiodimou et al., 2021), as already highlighted in Section 3.2.1.2. In relation to the source of isolation, some clear commodity source associations were seen (e.g. CC9 with meat/pork sources, CC573 with horticulture products).

However, it is important to note that a SNP cluster will include over-representation of isolates from specific source attribution investigations; for example, if pork meat was implicated in an outbreak related to a given SNP cluster, then this SNP cluster may be over-represented with isolates from the contaminated pork product, and the FoPE that produced it, since these would be specifically sampled at higher frequency. Thus, there could be a high number of isolates of a specific outbreak strain from pork-related sources, represented within that SNP cluster. Of the SNP clusters analysed, the majority of isolates were collected within the previous 10–15 years; it is notable, however, that some clusters included large proportions of isolates collected over extended timeframes. For example, isolates of the CC121 SNP cluster were collected over a period of more than 10 years. This suggests that certain clonal subgroups can occur stably and widespread over prolonged periods.

TABLE 3 Overview of genetic features of *L. monocytogenes* lineage I possibly linked to persistence.

CC, ST[a]	1	2	3	4	5	6	59	87	183 (ST382)	217	218	224	288	554
Globally spread[b]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Clinical & Environment[c]	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	No	No	No	Yes
Hypo/ hypervirulent[d]	Hypervirulent	Hypervirulent	/	Hypervirulent	/	Hypervirulent	/	Hypervirulent	Hypervirulent	/	/	/	/	/
LIPI-hypervirulent	LIPI-3	LIPI-3, LIPI-4	LIPI-3	LIPI-3, LIPI-4	/	LIPI-2, LIPI-3	LIPI-3	LIPI-3	LIPI-3, LIPI-4	LIPI-3, LIPI-4	LIPI-3, LIPI-4	LIPI-3	LIPI-3	LIPI-3
Stress survival/ response[e]	SSI-LMOf2365-0481	SSI-LMOf2365-0481	SS-1	SS-1	SS-1	/	/	/	/	/	/	SS-1	/	/
PMSC	/	PMSC *inlA*	/	/	PMSC *inlA*, *inlB* some	PMSC *actA*	/	/	/	/	/	/	/	/
Plasmid groups	Group 1, Group 2, Group 4	Group 1, Group 2, Group 1 & 2, Group 4	Group 1, Group 1 & 2	Group 4	Group 1, Group 2, Group 1 & 2	Group 1, Group 2, Group 1 & 2	/	/	/	/	/	/	/	/
Plasmid[f]	/	pLM33, pLM5578	Group 1 (pLMG1-7)	/	Group 1 (pLM33 (rep25), pLMG1-7, pLMG1-9, pLMG1-12), Group 2 SG2 (pLM80, pIMG2-8, pIMG2-10), LM-F-131	Group 1 (pLMST6, QAC efflux (emrE), resistance to amoxicillin, gentamycin)	Group 2 SG1 (pIMG2-13)	/	/	/	pLI100, j1776, pLM33	/	/	/
Adaption to disinfection	LGI 2, arsenic resistance (*arsA1*), QAC efflux (*bcrABC*), *cadA4C*	LGI 2, Arsenic resistance, efflux pump (*lde*, *mdrL*)	LGI 1, QAC efflux (*bcrABC*), cadA1C1_ Tn5422	efflux pump (*ladR*, *lde*, *mdrL*)	QAC efflux (*bcrABC*, *emrC*), efflux pump (*clpL*, *lde*, *mdrL*), cadmium resistance cass. (*cadA1*, *cadA2*, *cadA4*), *cadA*, *cadC*	QAC efflux (*bcrABC*), efflux pump (*lde*, *mdrL*), *cadA*, *cadC*	/	LGI 2, arsenic resistance	/	/	*cadA* and *cadC*	/	/	/
Biofilm marker[g]	*comK*	*comK*	*comK*	*comK*	/	/	/	/	/	/	*comK*	/	*comK*	/

Abbreviations: CC, clonal complex; ST, sequence type; PMSC, premature stop codon; SSI, stress survival islet; LIPI, *Listeria* Pathogenicity Island; QAC, quaternary ammonium compound; LGI, *Listeria* Genomic Island; /, unknown, not reported.

[a]Green cells: major human CC; blue cells: more linked to human; white cells: linked to human and food.

[b]Globally spread: a genotype that occurs worldwide in various niches (human, food, environment, animal).

[c]Clinical & environment: present in the dataset from the literature search in distiller SR and NCBI genome data comparison related to Figure 4.

[d]Hypervirulent, presence of additional *Listeria* Pathogenicity Islands (LIPIs) and involved in severe infection and documented outbreaks.

[e]SSI-1 (stress survival islet-1): adaption to acid and osmotic stress, bile stress in the stomach; single-gene insert LMOf2365_0481 instead of SSI-1; SSI-2 (stress survival islet-2): adaption to alkaline and oxidative stress.

[f]Group 1 and group 2 plasmids: typed by plasmid replication protein (*RepA* sequence). The group 2 plasmids are associated with resistance to heavy metals, other stress conditions and persistence.

[g]*comK* Prophage junction fragments as markers for persistence.

TABLE 4 Overview of genetic features of *L. monocytogenes* lineage II possibly linked to persistence.

CC, ST[a]	7	8	9	11	14	20	31	37	101	121	155	199	204	321	403
Globally spread[b]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Clinical & Environment[c]	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	No
Hypo/ hypervirulent[d]	/	/	Hypovirulent	/	Hypervirulent	Hypervirulent	Hypovirulent	/	/	Hypovirulent	/	/	/	/	/
LIPI-hypervirulent	LIPI-2-inlI	LIPI-2, LIPI-3	LIPI-2-inlI	/	/	/	LIPI-2-inlI	LIPI-2	LIPI-2-inlI	LIPI-2-inlI	/	/	/	/	/
Stress survival/ response[e]	SS-1	SS-1	SS-1	/	/	/	SS-1	SS-1	SSI-LMOf2365 -0481	SS-2, Alkaline and oxidative stress resistance	SS-1	SS-1	SS-1	SS-1	/
Truncated inlA	PMSC *prfA*	PMSC *actA*	PMSC *inlA*, PMSC *prfA*, PMSC *actA*	/	PMSC inlA	/	PMSC *inlA*	/	/	PMSC *inlA*	PMSC *inlA*	PMSC *inlA*	/	PMSC *inlA*	/
Plasmid groups	Group 1	Group 2	Group 1, Group 2, Group 1 & 2	Group 1	Group 1, Group 2	/	/	/	/	Group 2	Group 2	/	Group 1, Group 2, Group 1 & 2	Group 2	/
Plasmid[f]	Group 1 (pLMG1-6, pLMST6 (pLmN12-0935)), Group 1 (pLMG1-7), Plasmid pAUSMDU00000235, pIM80 (pCFSAN004330/ pLIS1/ pN1-011A/ pCFSAN021445)	Group 2 SG1 (pIMG2-1, pIMG2-11, pIMG2-12, pIMG2-5), Group 2 SG2 (pLI100 (rep26), pIMG2-4, pIMG2-7)	Group 1 (pLMG1-1, pLMG1-2, pLMG1-4, pLMG1-5, pLMG1-7, pLMG1-8, pLMG1-11, pLM33 (rep25)), Group 2 SG1 (pIMG2-6), pLM58	Group 1 (pLMG1-7, pLMG1-13)	/	/	Group 1(pLM33 (rep25))	/	/	Group 2, SG1 (pIM5578, pIMG2-2), Group 2 SG2 (pLI100 (rep26)), pLM6179 (pLM6179/ pGMI16–004/ pCFSAN022990)	Group 2 (pImG2-9), pIM80 (pN1-011A/ pCFSAN021445/ pCFSAN004330/ pLIS1)	Group 1 (pLMG1-10)	Group 2 (pImG2-10), pIM80 (pN1-011A/ pCFSAN021445/ pCFSAN004330/ pLIS1)	Group 2 SG1 (pIMG2-3)	Group 1 (pLMST6 (pLmN12-0935))
Adaption to disinfection	Tn6188-qaC/*lmo2082*, *emrC*	LGI 1, QAC efflux (*emrE*), cadA1C1_ Tn5422, Tn6188, Tn5422, *arsD*	LGI 1, LGI 2, Tn554- Arsenic resistance, *arsA*, *arsD*, QAC efflux (*bcrABC*), QAC efflux (*emrC*, *emrE*), Tn6188, Tn5422, cadA1C	/	/	/	*cadA1C*, *arsA*, *arsD*, *bCrB* and *bCrC* lack	/	LGI 1, LGI 3, QAC efflux (*emrC*), *cadA*, Tn916	QAC efflux-Tn6188 (*qacH*, *emrC*), tolerance to BC, cadA1C1_Tn5422, *cadA*, QAC efflux (*emrE*), efflux pump (*clpL*, *lde*, *mdrL*)	QAC efflux (*bcrABC*)	QAC efflux (*bcrABC*)	LGI 1, LG 2 (*TnyfbR-arsA2*), QAC efflux (*bcrABC*), TnILP, *cadA2C*, *cadA4C*, arsenic resistance cassette (*arsA*-1, arsA-2, *arsR*, *arsD*-1, *arsD*-2, *acr3*)	QAC efflux (*bcrABC*), Tn5422	/
Biofilm marker[g]	vip-lack, *comK*	*comK*, vip-lack	*comK*, *bapL*	/	*bapL*	*bapL*	vip-lack	/	*comk*	*comK*, *bapL*	*comK*	/	*comK*, *bapL*	*comK*	/

Abbreviations: CC, clonal complex; ST, sequence type; PMSC, premature stop codon; SSI, stress survival islet; LIPI, *Listeria* Pathogenicity Island; QAC, quaternary ammonium compound; LGI, *Listeria* Genomic Island; /, unknown, not reported.

[a]Blue cells: more linked to human; orange cells: more linked to food; white cells: linked to human and food.

[b]Globally spread: a genotype that occurs worldwide in various niches (human, food, environment, animal).

[c]Clinical & environment: present in the dataset from the literature search in distiller SR and NCBI genome data comparison related to Figure 4.

[d]Hypervirulent, presence of additional *Listeria* Pathogenicity Islands (LIPIs) and involved in severe infection and documented outbreaks.

[e]SSI-1 (stress survival islet-1): adaption to acid and osmotic stress, bile stress in the stomach; single-gene insert LMOf2365_0481 instead of SSI-1; SSI-2 (stress survival islet-2): adaption to alkaline and oxidative stress.

[f]Group 1 and group 2 plasmids: typed by plasmid replication protein (*RepA* sequence). The group 2 plasmids are associated with resistance to heavy metals, other stress conditions and persistence. *Tn* 6188, a novel transposon in *L. monocytogenes* conferring tolerance to BC. Tn5422, first-described natural transposon of *L. monocytogenes*, mediates plasmid-mediated cadmium resistance. Ars, arsenic resistance cassettes are comprised of three (arsRBC) to five (arsRDABC) genes that are transcribed into a single polycistronic mRNA. Cad, cadmium efflux P-type ATPase (cadA) and its repressor cadC, involved in plasmid-mediated cadmium resistance. QacH, transporter, a molecular mechanism leading to increased tolerance to BC. *emrE*, gene located on the Listeria Genomic Island 1 encodes for an efflux pump involved in BC tolerance. *bcrABC*, benzalkonium chloride resistance cassette.

[g]*comK* prophage junction fragments as markers for persistence. BapL, Biofilm-Associated Protein. Vip, is anchored to the *Listeria* cell wall by sortase A and is required for entry into some mammalian cells.

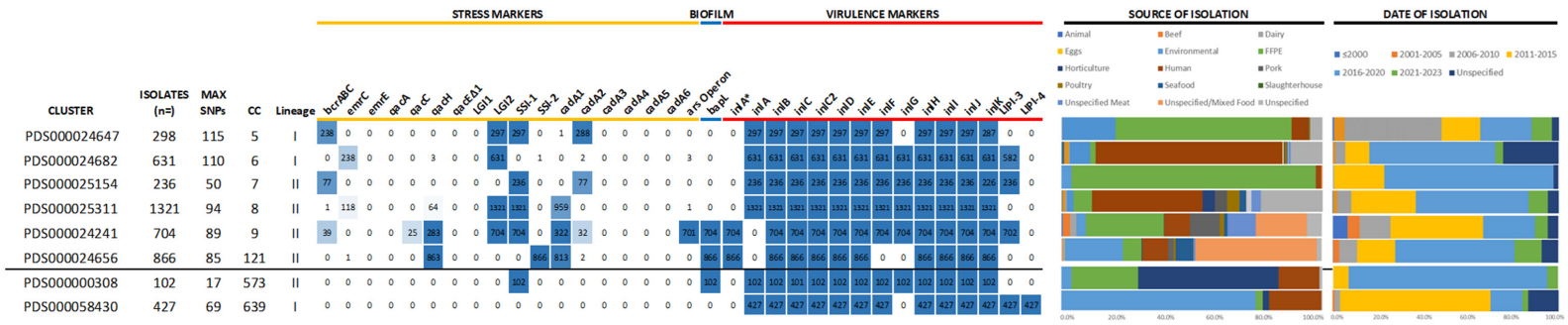

FIGURE 4 Overview of genetic features of selected SNP clusters from the NCBI Pathogen Detection database. This includes six SNP clusters related to CC more commonly associated with persistence in food processing environments (above the black line), and the two SNP clusters present in the database containing at least 100 isolates from CC which have not been identified as persistent in the literature screened for this scientific opinion (below the black line). *'inlA*'* indicates isolates harbouring an *inlA* gene with a premature stop codon, associated with a truncated InlA protein. Presence/absence is indicated by the heatmap, with numbers relating to the number of isolates in that cluster carrying a given marker. 'Max SNPs' refers to the largest SNP difference among isolates within that cluster in the NCBI Pathogen Detection database.

3.2.2 | *Salmonella enterica* subtypes and features

3.2.2.1 | *Subtypes linked to persistence*

There are over 2500 different *Salmonella* serotypes (Grimont & Weill, 2007). Human salmonellosis is caused by a relatively small number of serotypes, with many serotypes being host specific or unable to cause infection in humans. As in previous years, the most commonly reported *Salmonella* serovars in 2021 were *S*. Enteritidis (54.6%), *S*. Typhimurium (11.4%) and monophasic *S*. Typhimurium (1,4,[5],12:i:-) (8.8%), representing 74.8% of the confirmed human cases. The fourth and fifth serovars, *S*. Infantis (2.0%) and *S*. Derby (0.93%), were at the same levels as in 2020 and 2019, closely followed by *S*. Coeln (0.91%) (EFSA and ECDC, 2022).

A wide range of serotypes of *Salmonella* ($n = 36$) have been reported to be linked to persistence in the FFPE (see Figure 5). The greatest number of serotypes of persistent *Salmonella* were associated with meat processing plants, including serotypes of human health importance. In the meat processing sector serotypes reported as persisting in multiple plants were *S*. Typhimurium, *S*. Derby, *S*. Infantis, *S*. Heidelberg, *S*. Enteritidis, *S*. Agona, *S*. Rissen, *S*. Kentucky, *S*. Typhimurium 4,[5],12:i:- and *S*. Ohio (Arguello, Carvajal, et al., 2013; Bersot et al., 2021; Bertrand et al., 2010; Boubendir et al., 2021; Bridier et al., 2019; Charlebois & Horan, 2010; Corcoran et al., 2013; Dantas et al., 2020; Duggan et al., 2010; Gantzhom et al., 2014; Gu et al., 2020; Kawakami et al., 2019; Kuhn et al., 2013; Medina-Santana et al., 2022; Monte et al., 2020; Monte et al., 2022; Morganti et al., 2018; Piras et al., 2011; Piras et al., 2015; Prasertsee et al., 2019; Prendergast et al., 2011; Schroeder et al., 2016; Tadee et al., 2015; van Hoek et al., 2012; Vinueza-Burgos et al., 2019; Wang et al., 2014; Wingstrand et al., 2012; Zeng et al., 2021). Other serotypes described in single cases included *S*. Indiana, *S*. Larochelle, *S*. Livingstone, *S*. Muenchen, *S*. Panama, *S*. Paratyphi var Java, *S*. Saintpaul, *S*. Schwarzengrund and *S*. Weltevreden (Boubendir et al., 2021; Gantzhom et al., 2014; Hiko et al., 2016; Mannion et al., 2012; Ren et al., 2016; Wang et al., 2014; Zeng et al., 2021).

For LMF, *S*. Agona was reported in two studies (Hoffmann et al., 2020; Russo et al., 2013), and *S*. Bareilly, *S*. Bredeney, *S*. Mbandaka and *S*. Poona in single studies (Jones et al., 2019; Keaton et al., 2022; Labska et al., 2021; Viazis et al., 2015). Only Grasso et al. (2015) reported two serotypes in the same LMF premises, Anatum and Tennessee.

S. Bareilly (Kim et al., 2015), *S*. Tennessee (Jakociune et al., 2014) and *S*. Typhimurium (Moffatt et al., 2017) were also reported in individual studies related to persistence in the processing environment of egg and egg products.

In animal FePE 15 different serotypes were reported to persist, and many studies reported the persistence of more than one serotype. *S*. Anatum, *S*. Senftenberg, *S*. Mbandaka, *S*. Agona, *S*. Montevideo and *S*. Cubana were reported across multiple studies (Davies et al., 1997; Häggblom, 2012; Löfström et al., 2006; Moretro et al., 2003; Morita et al., 2022; Nesse et al., 2003; Nesse et al., 2005; Parker et al., 2019; Parker et al., 2022; Pellegrini et al., 2015; Trinetta et al., 2020; Wierup & Kristoffersen, 2014). Other serotypes reported included *S*. Agama, *S*. Liverpool, *S*. Livingstone, *S*. Lexington, *S*. Tennessee, *S*. Kedougou, *S*. Ohio, *S*. Singapore, *S*. 4,12,d:- and *S*. 13,23,i:- (Davies et al., 1997; Eriksson et al., 2005; Gosling et al., 2022; Parker et al., 2019; Wang et al., 2014; Wierup & Kristoffersen, 2014).

The identification of serotypes of human health importance as being persistent within the meat and egg and egg products processing environment is not surprising as food categories from these two sectors are the ones more frequently involved in FBOs of human salmonellosis (EFSA and ECDC, 2022). Interestingly, *S*. Agona was the only serotype to be found in persistence events across three different food and feed sectors, namely feed, meat and LMF (Corcoran et al., 2013; Dantas et al., 2020; Hoffmann et al., 2020; Russo et al., 2013; Wang et al., 2014).

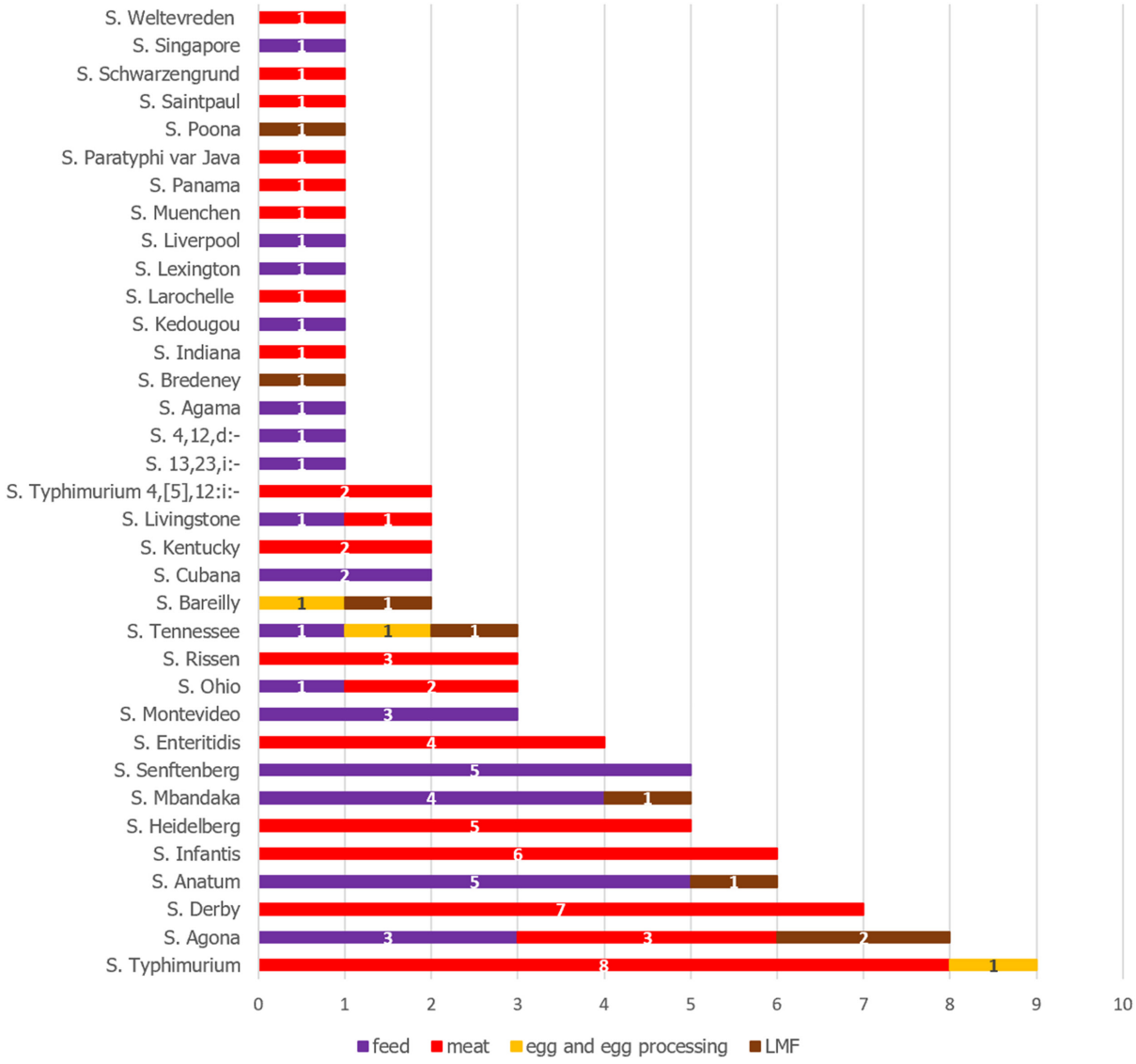

FIGURE 5 Overview of the various *Salmonella enterica* subtypes (serotypes) that have been found to persist in in the FFPE of each of the food and feed production and processing sectors. The numbers indicate the cases of persistence being identified from the experimental studies retrieved in the literature search for each serotype and sector. *Note*: Based on (Arguello, Carvajal, et al., 2013; Bersot et al., 2021; Bertrand et al., 2010; Boubendir et al., 2021; Bridier et al., 2019; Corcoran et al., 2013; Dantas et al., 2020; Davies et al., 1997; Duggan et al., 2010; Eriksson et al., 2005; Friesema et al., 2014; Gantzhom et al., 2014; Gosling et al., 2022; Grasso et al., 2015; Gu et al., 2020; Häggblom, 2012; Hiko et al., 2016; Hoffmann et al., 2020; Jakociune et al., 2014; Jones et al., 2019; Kawakami et al., 2019; Keaton et al., 2022; Kim et al., 2015; Kinross et al., 2014; Kuhn et al., 2013; Labska et al., 2021; Lienemann et al., 2011; Löfström et al., 2006; Mannion et al., 2012; Medina-Santana et al., 2022; Moffatt et al., 2017; Monte et al., 2020; Monte et al., 2022; Moretro et al., 2003; Morganti et al., 2018; Morita et al., 2022; Nesse et al., 2003; Nesse et al., 2005; Parker et al., 2019; Parker et al., 2022; Pellegrini et al., 2015; Piras et al., 2011; Piras et al., 2015; Prasertsee et al., 2019; Prendergast et al., 2011; Ren et al., 2016; Russo et al., 2013; Schroeder et al., 2016; Tadee et al., 2015; Trinetta et al., 2020; van Hoek et al., 2012; Viazis et al., 2015; Vinueza-Burgos et al., 2019; Wang et al., 2014; Wang et al., 2019; Wierup & Kristoffersen, 2014; Wingstrand et al., 2012; Zeng et al., 2021)

3.2.2.2 | *Features associated with persistence and link to subtypes*

The FFPE poses a variety of challenges to bacteria, including low pH, osmotic and heat stress, starvation, exposure to biocides, microbial competition, etc. In principle, any stress adaptation strategy that assists in survival of hazards in the FFPE, and/or selects resistant clones that can maintain viability longer than their susceptible isogenic counterparts, could (by default) be considered as candidate mechanisms of persistence. Such mechanisms are common to most hazards (Begley & Hill, 2015). *Salmonella* possesses a broad armoury of stress resistance mechanisms, located in the cell envelope or in the cytoplasm (Finn et al., 2013). An indicative (but not exhaustive) list of cell envelope-located mechanisms includes transporters of potassium (Kdp) or osmoprotectants, such as glycine and betaine (ProU, ProP, OsmU, belonging to the major facilitator superfamily permeases), and porins (OmpC), all assisting in survival in high salinity environments. Cytoplasm-located stress response features include the trehalose 6-phosphate synthetase (OtsA) for synthesis of the osmo-protective trehalose (at low a_w), or decarboxylases, e.g. lysine decarboxylase systems (cadB), and chaperones (e.g. GroESL, DnaK), for combating acid and heat stress, respectively (Begley & Hill, 2015; Finn et al., 2013). However, to identify which of these mechanisms and how they contribute to persistence, requires a systematic and targeted experimental approach, that combines genetic and phenotypic traits. In this assessment, an effort was made to capture both the persistence-related features (if any) of *Salmonella* and review the features that have been assessed particularly in persistent isolates.

In the available studies where persistent strains of *Salmonella* were isolated, the genotypic and phenotypic characterisation (when performed) suggested that isolates had one or more of the following features: (i) AMR and/or resistance to disinfectants, (ii) ability for biofilm formation, (iii) growth or survival capacity in foods produced in the FFPE where strains persist, (iv) harbourage of mobile genetic elements, mainly plasmids and (v) carriage of virulence genes and/or a confirmed cellular invasion phenotype.

It needs to be noted however that these studies describe selected traits of strains without essentially suggesting that these features are responsible for persistence. Besides, most of these, e.g. virulence, growth/survival and biofilm formation are typical to most infectious foodborne hazards, including *Salmonella*. In addition, not all studies isolating persistent strains evaluated the genotypic and phenotypic features of isolates, nor did they assess a common (and exhaustive) list of determinants possibly contributing to persistence. Overall, the set of genotypic and phenotypic characteristics of persistent strains assessed in the different literature reports evaluated seems to lack completeness. As such, it is difficult to deduce those features that are either indispensable for, or may markedly contribute to, persistence, alone or in combination with other key genotypic and phenotypic elements.

Strains of the most frequently persistent serotypes in the studies included in this opinion (*S.* Typhimurium, *S.* Agona) were identified as having characteristics enabling antimicrobial resistance, biofilm-forming ability, cell invasion and virulence (for *S.* Agona only).

There has been diverse evidence about the correlation between resistance or decreased susceptibility to antibiotics and biocides (disinfectants), suggesting a lack of a systematic mechanism consistent in all resistant isolates. Efflux pumps and modification of the membrane composition (e.g. increase of short-chain polysaccharide fractions of the LPS) are among the presumptive mechanisms shared between isolates resistant to alkaline disinfectants and cell wall/membrane-targeting antibiotics, e.g. *β*-lactams, cephalosporins and polymyxins (Dubois-Brissonnet, 2012; Gantzhom et al., 2014). In the same context, adaptation to some alkaline biocides, e.g. via exposure to sublethal levels, i.e. less than the *'in use'* concentrations, may select for decreased susceptibility to antimicrobials in *Salmonella*. In particular, Gantzhom et al. (2014) recorded decreased susceptibility to disinfectants, particularly the commercial formulation Incimaxx DES,[24] of strains belonging to serovars Livingstone, Typhimurium and Derby, from a pig processing plant. The fact that the resistance to antimicrobials and alkaline disinfectants share common mechanisms suggests that AMR could potentially assist in the selection of strains persisting in the FFPE via, among others, their resistance to C&D, but this needs to be further explored in the future in order to draw solid conclusions.

The AMR in strains of serovars Rissen and Infantis from studies in pork and poultry, respectively, was shown to be plasmid-mediated (Medina-Santana et al., 2022; Prasertsee et al., 2019). A proportion of 97.5% of *S.* Infantis isolates from the meat sector harboured the pESI-like mega plasmid that plays an important role in global AMR dissemination (Medina-Santana et al., 2022; Vinueza-Burgos et al., 2019).

The second most frequently studied phenotype for *S. enterica* persistent isolates was biofilm formation. (Dantas et al., 2020; Jakociune et al., 2014) studied together with AMR for *S.* Typhimurium, however no specific genes were reported (Piras et al., 2015). Biofilm community leads to cell ageing and induces general stress response mechanisms that help bacteria to maintain their viability and persist under adverse conditions (Alvarez-Ordóñez et al., 2019). This characteristic has been highlighted as a potential mechanism for persistence within the FePE (Milanov et al., 2017; Velhner et al., 2018), for months or even years (Prunic et al., 2016; Schonewille et al., 2012; Vestby et al., 2009). Strains from the serovars Enteritidis, Heidelberg, Ohio, Tennessee and Agona, isolated from polystyrene materials and canvas in poultry plants, and fittings in egg processing plants, were reported to form biofilms as well as being virulent (Dantas et al., 2020; Jakociune et al., 2014). This may be partly explained by the fact that invasion in epithelial cells requires pre-establishment of *Salmonella* on gut mucosa, which practically involves the expression of mechanisms for biofilm formation (Bai et al., 2021). Of note is that *S.* Tennessee strains persisting in egg processing plants had higher growth capacity in pasteurised egg product than the presumptive non-persistent strains (Jakociune et al., 2014). Quorum sensing (e.g. autoinducer 2 and 3) may further contribute to virulence expression of cells colonising abiotic or biotic surfaces, and thus, as part of biofilm communities (Horn & Bhunia, 2018). Nevertheless, as mentioned above, biofilm formation is a generic phenotype expressed by most *Salmonella* strains, regardless of being characterised as persistent or not. As such, this phenotype should not be viewed as a definite feature contributing to persistence. Regarding virulence, Rissen strains were genotypically considered less virulent than other serovars (Prasertsee et al., 2019). On the contrary, the pathogenicity island genes *avrA*, *ssaQ*, *mgtC*, *siiD* and *sopB* and the fimbrial gene *bcfC* were present in all Weltevreden and Agona strains inhabiting a poultry slaughterhouse (Dantas et al., 2020; Ren et al., 2016).

3.2.2.3 | *Analysis of clusters of related genome sequences in the NCBI Pathogen Detection database*

Considering the 36 *Salmonella* serotypes found in the literature search as linked to persistence in the FFPE, 25 had SNP clusters in the NCBI Pathogen Detection database with at least 100 isolates (Figure 6). The remaining 11 persistent serotypes did not have clusters meeting this criterion (i.e. 13,23,i:-; 4,12,d:-; Agama; Bredeney, Cubana, Larochelle, Lexington, Livingstone, Paratyphi var Java, Singapore and Tennesse). The two predominant serotypes reported most frequently in the database were Typhimurium and Enteritidis (89 and 55 SNP clusters with ≥ 100 isolates, respectively). These clusters typically include a diverse geographical distribution of isolates, and include samples from clinical, food, FFPE and environmental sources

[24]The disinfectant Incimaxx DES contains a mixture of peroxyacids and hydrogen peroxide as active ingredients.

(although clinical isolates are typically the most represented among individual SNP clusters). Of the top 8 serotypes most frequently identified as persistent among the studies retrieved in the literature search, 6 were also among the top 10 largest SNP clusters in the NCBI Pathogen Detection database, including strains from both clinical and environmental/other sources (*S*. Typhimurium, *S*. Infantis, *S*. Agona, *S*. Anatum, *S*. Heidelberg and *S*. Mbandaka), which evidences their clinical relevance and widespread distribution.

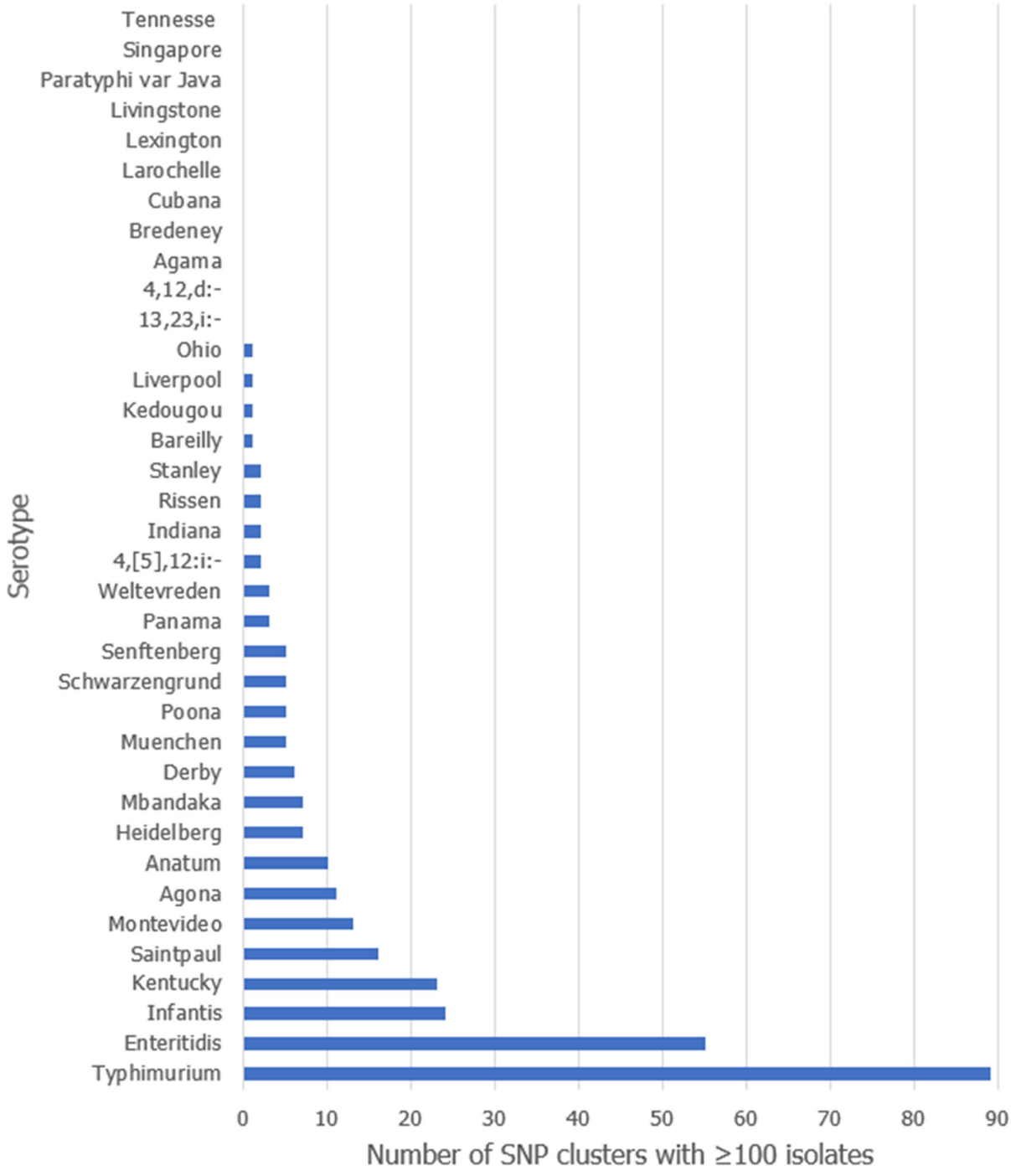

FIGURE 6 Number of SNP clusters in the NCBI Pathogen Detection database with at least 100 isolates, for *Salmonella* serotypes identified as persistent in the literature screening.

3.2.3 | *C. sakazakii* subtypes and features

3.2.3.1 | *Subtypes linked to persistence*

Studies retrieved in the literature search isolating and subtyping *C. sakazakii* strains from LMF (mainly powdered infant formula) manufacturing plants have identified indistinguishable strains persisting in the FoPE over wide time periods which have been characterised as belonging to some particular clonal complexes: CC1, CC3, CC4, CC40, CC58, CC64 and CC83 (Figure 7). Of these, CC1, CC64 and CC83 are the pathovars that have been reported as linked to persistence in the FoPE

on more than one occasion, considering published studies since 2010. In addition, review articles and book chapters have highlighted the frequent isolation of persistent *C. sakazakii* CC4 strains from powdered infant formula facilities. For example, Muller et al. (2013) undertook a 13-month survey of a powdered infant formula manufacturing plant in Switzerland and recovered indistinguishable strains from CC4 which were later found to have persisted for up to 4 years (2011–2016). Jacobs et al. (2011) analysed environmental and final product samples from a milk powder manufacturing plant in Germany over a 4-year period (2005–2009) from the spray-drying and roller-drying areas. The recovered strains were further profiled by Sonbol et al. (2013) as four ST of *C. sakazakii* including the pathovar CC4. A well-characterised persistent strain from a powdered infant formula production facility in Ireland initially recovered from the powdered infant formula production environment and persisting for at least 30 months was also from CC4 (Power et al., 2013). In other studies, retrieved from the literature, strain persistence was demonstrated using PFGE as a typing method, which provides information that is not interoperable or does not follow universally harmonised terminology for the subtypes.

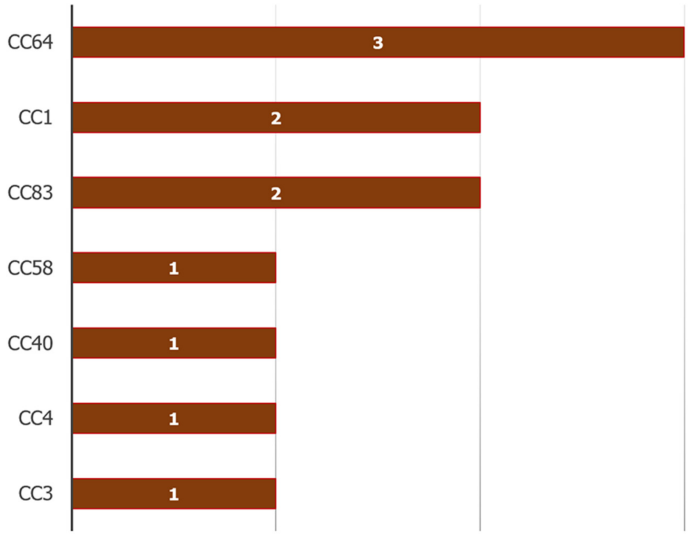

FIGURE 7 Overview of the various *C. sakazakii* subtypes (clonal complexes; CC) that have been found to persist in the FoPE of the low moisture food sector. The numbers indicate the cases of persistence being identified from the experimental studies retrieved in the literature search for each CC and sector. *Note*: Based on (Chase et al., 2017; Fei et al., 2015; Gan et al., 2021; Lu et al., 2019; Negrete et al., 2022; Pei et al., 2019; Yan et al., 2015).

3.2.3.2 | *Features associated with persistence and link to subtypes*

Various studies and review articles have speculated about the features responsible for the special ability shown by *C. sakazakii* to survive for long time periods and persist in the dry conditions of LMF FoPE and the following characteristics have been highlighted:

- The ability to form biofilms on a variety of abiotic surfaces including silicon, latex, polycarbonate, stainless steel, glass or polyvinyl chloride (Du et al., 2012; Lehner et al., 2005).
- A high desiccation resistance and a high heat tolerance, as compared to that of other related microorganisms, such as other members of the *Enterobacteriaceae* family. Survival under desiccation is related to the high osmotolerance mediated by the synthesis/accumulation of a range of compatible solutes, such as trehalose. Thermotolerance can be due to the presence of genomic islands (Orieskova et al., 2016). The locus of heat resistance is one of the genomic islands conferring heat resistance in *C. sakazakii* (Wang et al., 2021). Interestingly, desiccation resistance is positively correlated with heat resistance (Fakruddin et al., 2014) and biofilm formation, but apparently it is not linked to particular ST (Du et al., 2018).
- The production of a capsule that aids attachment in biofilm formation, provides resistance to biocides and contributes to survival following desiccation due to the entrapment of a shell of water within the capsule (Barron & Forsythe, 2007; Craven et al., 2010; Fei et al., 2015; Iversen et al., 2004; Osaili & Forsythe, 2009; Yan et al., 2015).
- The production of a yellow carotenoid pigment which stabilises cell membranes and removes reactive oxygen species providing protection against oxidative stress, direct UV-radiation and desiccation (Johler et al., 2010; Vojkovska et al., 2015).

Some of the experimental studies retrieved in the literature search have characterised persistent strains of *C. sakazakii* in relation to their biofilm formation ability and associated characteristics (mobility, congo red binding, cellulose production), and virulence potential. However, apart from disclosing some differences among strains from different CC, such as the ones observed in biofilm formation ability between CC1 and CC4 strains by Yan et al. (2015), these experiments do not allow to extract conclusions in relation to the presence of specific features associated with a higher persistence ability by some particular *C. sakazakii* subtypes. Some of the strains associated with persistence events in powdered infant formula processing

plants have been also characterised in detail by WGS (Chase et al., 2017; Negrete et al., 2022). These studies have shown that persistent strains from CC64 and CC83 bear multiple plasmids harbouring virulence genetic determinants (secretion systems gene clusters), prophages or arsenic resistance determinants, among others, which could be conferring to the host strain properties of relevance for environmental persistence.

3.2.3.3 | *Analysis of clusters of related genome sequences in the NCBI Pathogen Detection database*

Only 17 clusters contained genomes from both clinical and environmental/other sources, most of them with very few strains (in some cases just one clinical and one environmental strain), and only three cross-sectoral clusters contained > 10 genomes. Eleven out of these seventeen clusters of closely related genomes (64.7%) were typed as belonging to CC4, with three of the CC4 clusters spanning multiple years (1982–2005; 2004–2017; and 1950–1980, respectively). The other clusters contained strains from CC8 (three clusters; 17.6%), CC1, CC13 and unassigned (one cluster each; 5.9%). With the exception of CC13, clinical isolates have been noted among these CCs (Costa et al., 2021).

3.2.4 | Concluding remarks related to (sub)types and features

- There is a wide range (a total of 43 CC, belonging to two lineages) of ***L. monocytogenes*** subtypes reported to be involved in persistence in the FoPE. Persistence was most commonly reported for lineage II (with the top CC 121, 8, 9 covering 46% of persistence events for this lineage) followed by lineage I (with the top CC 5, 6, 2 covering 59% of persistence events for this lineage). These most commonly persisting CC are found in the FoPE from many sectors, with some exceptions (e.g. CC9 linked mostly to the meat sector). Of the top 10 CC most frequently identified as persistent, 5 were also among the top 10 largest SNP clusters in the NCBI Pathogen Detection database including strains from both clinical and environmental/other sources (CC6, CC8, CC9, CC121 and CC321), which evidences their clinical relevance and/or widespread distribution.
- Several phenotypic and genomic features for *L. monocytogenes* have been investigated in relation to persistence in the FoPE. The markers that have been identified as supporting improved environmental fitness, and possibly associated with persistence, are: stress survival islets SSI-1 and SSI-2, genomic islands LGI1 and LGI2, heavy metal (cadmium and arsenic) and biocide (*bcrABC, gacC, qacH, emrE* and *emrC*) resistance determinants, often located on mobile genetic elements (including genomic islands as is the case for *emrE*, transposons as is the case for *qacH* or plasmids), and bacteriophage regions (*comK*), globally linked to increased environmental robustness, tolerance to disinfection and/or biofilm formation. Different combinations of these genetic markers were found in large SNP clusters in the NCBI Pathogen Detection database from CCs frequently linked to persistence (e.g. CC121, CC9) but not from CCs not yet associated with persistence (e.g. CC573, CC639). However, it is not clear yet what is the individual contribution of each of these markers for persistence.
- A wide range of ***Salmonella*** serotypes (a total of 35) were reported to be involved in persistence in the FFPE, with *S*. Typhimurium and *S*. Agona being the ones most frequently reported. *S*. Typhimurium was mainly associated with persistence in the meat sector while *S*. Agona was linked to the LMF, meat and feed sectors. Other serotypes frequently reported as persistent were *S*. Anatum, *S*. Senftenberg and *S*. Mbandaka, mainly linked to the feed sector; and *S*. Derby, *S*. Heidelberg and *S*. Infantis, only linked to the meat sector.
- Of the top 8 serotypes most frequently identified as persistent, 6 were also among the top 10 largest SNP clusters in the NCBI Pathogen Detection database including strains from both clinical and environmental/other sources (*S*. Typhimurium, *S*. Infantis, *S*. Agona, *S*. Anatum, *S*. Heidelberg and *S*. Mbandaka), which provides further evidence for their clinical relevance and widespread distribution.
- The set of phenotypic and genomic features that have been investigated for *Salmonella* in relation to persistence in the FFPE is incomplete. Most of the studies focused on features inherent to most infectious foodborne hazards (e.g. AMR, virulence, growth/survival in foods and biofilm formation), and reported resistance of some persistent strains to one or more antimicrobials/disinfectants, carriage of plasmid-mediated virulence factors, biofilm formation ability or reduced susceptibility to alkaline disinfectants. As such, it is difficult to deduce certain features, that are either indispensable for, or may markedly contribute to, persistence, alone or in combination with other key genotypic and phenotypic elements.
- The subtypes of ***C. sakazakii*** most frequently isolated as persistent clones in the FoPE of powdered infant formula processing plants are the pathovars CC1, CC4, CC64 and CC83.
- *C. sakazakii* CC4 is the subtype most widely represented in the main clusters of related genome sequences (including strains from both clinical and environmental/other sources) of the NCBI Pathogen Detection database, evidencing its clinical relevance and widespread distribution.
- Several features have been associated with the ability of *C. sakazakii* to survive for long time periods and persist in the dry conditions of LMF FoPE, includingthe ability to form biofilms on a variety of abiotic surfaces; a high heat tolerance and desiccation resistance; the production of a capsule that aids attachment to surfaces, provides resistance to biocides and contributes to survival following desiccation; and the production of a yellow carotenoid pigment which stabilises cell membranes and provides protection against stress. However, none of these features seem to be specifically linked to those CCs most frequently isolated as persistent clones in FPE of powdered infant formula processing plants.
- Overall, no universal markers or features, responsible for persistence have been identified for any of the three bacterial hazards under consideration. Although the carriage of different combinations of genetic determinants linked to

increased environmental robustness possibly predisposes some particular subtypes to have a better chance of persisting in the FFPE, persistence is a multifactorial process that also depends on specific environmental conditions and risk factors (discussed below) to take place.

3.3 | Factors at facility level that increase the likelihood of persistence of the most relevant bacterial hazards in the FFPE (AQ4)

The contamination of FCS or NFCS is the first in a series of events that may lead to persistence, depending on the hygienic conditions in a FFPE and the capacity of the hazards to persist, as determined by their relevant genetic and phenotypic features (traits). As such, accidental actions, practices or hygiene failures that favour colonisation of surfaces, instead of preventing, eliminating or controlling it, are the primary risk factors that increase the likelihood of persistence of most of the relevant bacterial hazards in the FFPE (e.g. lack of hygiene barriers between unclean ('dirty') and clean(er) areas (i.e. inadequate zoning), uncontrolled movement of personnel or product flow, frequent receival of highly contaminated raw materials, poor hygienic design or hygienic status of processing equipment or ineffective C&D). The following paragraphs review the literature evidence as to why and how the aforementioned risk factors are pertained to the different hazards assessed, in certain food sectors, also detailing the niches (harbourage sites) where persistence has been reported to occur.

3.3.1 | *L. monocytogenes* in FoPE of four sectors

3.3.1.1 | *Environmental niches or site of persistent L. monocytogenes strains*

The retrieved studies frequently reported the site (or location) where the persistent strains were isolated but did not always further investigate the harbourage site (niche) or true location of the persistent strain. Therefore, in many studies the specific niche is not identified, due to limiting sampling, persistent clones found in several sample sites, etc.

L. monocytogenes was found to persist in a wide variety of sites in the FoPE, demonstrating a comparable split across FCS and NFCS. As can be seen in Figure 8, FCS sites were frequently identified which provide a direct opportunity for contact and thus contamination of the associated food products produced in the FoPE. The most common FCS site where the main subtypes of *L. monocytogenes* linked to persistence were isolated, across sectors, were conveyor systems/belts. Examples of other FCS sites where persistent *L. monocytogenes* was isolated are: crates/buckets/trays (fish and seafood, fruit and vegetables), carcass cutters/splitters and grinders (meat), gutting-, head/tail removing-, fileting- and skinning machines (fish and seafood), tables and slicing- and deboning machines (fish and seafood, meat), ice cream- and milk shaking machines and smear/brine (dairy), packaging lines and mycelium scraping machines[25] (fruit and vegetables). The top NFCS sites where persistent *L. monocytogenes* was isolated from were drains and floors. The repeated isolation of a clone from drains and floors may not always indicate that drains/floors are the niches (harbourage sites) of the persistent strain(s), as these sites may act as collector sites from other sources/niches.

In the review by Belias et al. (2022), persistent *Listeria* were most commonly isolated from floors (20 studies), drains ($N = 14$), conveyor belts ($N = 14$), slicers ($N = 9$ studies and additional 11 studies mentioned other cutting machines) and tables ($N = 8$). The authors stated that, while these sites are likely to harbour *Listeria*, the high number of studies that mentioned persistent *Listeria* being isolated from these top five sites may be biased by the fact that these sites were commonly sampled among all relevant studies.

[25]Machine used in mushroom production. Once inoculated, early on in the cultivation of mushrooms, surface scraping is performed before the mushrooms then grow and are harvested.

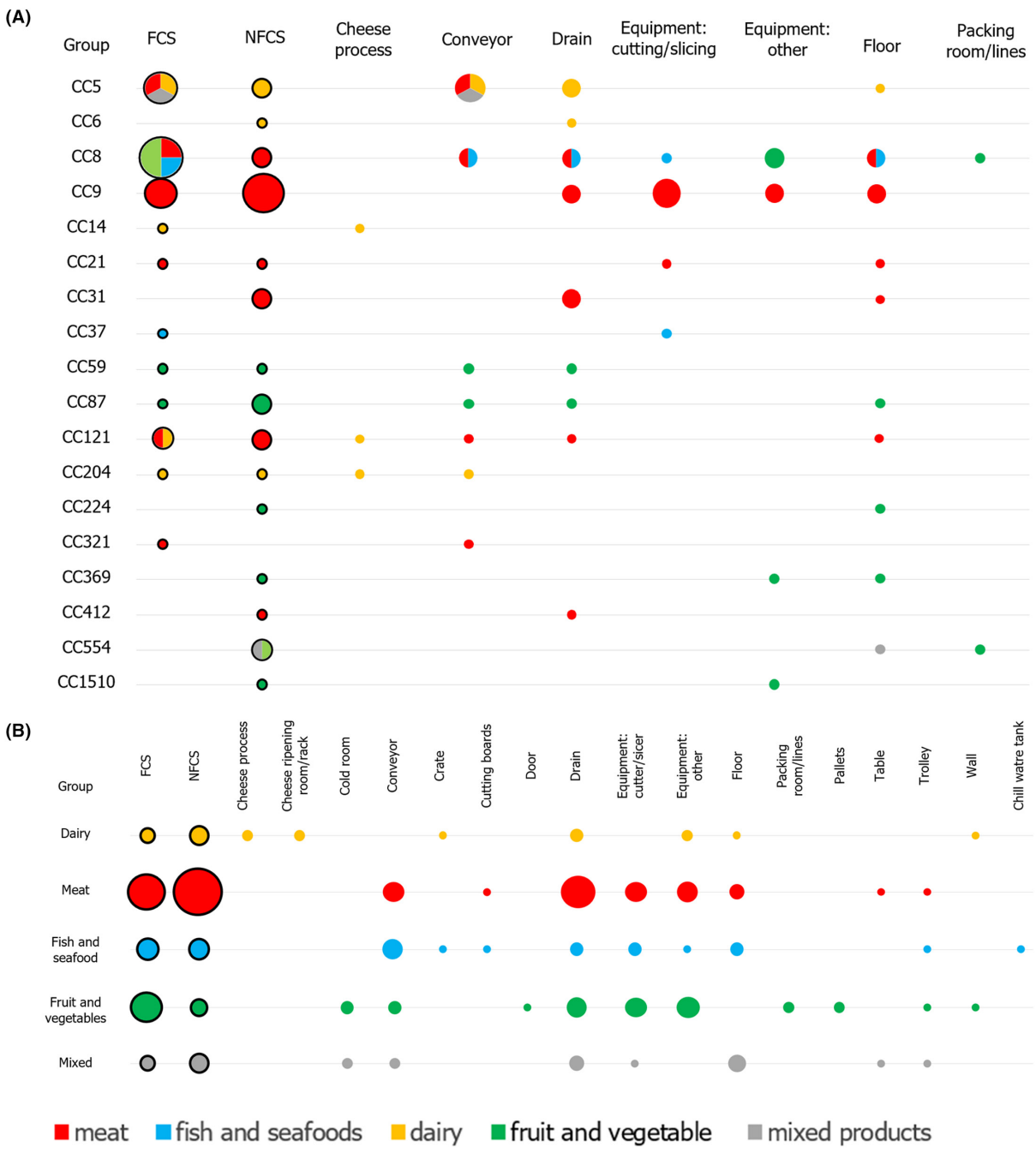

FIGURE 8 Sites associated with *Listeria monocytogenes* persistence in FoPE. The size of the bubble is proportional to the number of studies reporting persistence in a given site. (A) breakdown of persistence reported by clonal complex. (B) breakdown of persistence by sector. Site type (FCS or NFCS) is shown in green, specific site within the FoPE in yellow. *Note*: FCS, Food contact surface; NFCS, non-food contact surface; CC, clonal complex.

3.3.1.2 | *Risk factors for persistence of L. monocytogenes*

In general *L. monocytogenes* is ubiquitous in nature and there are multiple routes for its introduction to the FoPE. Factors that increase introduction to and spreading of *L. monocytogenes* in the FoPE may increase contamination, but it is often difficult to distinguish between repeated reintroduction and persistence. Factors that create niches where *L. monocytogenes* is protected against disinfection may increase the likelihood for persistence. In the review by Belias et al. (2022), equipment cleanability and lack of hygienic zoning were identified as the two most common risk factors for persistent *Listeria* by the included studies.

The risk factors or factors that were reported to increase the likelihood of persistence in the studies in our assessment (since 2010) were mainly related to poor hygienic design of equipment, inadequate C&D of facilities, inadequate zoning/hygienic barriers, raw materials and humidity.

Poor hygienic design of equipment. The most reported risk factor for persistence of *L. monocytogenes* was poorly designed equipment and machines, where moisture and nutrients can accumulate, thus creating a niche in the equipment where *L. monocytogenes* can persist. A list of equipment where persistence of *L. monocytogenes* has been observed is given in Section 3.3.1.1. Common for these niches is often that they are difficult to clean due to poor design and/or low accessibility and/or to the fact that they contain worn materials with for example scratches, crevices or porous materials.

Irregularities in the equipment and difficult to reach places are suggested to hold appropriate conditions for microorganisms to grow, adhere and adapt. Examples of persistent *L. monocytogenes* on difficult to clean equipment are a carcass splitter (Demaitre et al., 2021), a mycelium scraping machine (Sun et al., 2021), floor cracks (Chen et al., 2022), or porous wall, worn surfaces and cleaning tools (Guidi et al., 2022). Several outbreaks of listeriosis were linked to difficult to clean equipment where *L. monocytogenes* persisted. For the caramel apple outbreak in USA in 2014–2015 the outbreak strain was isolated from wear and damaged equipment, and a wooden bin (Angelo et al., 2017). A six-months outbreak in 2008 in Canada linked to delicatessen meats likely involved a slicer that was difficult to dismantle and clean (Currie et al., 2015).

Introduction or instalment of used and difficult to clean equipment is a risk factor for persistence of *L. monocytogenes*, as exemplified for conveyor systems (Fagerlund et al., 2016), a slicer (Fagerlund et al., 2020), and a dicing machine (Lundén et al., 2002). Also, the large *L. monocytogenes* outbreak with cantaloupes in the USA occurred after instalment of a line for washing and drying of melons, previously used for other agricultural products. The outbreak report refers to inadequate design that precluded effective C&D of the processing line as a likely source of the outbreak in addition to a lack of cantaloupe precooling (McCollum et al., 2013).

Inadequate C&D of facilities. Several studies report isolation of persistent clones of *L. monocytogenes* after C&D and conclude that inadequate C&D was the cause of persistence (Chen et al., 2022; Chen, Wang, et al., 2016; Veghova et al., 2017). Some studies pointed to poor C&D procedures (Beccalli et al., 2019; Camargo et al., 2015; Chen et al., 2022; Chen, Wang, et al., 2016) as a risk factor for persistence without providing more specific information, while a few studies provided details about the discrepancies in the procedures used. For example, a study from a meat processing facility in Slovakia identified inappropriate floor cleaning using a full-pressure steam system as a likely risk factor (Veghova et al., 2017).

In an outbreak in Switzerland in 2018, where the outbreak clone was found in the cheese processing environment, the dairy plant had disinfection shortcomings, but no further details were given (Nüesch-Inderbinen et al., 2021). In an outbreak in the USA with imported Ricotta salata cheese, cross-contamination between cheeses with the outbreak strain through cutting and repackaging was observed. The need for routinely using validated disinfection protocols and to clean and disinfect cutting equipment between blocks or wheels of cheese was stated (Heiman et al., 2016).

Self-reporting from five Italian meat processing plants showed that the most commonly neglected C&D hygienic actions were the following: the correct use of disinfectant concentration, the correct exposure time to cleaning agents, the control of rinsing water temperature, the appropriate use of cleaning nozzles and avoidance of aerosol formation (Conficoni et al., 2016). However, this study did not consider persistence.

Inadequate zoning/hygienic barriers. *L. monocytogenes* can be introduced to the processing environment through many routes, such as personnel, equipment, animals, dust, water and raw materials. To limit the introduction and spread of *L. monocytogenes* the industry is dependent on hygienic zones and barriers between outside and inside and between low- and high-risk areas in the processing facility. An increased introduction and/or spread of *L. monocytogenes* may not necessarily lead to persistence in the FoPE as many strains may be transient, but a high rate of introduction/spread of *L. monocytogenes* may increase the likelihood of a given strain to reach a niche and become persistent. For facilities or sectors with low or insufficient hygienic barriers there may be a continuous introduction of new *L. monocytogenes* strains to the facility, in some cases the same clones may be introduced over time making it difficult to distinguish between persistence in the FoPE of the plant or continuous reintroduction of the same strain (it may indicate persistence at a supplier or in raw materials/outdoor environment). There may be differences between and within sectors regarding zoning and hygienic barriers. The fruit and vegetable sector often has less strict barriers than other sectors. Departments for production of soft cheeses and RTE meat products typically have strict barriers against introduction from departments handling raw materials such as raw milk and meat. In a newly opened meat facility, a persistent *L. monocytogenes* strain was believed to be widespread in the facility due to the lack of hygienic barriers within the facility (Bolocan et al., 2016). Similarly, in a newly opened dairy processing plant, lack of hygienic barriers and uncontrolled personnel flow led to the spread of a persistent clone (ST204) within the building (Melero, Stessl, et al., 2019). A reconstruction event aimed at an expansion of the main building of a meat processing facility increased the probability of breaching hygienic barriers and has been linked to increased introduction of *L. monocytogenes* (Stessl et al., 2020). In addition, lack or failure of systems creating overpressure in RTE zones, may enable contaminated air to move directly from unclean areas to cleaner areas, which can contribute to the spread of *L. monocytogenes* within the establishment via air (aerosols) (Nastasijevic et al., 2017). In a survey of management practices at 32 food producers in Ireland, no management practices correlated with persistence, while separation of hygiene control areas correlated with a reduction in *L. monocytogenes* occurrence (Alvarez-Ordonez et al., 2018).

Raw materials. *L. monocytogenes* is commonly found in many raw materials and may be introduced to the FoPE through contaminated raw materials. It may be difficult to distinguish between persistent strains and strains repeatedly introduced with raw materials, the latter may indicate persistence earlier in the food chain or in the outdoor environment. However, repeated introduction will increase the contamination level and the likelihood of strains reaching niches where they can become persistent (EFSA BIOHAZ Panel, 2018). Raw materials that are not heat treated are more likely to contain *L. monocytogenes* and be a source for *L. monocytogenes* than heat-treated raw materials. In an outbreak linked to stone fruits in the USA in 2014, it was difficult to evaluate if the outbreak strain was persistent in the facility or was a transient contamination originating from sources outside the facility, e.g. fruit orchards (Chen, Burall, et al., 2016). Raw materials as a risk factor for persistence were found most commonly reported for the fruit and vegetable sector, but it is also a risk factor in other sectors. There are several reports of the same *L. monocytogenes* clone persisting in several processing plants in the meat sector. This has been shown for several subtypes (e.g. CC9, CC7 and CC19) in the Norwegian meat sector, and it has been suggested that such clones persisting in several processing plants can be regarded as pervasive (persistent strains isolated

from different processing plants). The original source of the pervasive strains was not reported, but it was suggested that raw meat from the same suppliers may have been the original source of introduction (Fagerlund et al., 2022). Also in the USA, the same persistent subtypes were found in several factories which received raw meats from the same slaughter plants (Berrang et al., 2010). Lucchini et al. (2023) traced the persistent strains back to the raw meat from slaughterhouses which are not inspected for *L. monocytogenes* and seem to be hot spots for its occurrence and persistence. In Germany, two outbreaks with the same *L. monocytogenes* clone but associated with meat products from two different producers, that had common suppliers, lead to the speculation that the outbreak strain could have been introduced to both plants with raw meat (Luth et al., 2020). In the salmon sector gutted salmon from a producer may be sold as raw material to producers of fileted or smoked salmon, and strains persisting in the salmon slaughterhouse may then be transferred via gutted salmon to fileting or smoking plants. In Norwegian salmon processing plants, pervasive clones were found in several processing plants (Fagerlund et al., 2022). In a cold smoked salmon processing facility in Ireland, which used raw (gutted) salmon as raw material, the same MLVA type was repeatedly isolated from the raw materials (Dass et al., 2010).

Humidity. *L. monocytogenes* is commonly isolated from humid niches where it may grow and persist, thus the presence of such humid niches is a risk factor. Ruckerl et al. (2014) reported intense humidity and steam and water residues on floors after C&D due to intensified cleaning activities and speculated on this as a possible risk factor for *L. monocytogenes* persistence on floor associated niches (floor, drains, boots). High humidity in cheese ripening rooms has also been suggested as a risk factor (Tirloni et al., 2020).

3.3.2 | *Salmonella enterica* in the FFPE of four sectors

3.3.2.1 | *Environmental niches or location of persistent Salmonella enterica strains*

As for *L. monocytogenes* (Section 3.3.1.1), the niche for persistence of *Salmonella* in the FFPE was often not identified in the studies retrieved, due to limiting sampling or due to persistent serotypes being found at several sampling sites. Sampling sites where *Salmonella* was repeatedly isolated, and therefore may favour persistence, were reported to be NFCS (e.g. mainly drains, whipping machines, evisceration or pre-cooling areas and personnel clothing) slightly more than FCS (e.g. feather plucking rubber fingers, evisceration equipment, plucking machines), although, in a number of these studies, the specific NFCS niche of persistent strain(s) is not clear. Figure 9 presents an overview of persistence of *Salmonella* in the FFPE in sites, where the hazard was isolated. Contrary to the relevant evidence for *L. monocytogenes*, the niches where persistent strains of *Salmonella* have been recovered are of lower resolution, pinpointing to broad (e.g. floors, drains, equipment, lairage, NFCS/FCS) rather than specific sites within a FoPE.

For the meat sector, where detailed, NFCS included floors, drains and matting; FCS included scalding, splitting and feather plucking equipment, and more generically the slaughter line.

For LMF the location of persistence was generally unclear; two studies identified the drying process as an area of persistence. Very few studies reported *Salmonella* persistence in egg and egg products, and those who did so were unclear about the location of persistence. Nonetheless, in egg processing plants, *Salmonella* is commonly isolated from various sites, including floor drains, breaker egg diverters or breaker egg belt surfaces. For feed, exact locations for persistence were often not reported, with data being collated as feed mill equipment or mill environment, or where sampling locations were provided, data for persistent serotypes was not available (Musgrove et al., 2008).

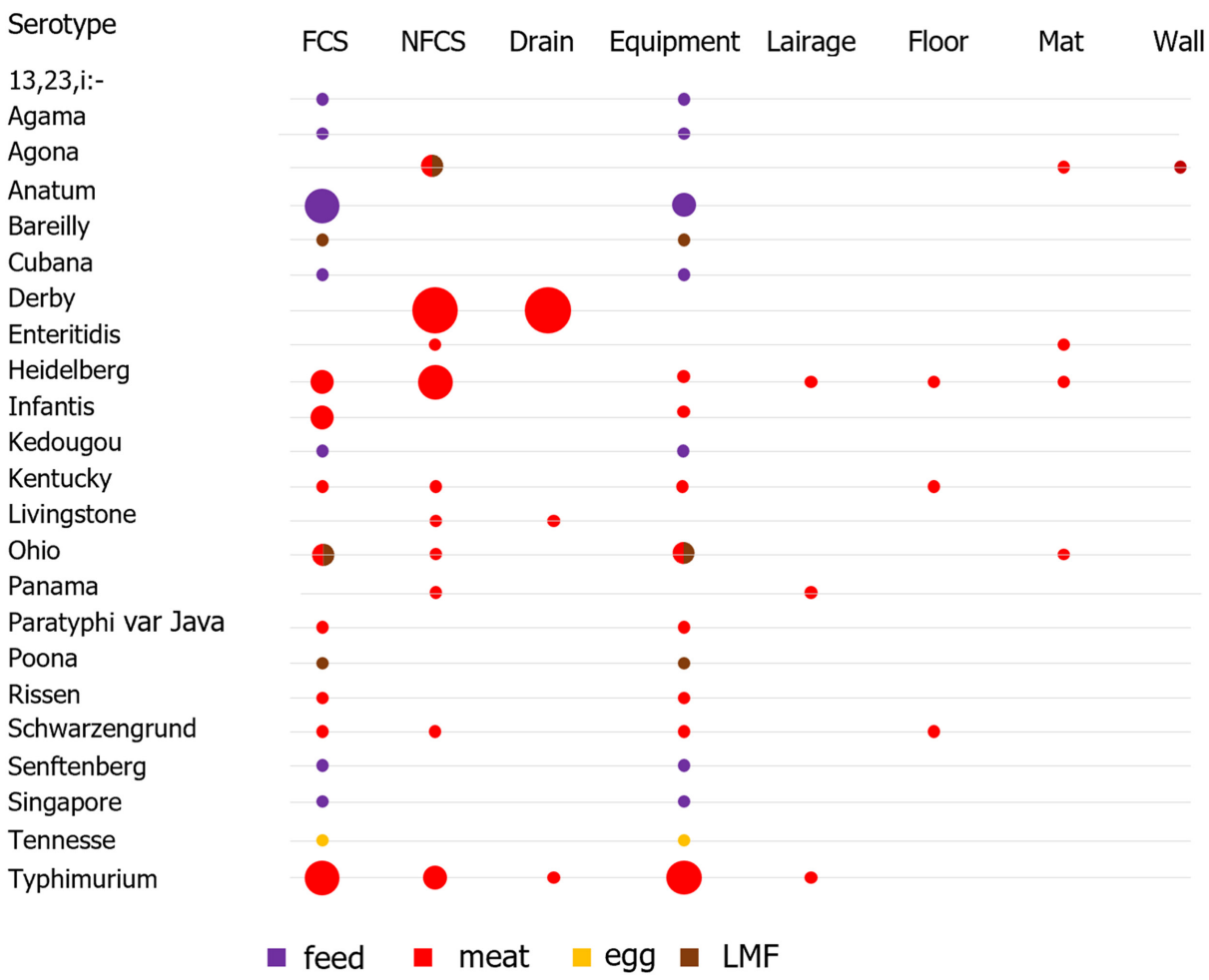

FIGURE 9 Sites associated with *Salmonella enterica* persistence in the FFPE, by serotype. Circle size is proportional to the number of isolates. The colours indicate the food or feed production sector. *Note*: FCS: food contact surface; NFCS: non-food contact surface; LMF: low moisture foods.

3.3.2.2 | *Risk factors for persistence of Salmonella enterica*

Salmonella can survive in dry and dusty conditions. Therefore, animal feed and LMF processing environments support persistence of this pathogen. However, *Salmonella* may also persist in the FoPE of high moisture foods, such as eggs, meat and poultry. Hygiene failures in the reception of raw materials, the zoning of the processing lines, i.e. insufficient separation of 'dirty' from 'clean' areas (especially in slaughter houses), with carry-over of the contamination to adjacent cleaner areas, deficiencies in the hygienic design of the equipment, including the use of food contact materials that may serve as surface of biofilms, lack of proper ventilation and flaws in the C&D protocols, are the primary risk factors for *Salmonella* persistence. The following paragraphs detail the literature evidence associated with each of the aforementioned risk factors considered contributory, particularly to the risk of *Salmonella* persistence in the FFPE. Their control may limit or prevent the persistence of this hazard in the relevant FFPE.

Raw materials and inadequate zoning/hygiene barriers. Contamination of pork during slaughter can occur both in the 'dirty' and the 'clean' zones (Arguello, Alvarez-Ordonez, et al., 2013; van Hoek et al., 2012) may eventually lead to persistence. In the dirty zone, the stages where carcasses are most prone to contamination include stunning and bleeding, dehairing and polishing (to a lesser extent). The contamination is mainly associated with the hides and the accumulation of organic matter in scalding water, or the dehairing and polishing equipment. In the clean zone, contamination may occur during evisceration, splitting, trimming and fabrication (preparing meat cuts and deboning) and is mainly linked to leakage of intestinal content and the use of improperly cleaned equipment (Arguello, Alvarez-Ordonez, et al., 2013). Once *Salmonella* enters a slaughterhouse, it may become part of the resident microbiota, inhabiting certain niches and constitute a renewable contamination reservoir, independent of incoming raw materials (Arguello, Alvarez-Ordonez, et al., 2013; van Hoek et al., 2012). In poultry slaughterhouses, the most critical contamination sites are the neck-cutting knife blade, the scald tank, the defeathering and the immersion chill tank (Hiett, 2010). Like pork, the source of poultry meat contamination is the equipment and the accumulated organic matter released from carcasses during slaughter, mainly during evisceration. Failure to replenish and disinfect water, or maintain sufficiently high temperatures (e.g. > 60°C for pork) in water-using processes, increases the likelihood of contamination (Arguello, Alvarez-Ordonez, et al., 2013; Hiett, 2010). Contamination of the lairage environment over time highlights the importance of hygienic handling of animals through the slaughter process, as this presents a risk of cross-contamination of such strains into food processing lines. Within the FFPE, reports of persistent strains detected on floors and mats also highlights the potential role of footfall of personnel around the FFPE as a means of cross-contamination; this highlights

the importance of PRPs covering workflows and hygiene, for example, the use of disinfecting foot baths, as highlighted below in Section 3.4.2.

Storing and handling raw materials and finished goods in the same area is a detrimental risk factor that, although rarely occurs nowadays, has been provenly associated with outbreaks, such as the US peanut butter outbreak in 2008–2009.[26]

Egg contact surfaces have been shown to protect *Salmonella* cells, especially within a biofilm community from direct contact with disinfectants (Yang et al., 2017). In particular, egg belts have been identified as one of the means by which *Salmonella* can spread from laying hen houses to egg processing plants (Murase et al., 2001).

The hygiene level of NFCS is also critical for preventing persistence, because persistent strains from NFCS have been linked to foodborne outbreaks. A typical example is the 2018 sweetened puffed wheat cereal outbreak in Illinois, caused by *S*. Mbandaka that originated from drains and the external surfaces of food processing equipment (Keaton et al., 2022).

Poor hygienic design of equipment. The use of equipment that is not well-designed and maintained poses a significant cross-contamination risk. Crevices in machinery, flooring and walls, and dead ends in piping are potential areas for pathogen accumulation and subsequent contamination of the final product, especially of LMF. An outbreak of *S*. Agona from a toasted oat cereal occurred due to an inadequate design of the manufacturing environment that was subsequently discovered upon investigation. In this example, the processing machinery was open to the atmosphere (Breuer, 1999). Jones (2011) suggested contamination in coolers may be elevated because condensation on interior surfaces increases moisture, which encourages *Salmonella* growth. In a major outbreak of salmonellosis linked to peanut butter and peanut paste in the US in 2008–2009, more than 700 cases of illness were reported, and the outbreak may have contributed to the death of nine individuals. The contamination of the product implicated in this outbreak was partly attributed to the peanut crackers used as raw materials and further to bad hygiene practices in the processing plant that either did not eliminate the incoming contamination or contributed to the establishment and dissemination of *Salmonella* in the processing environment. Examples of bad practices that could have played a role as risk factors for persistence in this outbreak are the flawed equipment maintenance and factors discussed in the following paragraphs, or already discussed above, e.g. insufficient ventilation, lack of lethality treatments and improper cleaning of containers and utensils.

Aeration/ventilation/dust. In feed and LMF processing plants, persistence can be favoured by insufficient aeration and ventilation or by the dispersion of dust. One study performed in a feed processing plant identified the intake pit as a location for persistence (or continued reintroduction) (Davies & Wray, 1997), another study reported persistent *Salmonella* in the pellet cooler and aspiration system (Häggblom, 2012). Even though the exact source of *Salmonella* contamination of peanut butter paste and peanut butter-containing crackers in the 2008 US outbreak was not clearly determined, it was concluded that *Salmonella* strains re-occurred in these products possibly due to a persistent contamination reservoir and prevalence in raw materials. In addition, maintaining moisture of the processing environment, especially of dry foods, as low as possible minimises the growth and spread of microbial contamination (Grasso et al., 2015).

Inadequate C&D of facilities. This risk factor applies to all sectors, and even more in those associated with foods of animal origin, especially slaughterhouses. The prolonged survival of *Salmonella* in the FoPE and the resistance to disinfectants may be further enhanced by the sheltering of cells in biofilms formed on FCS and NFCS that have been inadequately cleaned and disinfected (den Besten et al., 2016; van Hoek et al., 2012; Wang, 2019). The ability of *Salmonella* to form biofilms is widely recognised, with various serovars of *Salmonella* spp. being characterised as good biofilm formers. Flawed C&D may enhance formation and maintenance of living biofilms that can persist for long in FoPE. In addition, the reduction of potential commensal *Salmonella* competitors by decontamination interventions, e.g. in the poultry chain, in combination with the suppression of certain serovars by vaccination, has favoured the emergence of new serovars and their establishment in FoPE (Kipper et al., 2022; van Hoek et al., 2012). Inadequate C&D of cutting equipment, such as carcass splitters, may further enhance the survival and establishment of *Salmonella* (van Hoek et al., 2012). In dry food processing environments, such as in feed and LMF producing plants, dry C&D is recommended (Grasso et al., 2015). If chemical (wet) disinfection is needed, the methods should be reviewed and validated for its efficacy in removing the contamination, without accidentally increasing the condensation of water on FCS and NFCS that may support microbial growth.

3.3.3 | *C. sakazakii* in the FoPE of LMF sector

3.3.3.1 | *Environmental niches or location of persistent C. sakazakii strains*

C. sakazakii has been isolated from a wide range of powdered infant formula production environments, including roller dryers, spray dryers, drying towers, tanker bays, packing machines, air filters, vacuum cleaners, tubing, ventilators, fluidised-bed areas, powder lumps, floors, shoes, trucks or roofs, and has been shown to persist in various of these environments due to its resistance to desiccation and ability to survive spray drying (Barron & Forsythe, 2007; Osaili & Forsythe, 2009; Yan et al., 2013). In some studies, the persistent strain(s) were related to dryers and drying towers, vacuum cleaners, tubing or powder lumps, whereas the niche of the persistent strain(s) was not thoroughly investigated or was not clearly identified/reported in other studies. Overall, spray-drying, fluidised-bed drying and packing areas of production have been characterised as the main risk areas for contamination (Iversen et al., 2004; Mullane et al., 2007; Mullane et al., 2008). In addition, in the powdered infant formula production process, textile filters used for exhaust of spray-drying towers have been found to

[26] https://www.cdc.gov/salmonella/2009/peanut-butter-2008-2009.html

be a particularly risky location for colonisation by pathogenic bacteria, including *C. sakazakii*, which have caused contamination of the final product (Jacobs et al., 2011). Moreover, *C. sakazakii* has been isolated at high frequency from locations in the external factory environment which highlights the need for vigilance in preventing cross-contamination into spray-drying facilities from external sources.

3.3.3.2 | *Risk factors for persistence of C. sakazakii*

The contamination of powdered infant formula may take place via the addition of potentially contaminated heat labile ingredients including starches, proteins, lecithin or gums. Ingredient dry-mix processes are at higher risk of contamination of the final product. Alternatively, contamination can occur via the production environment. The risk factors identified are mainly related to inadequate zoning/hygienic barriers; aeration/ventilation/dust; and inadequate C&D of facilities.

Inadequate zoning/hygienic barriers. Several studies have reported hygienic failures as a risk factor associated with FoPE contamination and/or persistence by *C. sakazakii*, including violations of the hygienic zoning concept, apertures for the aeration of the plant, lack of proper control for the exchange of goods through roller shutters, or personnel, air and powder movement as significant routes of transmission.

Aeration/ventilation/dust. The opening of filters for mechanical cleaning at regular intervals has been reported as contamination source of the environment with contaminated milk powder (Jacobs et al., 2011). Certain components of powdered infant formula such as lactose, milk fats and proteins may have a protective effect on pathogens during drying and the residual nutrients on the equipment and other FoPE may facilitate formation of biofilms by *C. sakazakii* (Henry & Fouladkhah, 2019). Aerosolised organisms in dust particles are a route of dissemination of *C. sakazakii* in powdered infant formula production facilities. The use of vacuum cleaners in the production area in dry cleaning operations can facilitate the transmission of *C. sakazakii* from FoPE through dust (Pei et al., 2019).

Inadequate C&D of facilities. Dry cleaning of FoPE is the preferred option in the LMF sector and encompasses sweeping, brushing, scraping, vacuuming and wiping with cloths to remove residues. However, the use of limited amounts of water may be required in certain circumstances, for example through controlled wet cleaning followed by a rapid and thorough drying of the cleaned surfaces immediately after cleaning. Uncontrolled wet cleaning should be avoided. The presence of water in the dry FoPE of LMF, whether from wet cleaning, condensation or other sources, will lead to significant and rapid growth of the microorganisms colonising FoPE, especially if temperatures are optimal. Even small quantities of water may have a significant impact. Moist residues in spots and niches, such as cracks or crevices, which cannot be rapidly and thoroughly dried, have a particular risk of growth and hence may lead to the build-up of a reservoir of microorganisms. Cordier (2007) reported a rapid increase of *Enterobacteriaceae* counts following the presence of water in the environment. A thorough investigation carried out following an outbreak of *Cronobacter* spp. Coignard (2006) showed that incriminated lots contaminated with the same *C. sakazakii* subtype were manufactured over a period of about 6 months during which wet cleaning had taken place on several occasions. Condensation and water droplets may be generated through temperature gradients within the facility or within equipment. The cooling air for LMF such as powders comes into direct contact with the food. Significant differences between the air temperature and the temperature of the processing plant can cause condensation in ducts or on FCS if the relative humidity is not correctly maintained.

3.3.4 | Concluding remarks related to factors at facility level that increase the likelihood of persistence

- The most common sites where persistent ***L. monocytogenes*** was isolated are equipment (FCS), drains (NFCS), floors (NFCS) and conveyor belts (FCS). Repeated isolation of *L. monocytogenes* from drains and floors may sometimes indicate persistence elsewhere in the processing environment as drains/floors may act as collector sites. Specific equipment where persistence has been described include carcass cutters (meat sector), deboning machines (fish and seafood, meat), gutting, head/tail removing, skinning and fileting machines (fish and seafood), slicing machines (fish and seafood, meat), ice cream-milk shaking machines and smear/brine (dairy) or mycelium scraping machines (fruit and vegetable).
- The main risk factor for persistence of *L. monocytogenes* on FoPE is poor hygienic design of equipment/machines. This leads to niches which are difficult to clean and disinfect and where food debris and moisture can accumulate, and *L. monocytogenes* can persist. Inadequate C&D of facilities and inadequate zoning/hygienic barriers and introduction through raw materials may also lead to the introduction and spread of persistent clones in the processing environment increasing the likelihood of strains reaching niches where they can become persistent. In addition, humidity favours persistence of *L. monocytogenes*.
- Persistence of ***Salmonella*** is most frequently reported in meat and feed processing, where contamination and/or colonisation of equipment is frequently reported. Contaminated equipment such as cutting/slicing, feather plucking equipment, cyclones, etc. contact food/feed directly during processing, and can lead to cross-contamination of end products.
- The main risk factors for *Salmonella* persistence in a FFPE are: (i) inadequate zoning and hygiene barriers, that enables contamination of clean areas with residues from contaminated areas ('dirty'), especially in meat, poultry and egg processing plants; (ii) flawed hygienic design, which may lead to the harbouring of strains in niches, and protect them from exposure to disinfectants; (iii) lack of sufficient aeration/ventilation or presence of dust (especially in dry, LMF/feed processing environments); and (iv) the inadequate C&D of the facilities, which enables *Salmonella* to become a member of the resident microbiota and constitute a standing reservoir of recontamination of incoming product batches.

- The most common sites for persistent contamination with ***C. sakazakii*** are the drying and packing areas of production, while the dryers and drying towers, vacuum cleaners, tubing and powder lumps have been identified as harbourage sites for persistent strains in LMF (especially powdered infant formula) factories.
- The main risk factors for *C. sakazakii* persistence and/or cross-contamination in LMF factories are related to inadequate zoning/hygienic barriers (e.g. violations of the hygienic zoning concept, apertures for the aeration of the plant, lack of proper control for the exchange of goods or personnel, air and powder movement); aeration/ventilation/dust (e.g. airborne transmission by the opening of filters for mechanical cleaning or the use of vacuum cleaners in dry cleaning operations); and inadequate C&D of facilities (e.g. presence of water in the FoPE, whether from wet cleaning, condensation generated through temperature gradients within the facility or within equipment or other sources, which would allow the pathogen to grow).

3.4 | Measures for monitoring, preventing and/or controlling the persistence of the most relevant bacterial hazards in the FFPE (AQ5)

An overview of the measures for prevention and/or control of persistence of bacterial hazards in the FFPE can be found in Figure 10. FSMS are the result of the obligation to comply with the general hygiene rules mentioned in Regulation (EC) No 852/2004, the requirement to establish a permanent procedure based on the HACCP principles mentioned in the same Regulation, and general aspects such as the precaution principle and traceability mentioned in Regulation (EC) No 178/2002. Before implementing HACCP principles, general hygiene needs to be on point using basic rules necessary to operate hygienically (PRP). The number and type of PRPs depend on the sector, but overall, many of the proposed PRPs in the Commission Notice (EC) No 2016/C 278/01 contribute to prevent and/or control microbial persistence in the FFPE through avoiding the entry of the hazards to the processing plant or their spread across the facility.

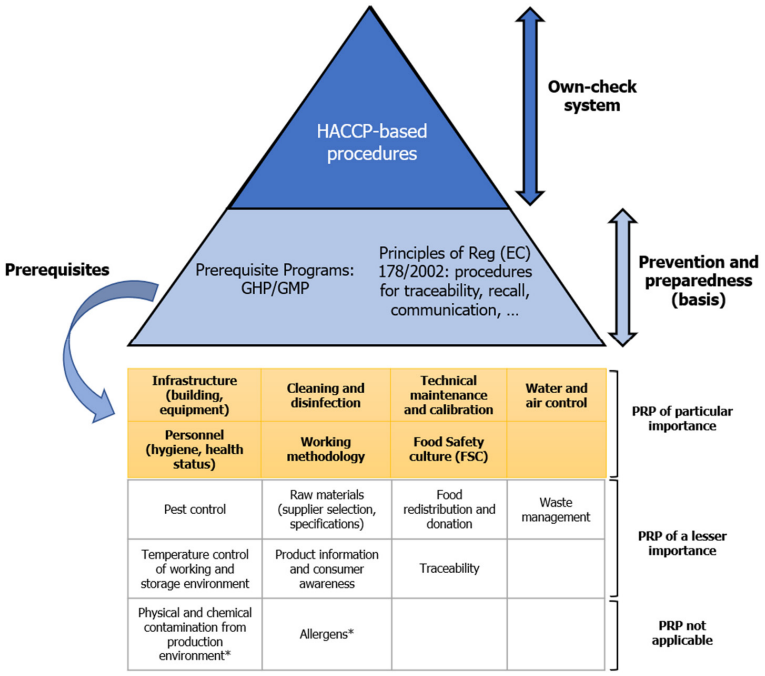

FIGURE 10 Measures for prevention and/or control of persistence of bacterial hazards in the FFPE (based on Commission Notice (EC) No 2016/C 278/01, EFSA BIOHAZ Panel (2017, 2020) and Tuytschaever et al. (2023)).

The verification that the FFPE is free from hazards is achieved through regular sampling and testing activities. When a biological hazard is detected, it is recommended to promptly react. A 'seek-and-destroy' approach has been frequently recommended for that (Belias et al., 2022; Malley et al., 2015; Tuytschaever et al., 2023). This is a systematic way to find sites or niches of persistent strains in food/feed processing plants, with the goal of eradicating them or mitigating their effect (Malley et al., 2015). It includes intensified sampling and testing, the introduction of measures to control the event and the continuation of the intensified monitoring programme. Alternatively, systematic 'root cause analyses' can be applied to identify the most probable factors/sites within the facilities contributing to the problem and define the most appropriate interventions to eliminate the pathogen from the premises, as proposed by Belias et al. (2021). Although the protocol for performing root cause analysis focused on elimination of *Listeria* in an apple packinghouse, the protocol and procedures are expected to be broadly applicable to different food processing operations. A key step for seek-and-destroy and root cause analyses is identifying the source of contamination, which is commonly referred as source tracking. To enable effective source tracking and to understand if persistence in the FFPE is involved, methods that are able to subtype isolates are

critical to provide the discriminatory power to determine if isolates coming from different environments or sampling times are the same or closely related to each other (Baert et al., 2021).

The subsections below describe the main activities for sampling and testing and the PRP activities that can be applied in processing plants to detect (Section 3.4.1) and prevent (Section 3.4.2) persistence, respectively, and summarise the evidence found in the literature on the interventions triggered by persistence events (Section 3.4.3).

3.4.1 | Sampling and testing in the FFPE

The regular microbiological testing of FFPE is widely recognised as a requirement in the production of many types of food as a critical component of any FSMS as a means of verification that food safety measures in place are effective. A well-designed environmental sampling and testing programme can allow manufacturers to early detect hazards that have become persistent and verify that existing control measures are effective to prevent or remove contamination or persistence. Moreover, trend analysis of results can serve as an early warning, which will allow producers to rectify problems before they become a serious risk (Bourdichon et al., 2021).

When designing an environmental sampling and testing programme, it is important to consider and fully document: (i) the identification of sample locations and the reasons why these were selected; (ii) the target organism(s); (iii) the frequency and timing of sampling and the number of samples; (iv) the sampling protocol and defined test methods; (v) and the recording and evaluation of results, with defined limits for acceptable and unacceptable results and follow-up actions in case of non-compliant results (Bourdichon et al., 2021; EFSA BIOHAZ Panel, 2020). Many guidance documents and literature review articles have been published providing recommendations for an effective routine testing in food processing environments and can be consulted for more detailed information on these aspects. Most of them apply to *L. monocytogenes*. Examples include, among others: (1) the EURL Lm Technical Guidance Documents on Sampling the Food Processing Area and Equipment for the Detection of *L. monocytogenes*' (EURL-L. monocytogenes and ANSES, 2023);[27] (2) the document from the Food and Drug Administration (FDA) 'Testing Methodology for *Listeria* species or *L. monocytogenes* in Environmental Samples' (FDA, 2015); and (3) the 'Guidance on environmental monitoring and control of listeria for the fresh produce industry' (UFPA, 2018). A previous EFSA scientific opinion on the public health risk posed by *L. monocytogenes* in frozen fruit and vegetables (EFSA BIOHAZ Panel, 2020) and technical report (EFSA, 2018), also provided recommendations on processing environment testing for *L. monocytogenes* in a freezing plant or handling facility for frozen vegetables. Most of the recommendations included in this former EFSA opinion are also fully applicable to processing plants from other food sectors. Likewise, guidance is also available in the Code of Hygienic Practice for Powdered Formulae for Infants and Young Children for the establishment of monitoring programmes for *Salmonella*, *Cronobacter* species and other *Enterobacteriaceae* in high hygienic processing areas and in powdered preparation units (CAC, 2008; FAO and WHO, 2008). The recent review by Tuytschaever et al. (2023) on *L. monocytogenes* in food businesses highlights the intervention strategies embedded in the food hygiene regulation and provides guidance on hygiene, control measures and FoPE testing strategies of *L. monocytogenes* in the food industry.

Specifically talking about persistence, the selection of sampling points should take into account areas that have tested positive previously or that are likely to be contaminated, such as wet areas, hard to reach places and poorly cleanable equipment, and FFPE more frequently linked to persistence for each specific hazard and production sector (see Section 3.3). It is recommended to randomly rotate sampling sites across sampling times. Furthermore, in the case of special events linked to increased risk of persistence (e.g. construction), a specific sampling plan should be developed to investigate the potential presence of harbourage niches that could be accessible due to the modification, even if temporary, of the process. Likewise, a specific sampling plan should be established following 'non-conformities', where intensified samplings around the initial contamination site, considering also different categories of product proximity, should be performed to assess how far the contamination is spread and to identify potential harbourage sites.

Although sampling and testing activities commonly include the enumeration of 'hygiene indicator' microorganisms (e.g. aerobic mesophilic counts, coliform and/or Enterobacteriaceae counts, yeast and mould counts, or *Listeria* spp. counts), to detect persistence, particular pathogenic microorganism(s) must be targeted, selected depending on the product being produced and its intended use. Considering the outcomes of AQ1 (Section 3.1), the most obvious choice would be *L. monocytogenes* in plants producing RTE foods from different sectors, *Salmonella* in facilities processing feed, meat, egg products or LMF and *C. sakazakii* in powdered infant formula processing plants.

Investigations require the detailed characterisation of isolates of the specific hazard(s) recovered from positive samples through subtyping methods with enough resolution, preferably through WGS-based subtyping schemes, which provide a higher resolution than traditional serotyping or older genotyping methods, previously considered as the gold standard, such as PFGE, MLST or MLVA typing. This detailed analysis will facilitate confirmation of the presence of a persistent strain and identification of its niche within the facility, providing very valuable information for the design of control approaches, and can also be used in outbreak investigations (Pightling et al., 2018). Storage of isolates over time will be advantageous when studying persistence, as isolates from different time periods can be typed and compared. For *L. monocytogenes* it has

[27]This update includes new features adding the presentation of important concepts, such as persistence, biofilm or viable but non-culturable (VBNC), and new guidance for practical implementation (link to data sheets and video tutorials).

been shown that more than one clone can be found in a single environmental sample. This means that it is advisable to type several isolates from each sample/enrichment to capture the whole diversity of the sample (Sullivan & Wiedmann, 2020).

Although pre-defined thresholds are often applied to identify case clusters and their potential sources in epidemiological surveillance, known outbreak-specific features such as pathogen mutation rate and duration of source contamination are rarely considered. Duval et al. (2023) developed a hypothesis-based model that estimates genetic distance thresholds and mutation rates for point-source single-strain food or environmental outbreaks. This forward model, applicable to foodborne or environmental source single point case clusters or outbreaks, is useful for epidemiological surveillance and may inform control measures.

Recent technological advances are paving the way to the design of novel approaches of environmental testing based on the untargeted analysis of the microbiome of FFPE through metagenomics, which can also allow the reconstruction and characterisation of Metagenome Assembled Genomes (MAGs) from multiple taxa in a single analysis, with huge potential benefits for source tracking and investigation of persistence. These approaches are still under development and have some associated challenges and limitations, partly discussed in a previous EFSA scientific opinion (EFSA BIOHAZ Panel, 2019. However, considering recent major improvements (Barcenilla et al., in press), it is foreseen that these innovative technologies will be integrated soon in environmental testing programmes.

Recent studies have shown that the number of samples to be taken can be calculated following mathematical or statistical approaches (Zoellner et al., 2018), and that the performance of effective testing programmes can be also validated through mathematical modelling. For instance, (Zoellner et al., 2019) applied an agent-based modelling, developing a model called EnABLe ('Environmental monitoring with an Agent-Based Model of Listeria') which allowed an in-silico approach to map *Listeria* persistence and dispersal and to evaluate interventions using a data-driven methodology. Likewise, for trend analysis, statistical tools implementing binomial tests based on subtype frequencies or on previous positive results have been applied to support identification and management of persistent *L. monocytogenes* contamination in smoked fish processing plants (Malley et al., 2013).

3.4.2 | Hygienic measures

Various activities of the PRPs, which include basic conditions and actions to maintain a hygienic environment, are necessary to avoid the entry of hazards into the processing plant or their establishment and/or spread within the facility, hence, to also reduce the risk of persistence. Among all the available PRPs, the following prerequisites are of particular importance in relation to bacterial persistence in the FFPE: infrastructure (building, equipment), C&D, technical maintenance and calibration, water and air control, personnel (hygiene, health status), working methodology and food safety culture. Some PRP (i.e. pest control, raw materials (supplier selection, specifications), food redistribution and donation, waste management, temperature control of working and storage environment, product information and consumer awareness, and traceability) are considered of a lesser importance in relation to microbial persistence in the FFPE, while two PRP (i.e. physical and chemical contaminations from production environment and allergens) are not applicable.

All these PRP activities may be specific for each production sector, or factory type, with some documents being available capturing the most relevant ones in some food productions, such as in the recent EFSA scientific opinion on the public health risk posed by *L. monocytogenes* in frozen fruit and vegetables (EFSA BIOHAZ Panel, 2020), which can be consulted for more detailed information on these aspects. More information can be found in the Commission Notice (EC) No 2016/C 278/01 and the recent review by Tuytschaever et al. (2023).

3.4.2.1 | *PRP infrastructure (building, equipment)*

According to Commission Notice (EC) No 2016/C 278/01, the proximity of potential contamination sources should be considered when assessing the risk from the location and surrounding areas. The factory lay-out should strictly separate contaminated (high risk) from clean areas (low risk). Floors and walls should be waterproof, non-absorbent, washable and without fissures. Automatic door opening should be considered. Defined storage facilities should be available for raw material. Attention should be paid to the possibilities whereby the use of equipment can result in (cross-) contamination of food aiming to prevent contamination: (i) of the equipment by the environment, e.g. condensation dripping from ceilings; (ii) within the food handling equipment, e.g. accumulation of food residues in slicing devices; (iii) by raw materials, e.g. separate equipment or clean and disinfect the equipment between uses, for example when using it for raw and cooked products.

Design and organisation of infrastructure, equipment and devices is thus important to prevent bacterial persistence in the FFPE, in particular the prioritisation of areas of a facility according to levels or required hygienic care (hygienic areas) and surfaces within each area designated into zones (also known as zoning). In addition, the following requirements related to the hygienic design of equipment are important: the materials used for construction, the surface roughness, the accessibility of all parts of the equipment for inspection, the self-draining (i.e. no liquid collection) and the absence of niches (e.g. welds should be flush and free of pits, occlusions and corrosion).

3.4.2.2 | *PRP cleaning and disinfection*

Cleaning and disinfection are critical operations to prevent bacterial persistence in the FFPE. According to Commission Notice (EC) No 2016/C 278/01, it needs to be considered what, when, how and by whom to clean and disinfect. Typical steps

should be the removal of visible dirt, followed by cleaning, rinsing, disinfection and rinsing again. Cleaning should start in high-risk areas and end in low-risk areas and different materials and equipment should be used for low and high-risk areas. Special attention must be paid to the contamination of already disinfected surfaces due to splash when rinsing other surfaces. Potable water and/or cleaning agent or disinfectant should be used as much as needed to gain the desired effect. The water should be at an appropriate temperature and the chemicals should be used as per the manufacturer's instructions using available technical information (e.g. instructions for use, active component, contact time, concentration). Visual checks on cleaning and sampling for analysis should be used.

Therefore, it is important to have a well-documented C&D programme, reporting if the equipment must be disassembled, the method of C&D (e.g. foam cleaning, cleaning-in place (CIP)), types and concentration of cleaning compounds and disinfectants, and times/temperatures/pressures to be used (PROFEL, 2019). The efficacy of the disinfection programme should be verified to avoid harbourage sites of bacterial pathogens. C&D is commonly carried out following wet cleaning approaches, as disinfectants require the presence of water or traces of moisture for the active agents to be effective. Nevertheless, rooms should be kept as dry as possible afterwards as moisture fosters growth and transfer of surviving bacteria. This is particularly important in plants producing LMF, where dry cleaning procedures are mostly followed. Dry cleaning can encompass a simple rinsing or flushing of the processing line with the subsequent product or with a neutral matrix. Alternatively, the removal of product residues with a vacuum cleaner followed by scraping or brushing of the FCS is performed in situations where simple flushing is insufficient. Vacuum cleaners can also be used to collect loose powder and dust residues as well as residues dislodged by brushing and scraping.

Particular attention should be paid to the cleaning step, as disinfection of an improperly cleaned surface is ineffective.

The rotation of disinfectants can be considered to avoid adaptation and development of tolerance or resistance by surviving bacteria.

Special attention should be paid to C&D of potential niches of persistent strains, for instance drains. Drains should be cleaned and disinfected in a manner that prevents contamination of other surfaces in the room. Utensils for cleaning drains should be easily distinguishable and be dedicated to that purpose to minimise the potential for contamination. Floor drains should not be cleaned during production. If a drain backup occurs in finished product areas, production should stop until the water has been removed and the areas have been cleaned and disinfected. Employees who have been cleaning drains should not contact or clean FCS without changing clothes, washing and disinfecting hands (FAO, 2007).

3.4.2.3 | *PRP technical maintenance and calibration*

A preventive maintenance plan, drawn with appropriate inspection frequencies to minimise the risks, is also important to prevent bacterial persistence in the FFPE. According to Commission Notice (EC) No 2016/C 278/01, the maintenance plan should be considered with a technical specialist. The plan should include 'emergency' procedures when equipment is defective and instructions for preventive replacement of seals, gaskets, etc. Attention should be paid to hygiene during maintenance operations. Calibration of monitoring devices is important in controlling food safety and hygiene.

Inspection of equipment for damage is critical and should be conducted during pre-operational checks and during preventive maintenance activities.

3.4.2.4 | *PRP water and air control*

Water quality is a relevant factor to be controlled to prevent transmission of hazards and their persistent colonisation of the FFPE. Large volumes of water are commonly used in some food processing sectors for washing, cooling and transport of food, among other uses, and for the cleaning of the FFPE. If the water quality is not well maintained, this can cause cross-contamination with biological hazards (Gil et al., 2015).

In addition to the quite detailed requirements in Chapter VII of Annex II to Regulation (EC) No 852/2004, according to Commission Notice (EC) No 2016/C 278/01, regular own microbiological and chemical analysis of water directly in contact with food (unless community potable water) should be carried out. If community water is held in a tank prior to use, the tank must be part of a regular cleaning schedule. At least clean water, or where applicable clean sea water,[28] should be used in other cases.

A water safety management plan needs to be elaborated in function of the source and quality of the water and the water disinfection technologies (PROFEL, 2019) and should include the sampling and analytical procedures for the verification of the quality of the water. Good manufacturing practices (GMP) and GHP related to a water management plan and the implementation of a water management system are critical to maintain the microbiological quality of the water used in handling and processing operations. This has been concluded for the post-harvest handling and processing operations of fresh and frozen fruit, vegetables and herbs. Identified hygienic practices included technical maintenance of infrastructure, training of staff and cooling of post-harvest process water and intervention strategies (e.g. use of water disinfection treatments and water replenishment) suggested to maintain the microbiological quality of process water. These water disinfection treatments must be undertaken following an appropriate water management strategy including validation, operational monitoring and verification (EFSA BIOHAZ Panel, 2023). Other examples are available for the safety and quality

[28]Clean water: 'water that does not compromise food safety in the circumstances of its use'. It is clean seawater (natural, artificial or purified seawater or brackish water that does not contain microorganisms, harmful substances or toxic marine plankton in quantities capable of directly or indirectly affecting the health quality of food) and fresh water of a similar quality according to the Regulation (EC) 852/2004.

of water use and reuse in the production and processing of fish and fishery products (FAO and WHO, 2023b) and dairy products (FAO and WHO, 2023a).

Regarding air quality, according to Commission Notice (EC) No 2016/C 278/01, ventilation systems should be kept clean. For high risk/care areas requiring air control, implementation of positive air pressure systems and appropriate air filtering systems should be considered. Condensation is mostly the result of poor ventilation and should be avoided in areas where food is being produced, handled or stored, especially if exposed or not packed.

Filtering air that enters production zones may also be an effective measure to avoid the entry of hazards to the processing plant or their spread across the facility (Podolak et al., 2010), hence to prevent their persistence. Filters should be cleaned and replaced on a regular basis and the system validated for removal of microorganisms (Mullane et al., 2008), especially when the air comes directly into contact with the food product.

3.4.2.5 | *PRP personnel (hygiene, health status)*

Training programmes for personnel in proper handling and cleaning practices are essential to raise awareness about persistence of bacterial hazards in the FFPE and assure hygienic practices are accomplished, mainly among the operators involved in C&D or maintenance activities. According to Commission Notice (EC) No 2016/C 278/01, personnel should be aware of hazards from gastro-intestinal infections, hepatitis and wounds and should report relevant health problems to the manager. Hands should be washed regularly and disinfected if necessary. Disposable gloves used hygienically can be effective in preventing cross-contamination when handling RTE foods.

Avoiding traffic of personnel between different areas (especially from dirty to clean areas) is also very important, as the personnel can transfer bacteria across the facility and to the end product.

3.4.2.6 | *PRP working methodology*

According to Commission Notice (EC) No 2016/C 278/01, clear instructions should be provided on proper operation of equipment. Work instructions or standard operation procedures (SOP)[29] should be clear, accurate and simple, and easily accessible.

Personnel should be supervised when following SOP for C&D procedures, with regular maintenance, inspection of cleaned and disinfected equipment and audits of the whole process. The development and use of SOP are an integral part of a successful quality system as they provide individuals with the information to perform a job properly and facilitate consistency in the quality and integrity of a product or end results. Traffic flow patterns for personnel, food products, food packaging materials and equipment need to be controlled.

3.4.2.7 | *PRP food safety culture (FSC)*

According to Commission Notice (EC) No 2016/C 278/01, the components of a FSC are (i) commitment of the management and all employees to the safe production and distribution of food; (ii) leadership in the production of safe food and to engage all employees in food safety practices; (iii) awareness of food safety hazards and of the importance of food safety by all employees in the business; (iv) open and clear communication between all employees in the business and (v) availability of sufficient resources to ensure the safe and hygienic handling of food.

3.4.3 | Actions triggered by persistence

Considering the recommendations made by various authors and in different guidelines to industry, the detection of bacterial persistence in a food processing plant, or even the suspicion, should trigger an immediate response, following a 'seek-and-destroy' or a 'root cause analysis', approach. Intensified sampling and testing (preferable including typing), following the general principles described in Section 3.4.1, can allow an assessment of how far the contamination is spread across the facility and identify of the niche. Vector sampling involves collection and testing of additional samples in various directions (i.e. vectors) from the sample location(s) that tested positive. If the initial positive samples were collected during processing, follow-up samplings after C&D can allow an assessment of the efficacy of the protocols applied and detect potential persistent contamination (Malley et al., 2015).

Different follow-up activities can be implemented with the aim of introducing measures to control the event, and the choice of control measures must be decided in a case-by-case manner, i.e. specific for each processing plant.

Finally, the continuation of the intensified monitoring programme is required to confirm the efficacy of the measures taken or the need to take new ones.

Relevant studies describing actions triggered by persistence with the aim to eliminate persistent *L. monocytogenes* from the processing environment are shown in Table 5. Actions included the introduction of new or specialised (deep) C&D (of more difficult to clean equipment) (Biguzzi et al., 2012; Blatter et al., 2010; Stessl et al., 2020); the implementation of workflows (Dalmasso & Jordan, 2013); the installation of a new drainage system with back-flow prevention (Jordan et al., 2012); the implementation of structural changes and renovations (Nakari et al., 2014); the control of the contamination of raw ingredients and the improvement of the compartmentalisation (Ortiz et al., 2010); the reduction of all contamination

[29] A set of written instructions that documents a routine or repetitive activity followed by an organisation.

pathways of the manufacturing area to the maximum extent practicable (Ortiz et al., 2014); the use of various chemical interventions (Malley et al., 2013), the implementation of one or a combination of hygienic measures (Murugesan et al., 2015) or the simultaneous implementation of various corrective actions (Pennone et al., 2020; Strydom et al., 2016).

More information can also be found in the review by (Belias et al., 2022) on factors that contribute to persistent *Listeria* in food processing facilities and relevant interventions, and (Malley et al., 2015), on the 'seek-and-destroy' approach related to *L. monocytogenes* process controls in the RTE meat and poultry industry.

Some of the interventions that can be implemented to control a persistence event rely on measures aimed at destroying hazards, thus avoiding their long-term establishment in the FFPE or facilitating the prompt removal. These bactericidal interventions can be classified considering their nature as chemical, physical or biological and are summarised below in Sections 3.4.3.1, 3.4.3.2 and 3.4.3.3, respectively. Examples are given of studies assessing their performance in industrial settings or on model systems very closely resembling real industrial settings.

TABLE 5 Actions triggered by persistence with the aim to eliminate persistent *Listeria monocytogenes* from the processing environment in relevant studies.

Study	Persistence locations	Interventions description	Impact of the intervention
Blatter et al. (2010)	Particular slicers and conveyor belts of a sandwich-producing plant	Specialised deep C&D of more difficult to clean equipment (details not provided)	*L. monocytogenes* was no longer found on slicers, conveyor belts or in products
Biguzzi et al. (2012)	The drying-cooling tunnel in a chicken wurstel (a frankfurter sausage made from chicken) production plant	New and more accurate C&D were introduced, including C&D of evaporative cooling pads, front and rear, and fan coolers, at the beginning and at the end of each working day in the slaughterhouse. Further on, the process was modified, including a pasteurisation step (72°C for 15 min) after the final packaging of the product	A contamination average of *L. monocytogenes* on carcasses dropped from about 23%–45% to 7% or below, with a maximum count of 10 CFU/g. After the additional precautionary actions (pasteurisation step), *L. monocytogenes* was detected in wurstel samples, but at a level that complied with the microbiological criteria
Dalmasso and Jordan (2013)	The processing area, the washroom, a storeroom and the packing room; drains, floors, wheels of trolleys or other mobile equipment, tables and boots in the changing room of a food processing facility	Drastic revision of C&D procedures and implementation of workflows (more rigorous cleaning procedures with additional staff, including use of peracetic acid as a disinfectant)	Two out of three persistent *L. monocytogenes* subtypes were eliminated and there was a reduction in the number of *L. monocytogenes* positive NFCS samples. The third subtype was still found, but its prevalence was reduced
Jordan et al. (2012)	Surface areas and drains in the storage rooms of an artisan RTE food processing facility	(i) Thorough cleaning of the facility, workflow changes and staff movement restrictions (ii) Installing a new drainage system with back-flow prevention and changing the cleaning detergent in use	(i) The problem with persistent *L. monocytogenes* was not resolved (ii) The problem was controlled but not solved
Malley et al. (2013)	Smoked fish processing plants	Interventions were implemented in two plants. Various of the interventions used were chemical interventions, including dry QAC granules on floors; treatment of drains with peracetic acid foam; weekly cleaning of drains with foamers, detergent and water; improved forklift fork disinfection; intensified room C&D; and daily drain foaming instead of weekly	Elimination of persistent *L. monocytogenes* was found extremely challenging, but a reduction in the number of samples positive for a given, presumably persistent subtype, was often achieved without complete elimination of the subtype from the plant
Murugesan et al. (2015)	A trench drain and a floor crack during the 1.3 years sampling period in a mushroom slicing and packaging facility	Improvements made to C&D procedures, including among other changes, use of granulated QAC product on the floor, filling and sealing floor cracks and larger crevices, manual cleaning of floors and equipment with brushes and low pressure hoses[a]	There was a significant reduction of *L. monocytogenes* in the washing and slicing area and packaging area. The persistent subtype was still isolated after implementation from the trench drain and a floor crack[b]
Nakari et al. (2014)	Two fishery production plants	Intensive cleaning procedures, structural changes and renovations	*L. monocytogenes* was no longer detected in the production environments or products of these two plants
Ortiz et al. (2010)	The environment and equipment in the cutting and manufacturing room, the slaughterhouse, the loin-marinating and grinding machine in an Iberian pork-processing plant producing RTE meat products	Controlling the contamination of raw ingredients, improving the compartmentalisation and changing the cleaning protocols	Two persistent *L. monocytogenes* PFGE types were eliminated from the processing plant, although eradication of other adapted strains was not achieved
Ortiz et al. (2014)	An Iberian pork-processing plant that produced RTE meat products	Reducing all contamination pathways of the manufacturing area (e.g. raw ingredient contamination, compartmentalisation of fresh meat environments and continuous quality control of machines when manufactured foods are produced) to the maximum extent practicable	Significant effect on subsequent contamination of manufactured products and was probably the cause of the elimination of two persistent *L. monocytogenes* PFGE types at the end of the study

(Continues)

TABLE 5 (Continued)

Study	Persistence locations	Interventions description	Impact of the intervention
Pennone et al. (2020)	Mushroom casing production	Various corrective actions were implemented[c]	*L. monocytogenes* positive samples during the mushroom casing production stage were 31% (18/39) and 22% (8/37) before and after corrective actions, respectively. After hygienic measures were adopted, *L. monocytogenes* was not detected in the first batch of casing samples, but 3 months later, the occurrence in the casing was 20%, suggesting that despite adopting harsher measures recontamination occurred
Stessl et al. (2020)	Drains (storage rooms, raw material processing areas, heat treatment areas, corridors) and overshoes	A basic and an intensified (deep) C&D event were performed. Returning to normal production after a construction event (wall breaks throughs were closed, return to normal work and C&D)	Initially persistent *L. monocytogenes* ST121 strains were identified. There was a decrease in *Listeria* prevalence after implementation of both cleaning and disinfection events. During the construction work more genetic lineage I strains were introduced into the factory (ST1, 6). After returning to normal production there was an increase in the initially detected persistent genetic lineage II strains (e.g. ST121)
Strydom et al. (2016)	An avocado processing facility	Introduction of new strategies (incl. a combination of hygienic measures, intensive environmental testing, structural interventions and higher quality of raw materials)[d]	A drastic reduction of *Listeria* spp. in final products and the processing facility was achieved

Abbreviations: C&D, cleaning and disinfection; NFCS, non-food contact surface(s); PFGE, pulsed-field gel electrophoresis; QAC, quaternary ammonium compound; RTE, ready-to-eat; ST, sequence type.

[a]e.g. adoption of QAC and hydrogen peroxide disinfectant, twice-a-week application of QAC product on the floor, removal of power hose for cleaning floors coupled with minimalisation of aerosol generation during floor cleaning, filling and sealing of floor cracks and crevices with cement sealer.

[b]A possible explanation for the prolonged survival below the floor surface was that *L. monocytogenes* persisted within the porous concrete matrix, which acted as a protection towards intermittent and short contact with surface C&D chemicals while a continuous supply of water and nutrients was available.

[c]e.g. washing procedures for the conveyor belts and transport lorries, the introduction of pools for boot disinfection at the entrance and the exit of all areas, structural interventions to contain more storage bays indoors rather than outdoors.

[d]The strategies included a combination of hygienic measures (double sanitation period of 10 min for fruits entering the facility, disposable aprons), intensive environmental testing (e.g. personnel, surfaces and equipment sampled three times/week during processing and after cleaning), structural interventions (physical separation of the hygiene box for avocado pulp handling from the rest of the facility, and enclosure of the box with only one opening coming directly from the high-risk boot captive and openings for the conveyor belts and waste disposal window, construction of a new canteen) and higher quality of raw materials (wash step with detergent and water rinse before disinfection, strict monitoring of fruit receiving upon the facility).

3.4.3.1 | *Chemical interventions*

Food industries rely on the use of cleaning agents and disinfectants to establish barriers to the entry of foodborne pathogens and avoid their colonisation of FCS and equipment. Most commonly a two-step approach is used with a cleaning phase followed by disinfection. For open C&D of surfaces and equipment, foaming agents are commonly used to facilitate sufficient contact time. Alkaline cleaners like chlorinated alkaline or acid cleaners are frequently used, while disinfectants contain one or more active compounds like alcohols, aldehydes, chlorine and chlorine releasing agents (e.g. sodium hypochlorite, chlorine dioxide), peroxygen compounds (e.g. hydrogen peroxide, peracetic acid), quaternary ammonium compounds (e.g. benzalkonium chloride), amphoterics, bases (sodium hydroxide, potassium hydroxide, sodium carbonate) or acids (mineral and organic acids). CIP (cleaning-in-place) is commonly used for closed systems (e.g. pipes and vessels in dairies), where acids and bases are the most widely used agents. The utilisation of cleaning agents incorporating enzymes, such as proteases, lipases, DNAses, amylases, cellulases or pectinases, can help degrade extracellular polymeric substances before disinfection, facilitating the removal of bacterial biofilms. For disinfection, whole room disinfection is also an option, where the room is filled with a fogging or gaseous disinfectant, e.g. hydrogen peroxide or ozone.

Studies under laboratory settings have assessed the survival of the main foodborne pathogenic bacteria (in planktonic state and/or as biofilms) to a wide range of industrially used disinfectants or their active compounds at their in-house use concentration, and some studies have shown that these are not always capable of completely inactivating target microorganisms when grown in single or multiple species biofilms, as reviewed by Alvarez-Ordóñez et al. (2019). A limitation of many studies is that disinfectants are tested directly on biofilms, without a prior cleaning step, since most disinfectants are designed to work on cleaned surfaces. Although these studies can provide the scientific basis on the most appropriate substances and optimal concentrations to be used in industrial settings, validation activities in real industry settings should be conducted. Validation of the formulations used is essential and may identify improper usage, leading to inadequate contact concentrations or contact times, for instance, through erroneous formulation, inappropriate storage, inadequate rinsing of recently cleaned and disinfected areas or biocide application to wet surfaces, with a consequent dilution of the compound to concentrations that may be sublethal for microorganisms. However, standardised protocols for validation of C&D in situ in processing plants are not available and the results of validation activities are not frequently published and/or made publicly available, contributing to a general lack of information on the efficacy of alternative chemical formulations in C&D regimes in food processing plants.

Numerous experimental studies, mainly undertaken in laboratory settings, have tested novel agents capable of removing bacterial biofilms (e.g. enzymatic agents) and identified novel antimicrobial compounds suggested to be included in formulations for use as green disinfectants. These include electrolysed water, plasma activated water, ozone and compounds of natural origin, including essential oils or extracts obtained from plants, foods and by-products and exerting either a direct bactericidal effect or an indirect biofilm inhibition activity mainly related to the inhibition of quorum sensing and their regulated phenotypes. Further details about research activities in these areas can be found in the reviews by Ashraf et al. (2014), Coughlan et al. (2016) and Alvarez-Ordóñez et al. (2019).

The scarce experimental studies, retrieved from the literature search, performed in industry settings (or in model systems closely resembling industry surfaces), testing chemical interventions to prevent or control contamination or persistence of hazards in the FoPE, all regarded *L. monocytogenes* (summarised in Appendix D).

The experimental studies in industry settings consisted of:

- testing disinfectants in different parts of a dessert-processing factory by (Campdepadros et al., 2012) and in blue crab meat and crab processing plants by (Pagadala et al., 2012).
- adding citric acid powder to floors where water tends to accumulate in meat processing plants (Moretro et al., 2017).
- ozonation as an adjunct to the disinfection regimes across all production areas in a cheese processing facility (Eglezos & Dykes, 2018).

The experimental studies in model systems closely resembling industry surfaces involved:

- using self-contained chlorine dioxide (ClO_2)-generating and delivery pods to disinfect floor drains; the authors concluded that commercially available ClO_2 pods may have practical utility for killing *L. monocytogenes* during periodic disinfection of floor drains (Berrang et al., 2017).
- adding antimicrobial additives in conveyor belt material, which may help to reduce *L. monocytogenes* on belts at low temperatures when food residues are absent, and belts are not rapidly dried (Chaitiemwong et al., 2010).
- comparing C&D protocols of rubber blades simulating procedures used in food processing, showing that rubber blades can be cleaned more efficiently than foam blades and leading to the recommendation of using a full procedure (detergent and rinse, followed by disinfectant) including a scrubbing step (Martinez et al., 2021).

3.4.3.2 | *Physical interventions*

In the experimental studies retrieved, physical interventions applied to prevent persistence or eliminate persistent strains from FoPE were mainly based on the use of non-ionising radiation and heat, with *L. monocytogenes* being the target microorganism for testing. These are summarised below and in Appendix D.

UV-C radiation has been tested to prevent or eliminate contamination of surfaces and the FoPE by *L. monocytogenes*. Bernbom et al. (2011) and Morey et al. (2010) concluded that UV light can be effectively used to reduce *L. monocytogenes*

contamination on food processing surfaces in a fish smoke house after the routine C&D procedure and on conveyor belts, respectively. **Heat treatments**, applied following different approaches, have been shown to be effective interventions to remove *L. monocytogenes* from the FoPE. Eglezos and Dykes (2011) used electrical air-blowing heaters to heat and dry out holding chillers used for post-cook commercial processed meats. The incorporation of two simple chiller heating protocols into these facilities' GMPs effectively reduced *Listeria* prevalence in chillers. Steam cookout of the floors of mushroom growing units can minimise the chances of contamination of subsequent batches, as shown by Pennone et al. (2020). Tobin et al. (2020) illustrated the feasibility of hot water disinfection treatments of commercial mushroom slicers to minimise *L. monocytogenes* food safety risks.

Some **novel non-thermal treatments** (e.g. plasma treatments) could also be applied for the decontamination of air or surfaces (Alvarez-Ordóñez et al., 2019; Coughlan et al., 2016), but have not been tested yet in industrial settings or in relation to microbial persistence in the FFPE.

3.4.3.3 | *Biological interventions*

Biological interventions to prevent persistence or eliminate persistent strains from the FoPE can be based on the use of live microorganisms or their metabolites for competitive exclusion or inactivation of the targeted hazards, or on the application of bacteriophages active against them. Some studies have highlighted that the structure of microbial communities in food processing facilities can impact their colonisation by pathogenic bacteria, mainly *L. monocytogenes*, and that influencing the microbiome in favour of certain species may limit the likelihood of product/process contamination with them (Fox et al., 2014). Moreover, in the production of some traditional fermented products, it has been shown that the development of ad hoc biofilms of lactic acid bacteria on vats used for production can reduce microbial variability in the product (Gaglio et al., 2016) and that natural biofilms present on shelves used for ripening can prevent the growth of hazards like *L. monocytogenes* (Mariani et al., 2011).

Biological interventions have been frequently tested as biocontrol strategies in laboratory settings to inhibit biofilm formation or remove biofilms formed by different pathogenic bacteria, where the antifouling activity of various lactic acid bacteria, mainly bacteriocin producers, bacteriocins and bacteriophage, has been monitored in detail (Alvarez-Ordóñez et al., 2019; Coughlan et al., 2016).

However, the efficacy of biological interventions to prevent or control contamination or persistence of hazards in the FoPE has been rarely tested in industry settings, and only for *L. monocytogenes*. For example, Zhao et al. (2013) illustrated that *L. monocytogenes* could be efficiently reduced using competitive exclusion (using a combination of *Lactococcus lactis* and *Enterococcus durans*) applied onto floor drains of a RTE poultry processing plant. Likewise, (Schobitz et al., 2014) showed that *L. monocytogenes* can be successfully eliminated from the walls of floor gutters in a salmon processing plant using a so-called 'biocontroller' composed of a thermally treated fermentate from two *Carnobacterium maltaromaticum* strains and a strain of *Enterococcus mundtii*, plus nisin. The application of bacteriophage P100 (Listex™) was shown to significantly reduce the incidence of *Listeria* spp. on NFCS in the RTE environment of refrigerated (4°C) and ambient (20°C) temperature facilities (Reinhard et al., 2020). More details about these experimental studies are shown in Appendix D.

The main advantage of biological interventions is that biocontrol agents have generally a rather narrow spectrum of activity. This would facilitate the design of more hazard-targeted interventions, which would better preserve harmless taxa within the processing environment microbiota, which at the long term can result in positive ecologic effects (Alvarez-Ordóñez et al., 2019).

3.4.4 | Concluding remarks related to measures for monitoring, preventing and/or controlling persistence

- A well-designed environmental sampling and testing programme is the most effective strategy to identify contamination sources and detect potentially persistent hazards. It should be designed following a risk-based approach, should be fully documented, defining limits for acceptable and unacceptable results and outlining follow-up actions in case of non-compliant results, and should be regularly revised based on trend analysis. Key aspects are the identification of sample locations; the target organism(s); the frequency and timing of sampling and the number of samples; the sampling protocol and defined test methods; and the recording and evaluation of results.
- The establishment of hygienic barriers and measures within the FSMS, during implementation of HACCP, is key to prevent and/or control bacterial persistence in the FFPE through avoiding the entry of the hazard(s) to the processing plant and/or their spread across the facility. The following prerequisites are of particular importance in relation to bacterial persistence in the FFPE: infrastructure (building, equipment), C&D, technical maintenance and calibration, water and air control, personnel (hygiene, health status), working methodology and food safety culture.
- To confirm the presence of a persistent strain and identify its niche within the facility, the testing programme must involve the detailed characterisation of isolates of the specific hazard(s) recovered from positive samples using subtyping methods with enough resolution, preferably through WGS-based subtyping schemes, which provide a higher resolution than traditional serotyping or older genotyping methods.
- Once persistence is suspected in a food or feed processing plant, a 'seek-and-destroy' approach has been frequently recommended, which includes:

i) intensified monitoring to assess how far the contamination is spread across the facility and to identify a potential harbourage site;
ii) the introduction of measures to control the event, which can involve from drastic decisions (e.g. closure of a line, change of equipment) to other progressive actions such as the intensification or change of the C&D scheme (including increased dismantling) or the introduction of a novel bactericidal intervention; and
iii) the continuation of the intensified monitoring programme to confirm the efficacy of the measure(s) taken or to identify the requirement for additional measures.

Alternatively, systematic 'root cause analyses' can be applied to identify the most probable factors/sites within the facilities contributing to the problem and define the most appropriate interventions to eliminate the pathogen from the premises.

- Successful actions triggered by persistence in the FoPE were found for *L. monocytogenes* only. For example, these included the introduction of new or specialised (deep) C&D, the implementation of workflows, the installation of a new drainage system; the implementation of structural changes and renovations; the control of the contamination of raw ingredients and the improvement of the compartmentalisation, or the simultaneous implementation of various corrective actions.
- There are several options of interventions with direct bactericidal activity that can be implemented to eliminate the persistent hazard(s). These interventions can be classified considering their nature as chemical (biocides, electrolysed water, plasma activated water, essential oils, natural extracts, directly applied or included in the formulation of conventional or novel (green) disinfectants,) physical (based on the use of non-ionising radiation, heat or novel non-thermal technologies) or biological (use of live microorganisms for competitive exclusion or their metabolites, or the application of bacteriophage). While some of these interventions are already in use in the food industry and their efficacy is known from practical experience or scientific studies, other interventions are not yet commercially available and/or their efficacy is not yet fully validated under industrial conditions. For the majority of the listed interventions, a thorough cleaning (often involving dismantling of equipment) is needed before the biocidal step, in order for the latter to be efficient.

3.5 | Perspectives of integrating the information gathered in risk assessment (AQ6)

It was considered that the aim of a risk assessment on the role of persistence for food safety PH risks could be to assess the number or percentage of foodborne illness cases attributable to persistence for a certain population, hazard and/or food product(s) and/or to estimate how different factors (e.g. the features of the hazard or the implementation of mitigation interventions) can impact on them. Such assessments can be performed by two approaches: a bottom-up (or forward) and/or a top-down (or backward) approach.

The bottom-up approach (food chain Quantitative Microbiological Risk Assessment (QMRA)) adheres to the standard microbial risk assessment paradigm and follows the agent through the food chain to produce a prediction of risk to human health. In the top-down approach, the level of risk associated with specific foods, hazards or their combinations is assessed based on information gathered from epidemiological systems such as disease reporting and outbreak databases (EFSA BIOHAZ Panel, 2012). The perspectives of these two approaches to assess the role of persistence for food safety PH risks are described and discussed below.

3.5.1 | Bottom-up approach and data needs

The bottom-up approach is based on the Codex Alimentarius guidelines for microbiological risk assessment (FAO and WHO, 2021a), and encompasses hazard identification, hazard characterisation, exposure assessment and risk characterisation. The transfer, growth and survival of a pathogen is modelled, and the risk (e.g. number of cases) is assessed using an exposure assessment and a DR relation. In the context of studying the role of persistence for food safety risks, this approach can be used for comparing the potential impact of persistence on the model estimates of the PH risks with that of other factors, for example bacterial growth potential in the food, storage conditions, food hygiene or virulence potential of the hazard.

Appendix E provides a detailed description of the perspectives for integrating persistence in a QMRA model. Available 'persistence' models are usually presented as transfer models or cross-contamination models, where the principle is that bacteria in the FoPE are transferred to a food being processed. Some generic – one- and two-compartment – models are presented in Appendix E, including a model with two linked compartments based on (Mokhtari & Van Doren, 2019). This model was adopted as the 'basic model for persistence' in this scientific opinion. The model dynamics are illustrated in Figure 11. This model considers:

- the continuous transfer of the hazard from a contaminated food contact surface shown as FoPE1 to food products (with transfer rate b_1) and to a non-food contact surface shown as FoPE2 (with transfer rate b_2) and the flow back from FoPE2 to FoPE1 (with transfer rate b_3)
- the change in population size in FoPE2 in the time frame between two food products passing in FoPE1, represented by the 'persistence parameter' a_2.

- A positive value implies net growth in FoPE2 with a growth rate a_2;
- When $a_2 = 0$, growth is equal to inactivation and there is no change in population size; and
- A negative value represents a net inactivation rate implying less than 100% survival.

Note that the chosen unit of time (i.e. the time between two food products being processed in FoPE1) implies that the model does not use a common growth or inactivation rate, expressed per minute or per hour.

- the decrease in population size in FoPE1 (which, if negative, might represent growth), also in the time frame between two food products passing in FoPE1, represented by c_1.

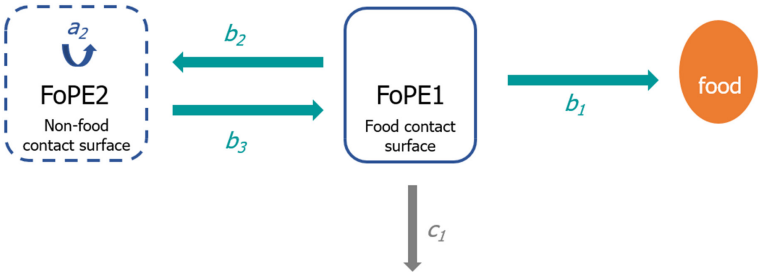

FIGURE 11 The two linked compartments model considered as the 'basic model for persistence' to evaluate the perspectives of QMRA to assess the role of persistence for food safety public health risks. From a contaminated food contact surface FoPE1 there is continuous transfer to food products (with transfer rate b_1) and to FoPE2 (with transfer rate b_2). There is also a flow back from FoPE2 to FoPE1 (with transfer rate b_3). The persistence parameter a_2 indicates the growth or inactivation rate in the hidden environment FoPE2, whereas there may be limited survival in FoPE1, described by inactivation rate c_1.

To better understand the basic model for persistence and how it can be used, two illustrative examples are presented below:

- Imagine a situation where a *L. monocytogenes* strain persists in a drain of the packaging room of a plant producing roast beef. Contamination starts at the drain and is transferred to a FCS in the packaging machine, contaminating the end product. The strain persisting in the drain belongs to CC9, has low virulence (with several truncations in virulence factors), but has an enhanced survival in the environment and resistance to C&D, due to the carriage of several efflux pumps, heavy metals and heat resistance genes and stress survival islet I. In this example, FoP1 is the packing machine, FoP2 is the drain in the packaging room, and the food product is roast beef. The decrease in population size in FoPE1 (c_1) is expected to be very low, while the change in population size in FoPE2 (the 'persistence parameter' a_2) is expected to be zero or positive. The transfer rates are not known.
- A *S.* Agona strain persists in the spray drier of a plant producing powdered infant formula. Contamination starts at the FCS of the spray drier, is then transferred to hidden compartments of the spray drier where it persists. The strain has unknown properties in terms of virulence, biofilm formation or survival in the environment. Considering the low moisture found both in the end product and on the surfaces of the spray drier, it is assumed the strain does not grow neither in the hidden compartments of the spray drier nor in the powdered infant product. In this example, FoP1 is the FCS of the spray drier, FoP2 is the hidden compartments of the spray drier, and the food product is the powdered infant product. The decrease in population size in FoPE1 (c_1) is expected to be very low, while the change in population size in FoPE2 (the 'persistence parameter' a_2) is expected to be zero. The transfer rates are expected to be very low.

This basic model for persistence is deterministic, assuming constant rates for transfer between environments and food, growth and inactivation in FoPE1 and/or FoPE2. This simplifies the model but may not be realistic. The alternative is to develop a stochastic model, which may be more realistic but more complex, containing more (unknown) parameters. Random stochastic variation can be included (assuming constant rates, but with discrete bacterial cells being sampled) or even the rates can vary with every transfer.

The basic model assumes that inactivation and growth take place gradually (at a constant rate). This is not necessarily realistic, as for example, with C&D, a larger amount of the bacterial contamination will be removed, which can be modelled by an instantaneous inactivation step, implemented by a few logs decrease in concentration. Typically, for persistence to occur, it can be assumed that the C&D is effective in FoPE1, but not in FoPE2. Similarly, growth may occur during a period that the food processing is halted, e.g. overnight, giving ample time for an increase in concentration, for example in a biofilm.

The persistence model can be used in conjunction with other models in a larger food chain model for QMRA. As an example, a QMRA model for *L. monocytogenes* was developed inspired by the production of cooked ham. In the *L. monocytogenes* model, persistence was considered to occur in a slicer. It was assumed that cooking of the ham inactivates all *L. monocytogenes*, that the slicer has been persistently contaminated with *L. monocytogenes* and that there is growth during storage of the ham in subsequent parts of the food chain. Another example, following the same food chain model structure, without the storage step, was explored for *Salmonella* in a LMF, where growth is not expected to occur in the food product during storage.

Performance of the QMRA model for *L. monocytogenes* is illustrated in Figure 12, that shows an example of the dynamics of the persistence model and the resulting impact on the risk per serving. Appendix E further illustrates the performance of the model and shows how such a model can be used to investigate the expected impact of several factors associated with persistence on the risk estimates, compared to other factors affecting the risk. Note that the QMRA model is a simplification in many respects, not only because stochastic processes in the FoPE are not included, but also because variability in storage times and temperatures is not included. It is to be expected that observed levels of contamination in the FoPE, observed concentrations in implicated food products and observed cases in an outbreak are much impacted by this stochasticity.

As an example, Figure 13 compares the PH risk estimates for a hypothetical set of strains with that of a strain that, in terms of persistence, is characterised by $a_2 = 0$, and has an average virulence and growth capacity during storage (the baseline shown in Figure 12). Here, a PH risk estimate is the expected number of human cases in an outbreak following the contamination of the FoPE modelled. The alternative strains are more persistent ($a_2 > 0$), more virulent and/or have a larger growth capacity during storage than the baseline strain. In this particular example, all these characteristics increase the PH risk estimate, where virulence seems to have most impact on the risk (the orange bar). It also shows that persistence, defined by an increased growth capacity in FoPE2, leads to a longer maintenance of the risk level (the grey bar), which means that, in the course of time, the bacterial load and the probability of illness per serving are unaltered (vertical blue line) or are decreasing slowly.[30] This maintenance (which in practice would lead to the observation of persistence) is to be expected for a strain that can grow in FoPE2. The example shows that more persistent strains are associated with larger risks, especially in the longer term, but also that factors like virulence and growth capacity in the food contribute much to these PH risks.

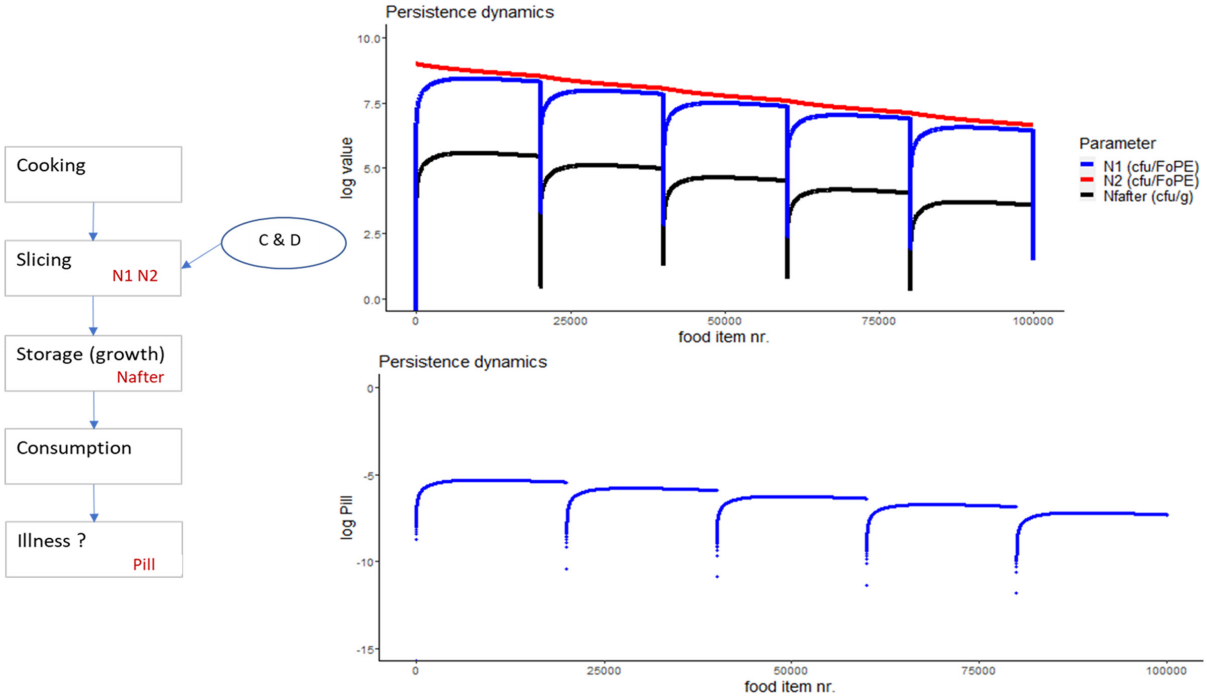

FIGURE 12 Performance of the baseline QMRA model with persistence for *L. monocytogenes* in a slicer. *Note*: The modelled process is shown at the left, the persistence model is included as the model for the slicer. The graphics at the right show the dynamics of (above) the levels of contamination in FoPE1 (blue line, N1) and FoPE2 (red line, N2) and the concentration in the food after storage (black line, Nafter), and (below) the probability of illness per serving of the food. The horizontal axis shows the consecutive food items processed. In this baseline, the persistence parameter is $a_2 = 0$, there is no growth or inactivation in the FoPE's. Every 20,000 food items there is C&D in FoPE1, resulting in a 5 log reduction of the contamination level. Further details and alternative scenarios are explained in Appendix E.

Which factor contributes most will depend on the specific case study and scenarios at hand. Although the figure may suggest that persistence is not a very important factor for the risk, this is not necessarily so. The baseline assumes that there is 100% survival (and not growth) in FoPE2, without an effect of C&D in this environment. This is a form of persistence as well, that is required to get an effect of growth during storage and virulence. A baseline without any persistence is not useful for the model comparison, as it is arbitrary and would give a very low risk. However, it may be realistic in many cases.

[30]In the model, an increasing risk (a grey bar beyond the blue vertical line in Figure 13) will in the course of time lead to a situation with unrealistically high concentrations and is not sustainable (see Appendix E).

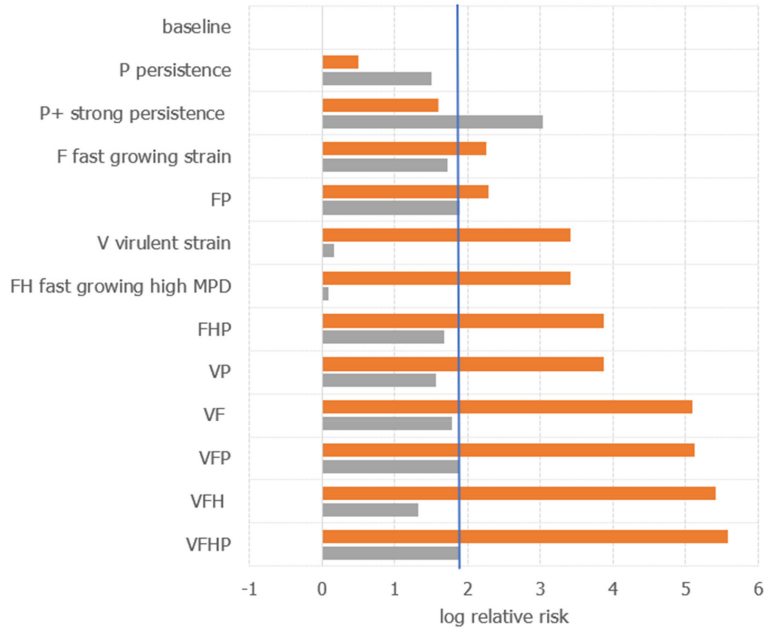

FIGURE 13 Results of the *L. monocytogenes* model, comparing a 'common' strain (baseline) with strains that are more persistent (P and P+), virulent (V) or fast growing in the food (F) (potentially with a higher maximum population density (FH)), or combinations thereof. *Note*: The orange bar is the relative risk (i.e. the log of the expected PH risk of the indicated strain divided by the expected PH risk of the baseline strain); the grey bar is the relative maintenance of risk of the indicated strain, which is the case ratio (the expected PH risk of the last 20% of food products divided by that of the first 20%) of this strain divided by the case ratio of the baseline strain; the blue vertical line indicates a relative maintenance of risk corresponding to 100% (a case ratio of 1, no change in PH risk associated to the first and last 20% of food products). For details, see Appendix E.

A bottom-up risk assessment approach is not only useful to assess and compare PH risks in different scenarios, it can also be particularly useful to clarify the potential role of persistence in food safety, and the importance of different elements of persistence. For example, the analyses described in Appendix E using a range of plausible input values for the different model parameters showed that:

- Persistence is a complex phenomenon, involving bacterial growth, inactivation and transfer, several compartments in the FoPE (such as FCS and NFCS and the food products themselves) and stochastic processes. A model of the process is necessarily a simplification, which will not allow an explanation of all observations of the process.
- It is feasible to include persistence in a QMRA model and explore its impact as compared to for example virulence or growth in the food product.
- Growth in the FoPE is required to explain a long-term outbreak of *L. monocytogenes* due to persistence of a strain in the FoPE. This was concluded using the cooked meat example presented above. Without growth in the FoPE, a high survival rate must be associated with low transfer rates to maintain a long-term presence of the hazard in the FoPE, meaning that relatively low initial doses will be attained. The QMRA model suggests, under the conditions used for model development, that this will not result in illness of consumers over a longer period of time.
- In the LMF example of *Salmonella*, the requirement for growth in the FoPE is less stringent because more infections are expected after exposure to much lower doses due to the non-linearity of the DR model.
- If growth in the FoPE is too extensive, the contamination of the environment will continuously increase and most likely result in early detection of the hazard in the FoPE or in the food, followed by interventions. There is actually a very small range of growth rates in FoPE 2 (persistence parameter a_2) that can explain long term persistence without a decrease or increase of the level of contamination in the FoPE, and a consequential decrease or increase of the associated PH risk. This suggests that the 'basic model for persistence' may be too simple to explain long-term persistence. This model is deterministic and does not include all the relevant biology, it lacks for example the complex mechanistic dynamics associated with biofilms.
- Stochastic processes can explain seemingly chaotic dynamics and the re-appearance and detection of a persistent strain over longer time periods. Although stochastic processes seem to play an important role in persistence in the FoPE, inclusion of stochasticity increases the model complexity and the number of model parameters for which data are lacking.
- In the process of developing the persistence model and the QMRA model, it became clear that the definition of persistence is crucial for conducting and communicating about a risk assessment on the role of persistence. Persistence, defined in this scientific opinion as '*the ability of a given organism to be established in niches within the FFPE for a long term, despite the frequent application of C&D*', is a characteristic of the hazard in a given environment that, in the model, is expressed as the persistence parameter a_2 and other model parameters. The values of these parameters defining persistence are an input of the model. However, in the literature, persistence is also referred to as for example 'repeated isolation of the same strain for months or even years at the same sites' which can be translated into a model output as continuous high levels of bacterial contamination in the FoPE (Larsen et al., 2014; Unnerstad et al., 1996). These continuous high levels can

result in a maintained level of risk that implies that cases can occur over a long period, potentially resulting in a long-term outbreak. The model analyses show that the input (the ability to be established for a long time) and output (long-term maintenance of high levels of contamination and an extended time period of increased risk) are strongly associated, but they are not the same.
- On top of that, the analyses show that persistence is not a black/white, yes/no, presence/absence concept, but it is gradual, as gradually increased persistence implies gradually better survival or longer presence in the FoPE, without a critical threshold. This has for example implications for the classification of strains as being persistent or not: the ability to be established is not present or absent, and will depend on an interaction between the strain, the specific FoPE and the implementation of hygienic measures such as C&D.
- There is a gap between the interpretation of molecular and genotypic data, as presented and discussed in Section 3.2, and QMRA. It would, for example, be highly relevant if the virulence as characterised by genotypic data could be translated to values of DR model parameters, such as the r-parameter for *L. monocytogenes*. At the moment, the virulence of subtypes that are considered 'hyper- or hypovirulent' can only hypothetically be translated in virulence in a QMRA model. The same is true for persistence: it is unclear how all features responsible for persistence (e.g. the carriage of some of the genetic markers identified in Section 3.2 as possibly associated with persistence) are to be translated in the a_2 parameter or other parameters in a persistence model.

The information gathered in the preceding part of this Scientific Opinion indicates for which pathogens persistence is considered to play an important role for specific food sectors. This clarifies for which hazard/food product combinations a QMRA involving persistence could be useful. Also, it may be feasible to develop QMRA for different strains of the same hazards, if the relevant traits of these strains in terms of growth and survival in the FoPE and the food, as well as the virulence, are characterised. So far, this information is however incomplete.

The data needs for risk assessment are large, as the PH risk is a function of many parameters, and the values of these parameters can only be obtained from bacterial concentration data that need to be frequently collected over a long period. On top of that, the parameter values are probably very process specific (Møller et al., 2016), which is in line with the observation that comparison of risk factors between food processing facilities is difficult (Belias et al., 2022). This challenges the data acquisition required for estimating the parameter values and model validation.

The increasing availability of genotypic data is scarcely applied for the purpose of risk assessment. These data are particularly useful to understand the molecular mechanisms of persistence and to identify strains and subtypes that can be associated with observed persistence and larger PH risks. However, so far, we have found no evidence of examples where this type of data has been applied in bottom-up risk assessments that aim to compare the reduction in PH risks that can be achieved by specific interventions or control measures. The application of this type of data in QMRA would require a translation of the genetic information into model parameter values that are required for risk assessment.

3.5.2 | Top-down approach and data needs

In a top-down approach, the assessment starts with an analysis of epidemiological human disease data, for example reported human sporadic listeriosis cases and/or outbreak cases. From these, to attribute the cases to their sources, the actual food source/vehicle carrying the pathogenic organism is to be retrieved. For the sporadic cases, this information is not available in the EU-wide databases (TESSy). The EFSA zoonoses database includes data on 'strong and weak' evidence FBOs (as defined in the Directive 2003/99/EC) occurring in MSs, including those caused by any virus, bacterium, alga, fungus, parasite and their products, toxins and biological amines (e.g. histamine), not just zoonotic agents. It captures the causative agent and the food vehicle. It is mandatory to report the food vehicle as a general food vehicle category (e.g. 'Eggs and egg products') and since 2020 more details about the food vehicle can be reported e.g. 'Cheeses, made from unspecified milk or other animal milk -unspecified -made from raw or low heat-treated milk'. Optionally (and this was the only possibility before 2020), a free text data element can be used to give more detailed information on the food vehicle (for example 'salad of raw carrots'). Unfortunately, the actual food is for many (past) outbreaks not available. More information about the reporting on FBOs can be found in the technical report titled 'Zoonoses, antimicrobial resistance and food-borne outbreaks guidance for reporting 2022 data' (EFSA, 2023).

To assess the proportion of human cases that can be attributed to persistence in the FoPE for different food categories, not only the food vehicle needs to be identified, but also whether there is persistence involved. As persistence implies long term survival in the environment, it is mainly of interest for its potential to cause long-term outbreaks, so the focus would be on these. The aim of the assessment would therefore be to identify the percentage of outbreaks for a specified food that is associated with persistence. Unfortunately, this is not (well) documented in the reporting of outbreaks. For example, in the EFSA zoonoses database, contributory factors can be reported in the optional data element. Contributory factors may include deficiencies in food handling or the use of contaminated material. Such contributory factors leading to FBOs are frequently unknown, and it should be considered that 'persistence' does not have a common definition. For example, long term isolation of the same strain in a food product is not necessarily a result of its persistence in the FoPE but can also be caused by repeated reintroduction in the FoPE, which, in this Scientific Opinion, is not interpreted as persistence. It should be noted that it is currently possible to report under 'General' two different terms: 'Continuation of an outbreak reported last/previous year' and 'Part of multi-country outbreak'.

A top-down risk assessment approach for persistence would require a database of outbreaks for the causative agent under investigation that allows identification of the food vehicle, but also gives an indication of the likelihood that such outbreak is due to persistence in the FoPE, using an agreed unambiguous definition of persistence. With an estimate of the percentage of outbreaks associated with persistence obtained in this way, the relative risk of persistence for specific hazards and/or food products (or categories) could be assessed.

A top-down approach can also be used to study to what extent 'persistent strains', i.e. genotypes associated with persistence, are found in outbreaks and/or in sporadic cases. As the bottom-up risk assessment model showed, attributes like virulence and growth capacity in foods may also be important for strains to be linked with outbreaks. If the genetic characteristics of outbreak strains are known, the relative importance of persistence can be assessed. This requires the availability of genetic data of outbreak strains together with the characteristics of the outbreaks for which they are found. Unfortunately, however, despite an increased use in last years of WGS for outbreak investigation, the strains involved in cases of infection or outbreaks are not always characterised in detail, and therefore it is not easy to know whether they have some features likely related to persistence. Such an approach would require the use of genotypic persistence markers (e.g. particular genes associated with increased survival in the FoPE) truly inducing phenotypic persistence. Unfortunately, however, the '*presence or absence of genes thought to promote persistence, was not found to be useful for predicting persistence*' in previous reports (Møller Nielsen et al., 2017) and, as highlighted in Section 3.2, no universal markers or features, responsible for persistence can be identified yet with the available evidence.

3.5.3 | Concluding remarks related to perspectives of integrating the information gathered in risk assessment

- Risk assessments may be performed for different relevant combinations of hazard and food product to assess the relative PH risks that can be associated with persistence.
- For that purpose, in bottom-up food chain QMRA, transfer or cross-contamination models involving one or two (linked or not) FoPE compartments may be used. In this assessment, a basic two linked compartments model for persistence has been developed and its performance explored using a range of plausible input values for the model parameters.
- In a simple, hypothetical example, it was shown that this type of model can be used to study the role of persistence in the PH risk for a specific food production process. However, since the dynamics of persistence and its role in PH risk is affected by complex processes, linked to the factors listed in Section 3.3, the current model may be too simple to capture important biological processes, such as biofilm formation.
- With the currently available data, top-down risk assessment, where food vehicles are linked to sporadic human cases and outbreaks, cannot be used to assess the relative PH risk that can be associated with persistence. It would require that the occurrence of persistence in FoPE is collected and reported along with the other outbreak data.
- Risk assessment cannot fully exploit the data gathered to support answering the previous AQs, and the data needs for risk assessment are not well covered. There is a need for a better translation of genotypic information of strains into phenotypic characteristics that can be converted into parameters of risk assessment models, as well as for extensive quantitative data to describe the dynamics of transfer, survival and growth of bacterial hazards in different FoPE niches.
- The ultimate objective of risk assessment is to provide decision support for risk managers. Risk managers would benefit from the knowledge of the relationship between persistence in the FoPE (as defined in this scientific opinion) and PH risk, especially if the risk assessment approach allows an assessment of the efficacy of intervention measures that reduce persistence and the associated risks. In that respect, the risk assessment model structure described here can help to suggest potential mitigation strategies against persistence of biological hazards in the food chain, whereas it is unclear how the current knowledge about identified relevant (sub)types and their main features can be practically used in risk management. So far, there seems no solid basis to manage strains differently depending on their subtype or genetic features.
- It is important to apply clear definitions of persistence in all studies that involve persistence (observational, experimental, epidemiological, etc.) and it would be preferable to use the same unambiguous definition for all of them.

3.6 | Knowledge gaps and priorities for future research (AQ7 and 8)

The knowledge gaps and the recommendations/priorities for future research related to bacterial food safety hazards associated with persistence in the FFPE are shown in Table 6. Most of the recommendations for research would involve activities in industrial settings. Therefore, their feasibility will depend on the availability and willingness of food industries to share data on persistence in their facilities and participate in research actions aimed at improving the knowledge on persistence in the FFPE. As an alternative, some of the recommended research activities could be performed using industrial-like model systems of certain niches (e.g. drains, conveyors, slicers), where different strains (including knock-out variants), environmental conditions and potential interventions can be tested, which would also allow generating quantitative data to describe the dynamics of transfer, survival and growth of bacterial hazards and to obtain strain- or subtype-specific parameter value estimates for QMRA.

TABLE 6 List of knowledge gaps and priorities for future research related to bacterial food safety hazards associated with persistence in the FFPE.

Knowledge gaps	Recommendations/priorities for research
In many studies the source of the contamination is unclear, especially the relative importance of persistence versus reintroduction	Longitudinal detailed, well-designed and reported studies for the most relevant hazards associated with persistence addressing multiple sources (e.g. raw materials, primary production settings from suppliers, multiple FFPE within the processing plant, end products, water, personnel, etc.) to distinguish persistence and reintroduction, and collecting metadata associated with the matrices, the process, the suppliers, the end product etc. Such studies will benefit from a common use of the term persistence and from harmonised approaches and SOPs for bacterial typing in relation to persistence using preferably WGS
Limited knowledge on the importance of persistence in the FFPE (and on niches and features of persistent strains) for bacterial hazards other than *L. monocytogenes*	Studies targeting hazards other than *L. monocytogenes* regarding their possible persistence in the FFPE. Such studies can particularly focus on *S. enterica* and *C. sakazakii*, given their relevance in the FFPE from specific food and feed sectors and the insufficient data available on their features linked to persistence, but can also consider other hazards for which studies addressing the FoPE are very scarce (e.g. *Campylobacter*, pathogenic *E. coli*, *S. aureus* or *B. cereus s. l.*). They can include a detailed genomic and phenotypic characterisation of strains of the main subtypes recovered from FFPE
Contribution of specific genetic markers and their link to phenotypes associated with persistence for the most relevant bacterial hazards and/or subtypes Relationship between AMR and biocide resistance of pathogens and its relevance for persistence of hazards in the FFPE	Systematic studies with persistent and presumed non-persistent strains harbouring specific genotypic markers with detailed characterisation of phenotypes relevant for persistence for QMRA. Such studies would benefit from the availability of a validated panel of persistent and presumed non-persistent strains and of industrial-like model systems of certain niches (e.g. drains, conveyors, slicers), where different strains (including knock-out variants) and environmental conditions can be tested
Assessment of persistence in the FFPE from a microbial community (microbiome) perspective	Ad hoc ecosystem studies at industry level longitudinally analysing the main niches linked to persistence of hazards in the FFPE following a holistic approach, with characterisation of the resident microbiome, to understand the dynamics of the interactions between food and environmental microbiomes, and of a wide range of physico-chemical parameters and with determination of the features of persisting strains
Factors promoting persistence at facility level in different sectors	Various facility-specific studies specifically designed for the identification of risk factors for persistence in different sectors
Efficacy of interventions at industrial scale to control/remove persistent strains from the main biological hazards	Systematic studies, ideally at industrial scale or using industry-like model systems, monitoring the impact of interventions in reducing or preventing persistence, particularly targeting the identified sites/niches. Such studies would benefit from: (i) the availability of surrogate organisms of persistent pathogenic strains that could be tested in processing plants, (ii) of industrial-like model systems of certain niches (e.g. drains, conveyors, slicers), (iii) and the development of harmonised protocols and/or rapid methods to in situ assess the efficacy of C&D, to be used for validation/operational monitoring/verification activities in relation to the control of persistence in processing plants
Translation of genotypic information into phenotypic characteristics that can be further converted into parameters of risk assessment models	Research into the efficient use of available data to improve risk assessment models and subsequently support risk management
Extensive quantitative data to describe the dynamics of transfer, survival and growth of bacterial hazards in different FFPE niches	Studies generating quantitative data to describe the dynamics of transfer, survival and growth of bacterial hazards in different FFPE niches and to define strain- or subtype-specific parameters for QMRA

Abbreviations: AMR, antimicrobial resistance; C&D, cleaning and disinfection; FCS, food contact surface; FFPE, food and feed processing environment; FoPE, food processing environment; NFCS, non-food contact surface; PH, public health; QMRA, quantitative microbiological risk assessment; WGS, whole genome sequencing.

4 | CONCLUSIONS

ToR1 (AQ1). To identify the most relevant microbiological food safety hazards associated with persistence in the FFPE

- The most relevant bacterial food safety hazards associated with persistence in the FFPE of each of the considered sectors in the EU/EEA are:
    - *S. enterica* in the feed for food animal production sector;
    - *L. monocytogenes* and *S. enterica* in the meat processing sector;
    - *L. monocytogenes* in the fish and seafood processing sector;
    - *L. monocytogenes* in the dairy sector;
    - *S. enterica* in the eggs and egg processing sector;
    - *L. monocytogenes* in the fruit and vegetables processing sector; and
    - *S. enterica* and *C. sakazakii* in the LMF sector.
- Other bacterial hazards were either not of highest PH relevance in the specified/specific sector or were of highest PH relevance but not considered as most relevant bacterial food safety hazards associated with persistence in the FFPE in the specified/specific sector based on the available information.

ToR2 (AQ2-3). To identify the main (sub)types of the most relevant hazards involved in persistence and the main features responsible for their persistence in the FFPE

- For the three most relevant hazards, there is a wide range of subtypes reported to be involved in persistence in the FFPE. Some specific subtypes are more commonly reported as persistent:
    - for *L. monocytogenes*, especially CC 121, CC8, CC9 from lineage II and CC 5, CC6, CC2 from lineage I;
    - for *S. enterica*, *S.* Typhimurium and *S.* Agona; and also *S.* Derby, *S.* Anatum, *S.* Infantis, *S.* Heidelberg, *S.* Mbandaka and *S.* Senftenberg; and
    - for *C. sakazakii*, CC64, CC1, CC83 and CC4.

- Some of these subtypes (CC6, CC8, CC9, CC121 and CC321 for *L. monocytogenes; S.* Typhimurium, *S.* Infantis, *S.* Agona, *S.* Anatum, *S.* Heidelberg and *S.* Mbandaka for *S. enterica*; and CC4 for *C. sakazakii*) have clinical relevance and are widely distributed according to the analysis of clusters available in the NCBI Pathogen Detection database.
- For *L. monocytogenes*, some markers have been identified as possibly associated with persistence: stress survival islets SSI-1 and SSI-2, genomic islands LGI-1 and LGI-2, heavy metal (cadmium and arsenic) and biocide (*bcrABC, qacC, qacH, emrE* and *emrC*) resistance determinants, often located on mobile genetic elements (mainly plasmids), and bacteriophage regions (*comK*), globally linked to increased environmental robustness, tolerance to disinfection and/or biofilm formation.
- The set of phenotypic and genomic features that have been investigated for *Salmonella* and *C. sakazakii* in relation to persistence in the FFPE is incomplete. As such, it is difficult to deduce certain features, that are either indispensable for, or may markedly contribute to, persistence, alone or in combination with other key genotypic and phenotypic elements.
- For *Salmonella*, most studies focused on features inherent to most infectious foodborne hazards (e.g. AMR, virulence, growth/survival in foods and biofilm formation), and reported resistance of some strains to one or more antimicrobials, carriage of plasmid-mediated virulence factors, biofilm formation ability or reduced susceptibility to alkaline disinfectants.
- Several features have been associated with the ability of *C. sakazakii* to survive for long time periods and persist in the dry conditions of the LMF FoPE, including the ability to form biofilms on a variety of abiotic surfaces; a high heat tolerance and desiccation resistance; the production of a capsule that aids attachment to surfaces, provides resistance to biocides and contributes to survival following desiccation; and the production of a yellow carotenoid pigment which stabilises cell membranes and provides protection against stress. However, none of these features seem to be specifically linked to particular subtypes frequently associated with persistence.
- No universal markers or features, responsible for persistence have been identified. Although the carriage of different combinations of genetic determinants linked to increased environmental robustness possibly confers the ability to persist on particular subtypes, persistence is a multifactorial process that also depends on specific environmental conditions and risk factors (discussed below).

ToR3 (AQ4). To identify the risk factors at facility level responsible for the persistence of the most relevant hazards in the FFPE

- The main risk factor at facility level responsible for the persistence of the three bacterial hazards in the FFPE is poor hygienic design of equipment and machines. This leads to niches (or harbourage sites) which are difficult to clean and disinfect and where food debris and moisture can accumulate, and the hazards can survive and persist. Examples of such niches on FCS are slicers and cutters for *L. monocytogenes*, feather plucking- and evisceration equipment for *Salmonella* and dryers and drying towers for *C. sakazakii*.
- Other important factors are: (i) inadequate zoning and hygiene barriers, that enables the spread of contamination from contaminated to clean areas; (ii) inadequate C&D of the facilities; (iii) introduction of the hazards through raw materials, which may lead to the colonisation and spread of persistent clones in the processing environment; and (iv) humidity, which favour persistence.
- Specifically for hazards of relevance in dry (LMF/feed) processing environments (*S. enterica* and *C. sakazakii*), additional risk factors are airborne transmission through dust, the limited use of disinfectants due to dry cleaning operations, or the presence of water in the FoPE, whether from wet cleaning, condensation generated through temperature gradients within the facility or within equipment, or other sources.

ToR4 (AQ5). To assess available and enhanced measures or interventions for monitoring, preventing and/or controlling the persistence of the most relevant microbiological food safety hazards in the FFPE

- A well-designed environmental sampling and testing programme, following a risk-based approach, is the most effective strategy to identify potential contamination sources and detect potentially persistent hazards.
- The establishment of hygienic barriers and measures within the FSMS, during implementation of HACCP, is key to prevent and/or control bacterial persistence in the FFPE through avoiding the entry of the hazard(s) to the processing plant and/or their spread across the facility. The following prerequisites are of particular importance: infrastructure (building, equipment), C&D, technical maintenance and calibration, water and air control, personnel (hygiene, health status), working methodology and food safety culture.

- The confirmation of the presence of a persistent strain, and the identification of its niche within the facility, require the detailed characterisation of isolates of the specific hazard(s) recovered from positive samples using subtyping methods with enough resolution, preferably WGS-based subtyping schemes.
- Once persistence is suspected in a food and feed processing plant, a 'seek-and-destroy' approach has been frequently recommended, which includes: (i) intensified monitoring; (ii) the introduction of measures to control the event; (iii) and the continuation of the intensified monitoring programme to confirm the efficacy of the measures taken or to identify the requirement for additional measures. Alternatively, systematic 'root cause analyses' can be applied to identify the most probable factors/sites within the facilities contributing to the problem and define the most appropriate interventions to eliminate the pathogen from the premises.
- Successful actions triggered by persistence of *L. monocytogenes* in the FoPE, for example, included the introduction of new or specialised (deep) C&D, the implementation of workflows, the installation of a new drainage system; the implementation of structural changes and renovations; the control of the contamination of raw ingredients and the improvement of the compartmentalisation, or the simultaneous implementation of various corrective actions.
- Some options of interventions to eliminate the persistent hazard(s) with direct bactericidal activity and of different nature (i.e. as chemical (e.g. use of biocides), physical (e.g. heat or novel non-thermal technologies) or biological (e.g. competitive exclusion, phage)) are described but in some cases these are not yet commercially available and/or their efficacy is not yet fully validated under industrial conditions.

ToR5 (AQ6-8). To identify knowledge gaps and priorities for future research and develop the perspectives of integrating the information gathered in the previous ToR in risk assessment

- Perspectives are provided for the use of risk assessment for relevant combinations of hazard and food product to assess the relative PH risks that can be associated with persistence, based on bottom-up and top-down approaches.
    - The proposed basic model for persistence to be used in bottom-up food chain QMRA can be used to study the role of persistence in the PH risk for a specific food production process. The dynamics of persistence and its role in PH risk will however be very food process specific, and the current model may be too simple to capture important biological processes, such as biofilm formation.
    - With the currently available data, top-down risk assessment cannot be used to assess the relative PH risk that can be attributed to persistence.

- Risk assessment cannot fully exploit the data gathered to support answering the AQs of this scientific opinion, and the data needs for risk assessment are not well covered. Application of these data would require better translation of genotypic information of strains into phenotypic characteristics that can be converted into parameters of risk assessment models, as well as extensive quantitative data to describe the dynamics of transfer, survival and growth of bacteria in the FoPE.
- Nine specific knowledge gaps have been identified and translated into recommendations for research for filling those knowledge gaps.
- Most of these recommendations would involve activities at industry settings, but some of the research activities could be performed using industrial-like model systems of certain niches, where different strains, environmental conditions and potential interventions can be tested.
- These research activities would enable to establish the contribution of specific genetic markers and their link to phenotypes associated with persistence, and to monitor the impact of particular interventions in reducing or preventing persistence. They would also allow the generation of quantitative data to describe the dynamics of transfer, survival and growth of bacterial hazards and to define strain- or subtype-specific parameters for QMRA.

5 | RECOMMENDATIONS

- To apply clear definitions of persistence in all involved research areas (observational, experimental, epidemiological, etc.), aiming at the same unambiguous definition for all of them.
- The environmental sampling and testing programme should be robust and carefully planned by the food business operators and ensure an adequate surveillance of higher risk niches for target bacterial hazards, preferably supported by molecular subtyping, to control contamination by persistent strains.
- During outbreak investigation, to optimise the sampling strategy (e.g. frequency, critical points) and to improve data reporting of official and industrial sampling, in order to strengthen the link between FFPE and the outbreak.
- To promote the use of interoperable standards to collect and report metadata associated with WGS data to ensure auditability, to streamline data sharing and to reduce uncertainty.
- To promote the open access both to WGS data, and to complete and unambiguous associated metadata related to the strain isolation, including strains from both industrial and official sampling, respecting data confidentiality and the interests of different partners in the food chain, for investigating persistence in the FFPE.

GLOSSARY

Biocide	a chemical substance or microorganism intended to destroy, deter, render harmless or exert a controlling effect on any harmful organism by chemical or biological means.[31]
Cleaning	the removal of soil, food residue, dirt, grease or other objectionable matter.[32]
Critical control point(s) (CCP)	a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level. Most typical CCP to control microbiological hazards are temperature requirements e.g. the time/temperature conditions to reduce or eliminate a hazard (e.g. pasteurisation). Other CCP may be checking for micro-lesions in canned food, checking for physical hazards by sieving or metal detection or checking time/temperature of frying oil to avoid chemical process contaminants (EU Commission Notice, 2022/C355/01.[33]
Disinfecting/disinfection	to destroy or irreversibly inactivate specified fungi, bacteria and/or viruses, but not necessarily bacterial spores
Disinfectant	chemical agent or combination of chemical agents that is used on inanimate objects or surfaces. Some chemicals may function as both sanitisers and disinfectants. Disinfectants can be sporostatic but are not necessarily sporicidal. Within the remit of this opinion, disinfectant agents are defined as those decontamination agents applied to eliminate microorganisms on surfaces
Food Safety Management system (FSMS)	Prerequisite programmes, supplemented with control measures at CCP, as appropriate, that when taken as a whole, ensure that food is safe and suitable for its intended use. The FSMS is also the combination of control measures and assurance activities. The latter aims at providing evidence that control measures are working properly such as validation and verification, documentation and record keeping (EU Commission Notice, 2022/C355/01[33])
Good Hygiene Practices (GHP)	Fundamental measures and conditions applied at any step within the food chain to provide safe and suitable food. GHP include also good manufacturing practice(s) (GMP, stressing correct work methodologies e.g. correct dosage of ingredients, appropriate processing temperature, checking that packages are clean and non-damaged), good agriculture practice(s) (GAP, e.g. use of water of appropriate quality for irrigation, all in/all out system in animal rearing), good veterinarian practice(s) (GVP), good production practice(s) (GPP), good distribution practice(s) (GDP) and good trading practice(s) (GTP) (EU Commission Notice, 2022/C355/01[33])
Monitor	The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control (EU Commission Notice 2022/C355/01[33])
Niche	the harbourage site of persistent strains
Operational Prerequisite Programme(s) (oPRP)	control measure or combination of control measures applied to prevent or reduce a significant food safety hazard to an acceptable level and where action criterion and measurement or observation enable effective control of the process and/or product. They are typically linked to the production process and are identified by the hazard analysis as essential, in order to control the likelihood of the introduction, survival and/or proliferation of food safety hazards in the product(s) or in the processing environment (EU Commission Notice, 2022/C355/01[33])
Prerequisite programme(s) (PRP)	Preventive practices and conditions including all GHP, as well as other practices and procedures such as training and traceability, that establish the basic environmental and operating conditions that set the foundation for implementation of HACCP-based procedures (EU Commission Notice, 2022/C355/01[33])

[31]CAC (Codex Alimentarius Commission), 2003. Code of Hygienic Practice for Fresh Fruits and Vegetables CXC 53–2003 p. 1–39.

[32]CAC (Codex Alimentarius Commission), 2022. General Principles of Food Hygiene CXC 1–1969 p. 1–38.

[33]European Commission, 2022. Commission Notice on the implementation of food safety management systems covering Good Hygiene Practices and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (2022/C 355/01). 16.9.2022, p. 1–58. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52022XC0916(01)

Presumed non-persistent strain	a strain that has been identified as sporadically (not repeatedly) contaminating the FFPE of a processing plant, as a more intensified or a longer sampling campaign could result in their repeated isolation from the FFPE
Persistent strain	a strain found to be established in niches within the FFPE for a long term, despite the frequent application of C&D. It requires prolonged existence usually with multiplication of the microorganism in the specific FFPE. It is a phenomenon which may lead to recurrent food contamination events and is normally detected through the repeated isolation from the same premises or equipment on different dates (spanning months or years) of strains that are subsequently identified as highly related subtypes (as determined by phenotypic or genotypic methods). Persistence does not include continuous reintroduction in the facility of the same organism, although in practice it is often not possible to distinguish between both phenomena
Pervasive strain	a persistent strain isolated from different processing plants
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).[34]
Site	the location of persistent strains (sampling sites positive for persistent strains)
Surveillance	the systematic ongoing collection, collation and analysis of information related to food safety and the timely dissemination of information for assessment and response as necessary (FAO, 2022).[35]
Validation	Obtaining evidence that a control measure or combination of control measures, if properly implemented in the HACCP-based procedures and by the oPRP, can control the hazard to a specified outcome. Revalidation may be required in case of changes affecting the control measure. Detailed examples can be found in CAC/GL 69-2008[36] (EU Commission Notice, 2022/C355/01[33])
Verification	The application of methods, procedures, tests and other evaluations, in addition to monitoring to determine compliance with the HACCP-based procedures, i.e. to determine whether a control measure is or has been operating as intended. Verification is conducted periodically to demonstrate that the HACCP system and the management of the oPRP are working as planned (EU Commission Notice 2022/C355/01[33])

ABBREVIATIONS

ADI	arginine deiminase
AFLP	amplified fragment length polymorphism
AhpCF	alkyl hydroperoxidase
AMR	antimicrobial resistance
AQ	assessment question(s)
ATR	acid tolerance response
BapL	biofilm associated protein
BC	benzalkonium chloride
BIOHAZ	Panel EFSA Panel on Biological Hazards
CAC	Codex Alimentarius Commission
Cat	catalase
CC	clonal complex(es)
CDC	Centers for Disease Control and Prevention (United States of America)
cgMLST	core genome multi-locus sequence type
CEA	Controlled Environment Agriculture
CFU	colony forming unit(s)
CIP	cleaning-in place

[34]Environmental Protection Agency (EPA). (n.d.). What are antimicrobial pesticides? https://www.epa.gov/pesticide-registration/whatare-antimicrobial-pesticides

[35]FAO (Food and Agriculture Organization), 2022. *Listeria monocytogenes* in ready-to-eat (RTE) foods: attribution, characterisation and monitoring. 202 pp. Available online: https://www.fao.org/3/cc2400en/cc2400en.pdf

[36]CAC (Codex Alimentarius), 2008. Guidelines for the validation of food safety control measures. CAC/GL 69-2008.p.1–16.

CP	control point(s)
Csp	cold shock protein
CT	clonal type(s)
C&D	cleaning and disinfection
DALY	disability adjusted life year
DR	dose–response
ECDC	European Centre for Disease Prevention and Control
EMP	environmental monitoring programme(s)
EnABLe	Environmental monitoring with an Agent-Based Model of Listeria
ESBL	Extended Spectrum Beta-Lactamase
FAO	Food and Agriculture Organization of the United Nations
FBO	foodborne outbreak(s)
FBOp	food business operator(s)
FCS	food contact surface(s)
FDA	Food and Drug Administration (United States of America)
FePE	feed processing environment(s)
FFPE	food and feed processing environment(s)
FoPE	food processing environment(s)
fri	ferritin-like protein
FSANZ	Food Standards Australia New Zealand
FSIS	United States Department of Agriculture (USDA), Food Safety and Inspection Services
FSMS	food safety management system(s)
GAD	glutamate decarboxylase
GMP	good manufacturing practice(s)
HACCP	hazard analysis and critical control points
HUS	haemolytic uremic syndrome
JNS	joint notification summary
LGI	Listeria Genomic Island
LIPI	Listeria Pathogenicity Island
LMF	low moisture food
MAG	Metagenome Assembled Genome
MLST	multi-locus sequence typing
MLVA	multi-locus variable number tandem repeat analysis
MLVST	multi-virulence-locus sequence typing
MPD	maximum population density
NCBI	National Center for Biotechnology Information
NFCS	non-food contact surface(s)
oPRP	operational prerequisite programme(s)
PFGE	pulsed-field gel electrophoresis
PH	Public health
PMSC	premature stop codon
PRP	prerequisite programme(s)
QAC	quaternary ammonium compound
QMRA	quantitative microbiological risk assessment
RAD	restriction site-associated DNA
RASFF	Rapid Alert System for Food and Feed
ROA	rapid outbreak assessment(s)
ROS	reactive oxygen species
RR	relative risk
RTE	ready-to-eat
SNP	single nucleotide polymorphism
Sod	superoxide dismutase
SQ	sub-question(s)
SSI	stress survival islet
ST	sequence type(s)
STEC	Shiga toxin-producing *Escherichia coli*
Ti/Ab	title/abstract
ToR	Terms of Reference
US FDA	United States Food and Drug Administration
VBNC	viable but non-culturable
wgMLST	whole genome multi-locus sequence type

WGS whole genome sequencing
WHO World Health Organization

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CONFLICT OF INTEREST
If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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The designations employed and the presentation of material on any maps included in this scientific output do not imply the expression of any opinion whatsoever on the part of the European Food Safety Authority concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Literature searches, screening and data extraction

A.1 | Literature searches for the assessment of the persistence of microbiological hazards in food production and processing environments

The search was performed on 17 October 2022 in the Web of Science™ Core Collection (1975–present) to retrieve papers, review papers, book chapters and books related to the persistence of microbiological hazards in food production and processing environments. The search string can be found in Annex A (protocol; Table A3). It was further restricted based on the record characteristics: only in English, post 2010 for the publication year and document types Article or Review Article or Book Chapters.

The EndNote file was transferred into DistillerSR® Web-Based Systematic Review Software (Evidence Partners, Ottawa, Canada) for the selection procedure.

The screening was undertaken in three steps:

(1) Screening of titles (for records classified as primary research studies only)

Screening of title was done by an EFSA staff member to identify obviously irrelevant studies, to be excluded from the assessment, and potentially relevant or unclear studies, to be moved to title/abstract (Ti/Ab) screening.

(2) Screening of titles and abstracts

Screening of titles and abstracts was done in duplicate to identify records containing:

- irrelevant studies, to be excluded from the assessment; and
- potentially relevant or unclear studies, to be moved to full-text screening.

This was done by EFSA staff and Working Group members in duplicate (i.e. each Ti/Ab was screened for relevance by two reviewers). If there were doubts or divergences between the two reviewers, it was solved by discussion.

A pilot sample of 25 records of primary research studies were screened by the two EFSA staff members and six Working Group members involved in the screening. Disagreements were discussed, which helped to clarify the questions.

The question posed was:

Does the study/review include one of the below:

- *sampling in a FoPE (one or more of this list: meat processing (including slaughterhouses and processing plants), fish and seafood processing, dairy processing, egg and egg products processing, fruit and vegetable processing, including Controlled Environment Agriculture (CEA)/production through indoor hydroponic operations and low moisture food (LMF) processing) with analysis of a hazard (one or more of this list: Salmonella* spp., *Listeria monocytogenes, pathogenic Escherichia coli, Campylobacter jejuni, Campylobacter coli, Clostridium botulinum, Clostridium perfringens, Bacillus cereus, Staphylococcus aureus, Vibrio parahaemolyticus, Cronobacter sakazakii) or subtypes*
- *features of strains associated with persistent events*
- *risk factors at facility level in a food processing environment related to persistence*
- *interventions at facility level in a food processing environment related to persistence*
- *Interventions at laboratory level related to persistence, for example to remove biofilms*
- *a study related to an outbreak in a food processing environment related to persistence*.

If yes, or unclear, it was asked to classify the study.

The study/review includes:

- *Sampling in a food processing environment with analysis of a hazard or subtypes*
- *Features of strains associated with persistent events*
- *Risk factors at facility level in a food processing environment related to persistence*
- *Interventions at facility level in a food processing environment related to persistence*
- *A study related to an outbreak in a food processing environment related to persistence*
- *Interventions at laboratory level related to persistence, for example to remove biofilms*

Does the study concern one of the below sectors/processing environments?

- *meat processing (including slaughterhouses and processing plants)*
- *fish and seafood processing*
- *dairy processing*
- *egg and egg products processing*
- *fruit and vegetable processing, including Controlled Environment Agriculture (CEA)/production through indoor hydroponic operation*
- *low moisture food (LMF) processing*
- *none of the above*
- *unclear*

Does the study concern one of the below hazards?

- *Bacillus cereus*
- *Campylobacter jejuni or Campylobacter coli*
- *Cronobacter sakazakii*
- *Clostridium botulinum or Clostridium perfringens*
- *Listeria monocytogenes*
- *pathogenic Escherichia coli*
- *Salmonella spp.*
- *Staphylococcus aureus*
- *Vibrio parahaemolyticus*
- *none of the above*
- *unclear*

(3) Screening of full-text documents

Screening of full-text documents was done in single by one of the Working Group members or EFSA staff in two parts:

Part I: to further identify records to be excluded based on criteria related to report characteristics (e.g. full text not available, not in English; other than primary research study or review) and to classify them as primary research study or review.

Part II: to confirm the relevance of the study based on the full text. The question posed was: *Does the study/review include one of the below [for one or more of this list: Salmonella* spp., *Listeria monocytogenes, pathogenic Escherichia coli, Campylobacter jejuni, Campylobacter coli, Clostridium botulinum, Clostridium perfringens, Bacillus cereus, Staphylococcus aureus, Vibrio parahaemolyticus, Cronobacter sakazakii] Pls select*.

- *Repeated sampling with the same subtype found at different sampling occasions in a food processing environment with analysis of one of the hazards*
- *Features of strains associated with persistent events*
- *Risk factors at facility level in a food processing environment related to persistence*
- *A study related to an outbreak in a food processing environment related to persistence*
- *Interventions at laboratory level related to persistence, for example to remove biofilms (if this selected: study to be flagged for potential later use)*
- *None of the above (if this selected: study to be excluded)*

Then, two questions were posed to further classify the studies.

Which of below sectors/processing environments?

- *meat processing (including slaughterhouses and processing plants)*
- *fish and seafood processing*
- *dairy processing*
- *egg and egg products processing*
- *fruit and vegetable processing, including Controlled Environment Agriculture (CEA)/production through indoor hydroponic operation*
- *low moisture food (LMF) processing*
- *none of the above (in case of a laboratory study)*

Which of the below hazards does the study include?

- ▫ *Bacillus cereus*
- ▫ *Campylobacter jejuni or Campylobacter coli*
- ▫ *Cronobacter sakazakii*
- ▫ *Clostridium botulinum or Clostridium perfringens*
- ▫ *Listeria monocytogenes*
- ▫ *pathogenic Escherichia coli*
- ▫ *Salmonella spp.*
- ▫ *Staphylococcus aureus*
- ▫ *Vibrio parahaemolyticus*

Data extraction

Data were extracted from the eligible full-text documents when the study concerned the most relevant bacterial food safety hazards associated with persistence in the FFPE of the specific food production and processing sectors; i.e. *S. enterica* in the food animal production, meat processing, eggs and egg processing and LMF sectors; and *L. monocytogenes* in the meat processing, fish and seafood processing, dairy and fruit and vegetables processing sectors; and *C. sakazakii* in the LMF sector.

A pre-defined data extraction form in DistillerSR was used and data extraction was performed by a Working Group member or EFSA staff. Data extraction consisted of gathering information about the country where sampling took place, the sector and plant within the sector, the reason for sampling, the hazard level 1 (i.e. *Salmonella*, *Listeria* or *Cronobacter*), the hazard level 2 (i.e. serovar of *Salmonella*, *L. monocytogenes* or *C. sakazakii*), the hazard level 3 (subtype), the location of the persistence (i.e. non-food and/or food contact surface) with further details, if available, the typing method(s) used, the features of persistence investigated, the risk factors for the persistence, the interventions, the number of sampling events, the sampling period and period of persistence.

The reviews that were eligible at full-text level and the extracted data from the primary research studies have been made available through the Knowledge Junction under https://doi.org/10.5281/zenodo.10299549.

A.2 | Literature searches for the assessment of the persistence of microbiological hazards in feed production and processing environments

The search was performed on 17 January 2023 in the Web of Science™ Core Collection (1975–present) to retrieve papers, review papers, book chapters and books related to the persistence of microbiological hazards in feed production and processing environments. The search string can be found in Annex A (protocol; Table A3). It was slightly revised to ensure more coverage by adding an additional string using a search based on the use of *Salmonella* and feed in the title. The search was restricted based on the record characteristics: only in English, post 2010 (for the broader title search only) for the publication year and document types Article or Review Article or Book Chapters.

The EndNote file was transferred into DistillerSR® Web-Based Systematic Review Software (Evidence Partners, Ottawa, Canada) for the selection procedure.

The screening was undertaken in three steps:

(1) Screening of titles

Screening of titles was done by an EFSA staff member to identify obviously irrelevant studies, to be excluded from the assessment, and potentially relevant or unclear studies, to be moved to Ti/Ab screening.

(2) Screening of titles and abstracts

Screening of titles and abstracts was done in duplicate to identify records containing:

- irrelevant studies, to be excluded from the assessment;
- potentially relevant or unclear studies, to be moved to full-text screening.

This was done by EFSA staff and a Working Group member in duplicate. Doubts or divergences between the two reviewers were solved by discussion.

The question posed was: *Does the study/review address the persistence of Salmonella* spp. *in feed processing environments, considering one of the below?*

- ▫ *repeated sampling with the same subtype found at different sampling occasions in a feed processing environment with analysis of Salmonella spp.*

- *features of strains associated with persistent events*
- *risk factors at facility level in a feed processing environment related to persistence*
- *interventions at facility level in a feed processing environment related to persistence*

(3) Screening of full-text documents

Screening of full-text documents was done in single by a Working Group member to confirm the relevance of the study based on the full text. The question posed was: *Does the study/review address the persistence of Salmonella* spp. *in feed processing environments, including one of the below? Pls select*.

- *Repeated sampling with the same subtype found at different sampling occasions in a feed processing environment with analysis of Salmonella spp.*
- *Features of strains associated with persistent events*
- *Risk factors at facility level in a feed processing environment related to persistence*
- *Interventions at facility level in a feed processing environment related to persistence*
- *None of the above (if this selected: study to be excluded)*

Data extraction

Data were extracted from the eligible full-text documents when the study concerned the most relevant bacterial food safety hazard associated with persistence in the FFPE of the feed production and processing sector, i.e. *S. enterica*. A predefined data extraction form in DistillerSR was used and data extraction was performed by a Working Group member.

The reviews that were eligible at full-text level and the extracted data from the primary research studies have been made available through the Knowledge Junction under https://doi.org/10.5281/zenodo.10299549.

APPENDIX B

Uncertainty analysis

TABLE B.1 Potential sources of uncertainty linked to specific assessment questions (AQs) in the assessment of the persistence of microbiological hazards in food and feed production and processing environments of the various food and feed production and processing sectors in the EU/EEA.

AQ	Source or location of the uncertainty	Nature or cause of the uncertainty
AQ1	Food sectors: Incomplete information in the data derived from the various sources	Especially for the 'strong evidence' FBO from the EFSA zoonoses database and to a lesser extent for the ROA and JNS, the link between cases/outbreaks and the incriminated food is not always established or reported and sometimes the general description of the food does not allow to accurately understand the type of food incriminated. The RASFF database is not an epidemiological surveillance system as this system is primarily a communication facility enabling many food safety risks to be averted to prevent further spread of the risk over Europe. As it provides some understanding of the types of hazards typically detected in particular foods, it was used to complement the FBO data
AQ1	Feed for food animal production sector: Incomplete information in the notifications extracted from the RASFF database	In the feed sector, only the RASFF data was used as FBO are only very rarely linked to the feed sector. Also, the former scientific opinion of the BIOHAZ panel on Microbiological Risk Assessment in feedingstuffs for food-producing animals for both public health and animal health (EFSA BIOHAZ Panel, 2008) was consulted
AQ1	Insufficient tracing of the contamination source in outbreak investigations	In most outbreak investigations there is a lack of clear information on whether the contamination source is the FoPE and whether the involved strains are persisting in the FoPE
AQ1	Lack of studies addressing the FoPE for some particular hazards and insufficient or inefficient sampling of FoPE in environmental monitoring studies	Persistent clones may be not detected due to lack of field data or to inefficient sampling (using a low frequency of sampling, an inefficient sampling method, not sampling relevant areas or sample types, absence of longitudinal sampling, etc). This may be particularly the case for those hazards for which studies addressing the FoPE are very scarce (e.g. *Campylobacter*, *S. aureus*, *B. cereus s. l.*)
AQ1	Origin of isolates	It is difficult to evaluate if re-isolation of the same clone over time is due to persistence or reintroduction (or both)
AQ2	Use of typing methods which provide information that is not interoperable or does not follow universally harmonised terminology for the subtypes	Only persistent clones identified through some subtyping methods (such as serotypes, epidemic clones, lineages, clades, spa types, *panC* types, clonal complexes, MLST types, wgMLST types and cgMLST types) can be unequivocally assigned to known subtypes. As a result, some relevant subtypes may be missed in the assessment
AQ2	Use of typing methods with low resolution. Typing method does not provide enough detail to confirm persistence of the same strain over time	In some studies, the use of typing methods with low resolution makes it uncertain whether isolates belong to the same clone or several clones. This is particularly relevant for *Salmonella*, where serotyping is the typing method frequently used. Serotyping methods used for typing of *Salmonella* lead to the assumption that repeated isolation of the same serotype over time is equivalent to persistence
AQ2	Insufficient characterisation of persistent strains	Many of the studies that report isolation of the same subtype along time in the FFPE did not characterise the persistent strain phenotypically or genotypically through WGS to identify features associated with persistence. As a result, there is no sufficient knowledge on the features of some of the subtypes identified in persistence events
AQ2	Use of presumed non-persistent strains as control strains in experiments trying to identify strain features associated with persistence in the FFPE	Some of the studies characterising features of persistent strains use presumed non-persistent strains as control. Sometimes they belong to other CC or serotypes and there is uncertainty on whether those strains could also persist under different circumstances or following different sampling approaches
AQ2	Lack of harmonisation among studies and of relevant model systems to test if particular features are involved in persistence	Different studies assess diverse genotypic and phenotypic features, without an exhaustive set of features shared between studies to enable the identification of features that are indispensable for persistence. It is difficult to assess whether features that seem to be statistically correlated to persistent subtypes play a role in persistence in the FFPE, as pathogenic strains cannot be added to the FFPE to test hypotheses and it is uncertain whether studies performed in laboratory settings reflect the situation in the FFPE satisfactorily
AQ2	Biased representation of subtypes in WGS databases	Databases such as the NCBI Pathogen Detection database are biased in the sense that they contain an uneven number of genomes from different sources, countries, subtypes, etc, reflecting particular sequencing efforts carried out by different instances to investigate specific contamination or outbreak events. Thus, the number of genomes available for the different subtypes will not necessarily reflect the relevance of that subtype

(Continues)

TABLE B.1 (Continued)

AQ	Source or location of the uncertainty	Nature or cause of the uncertainty
AQ3	Harbourage site (or niche) of the persistent strain(s)	In many of the studies reporting repeated isolation along time of the same subtype the actual niche (harbourage site) of the persistent strain was unclear, either because it was not investigated in detail or because the persistent strain was isolated from multiple sources within the processing plant. Hence, niches were not always identified. In addition, repeated isolation from a FFPE such as conveyers, drains, floors may not always indicate persistence on that specific surface as the original niche for persistence may be another niche shedding or leaking bacteria to that surface
AQ4	Lack of studies specifically designed for the identification of persistence risk factors	Many of the identified risk factors are reported in studies reflecting personal opinion or expertise of the authors or discussion around available literature, but there is scarcity of studies specifically designed to identify risk factors for persistence
AQ4	Risk factors for persistence or cross-contamination	It is sometimes difficult to understand/interpret from the available literature whether some of the risk factors for a particular hazard are true risk factors for persistence of such hazard or just risk factors for the entry of the pathogen in the facility of for the cross-contamination of the end product
AQ5	Scarcity of studies testing interventions in industry settings	While there is detailed information in the literature testing a wide range of interventions as alternatives for the control of biofilms of hazards in lab settings, only a few studies report results on testing interventions to address persistence of hazards in industry settings
AQ5	Hazards targeted in the interventions	Interventions tested at industry level sometimes follow indicator microorganisms (e.g. *Listeria* spp.) instead of a particular hazard. In other occasions, the effect of the intervention is assessed by monitoring changes in the incidence or prevalence of the hazard, but with no further characterisation of the hazard. Therefore, it is unclear whether the intervention is capable of preventing or controlling persistence. Interventions reducing the numbers of indicator microorganisms or the incidence/prevalence of a hazard in the industry are expected to also prevent/control the persistence of hazards
AQ6	Deterministic versus stochastic modelling	In deterministic models we omit stochastic processes and leave out heterogeneity in processing. Neither do we include uncertainty in model parameter estimates. Models including all these aspects would become highly complex. Studying their perspectives was beyond the scope of this Scientific Opinion
AQ6	Options for risk assessment approaches	Many risk assessment approaches exist. We only explored bottom-up food chain QMRA and did not apply methods like Bayesian modelling, machine learning or agent-based modelling. These may have perspectives that have not been covered

Abbreviations: AQ, assessment question; CC, clonal complex; cgMLST, core genome multi-locus sequence type; C&D, cleaning and disinfection; FBO, foodborne outbreak; FFPE, food and feed processing environment; FoPE, food processing environment; JNS, joint notification summary; MLST, multi-locus sequence type, NCBI, National Center for Biotechnology Information; NFCS, non-food contact surface; QMRA, quantitative microbiological risk assessment; RASFF, Rapid Alert System for Food and Feed; ROA, rapid outbreak assessments; wgMLST, whole genome multi-locus sequence type; WGS, whole genome sequencing.

APPENDIX C

Bacterial pathogens most relevant for public health in the food and feed production and processing sectors

C.1 | Feed for food animal production sector

Considering the notifications for feed from the RASSF database since 2010, more than 99.5% of the 1511 notifications were associated with *Salmonella enterica* subsp. enterica (1504 notifications). Most notifications were for the serovars *S.* Senftenberg (*N* = 138), *S.* Mbandaka (*N* = 124), *S.* Agona (*N* = 116), *S.* Tennessee (*N* = 87) and *S.* Infantis (*N* = 58). There were three notifications for *Clostridium perfringens* in fish feed, two for *Brucella* in frozen hare by-products, one for *Bacillus cereus s. l.* in a bacterial feed additive for pigs produced by *Corynebacterium glutamicum*, and one for the possible presence of *Bacillus anthracis* in beef bones for feed.

In the scientific opinion of the BIOHAZ panel on Microbiological Risk Assessment in feedingstuffs for food-producing animals for both public health and animal health, the panel identified *Salmonella* spp. as the major hazard for bacterial contamination of animal feed. *L. monocytogenes*, *E. coli* O157:H7 and *Clostridium* spp. are other hazards for which feed was regarded a far less important source (EFSA BIOHAZ Panel, 2008).

C.2 | Meat sector, excluding LMF sector

Strong evidence FBO data at EU level since 2010 showed that the most relevant pathogen involved in outbreaks related with meat and meat products was *S. enterica* subsp. enterica (567 of 1050 outbreaks reported, involving 10,865 human cases). *S.* Enteritidis was the most common serovar identified, causing 230 of the outbreaks, with 3298 human cases and 5 deaths. This was followed by *S.* Typhimurium, which caused 149 outbreaks, involving 3467 cases and 6 deaths. Pork meat, broiler meat and meat and meat products were the most relevant vehicles of infection. *Clostridium* spp. was the second pathogen in importance (196 of 1050 outbreaks with 6160 cases involved), with bovine meat, pork meat and broiler meat most represented in outbreaks. Of these, *C. perfringens* predominated, causing 168 of the outbreaks with 6083 cases. In addition, all 9 deaths were due to *C. perfringens*. *C. botulinum* caused all other outbreaks (28), with 77 cases involved. The third pathogen was *Campylobacter* spp. (177 of 1050 outbreaks, involving 7936 cases and 2 deaths), with broiler meat products as the main vehicle of infection. The species of *Campylobacter* was not reported for most of the outbreaks (111 of the 177 outbreaks); when available, *C. jejuni* was the most common (62 outbreaks, 6201 cases and 1 death). Other hazards associated with outbreaks were *B. cereus s. l.* (43 outbreaks), pathogenic *E. coli* (26 outbreaks), *L. monocytogenes* (18 outbreaks), *S. aureus* (14 outbreaks, with an additional 2 *Staphylococcus* spp. outbreaks not attributed to a species), *Yersinia enterocolitica* (5 outbreaks), *Shigella* spp. (2 outbreaks) and *Francisella* (1 outbreak). *S. enterica* subsp. *enterica* was responsible for the highest number of hospitalisations (2177 out of 2834 hospitalisations including all bacterial hazards) while *L. monocytogenes* caused the highest number of deaths related to meat and meat products consumption (37 out of 67 deaths including all bacterial hazards).

Five out of the seven multi-country outbreaks published as ROAs since 2012 and linked to meat were caused by *S. enterica* subsp. *enterica*. One outbreak of *S.* Stanley was linked to turkey meat consumption (EFSA and ECDC, 2012; ECDC and EFSA, 2014c), another one of *S.* Enteriditis was linked to poultry products (ECDC and EFSA, 2021c), one outbreak of monophasic *S.* Typhimurium was linked to meat products (ECDC and EFSA, 2014a), one of *S.* Mbandaka was likely linked to RTE chicken products (ECDC and EFSA, 2022c) and one of *S.* Virchow was likely linked to kebab meat products containing chicken meat (ECDC and EFSA, 2023b). *L. monocytogenes* was involved in one multi-country outbreak linked to RTE meat products (ECDC and EFSA, 2019b), while a *B. anthracis* outbreak was linked to bovine meat contamination (EFSA and ECDC, 2015).

All four multi-country outbreaks reported as a JNS were caused by *S. enterica* subsp. enterica. An outbreak of *S.* Bredeney was caused by a chilled cooked pork preparation, while an outbreak of *S.* Typhimurium was caused by marinated pork and minced beef (European Commission, 2020). Another outbreak was caused by *S.* Agona in kebab meat and *S.* Enteriditis was the serotype involved in the last multi-country outbreak possibly linked to poultry products (European Commission, 2021).

S. enterica subsp. enterica has been the main bacterial pathogen involved in the RASFF notifications for meat and meat products with a total of 4644 notifications (79.1%). The top three serovars implicated were *S.* Enteritidis (795 notifications, comprising 22% of *S. enterica* subsp. enterica notifications, 17% of total RASFF notifications), followed by *S.* Infantis (364 notifications, 10% of *Salmonella* notifications and 8% of total RASFF notifications) and *S.* Typhimurium (333 notifications, 9% of *Salmonella* notifications and 7% of total RASFF notifications). Pathogenic *E. coli* was notified 460 times (9.9%). *L. monocytogenes* was the third pathogen with a total of 388 notifications (8.4%). *Campylobacter* spp. was also relevant with 103 notifications (2.2%). Of these, *C. jejuni* was most common (52 notifications), followed by pathogenic *C. coli* (18 notifications). This was predominantly associated with poultry meat and poultry meat products. Other pathogens reported with less than 1% of notifications were *Clostridium* spp., *Staphylococcus* spp., possible presence of *B. anthracis*, *Y. enterocolitica*, *Mycobacterium tuberculosis* and *Brucella* spp.

Considering outbreak data from outside the EU, 24 of 39 confirmed or suspected reported outbreaks from USA reported by CDC from 2010 to 2022 from meat and meat products were caused by *S. enterica* subsp. enterica. Pathogenic *E. coli* was involved in 11 outbreaks and four outbreaks were caused by *L. monocytogenes*; three of them related with RTE meat

products consumption. Six outbreak reports were retrieved from Health Canada in the period from 2015 to 2022, with *S. enterica* subsp. enterica involved in five of those linked to the consumption of chicken meat and *L. monocytogenes* in a single outbreak connected with cooked diced chicken.

The particular relevance of the pathogens more frequently involved in outbreaks linked to meat and meat products is further supported by previous studies and literature analyses (Lianou et al., 2023; Sofos, 2008).

C.3 | Fish and seafood sector, excluding LMF sector

Strong evidence FBO data at EU level since 2010 showed that outbreaks associated with fish and seafood caused by bacteria were associated with various serovars of *S. enterica* subsp. enterica (47 of 115 outbreaks of which 11 with *S.* Enteritidis; involving 1657 human cases), *B. cereus s. l.* (20 outbreaks with 159 cases), *L. monocytogenes* (19 outbreaks with 145 cases), *C. botulinum* or *C. perfringens* (14 outbreaks with 313 cases), *Vibrio parahaemolyticus*, all in crustaceans, shellfish, molluscs and products thereof (7 outbreaks with 127 cases), pathogenic *E. coli* (3 outbreaks with 57 cases), *Campylobacter* (3 outbreaks with 43 cases), *Enterococcus* (1 outbreak with 3 cases) and *S. aureus* (1 outbreak with 2 cases). *S. enterica* subsp. enterica and *L. monocytogenes* caused the highest number of hospitalisations (102 and 90, respectively, of 265 hospitalisations including all bacterial hazards) and *L. monocytogenes* the majority of the deaths (14 of 18 deaths including all bacterial hazards).

Five of the six multi-country outbreaks reported either as ROA (2012–2022) or JNS (2019–2021) were caused by *L. monocytogenes* and linked to cold smoked trout and cold smoked/marinated salmon (EFSA and ECDC, 2018a; ECDC and EFSA, 2019a) (European Commission, 2019, 2021) and one outbreak was caused by *C. botulinum* (botulism) and linked to dried and salted roach (EFSA and ECDC, 2016c).

The majority (64.5%) of the 864 RASFF notifications on bacterial pathogens in fish and seafood products were associated with *L. monocytogenes* (557 notifications, mostly fish and fish products; in particular, cold smoked salmon products), *S. enterica* subsp. enterica (217, in a wide range of fish and seafood products) or *Vibrio* (73, mostly crustaceans and products thereof; shrimp products).

Regarding outbreak data from outside the EU, of the eight confirmed or suspected reported outbreaks from bacteria from USA reported by CDC from 2012 to 2022 from fish and seafood products, five were caused by *S. enterica* subsp. enterica (raw tuna, cooked shrimp, raw and cooked seafood) and two by *V. parahaemolyticus* (raw shellfish and fresh crab meat). Health Canada reported two outbreaks of *V. parahaemolyticus* caused by (raw) shellfish in the period 2014–2022, while FSANZ published a notification on *V. parahaemolyticus* and raw Pacific oysters.

C.4 | Dairy sector, excluding LMF sector

Since 2010, 279 strong evidence FBOs associated with milk and milk products with 3053 human cases have been documented. The bacterial hazards most often associated with these outbreaks in the dairy sector in the EU since 2010 were: *S. enterica* subsp. enterica ($N = 128$), *Campylobacter* spp. ($N = 89$), pathogenic *E. coli* ($N = 25$), *S. aureus* ($N = 19$), *B. cereus s. l.* ($N = 7$) and *L. monocytogenes* ($N = 5$). Consumption of milk ($N = 237$) and cheese ($N = 287$) contaminated with zoonotic pathogens resulted in 610 hospitalisations. Among these reported outbreaks, patients after the consumption of cheese suffered of infections with *S. enterica* subsp. enterica ($N = 144$), *S. aureus* ($N = 57$) and *L. monocytogenes* ($N = 51$). Individuals infected with *L. monocytogenes* ($N = 14$), pathogenic *E. coli* ($N = 3$) or *S. enterica* subsp. enterica ($N = 3$) through consumption of milk and milk products, died as a result of the disease. Serovars Typhimurium, Enteritidis and Newport, of *S. enterica* subsp. enterica, were linked to strong evidence FBOs transmitted by cheese ($N = 88$), followed by *S. aureus* ($N = 14$), pathogenic *E. coli* ($N = 10$) and *L. monocytogenes* ($N = 5$). FBOs linked to milk consumption were mainly caused by *Campylobacter* spp. ($N = 81$), *S. enterica* subsp. enterica (12), pathogenic *E. coli* ($N = 10$) and *B. cereus s. l.* ($N = 4$). In the category dairy products other than cheese, *S. enterica* subsp. enterica ($N = 28$), *Campylobacter* spp. ($N = 5$), pathogenic *E. coli* ($N = 4$) and *S. aureus* ($N = 3$) were involved in the strong-evidenced FBOs. *C. perfringens* was linked to two FBOs and *Y. pseudotuberculosis* and *Shigella flexneri* to one FBO each transmitted by raw milk, dairy products and cheeses.

In RASFF notifications since 2010, 95% ($N = 487/512$) of those reported in the dairy products sector were associated with cheese. In the cheese category, most notifications for pathogenic bacteria were associated with *L. monocytogenes* (66.3%), followed by pathogenic *E. coli* (19.3%) and *S. enterica* subsp. enterica (12.1%). Notifications linked to bacterial contamination in milk were linked to *L. monocytogenes* (41.6%) and *B. cereus s. l.* (33.3%). Recent notifications in the RASFF portal (May and August 2022) are linked to FBOs suspected to be caused by *S.* Dublin in chilled raw milk cheese from France and *L. monocytogenes* ST155 in Asiago Pressato cheese, respectively.

A STEC O26 multi-country outbreak transmitted by cheese was reported in a ROA report (EFSA and ECDC, 2016b). It affected 25 humans with 19 haemolytic uraemic syndrome (HUS) cases with a lethal outcome. A JNS was related to *S.* Newport cases, potentially associated with French goats' cheese (European Commission, 2019). FBOs published in Eurosurveillance included a *L. monocytogenes* outbreak in cheese in 2015 (Magalhaes et al., 2015), and infections by STEC O26:H11 in children (Germinario et al., 2016) and by *S. Dublin* both after bovine raw milk products consumption (Ung et al., 2019).

CDC reported 13 outbreaks linked to cheeses (2006–2022). The majority were caused by *L. monocytogenes* ($n = 10$), and others were linked to STEC ($N = 1$) and *S. enterica* subsp. enterica ($N = 2$) transmitted by fresh and soft cheese. Raw milk

was the outbreak vehicle in a single outbreak linked to *L. monocytogenes*. FSANZ reported on a single outbreak linked to *L. monocytogenes* in soft cheeses.

In the U.S., multistate outbreaks with *L. monocytogenes* in pregnant Hispanic women have been linked to Mexican fresh cheese made from pasteurised milk for decades (Jackson et al., 2011; Palacios et al., 2022). Recently, multistate outbreaks linked to raw milk contaminated by *L. monocytogenes* have been reported by CDC (Nichols et al., 2019; Sebastianski et al., 2022).

Other documented multi-country FBOs, as the Quargel outbreak in Austria, were more often transmitted by raw milk or pasteurised cheeses, either by cross-contamination or recontamination events (Desai et al., 2019; Fretz et al., 2010; Gould et al., 2014). In Europe, most dairy-related outbreaks are caused by surface-ripened cheeses and raw milk cheeses made by small-producers (Filipello et al., 2020; Johler et al., 2015; Napoleoni et al., 2021; Robinson et al., 2020; Ung et al., 2019)

The EFSA Opinion related to the consumption of raw drinking milk (EFSA BIOHAZ Panel, 2015) already shows the range of pathogens that can accumulate in raw milk and pose a risk to consumers. In addition to the previously mentioned pathogens, *M. bovis* and *B. melitiensis* are named as relevant pathogens. The source of pathogen entry takes place in several ways, with few pathogens being excreted through the infected udder of farm animals (e.g. *S. aureus*, *B. melitensis*, *M. bovis*) (Collins et al., 2022; Rossetti et al., 2022; Ruegg, 2017). Raw milk is far more often contaminated by the farm environment (*L. monocytogenes*) or faeces (*Salmonella* spp., pathogenic *E. coli*, *Campylobacter* spp.) (Bangieva & Rusev, 2017; Christidis et al., 2016; Hussein & Sakuma, 2005). The lack of removal of biofilms in the equipment used for milk production and further processing also plays a major role (Bai et al., 2021; Carrascosa et al., 2021; Chlebicz & Slizewska, 2018). The initial concentration in raw milk is at the detection limit for most pathogens, so highly contagious microorganisms (pathogenic *E. coli, Brucella, Coxiella, Mycobacterium*) play a major role in farm related outbreaks (Cutler, 2014; Gale et al., 2015; Pexara et al., 2018; Sahu et al., 2021; Valkovska et al., 2021; Verraes et al., 2015; Zastempowska et al., 2016).

C.5 | Egg sector, excluding LMF sector

According to the assessment undertaken by the EFSA BIOHAZ Panel on the public health risks of table eggs due to deterioration and development of pathogens, *S.* Enteritidis is considered the only pathogen posing a major risk of egg-borne diseases in the EU, and while other different microorganisms can be found on or in eggs, eggs are not a significant vehicle for foodborne disease other than for *Salmonella* (EFSA BIOHAZ Panel, 2014a). This *Salmonella* serovar is recognised to be the major pathogen related to egg-borne disease because of its ability to contaminate the interior of intact eggs during their formation within the body of infected hens.

The revision of other data sources used in the current assessment showed that the conclusions of the former EFSA assessment remain valid. Indeed, most strong evidence FBOs at EU level since 2010 linked to eggs and egg products were caused by *S. enterica* subsp. enterica (1137 out of 1153 outbreaks, involving 12,502 cases). 73.4% of those salmonellosis outbreaks were known to be linked to *S.* Enteritidis. Other serovars of *S. enterica* subsp. enterica involved in outbreaks were *S.* Typhimurium ($N = 41$), *S.* Infantis ($N = 3$), *S.* Newport ($N = 2$), *S.* Virchow ($N = 2$), *S.* Kottbus ($N = 1$), *S.* Mbandaka ($N = 2$) and *S.* Muenchen ($N = 1$), while 20% of the outbreaks were linked to untyped *Salmonella*. Other biological hazards, apart from *S. enterica* subsp. enterica, involved as causative agents of occasional outbreaks linked to eggs and egg products were *B. cereus s. l.* (11 outbreaks), and *Campylobacter* spp., *C. perfringens*, pathogenic *E. coli*, *S. flexneri* and *S. aureus*, with one outbreak each. Unfortunately, additional information is only available for one of the 11 outbreaks linked to *B. cereus s. l.*, which was linked to pancake.

All six multi-country FBOs published as rapid outbreak assessments and involving eggs and egg products were caused by *S.* Enteritidis linked to German eggs (ECDC and EFSA, 2014b), Polish eggs (EFSA and ECDC, 2016a, 2017b, 2017c; ECDC and EFSA, 2020a) and eggs and egg products (ECDC and EFSA, 2022b). A JNS was related to a FBO suspected to be caused by *S.* Enteritidis in eggs from Poland (European Commission, 2020).

All but one of the 99 RASFF notifications on bacterial pathogens in eggs and egg products concerned *S. enterica* subsp. enterica (99%). These were linked to eggs ($N = 65$), liquid egg ($N = 31$) and other egg products ($N = 2$). There was one notification of *L. monocytogenes* in other egg products (i.e. frozen omelette strips).

Outbreak data from outside of the EU included five outbreaks of *S.* Enteritidis and other serovars of *S. enterica* subsp. *enterica* in shell eggs (CDC and FSANZ), and a single outbreak of *L. monocytogenes* in hard boiled eggs (CDC).

C.6 | Fruit and vegetable sector, excluding LMF sector

Under natural conditions (e.g. pre- or post-harvest storage of non-injured fruits and vegetables), the outer layer of the plant tissue consists of a hydrophobic surface providing a natural barrier for microorganisms (Beuchat, 2002; Brackett, 2007). As such, the microbiological safety risks in the consumption of fruit and vegetables are linked to their contamination with enteric pathogens, mainly via soil or water used in agriculture and/or post-harvest handling and processing operations of fresh produce, although other risk factors have been identified (FAO and WHO, 2021b). Among the key bacterial hazards, pathogenic *E. coli*, *S. enterica* subsp. enterica and *L. monocytogenes* have been the most common cause of disease outbreaks (EFSA BIOHAZ Panel, 2014b, 2020; Schierstaedt et al., 2020; Bell et al., 2021). Other bacterial pathogens which are less frequently associated with fresh produce outbreaks, including emerging ones, are *Arcobacter, Bacillus* and *Campylobacter* (Bell et al., 2021). *S. enterica* subsp. enterica and pathogenic *E. coli* can be found in the faeces of livestock and therefore are

very likely to contaminate soil, irrigation water and finally, leafy vegetables (Bell et al., 2021). *L. monocytogenes* is ubiquitous in the production and processing environment and may occur in whole fresh, fresh-cut or frozen fruits and vegetables, as a result of environmental (post-process) contamination (EFSA BIOHAZ Panel, 2020).

According to the FBO data at EU level (period 2010–2020), the main body of strong evidence for outbreaks by bacterial hazards linked to fruits and vegetables is associated with the following sub-categories: (i) fruit, berries and juices and other products thereof (16 outbreaks and 346 cases in total), (ii) herbs and spices (15 outbreaks and 572 cases) and (iii) vegetables (146 outbreaks and 8320 cases). *S. enterica* subsp. *enterica*, *B. cereus s. l.*, *Clostridium (botulinum* and *perfringens)* and pathogenic *E. coli* were the main causative agents, being responsible for 69 outbreaks (2197 cases), 36 outbreaks (849 cases), 24 outbreaks (252 cases) and 21 outbreaks (5139 cases), respectively. Fruits, berries and juices outbreaks were mainly linked to *S. enterica* subsp. enterica (13 out of a total of 15 outbreaks), while (fresh-cut) vegetables, juices and products thereof outbreaks were mainly linked to *S. enterica* subsp. enterica, *B. cereus s. l.*, *Clostridium* and pathogenic *E. coli* (52, 29, 24 and 18, respectively, out of a total of 146 outbreaks). Other hazards associated with vegetables and fruits outbreaks since 2010 include *L. monocytogenes* ($N = 8$), *Shigella sonnei* and *flexneri* ($N = 6$), *Y. enterocolitica* ($N = 5$), *Aeromonas hydrophila* ($N = 3$) and *Campylobacter* unspecified ($N = 2$).

Different serovars of *S. enterica* subsp. enterica have been involved in multi-country outbreaks. In particular, a *S.* Agona contamination possibly linked to RTE food products containing cucumbers caused a five-countries outbreak with 25 cases between 2014 and 2016 and another 122 cases from January 2017 till July 2018 (EFSA and ECDC, 2018d). *S.* Braenderup ST 22 was identified as the causative agent of an outbreak presumably linked to Gallia melons, imported from a Honduran producer, with 348 cases in 12 EU/EEA countries and the UK (ECDC and EFSA, 2021a). Between August 2022 and 12 July 2023, *S.* Senftenberg ST14 caused a multi-country outbreak possibly linked to cherry-like tomatoes in 11 EU/EEA countries and the UK, with 92 cases reported of which one fatality (ECDC and EFSA, 2023a).

A listeriosis multi-country outbreak with 47 cases as of June 2018, caused by serogroup Ivb, ST6, in 4 MSs and UK, was traced to frozen corn and possibly other frozen vegetables, such as green beans and spinach, produced in a freezing plant in Hungary during the 2016–2018 production seasons (EFSA and ECDC, 2018b).

The total number of RASFF notifications on bacterial pathogens in the fruit and vegetable sector (excluding LMF) are 539 for more than 65 different serovars of *S. enterica* subsp. enterica, 61 for *L. monocytogenes*, 35 for pathogenic *E. coli*, 19 for *Bacillus* (18 for *B. cereus s. l.* and 1 for *B. subtilis*), 12 for *Campylobacter* spp., 6 for *C. botulinum* or *C. perfringens*, 3 for *Shigella sonnei* and 1 for *Y. enterocolitica*. RASFF notifications for *S. enterica* subsp. enterica and *L. monocytogenes* mainly relate to various fresh and frozen fruits, vegetables and herbs.

Regarding the outbreak data from outside EU, CDC has reported 33 salmonellosis outbreaks since 2006, with papayas, cantaloupes, fresh-cut melons, tomatoes, mushrooms and various fresh-cut leafy salads being identified as vehicles of contamination with one or more of 28 different serovars of *S. enterica* subsp. enterica. In the same period CDC has identified 17 outbreaks due to O157- and non-O157 STEC strains in fresh-cut vegetable salads and 7 listeriosis outbreaks (since 2011) linked to packaged salads, mushrooms, bean sprouts and cantaloupes. In 2021, a multistate outbreak of *S.* Typhimurium linked to packaged leafy greens produced at a Controlled Environment Agriculture (CEA) indoor hydroponic operation took place in USA (FDA, 2022). In 2022, FDA listed numerous outbreaks associated with leafy greens and fruits (e.g. romaine lettuce, other packaged salads, peaches, fresh-cut cantaloupe and strawberries), contaminated with *L. monocytogenes*, *S.* Javiana, *S.* Typhimurium, *S.* Enteritidis and *E. coli* O157:H7. FSANZ has reported a single outbreak linked to rock melons with *S. enterica* subsp. enterica. Finally, Health Canada reported 12 outbreaks since 2015, due to consumption of fresh and frozen fruits and vegetables. More specifically, seven outbreaks were linked to fresh-cut salads contaminated with pathogenic *E. coli*, and a single listeriosis outbreak was linked to packaged salads, while four salmonellosis outbreaks were caused by contaminated frozen kernel corn, imported peaches and red onions and English cucumbers.

Although not yet officially linked to outbreaks caused by consumption of fresh fruits and vegetables, there is an increasing body of evidence that *Arcobacter* species, particularly *A. butzleri* and *A. cryoaerophilus*, are emerging zoonotic pathogens with remarkable occurrence in RTE fresh-cut salads (Ramees et al., 2017). *Arcobacter* is a *Campylobacter*-like organism, in terms of phenotypic responses at detection, a fact that introduces a systematic bias in the detection with culture-based methods and is known to cause acute or prolonged (chronic) watery diarrhoea combined with abdominal pain (Figueras et al., 2014; Mottola et al., 2021). Reported prevalence of the above two *Arcobacter* species in leafy greens can be as high as 14%–27% (Mottola et al., 2016; Mottola et al., 2021).

C.7 | LMF sector

Low moisture foods (LMF) are foods that are naturally low in moisture or are produced from foods with high moisture through drying or dehydration processes. They all show a low water activity (a_w), which contributes to a long shelf life. Indeed, LMF are commonly defined as any food item that has a a_w level of less than 0.85. However, despite they normally do not support the growth of pathogenic microorganisms given their low a_w, they are frequently involved in outbreaks of foodborne illnesses due to the low infectious dose of some of the microorganisms that may survive in the food product or to possible subsequent temperature abuse after rehydration that will allow the contaminant organisms to grow.

A wide range of foods and food products can be considered as LMF. A recent assessment by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) for ranking LMF using multi criteria decision analysis in support of microbiological risk management established the following categorisation of LMF (FAO and WHO, 2022):

1. cereals and grains;
2. confections and snacks;
3. dried fruits and vegetables;
4. dried protein products (dairy powders, egg powders, dried fish and fish meal/flour, gelatin and meat powders);
5. honey and preserves;
6. nuts and nut products;
7. seeds for consumption;
8. spices and dried aromatic herbs (including teas); and
9. specialised nutritional products (food supplements).

Powdered formulae for infants and young children were not included among the categories as the hazards and risks associated with these products had been previously reviewed by FAO and WHO (FAO and WHO, 2004; CAC, 2008). In addition, oils intended for use in food were not considered in the exercise. Remarkably, although 'cereals and grains' scored first in the risk ranking exercise, mainly due to the international trade and food consumption criteria, 'dried protein products', which were ranked second, stood out in terms of burden of disease. This was influenced by a couple of very large outbreaks associated with dried dairy products, which led to a high disability adjusted life year (DALY) calculation for this category. Likewise, 'nuts and nut products' also showed a higher burden of disease in terms of DALYs. The burden of disease was estimated considering data extracted from a scoping review on microbiological hazards in LMF, which showed that *S. enterica* subsp. enterica was implicated in most of the outbreaks and accounted for 44.9% of disease outbreaks across LMF categories, followed by *B. cereus s. l.* (25.7%), *C. botulinum* (15.0%), *S. aureus* (7.5%), *C. perfringens* (4.7%) and pathogenic *E. coli* (2.3%). In fact, *S. enterica* subsp. enterica was responsible for 93% of the outbreaks linked to 'confections and snacks' (mainly related to chocolate), 100% for 'dried fruits and vegetables', 46.1% for 'dried protein products', 80% for 'nuts and nut products', 100% for 'seeds for consumption', 46.4% for 'spices and dried aromatic herbs' (including teas) and 13.8% for 'cereals and grains'. The scoping review also evidenced that some of the microbiological hazards are mainly associated with some particular LMF sub-categories, such as *B. cereus s. l.*, involved in 61.1% of the outbreaks due to 'cereals and grains' (mainly related to cooked rice and pasta dishes) and 35.7% of the ones associated with 'spices and dried aromatic herbs (including teas)', or *C. botulinum*, causative agent of all but one outbreaks due to 'honey and preserves', linked to infant botulism cases through honey consumption.

Similar conclusions can be drawn from the revision of other data sources used in this assessment.

Strong evidence FBO data at EU level since 2010 showed that most outbreaks associated with cereal products including rice and seeds/pulses (nuts, almonds) were caused by *B. cereus s. l.* (51 out of 72 outbreaks, with 676 associated cases) and *S. enterica* subsp. enterica (12 out of 72 outbreaks, with 259 associated cases), with *C. perfringens* (5 outbreaks), *C. botulinum* (1 outbreak), *S. aureus* (1 outbreak) and *L. monocytogenes* (1 outbreak) being also occasional causative agents of outbreaks. In relation to sweets and chocolate, *S. enterica* subsp. enterica caused most of the outbreaks reported (93 out of 105, with 990 associated cases), with *B. cereus s. l.* (2 outbreaks), *S. aureus* (1 outbreak) and *Vibrio* spp. (1 outbreak) being involved in occasional outbreaks.

All multi-country outbreaks reported as ROA reports and involving LMF were caused by *S. enterica* subsp. enterica and included outbreaks of *S.* Agona and *S.* Poona infections linked to infant formula (EFSA and ECDC, 2018c; ECDC and EFSA, 2019c), *S.* Typhimurium and *S.* Anatum linked to Brazil nuts (ECDC and EFSA, 2020b), *S.* Amsterdam, *S.* Havana, *S.* Kintambo, *S.* Mbandaka, *S.* Orion and *S.* Senftenberg linked to imported sesame-based products (ECDC and EFSA, 2021b), and monophasic *S.* Typhimurium linked to chocolate products (ECDC and EFSA, 2022a). Between March 2016 and May 2017, a new serovar of *S. enterica* subspecies *enterica* with antigenic formula 11:z41:e,n,z15 infected 47 individuals in five EU countries through consumption of sesame seeds (EFSA and ECDC, 2017a). Five JNS notifications were associated with a multi-country outbreak by *S.* München, with sesame seeds from Sudan being, yet weakly, the suspect vehicle of contamination, as the same strain of the above serovar was involved in all five notifications (European Commission, 2020).

Outbreak data from outside of the EU included outbreaks of different serovars of *S. enterica* subsp. enterica linked to peanut butter, tahini, snacks cereal, dried coconut, pistachios, nuts and nut butter, and chia seed powder (20 outbreaks reported by CDC and one by Health Canada), pathogenic *E. coli* in flour and flour products, soynut butter and hazelnuts (4 outbreaks reported by CDC and one by Health Canada) and *Cronobacter sakazakii* in powdered infant formula (FDA). FSANZ also reported an outbreak linked to whey protein concentrate possibly contaminated with *C. botulinum*.

The majority of the 2216 RASFF notifications for bacterial pathogens for the product categories considered LMF concerned *S. enterica* subsp. enterica (93.0%). *Bacillus*, mainly *B. cereus s. l.*, was found in 4.5% of the notifications, and concerned herbs and spices in about half of those notifications. Between 0.6 and 0.8% of LMF notifications were for *C. sakazakii* (mainly dietetic foods, food supplements and fortified foods), *L. monocytogenes* (mainly in cereals and bakery products and nuts, nut products and seeds) and pathogenic *E. coli* in various groups of LMF. About 0.3% were for *S. aureus* (mainly in cereals and bakery products) while 0.2% were for *C. perfringens* (of which three for dried herbs and spices).

APPENDIX D

Interventions tested to eliminate *Listeria* from the processing environment

Studies assessing the performance of chemical, physical or biological interventions to destroy *Listeria* from the processing environment in industrial settings or on model systems very closely resembling real industrial settings are shown in Tables D.1–D.3.

TABLE D.1 Chemical interventions tested to eliminate *Listeria* from the processing environment in relevant studies.

Study	Possible persistence locations	Intervention description	Intervention conclusions
In industrial settings			
Campdepadros et al. (2012)	FCS and NFCS of different parts of a dessert-processing factory	Two disinfection protocols[a]	*L. monocytogenes* was not detected on FCS while its identification was restricted to NFCS (mostly the floor). Both disinfection protocols reduced the *L. monocytogenes* load but did not eradicate the microorganism
Moretro et al. (2017)	Floors where water tends to accumulate in two meat processing plants	Addition of citric acid powder to five floors and a floor gutter once a day during 4 weeks after disinfection but before start of production	There was a reduction of *L. monocytogenes* positive floors from 59% to 13% after 4 weeks. In the citric acid test period, *L. monocytogenes* was eradicated from all floor areas, except for a floor that was positive in three out of four samplings
Pagadala et al. (2012)	Seven blue crab meat and crab processing plants (the most common sites positive for *L. monocytogenes* were raw crab coolers and receiving docks)	Feedback provided to processors for improving C&D practices; for example, switching to more aggressive alkaline detergents for removal of biofilms	There was a significant reduction in the number of positive *L. monocytogenes* samples (samples were taken from crab meat and environment)
Eglezos and Dykes (2018)	All production areas in a cheese processing facility	Ozonation[b] as an adjunct to the C&D regimes	*L. monocytogenes* isolations were significantly reduced in all areas from 15% (27/180) in the samples taken pre-ozonation (i.e. taken after the end of the deep clean monthly disinfection) to 1.67% (3/180) in the post-ozonation samples
Using model systems			
Berrang et al. (2017)	Uninoculated PVC floor drains and *L. monocytogenes*-inoculated laboratory scale model PVC floor drains	Disinfection using self-contained chlorine dioxide (ClO_2)-generating and delivery pods. Drains were exposed to ClO_2 for 4 h or 24 h	There was a significantly reduction of *L. monocytogenes* counts in standing water[c] and the inner surface of treated drains.[d] A 24-h treatment was more effective than a 4-h treatment. The most efficient treatment eliminated viable *L. monocytogenes* in the drain liquid and lead to > 6 log decrease in attached *L. monocytogenes*
Chaitiemwong et al. (2010)	Conveyor belt	The conveyer belt was composed of polyester fabric impregnated with either thermoplastic polyurethane (control) or with thermoplastic polyurethane and the antimicrobial compound HyGUARD®[e]	The *L. monocytogenes* reduction on belt material with antimicrobial additives was greater than on belt material without additives when the surfaces were wet. The presence of food debris neutralised the effect of the antimicrobials. This suggests that the antimicrobial additives in conveyor belt material could help to reduce *L. monocytogenes* on belts at low temperatures when food residues are absent, and belts are not rapidly dried
Martinez et al. (2021)	*L. innocua* inoculated rubber or foam blade squeegees	Different C&D protocols simulating cleaning procedures used in food processing[f]	Rubber blades were cleaned more efficiently than foam blades, possibly due to reduced bacterial attachment to the structure. A full procedure (detergent and rinse, followed by disinfectants) including a scrubbing step was recommended

TABLE D.1 (Continued)

Abbreviations: C&D, cleaning and disinfection; FCS, food contact surface; NFCS, non-food contact surface; PVC, polyvinyl chloride.

[a]Protocol A: Applied daily. Visible dust was removed by manual scanning. The surface was rinsed with warm water (45–50°C) at low pressure. Easyfoam VF32 (diluted to 3%–4%) was applied and left for 15–20 min followed by manual scrubbing and abundant rinsing with tap water. Suredis VT 01 L (diluted to 1%–2%) was applied and left for 15–20 min. Finally, it was rinsed abundantly with tap water. Weekly air disinfection was performed after work (Saturday afternoon to Sunday morning). Protocol B: Once every 3 weeks. Visible dust was removed by manual scanning. The surface was rinsed with warm water (45–50°C) at low pressure. Easyfoam VF32 (diluted to 3%–4%) was applied and left for 15–20 min followed by manual scrubbing, and abundant rinsing with tap water. Aciplusfoam VF59 (diluted to 3%–4%) was applied and left for 15–20 min. It was then scrubbed manually and rinsed abundantly with tap water. Suredis VT 01 L (diluted to 3%–4%) was applied and left for 15–20 min. Finally, it was rinsed abundantly with tap water.

[b]5 L/min with a concentration of 20 g/min for 15 min on Mon - Fri and 120 min on Sat – Sun.

[c]Planktonic cells remaining viable in the liquid.

[d]Viable attached cells on the inner surface.

[e]With substances silver zeolite, aluminium oxide, calcium oxide, magnesium oxide, zinc pyrithione, oxybisphenoxarsine or a combination of those.

[f]The detergents used were quaternary ammonium (550 ppm), a chlorine-based disinfectant (600 ppm), a peracetic acid solution (2025 ppm) or a chlorinated alkaline detergent (20,000 ppm).

TABLE D.2 Physical interventions tested to eliminate *Listeria* from the processing environment in relevant studies.

Study	Possible persistence locations	Intervention description	Intervention conclusions
UV-C radiation			
In industrial settings			
Bernbom et al. (2011)	Food processing surfaces in a fish smoke house after the routine C&D procedure	Ceiling-mounted UV-C light (wavelength 254 nm)	*L. monocytogenes* positive samples were reduced from 44% (30/68) before the 48-h exposure to 12% (8/68). After 7 h of exposure, positive samples reduced from 34% (23/68) to 26% (18/68). Laboratory experiments showed that UV-C light is a useful extra bactericidal step and that it, as all disinfecting procedures, is hampered by the presence of organic material
Using model systems			
Morey et al. (2010)	Four types of conveyor belts made of different materials	UV light applied at 5.53 and 5.95 mW/cm^2 for 1 and 3 s	*L. monocytogenes* was significantly reduced on all belt types irrespective of UV light intensities and exposure times. More survival was found at the lowest light intensity on all conveyor belts. Populations were reduced to below detection limits on three types of belts after exposure to the highest UV light intensity for 3 s
Heat treatments			
In industrial settings			
Eglezos and Dykes (2011)	Holding chillers used for post-cook commercial processed meats in facilities	Heating interventions using electrical air-blowing heaters at each of the two facilities, with 2 weeks of post-intervention sampling after each treatment	The *Listeria* prevalence in chiller A was significant reduced using air temperatures of 37°C for 36 h from 10.6% (19/180) to 1.7% (3/180), while it was reduced in chiller E using 50°C for 2 h from 7.8% (7/90) to 0% (0/90)
Pennone et al. (2020)	The floors of mushroom growing units	Steam cookout processes	There was a significant reduction in the number of positive *L. monocytogenes* samples. Before cookout, the incidence averaged 63% (75% of the floor swabs and 45% of the spent substrate samples positive), while after a first cookout, the average was 40% (67% of the floor swabs positive and no positives in the spent substrates). 19% of floor swabs taken after a second cookout were found positive
Using model systems			
Tobin et al. (2020)	Detachable mushroom slicer heads from industrial food slicing equipment	Hot water disinfection using water at 55°C, 65°C or 75°C for 93, 16.4 or 6.5 min	Complete elimination of *L. innocua* cells from each slicer head treatment

Abbreviations: C&D, cleaning and disinfection; PVC, polyvinyl chloride; UV, ultraviolet.

TABLE D.3 Biological interventions tested to eliminate *Listeria* from the processing environment in relevant studies

Study	Possible persistence locations	Intervention description	Intervention conclusions
In industrial settings			
Reinhard et al. (2020)	The RTE environment of refrigerated (4°C) and ambient (20°C) temperature facilities producing RTE meat and poultry products, RTE bakery items and assembled RTE sandwiches	Bacteriophage P100 (Listex™) using two different application strategies[a]	There were significant reductions (ranging from 32% to 44.4%) in the fraction of NFCS samples positive for *Listeria* spp. using both application strategies
Schobitz et al. (2014)	The walls of floor gutters in a salmon processing plant	A so-called 'biocontroller' consisting of a thermally treated fermentate from two *Carnobacterium maltaromaticum* strains and a strain of *Enterococcus mundtii*, plus nisin at 1000 IU/mL, entrapped in an alginate matrix supported by a mesh-type fabric	*L. monocytogenes* was successfully eliminated from the walls of the floor gutters in five out of the seven trials. The two failures were linked to loss of contact of the biocontroller with the side wall of the floor gutter
Zhao et al. (2013)	Six floor drains of a RTE poultry processing plant that were consistently found *Listeria* positive	Competitive exclusion using a combination of *Lactococcus lactis* and *Enterococcus durans* applied for 4 weeks[b]	*L. monocytogenes* could be efficiently reduced; it was not found in five of the floor drains after the first week of treatment and all floor drains were negative after 8 weeks of treatment, whereas control drains were still *Listeria* positive

Abbreviations: IU, international Units; NFCS, non-food contact surface; RTE, ready-to-eat.

[a]A moderate application applied as a single treatment every 24 h over three consecutive days (2×10^7 PFU/mL) and an intensified application applied once every 6 h over a 24 h period (1×10^8 PFU/mL).

[b]4 times a week during the first week and twice-a-week for the following 3 weeks.

APPENDIX E

QMRA module for persistence

E.1 | Introduction

To better understand the importance of persistence for public health (PH) risks related to microbial food safety, as compared to other factors such as bacterial growth and virulence, we developed a model that describes the process, and included it in a hypothetical food chain model for risk assessment. The aim of the modelling is to:

- provide a generic mathematical description of the persistence process, that can be integrated in a quantitative microbiological risk assessment (QMRA) and is as simple as possible, whilst capturing the essential characteristics of persistence in a food processing environment (FoPE);
- explore the model, to increase our understanding of the essentials of the persistence process;
- explore the importance of persistence compared to other factors such microbial growth, survival and virulence; and
- compare strains with different characteristics in terms of persistence and other factors, by including the possibility to make the model 'strain specific', as specific persistent strains are well known.

As AQ6 requires to develop perspectives, and not to perform a risk assessment per se, an exploration of the model was pursued while a concrete case study was not performed.

Previously, for the development of QMRA models, Nauta (2007) defined a set of basic processes that typically describe events leading to changes in concentrations of bacteria in the food chain: growth and inactivation; and mixing, partitioning, cross-contamination and removal. Of these, persistence seems to involve cross-contamination (bacterial transfer), decreased inactivation (increased survival) and probably growth in the FoPE.

In general, persistence can be observed if the following steps hold:

1. the FoPE is contaminated, either from (raw) food material entering the environment or through (re-)contamination from the FoPE itself (e.g. through workers or equipment). This is a single event. Repeated contamination events may induce different instances of persistence;
2. there is prolonged survival (no inactivation or removal, as intended in the processing) and/or growth in the FoPE; and
3. there is bacterial transfer (cross-contamination) from the FoPE to the food product, as otherwise there is no PH effect for consumers.

The second step is the critical step, as here, in the food production process, it is foreseen that the contamination is reduced or eliminated, so the contamination is only temporal. For persistence to occur, the bacteria must prevail in the FoPE longer than anticipated in the hygienic design of the food production process. Persistence can be due to a processing step which is explicitly included to inactivate or remove potential contamination and is not effective (due to process parameters or the typical characteristics of the bacterial strain), or due to an incident in an otherwise safe processing step (something unexpected in the FoPE or 'colonisation' due to the typical characteristics of the bacterial strain). Typical for persistence is also that it occurs over a long period. In general, this 'long period' is not well defined.

Obviously, when there is bacterial growth in the FoPE, for example in a biofilm, there can be a continuous influx of bacteria. A priori, it is unclear to what extent bacterial growth in the second step is required to observe a persistent strain over a long period. This can be evaluated by the model.

Below, in Section E.2, we first provide an overview of potential persistence models for QMRA, based on literature known to the Working Group, and select a model that was considered suitable. Next, in Section E.3, simple QMRA food chain models are constructed for *Listeria monocytogenes* in a product like cooked ham and *Salmonella* in a low moisture food. Then, in Section E.4, the performance of these models is explored. The results of Section E.4 are discussed in Section E.5.

E.2 | Potential persistence models for QMRA

Although few models in the literature are explicitly made to describe persistence in the FoPE, some available models can be applied for that purpose. Usually, these models are presented as transfer models or cross-contamination models, where the principle is that bacteria in the FoPE are transferred to a food being processed. As these models are usually developed to describe a specific process for a specific (type of) food product, they contain specific factors or transfer routes. Here, we first provide some generic models, derived from published models (Sections E2.1-E2.3). Next, based on these, a basic deterministic persistence model is presented (Section E2.4) with variants that allow inclusion of stochasticity (Section E2.5) and instantaneous increase and decrease in population size, due to, for example, growth overnight or C&D (Section E2.6).

E.2.1 | One compartment model

The first model describes a FoPE that contains N bacteria (due to a single accidental contamination) that can be transferred to a food product that is processed in the FoPE (See Figure E.1). It holds the basic assumption of a fixed transfer rate. (Chaitiemwong et al., 2014), for example, use this as a model for transfer from a slicer; it is a simplified version of, for example, the broiler processing plant model described by (Nauta, 2005). In principle, there is a continuous flow of consecutive food products. At step *I* in the process, with a transfer rate b_1 and N_i bacteria in the FoPE, the number of bacteria on the food product is $b_1 N_i$ and the bacterial number in the FoPE reduces to $N_{i+1} = N_i(1 - b_1)$. The transfer rate can be interpreted as the probability for a bacterial cell in the FoPE to be transferred to the food product. The model assumes that the expected number of bacteria is transferred, so no stochasticity is included.

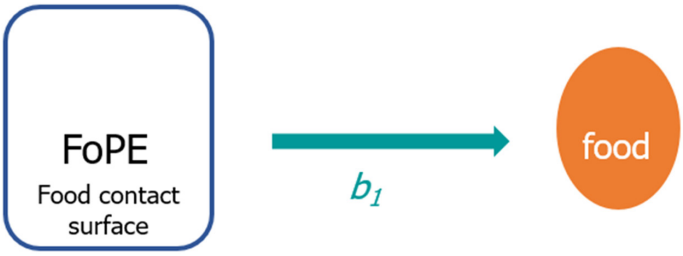

FIGURE E.1 One compartment model. From a contaminated FoPE there is continuous transfer to food products (it is assumed that there is a constant flow of consecutive food products, at step i the ith food product passes through the FoPE).

An example of the dynamics of the process is given in Figure E.2. It shows a loglinear decline of the bacterial numbers in both the FoPE and on the consecutive food products.

This model shows no typical persistence behaviour. The *jth* food product passing through the environment is contaminated with $N_0 b_1 (1 - b_1)^{j-1}$ bacteria.

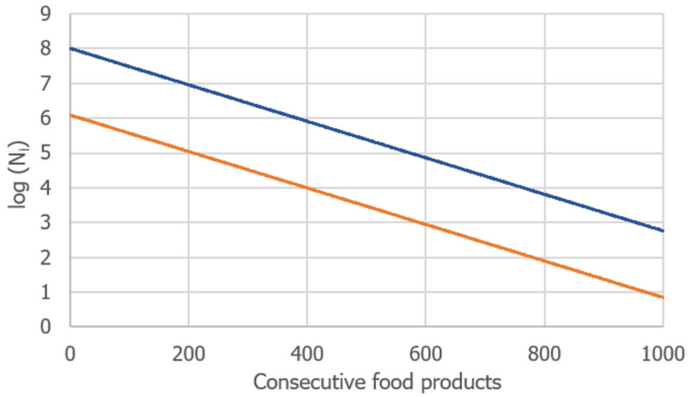

FIGURE E.2 One compartment dynamics, $N_0 = 10^8$ CFU, $b_1 = 0.012$. Blue line: log(N_i) in the FoPE. Orange line: Number of bacteria on the consecutive food products (in log CFU).

E.2.2 | Model with two separate compartments

After performing experiments with meat grinders, it was found that this one compartment model cannot explain observations, which show tailing (Møller et al., 2012; Møller et al., 2016). An alternative model was proposed, with two compartments representing two different FoPE (see Figure E.3). With this model the observations could be explained well. Here we present a simplified version where there is only transfer from the environments to the food products and not vice versa. FoPE1 and 2 are contaminated with $N_{1,i}$ and $N_{2,i}$ bacteria at step *i*, transfer rates are b_1 and b_2, so $N_{f,i} = N_{1,i-1} b_1 + N_{2,i-1} b_2$.

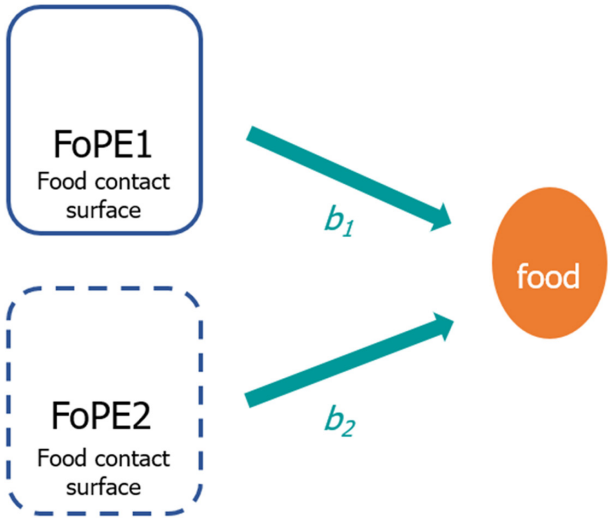

FIGURE E.3 Two separate compartments model. From two (simultaneously) contaminated FoPE there is continuous transfer to food products, with different rates.

An example of the dynamics of the process is given in Figure E.4. With this model, the tailing as observed in several experiments is reproduced (Møller et al., 2016).

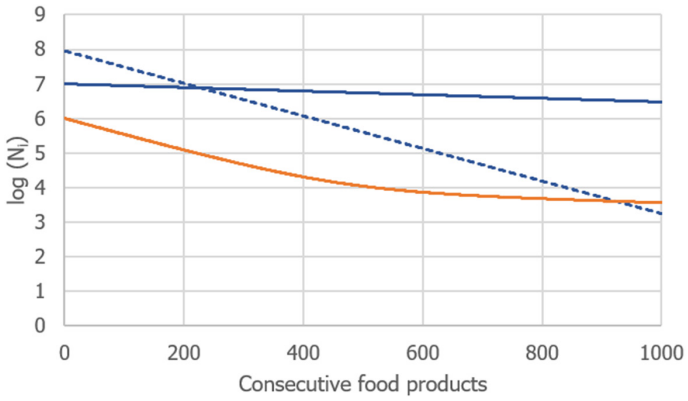

FIGURE E.4 Two separate compartment dynamics, $N_{1,0} = 10^7$ CFU, $N_{2,0} = 10^{7.95}$ CFU, $b_1 = 0.012$, $b_2 = 0.0108$. Blue line: log(N_i) in the FoPE1 (solid) and 2 (dashed). Orange line: Number of bacteria on the consecutive food products (in log CFU), showing tailing.

This model can explain the tailing that is often observed and can therefore explain persistence over a longer time than the one compartment model. The *jth* food product passing through the environment is contaminated with $N_{1,0}b_1(1-b_1)^{j-1} + N_{2,0}b_2(1-b_2)^{j-1}$ bacteria.

The identification of the two separate environments in the FoPE may be challenging (Møller et al., 2020). Furthermore, it is questionable whether two of those separate environments with direct transfer to the food products exist in every FoPE where persistence occurs.

E.2.3 | Model with two linked compartments

A third option for a model is inspired by the agent-based model for persistence presented by (Mokhtari & Van Doren, 2019). They describe the persistence as a process with two linked environments in the FoPE, one with bacterial cells on more accessible areas (FoPE1) and one with bacterial cells harboured in holes and cracks that are difficult to clean and disinfect (FoPE2) (Figure E.5). FoPE1 can be identified as a food contact environment and FoPE2 as a hidden environment. We here omit the C&D and present the principle of their model as a model with two linked compartments.

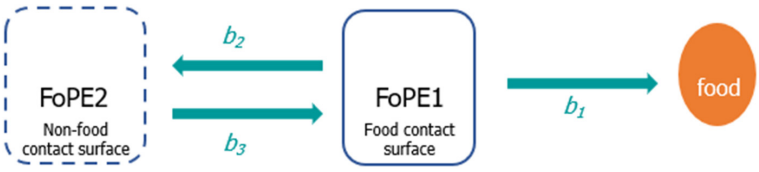

FIGURE E.5 Two linked compartments model. From a contaminated FoPE1 there is continuous transfer to food products and to FoPE2. There is also a flow back from FoPE2 to FoPE1.

In this model, transfer to the food products is only from FoPE1, whereas there is exchange between FoPE1 and FoPE2, with two transfer rates, b_2 and b_3

The model system reads as:

$$N_{f,i} = N_{1,i-1} b_1$$

$$N_{1,i} = N_{1,i-1}\left(1 - b_1 - b_2\right) + N_{2,i-1} b_3$$

$$N_{2,i} = N_{1,i-1} b_2 - N_{2,i-1} b_3$$

An example of the dynamics of the process is given in Figure E.6. Again, tailing is observed.

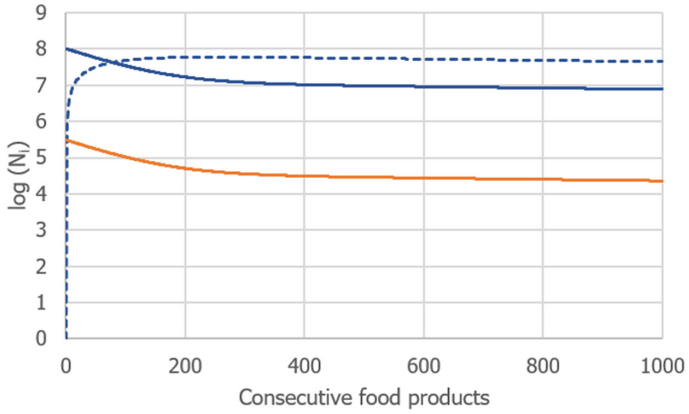

FIGURE E.6 Two linked compartment dynamics, $N_{1,0} = 10^8$ CFU, $N_{2,0} = 0$ CFU, $b_1 = 0.003, b_2 = 0.009, b_3 = 0.002$. Blue line: log($N_i$) in the FoPE1 (solid) and the FoPE2 (dashed); orange line: number of bacteria on the consecutive food products (in log CFU), showing tailing.

This model can explain the tailing that is often observed, and thus explain persistence over a longer time than the one compartment model. It requires one more parameter than the two separate compartments model but may be more realistic in the assumptions that only one FoPE is originally contaminated, and that there is a 'hidden' environment (such as a drain, or an inaccessible component of the equipment, difficult to clean and disinfect) that is connected to the other one, but not directly to the food products.

Note that it is assumed that the initial contamination occurs in FoPE1, which is in direct contact with the food that could be the source of the contamination.

E.2.4 | Basic model for persistence

The Working Group members agreed that the two linked compartments model is the most realistic, both in assumptions and performance.

The version of this model described in Section E.2.3 only contains transfer between environments and food but does not include growth potential or inactivation (less than 100% survival), which are both considered important for persistence. These are included in a more complete version of the model, which is presented in Figure E.7. This version of the model is considered as the 'basic model for persistence' in this Scientific Opinion as it includes the relevant characteristics leading to persistence; other versions of the model, with small modifications, can be used to describe more realistic case studies.

The 'persistence parameter' a_2 represents the change in population size in FoPE2 in the time frame between two food products passing in FoPE1, which is presumably very short. It could be estimated from observed growth in a certain time period, if the processing speed is known. A positive value implies growth in FoPE2, so a_2 represents a growth rate which will result in increased persistence. When $a_2 = 0$, there is 100% survival, and only transfer to and from FoPE2 influences changes in the population size in FoPE2. A negative value of a_2 represents an inactivation rate implying less than 100% survival. The inactivation rate parameter c_1 represents the decrease in population size in FoPE1 (which, if negative, might represent growth), also in the time frame between two food products passing in FoPE1.

The model system reads:

$$N_{f,i} = N_{1,i-1} b_1$$

$$N_{1,i} = N_{1,i-1}\left(1 - b_1 - b_2 - c_1\right) + N_{2,i-1} b_3$$

$$N_{2,i} = N_{1,i-1} b_2 + N_{2,i-1}\left(a_2 - b_3\right)$$

From these model equations, it can derived that the system is in equilibrium (i.e. constant values for N_1 and N_2) if

$$a_2 = \frac{b_3(b_1 + c_1)}{b_1 + b_2 + c_1}$$

The total population size ($N_1 + N_2$) is increasing if a_2 is larger and decreasing if a_2 is smaller. It is likely that $a_2 < b_3$, as otherwise growth dominates the process too much.

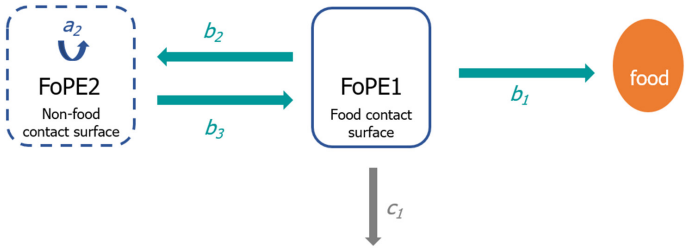

FIGURE E.7 Extended version of the two linked compartments model depicted in Figure E.6. Persistence parameter a_2 indicates the growth or inactivation rate in the hidden environment FoPE2, whereas there may be limited survival in FoPE1, described by inactivation rate c_1.

E.2.5 | Stochastic versions of the model

The basic model for persistence above is deterministic, assuming constant rates for transfer between environments and food, growth and inactivation. This simplifies the model but may not be realistic. The alternative is to develop a stochastic model, which may be more realistic but will contain more (unknown) parameters.

Random stochastic variation can be included. Instead of constant transfer and inactivation rates, for every passing food item, the number of bacteria transferred or inactivated is sampled from a binomial distribution $Bin(N, p)$, where N is N_1 and p is rate b_1, b_2 or c_1; or N is N_2 and p is rate b_3. This assumes constant rates, but discrete bacterial cells are sampled, so the model does not operate with expected values only.

Stochasticity can be taken one step further, as even the rates can vary with every transfer. This increases the variation in numbers of transferred and inactivated bacterial cells. A Betabinomial distribution can be used, which is similar to a Binomial distribution, but with rates that have a $Beta(kp, k(1-p))$ distribution, where k is a parameter $(0, \infty)$. This gives increasing variability in transfer or inactivation with increasing k; the model is similar to the one described by (Nauta, 2005), for clustering of cells during a partitioning process.

The persistence parameter a_2 could be sampled from, for example, a Normal distribution with mean $a_{2,mean}$ and standard deviation $a_{2,sd}$.

Figure E.8 shows an example of the change in dynamics when a stochastic model is applied. With high concentrations, the use of the binomial model does not add much variation. With the use of the betabinomial model the system gets more unpredictable, as transfer goes in 'bursts'. This may be realistic, but complicates the model analysis considerably, both in terms of input requirements, potential outputs and scenario analysis.

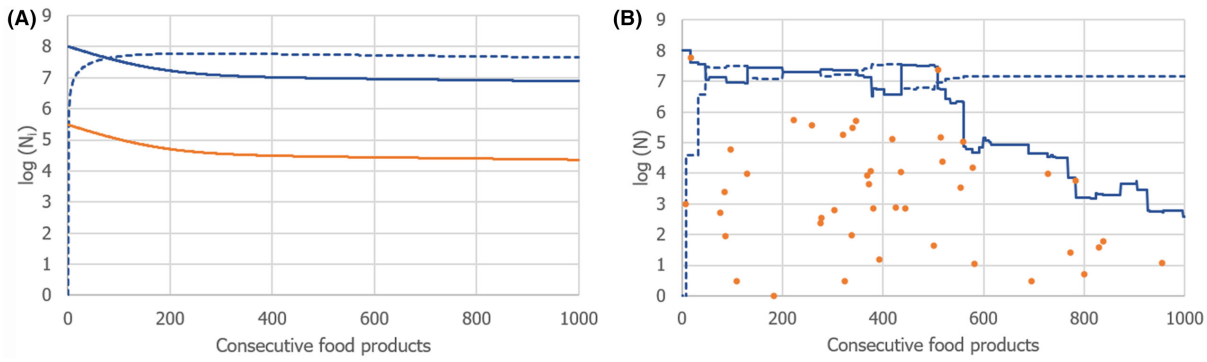

FIGURE E.8 Examples of results of the stochastic version of the two linked compartments model, comparable to Figure E.6. Figure A applies the binomial model; figure B applies the BetaBinomial model, with $k = 1$. Blue line: log(N_i) in the FoPE1 (solid) and the FoPE2 (dashed); orange line: number of bacteria on the consecutive food products (in log CFU). Note that in these examples, the initial contamination is in FoPE1, not in FoPE2.

Although a stochastic model would be more realistic, it was not further evaluated because it gives a model with even more unknown parameters and its analysis requires more resources, so it complicates the model analysis considerably.

E.2.6 | Instantaneous inactivation and growth

The basic model assumes that inactivation and growth take place gradually, at a constant rate. This is not necessarily realistic or desirable. With C&D, a larger amount of the bacterial contamination will be removed, which can be modelled by an instantaneous inactivation step, implemented by a few logs decrease in concentration. Typically, for persistence to occur, it can be assumed that the C&D is effective in FoPE1, but not in FoPE2. This represents a situation where the food business operator's hygienic measures are targeted at the food contact areas that are in direct contact with the food, whereas 'hidden' areas may be missed (Mokhtari & Van Doren, 2019). Similarly, growth may occur during a period that the food processing is halted, e.g. overnight, giving ample time for increase in concentration, for example in a biofilm.

These phenomena can be modelled by introduction of a log increase or decrease after processing of a certain number of food products. Figure E.9 shows an example of a sudden 5 log decrease in FoPE1, and the limited effect it has on the contamination of the processed food products.

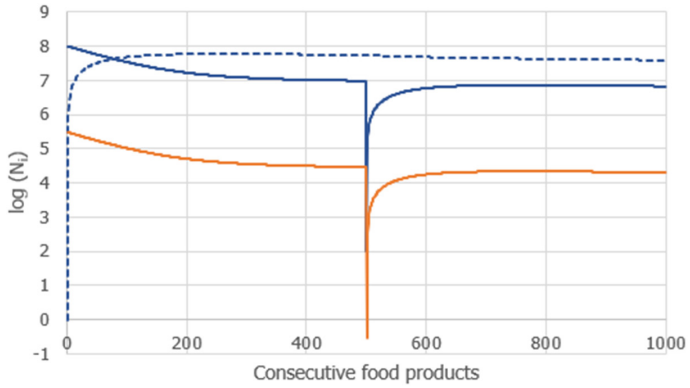

FIGURE E.9 Example of the effect of C&D after processing 500 food products, in the two linked compartments model as shown in Figure E.6. The concentration in the FoPE abruptly decreases, but increases again due to contamination from FoPE2, in which the C&D was not effective. Blue line: log(N_i) in the FoPE1 (solid) and the FoPE2 (dashed); orange line: number of bacteria on the consecutive food products (in log CFU). Note that in this example, the initial contamination is in FoPE1, not in FoPE2.

E.3 | A QMRA food chain model with persistence

The persistence model is to be used in conjunction with other models in a larger food chain model for QMRA. As an example, we applied a simple food chain, inspired by the production of cooked ham that is potentially contaminated with *L. monocytogenes*. Persistence was considered to occur in a slicer, as illustrated in Figure E.10.

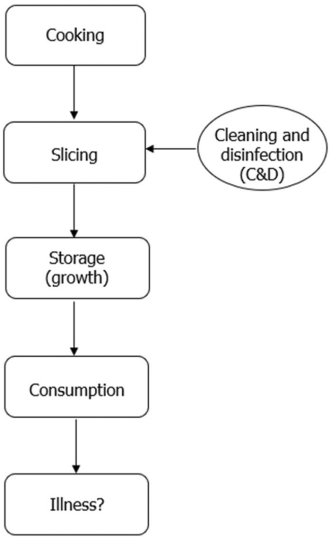

FIGURE E.10 QMRA food chain model involving persistence in the slicer.

We assume that cooking (the first step) inactivates all *L. monocytogenes*, and either a component of the slicer in contact with the ham (FoPE1) or a FoPE in contact with this part of the slicer (FoPE2) has been accidently contaminated with *L. monocytogenes* that became **persistent**. After that we can get **growth** during storage, exposure and possibly illness, as defined by a **dose–response** (DR) model. Additionally, some assumptions on the population considered (number of consumers and servings, serving sizes etc.) are required.

As an alternative, we also defined a model for *Salmonella* in a LMF, where growth is not expected to occur in the food product during storage. This follows the same food chain model structure, without the storage step.

The model described in Section E.2.4 was applied as a persistence model. The food products passing through FoPE1 were assumed to have a weight of 500 g. Below, the growth model for *L. monocytogenes* and DR models for *L. monocytogenes* and *Salmonella* are described.

E.3.1 | Growth model for *L. monocytogenes* growth during storage

The model for growth of *L. monocytogenes* during storage of the food product after slicing is taken from (Pouillot & Lubran, 2011), the triphasic linear model without lag, given by

$$\log N_{end} = min\left(log_{N_0} + \frac{\mu}{ln_{10}} \times t, MPD\right),$$

$$\text{with } \mu = \mu_{ref}\left[\frac{(T - T_{min})}{(T_{ref} - T_{min})}\right]^2,$$

and $T_{min} = -2.86\,°C$; $T_{ref} = 25\,°C$; $\mu_{ref} = 6.24$ per day and $MPD = 7.27$ log CFU / g.

As default, we take $T = 6\,°C$ and $t = 5$ days. Portion size is assumed to be 50 g.

E.3.2 | Dose–response models

E.3.2.1 | Dose–response model for *L. monocytogenes*

The lognormal-Poisson DR model for *L. monocytogenes* that was developed by (Pouillot et al., 2015) and later applied by (EFSA BIOHAZ Panel, 2018) provides models for specific population groups, depending on susceptibility, and includes specific terms describing variability between hosts in these population groups and between strains. The probability of illness after consumption of one cell of *L. monocytogenes* is expressed through the *r*-value. The standard deviation of the *r*-value is assumed to be the same for all the population groups. It is summarising the variability between *L. monocytogenes* strains and host individual susceptibilities. With a given mean value for the log *r*-value (typical for the exponential DR model) for each population group, the standard deviation of this log *r*-value between hosts is 0.55 and between strains is 1.52 (Pouillot et al., 2015).

For the healthy population (mean log $r = -14.11$) this allows the derivation of the log *r*-value distribution of a typically virulent strain. According to (Pouillot et al., 2015), the variability in mean log r-values between strains can be described by a Normal (–14.11, 1.52) distribution. A virulent strain can then be characterised as having a mean $\mu = -10.574$ (the 99%-ile of the between strain distribution) and $sd = 0.55$.

Implementation of the lognormal-Poisson DR model requires stochastic modelling, which is rather cumbersome and not needed. For our purposes, it was sufficient to use this model to establish a DR model providing mean probabilities of illness as a function of dose, obtained by Monte Carlo simulation with 100,000 iterations for a series of doses ranging from 10^0 to 10^8.

$$P_{ill,healthy\ pop,virulent\ strain} = D \times 10^{-10.229}$$

This is an approximation of the original model, but, as shown in Figure E.11, it seems to be appropriate as the correlation is perfect ($r^2 = 1$).

The DR model for the whole population can be derived in a similar way. Here, we use the definition of population subgroups given by Pouillot et al. (2015) and assume that all population groups consume the same amount of cooked ham. Mean values of log *r* are sampled on the basis of the relative sizes of the different population groups, to obtain a new (empirical) distribution of *r*-values for the same virulent strain. This distribution is applied in a Monte Carlo simulation with 1000,000 iterations for a series of doses ranging from 10^0 to 10^{10}, for 200 doses with step size 0.05 for the log dose, resulting in:

$$\log P_{ill,\ whole\ pop,virulent\ strain} = -9.038 + 0.9922 \times log_D - 0.00523 \times log_D{}^2 + 0.00352 \times log_D{}^3 - 0.00043 \times log_D{}^4$$

which is also a well-fitting approximation (see Figure E.11).

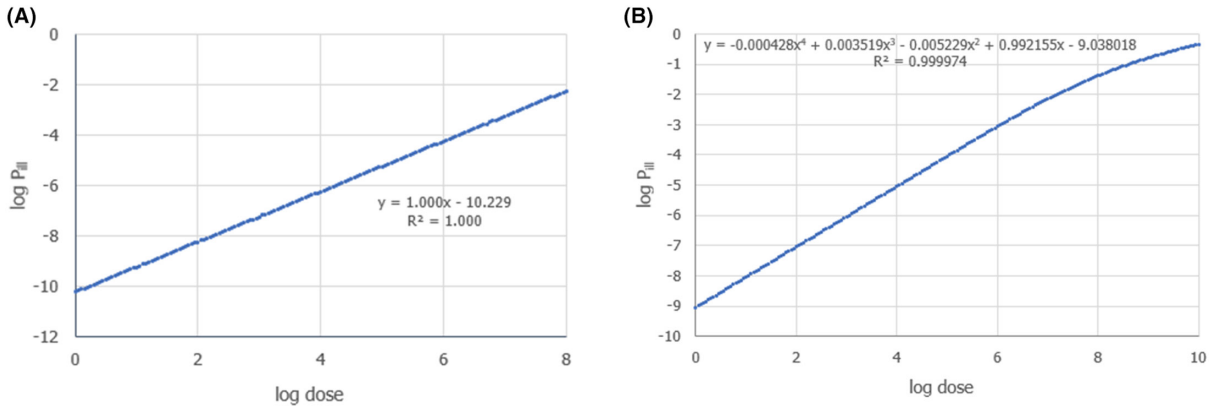

FIGURE E.11 Data points and fitted line of the dose–response model to mean probabilities of illness obtained for 160 and 200 doses, using the (Pouillot et al., 2015) DR model for a virulent strain and healthy people in the general population (A) and the whole population, including susceptible population groups (B).

With the same methodology, models can be derived for the 'median' strain (50% of the virulence scale, $\mu = -14.11$, for the healthy population) as

$$P_{ill,healthy\ pop,average\ strain} = D \times 10^{-13.766}$$

and

$$\log P_{ill,whole\ pop,average\ strain} = -12.585 + 1.00795 \times log_D - 0.004417 \times log_D{}^2 + 0.000831 \times log_D{}^3 - 0.00005 \times log_D{}^4$$

E.3.2.2 | Dose–response model for *Salmonella*

Previously, the (EFSA BIOHAZ Panel, 2014a) applied a Beta Poisson DR model for *Salmonella* in their opinion on *Salmonella* in eggs, based on (Thomas et al., 2006).

$$P_{ill} = 1 - \left(1 + \frac{D}{50.60408}\right)^{-0.149344}$$

In this model, variation between strains is not explicitly included, so strain virulence cannot be described.

Typically, *Salmonella* requires much smaller dose than *L. monocytogenes* to cause human infection and illness. The shape of the DR relation is not linear.

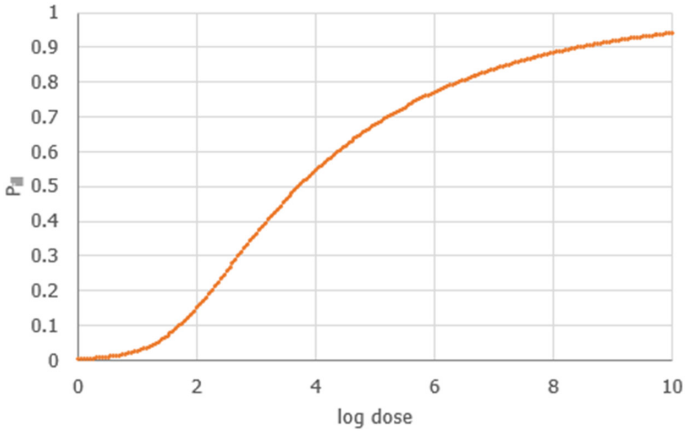

FIGURE E.12 Dose–response relation for probability of illness after exposure to *Salmonella*, based on (Thomas et al., 2006).

Other and more recent DR relations for *Salmonella* are published (for example Teunis, 2022), but it is outside our scope to investigate those.

E.4 | Model performance

The *L. monocytogenes* and the *Salmonella* QMRA food chain models have been implemented in R and are available as R shiny apps through the Knowledge Junction under https://doi.org/10.5281/zenodo.10299477. (see Section E.4.2.1).

The basic persistence model (Section E.2.4) has five unknown parameters (a_2, b_1, b_2, b_3 and c_1), and unknown values of the initial conditions. For all of them we have no or very little data available. Exploration of the model gives many possibilities for (combinations of) inputs. In general, for risk assessment, it would be of interest to know which (combinations of) parameter values characterise observations of persistence and long-term outbreaks, i.e. high concentrations in the food and/or cases of human illness over a long time period. Such (combinations of) parameter values of the persistence model would allow a comparison of risk estimates found with high persistence versus high virulence or large growth capacity and could identify parameters to be targeted for interventions. It is expected that the 'persistence parameter' a_2, which expresses the survival and/or growth in FoPE2, is of major influence, but a priori, the role of the transfer parameters (b_1, b_2, b_3) is less clear.

Below, the results of the *L. monocytogenes* model are shown and then results of the *Salmonella* model are summarised, for comparison, in Section E.5.3. Before the influence of the persistence model parameters is explored in Section E.4.2, it was first considered to what extent growth is required to explain persistence. This question is not only important for a general understanding of the persistence phenomenon, but also to evaluate whether the persistence model can be simplified by omitting growth, whilst still being able to describe what is observed when persistence is found.

E.4.1 | The role of growth in persistence

If we consider a simple version of the two linked compartments persistence model, i.e. the one shown in Figure E.5, without growth or inactivation in FoPE2 ($a_2 = 0$) and with full survival in FoPE1 ($c_1 = 0$), it is easy to see that all the bacteria that enter the system at $t = 0$ will eventually be transferred to the food. This can be a long-lasting process, when the transfer rates are low, especially when b_1 and/or b_3 are low. However, in that case, the number of cells transferred to the food are also low, as is the probability of illness after consumption of such a food product. Given the (approximate) linearity of the DR relation of *L. monocytogenes* (Figure E.11), under the same conditions in other parts of the food chain included in the model, the total expected number of cases from a single contamination event of FoPE1, initiating the process, is the same, independent of the transfer rates. Hence, without growth, and without removal of contamination in the food production process, the model predicts that transfer rates and the consequential duration of the presence of contamination in the FoPE have no effect on the expected number of human illness cases, as all bacteria end up in the food. In that case the contamination is a food safety problem, but the potentially observed persistence due to survival and transfer between compartments of the FoPE does not increase the PH risk.

If the DR relation is not linear (such as for *Salmonella*), persistence due to low transfer rates will (slightly) increase the expected number of cases, as, due to the shape of the DR models that follow the single-hit concept (and on biological grounds reject the minimum infectious dose concept), a few exposures to high doses will give less cases than many exposures to low doses, for the same total number of bacteria in all doses together. Note, however, that low levels of contamination on the food and a series of seemingly sporadic cases that occur over a longer period of time, may not be observed in surveillance of food or surveillance of human cases, and therefore the persistence may not be observed either. Similarly, high levels of contamination and a few cases occurring in a short time period, may be more easily found. This, counterintuitively, might imply that, without bacterial growth in the FoPE and with a non-linear DR relationship, persistence may *reduce* the observed contamination of food and the notification of outbreaks, whilst simultaneously actually increasing the number of cases, although that may go unnoticed.

If there is limited survival in FoPE1 (and/or FoPE2, i.e. $a_2 < 0$ and/or $c_1 > 0$), due to hygienic measures, such as regular C&D or other good hygienic practices, without growth, the overall PH risks are lower as compared to a situation with immediate transfer of contamination to a food product. So, the opposite of what we would call persistence (long term observation of the same strain in the FoPE) would theoretically give a higher PH risk. If, on the other hand, we define 'persistence' as a strain specific characteristic, so that persistent strains are those that are not removed with common C&D practices, 'persistence' increases the risk.

These findings imply that, in general, even though persistence without growth, as modelled by this simple version of the model, can result in the finding of positive FoPE samples over a longer time, it will not result in a higher risk than the alternative where the bacteria do not persist in the environment, but contaminate the food anyway. So, from a theoretical perspective, growth (increase of numbers of bacteria, potentially in biofilms) in the environment seems to be important for persistence to have a significant PH impact.

E.4.2 | Examples of model performance

E.4.2.1 | R shiny apps

The R shiny apps give many opportunities to explore the model. The user defines the transfer parameters b_1, b_2, b_3 and the persistence parameter a_2, which as a default is put at $a_2 = 0$ (no growth or inactivation in FoPE2), as well as the exposed population (only the healthy population or the whole population), the strain type considered (virulent or average) and the place of first contamination (FoPE1 or FoPE2).[37] Other inputs can be defined after clicking the 'Show more input' button. The output shows graphs with the dynamics of N_1 (population size in FoPE1), N_2 (population size in FoPE2) and $N_{f,after}$, the

[37] virulence and population are not included in the *Salmonella* model.

concentration in the food after storage; and of P_{ill}, the probability of illness per serving. Below it, inputs and outputs are summarised.

There are several options to include growth and inactivation:

- continuous growth or inactivation in FoPE2, parameter a_2. As growth in a_2 typically characterises persistence in the FoPE, this is the main persistence parameter;
- instantaneous increase, e.g. after a period where the food production is stopped, every 'interval for growth boost' food products, with an indicated log increase;
- instantaneous decrease in FoPE1 only, e.g. regular C&D that does not affect FoPE2, every 'C&D (FoPE1) food products', with an indicated log decrease;
- instantaneous decrease in FoPE1 and FoPE2, e.g. special thorough C&D that affects both FoPE, every 'thorough C&D (both FoPE) food products', with an indicated log decrease;

A 'standard run' of the *L. monocytogenes* model, as in Section E.4.1, would give the output as shown in Figure E.13. The model parameter values are $a_2 = 0; b_1 = b_2 = b_3 = 10^{-4}; c_1 = 0; logN_0 = 9$.

E.4.2.2 | Scenarios in the *L. monocytogenes* model

The effect of single parameters:

- Continuous growth in FoPE2: the situation with $a_2 = 0$ has been discussed in Section E.4.1. If a_2 is lower than the equilibrium value, the total number of bacteria in the system is decreasing. Persistence can take a very long time, definitely if it is close to the equilibrium, but the bacterial population will die out at some point. If a_2 is larger than the equilibrium value, the number of bacteria in the system will increase, and so will eventually the probability of illness per food product. This is not sustainable as at some point the bacterial numbers and numbers of cases will get too high. If this would occur in reality, it would be observed, and measures would be taken.
- A typical model output with $a_2 = 0.00008$ (which is above the equilibrium value) is shown in Figure E.14. Concentrations increase, and so does the probability of illness.[38]
- C&D, resulting in instantaneous decreases in FoPE1 only, does not affect the dynamics very much. FoPE1 is quickly filled up with bacteria from FoPE2 (as in Figure E.9). Here, one should realise that the axis is on a log scale. It shows that ineffective C&D, where a hidden area is not reached, is indeed inefficient (See Figure E.15).
- Thorough C&D resulting in instantaneous decreases in both FoPE1 and FoPE2 is effective. Despite a lower log reduction, Figure E.16 shows that the risk can be reduced to zero in this case.

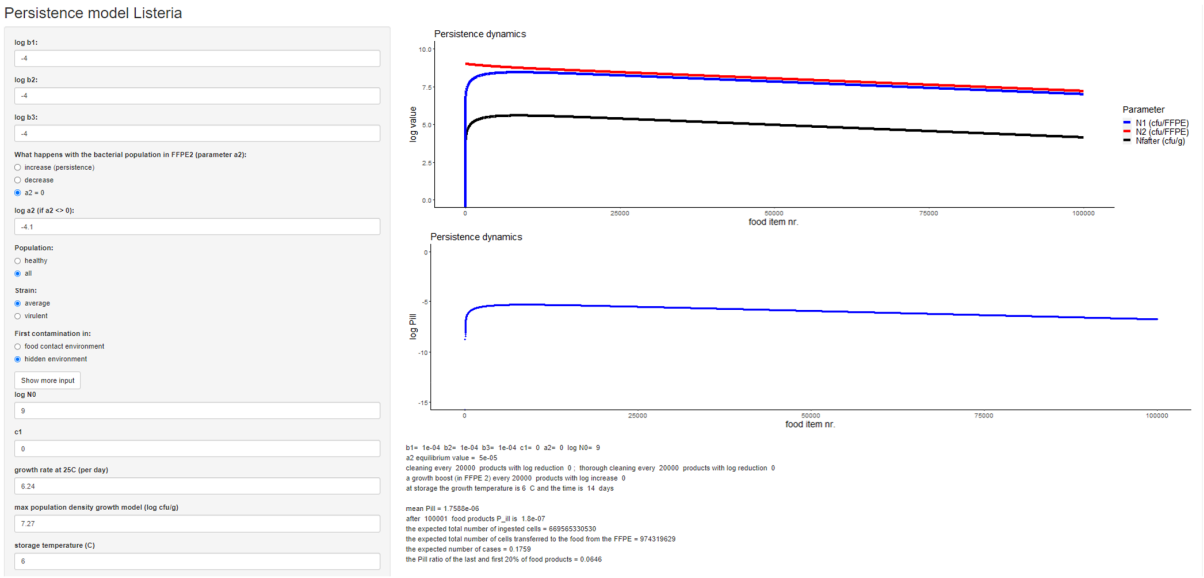

FIGURE E.13 Input and output of the model using the app, given default parameter values $b_1 = b_2 = b_3 = 10^{-4}; c_1 = 0; logN_0 = 9$) as described in Section E.4.2.1, without growth and with full survival ($a_2 = 0$). Initial contamination takes place in FoPE2.

[38]With growth in FoPE2, a complication in the model can occur: The growth model for storage has an MPD (maximum population density) which implies that, for the same growth conditions, increase in concentration in the food product before storage will not always lead to the same increase in the concentration after storage. The increase gets lower with higher initial concentration. However, if the food product is more heavily contaminated than the MPD, the concentration after storage shows an increase again as well, even without growth. This looks odd but is a consequence of the model formulation. Another complication may be that the dose is larger than 10 log CFU. In that case the DR model is no longer valid. However, obviously, in that case the concentration in the food is unrealistically high anyway.

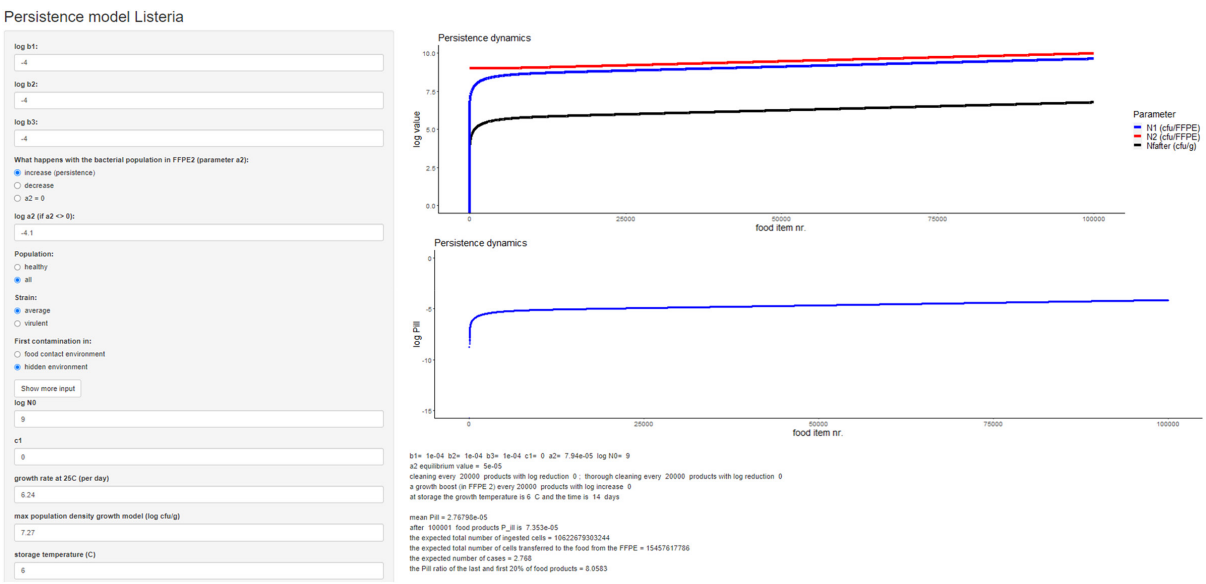

FIGURE E.14 The model of Figure E.13 with $a_2 = 0.00008$.

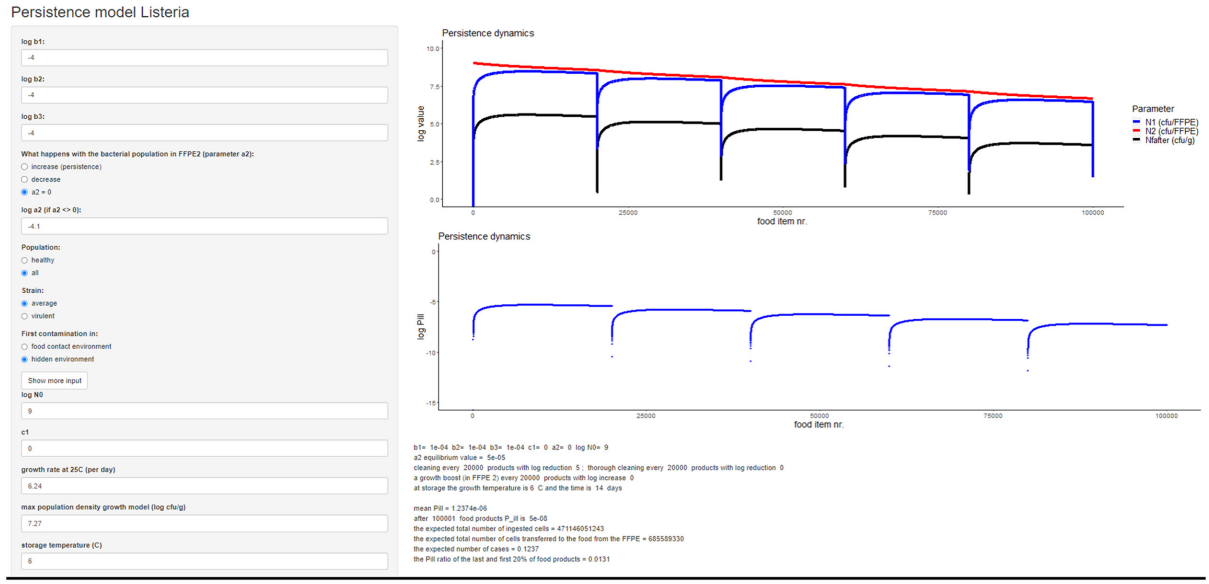

FIGURE E.15 The model of Figure E.13 with a log decrease of 5 log in FoPE1 every 20,000 food products shows that, in this case, C&D is not very effective, despite the 5 log reduction.

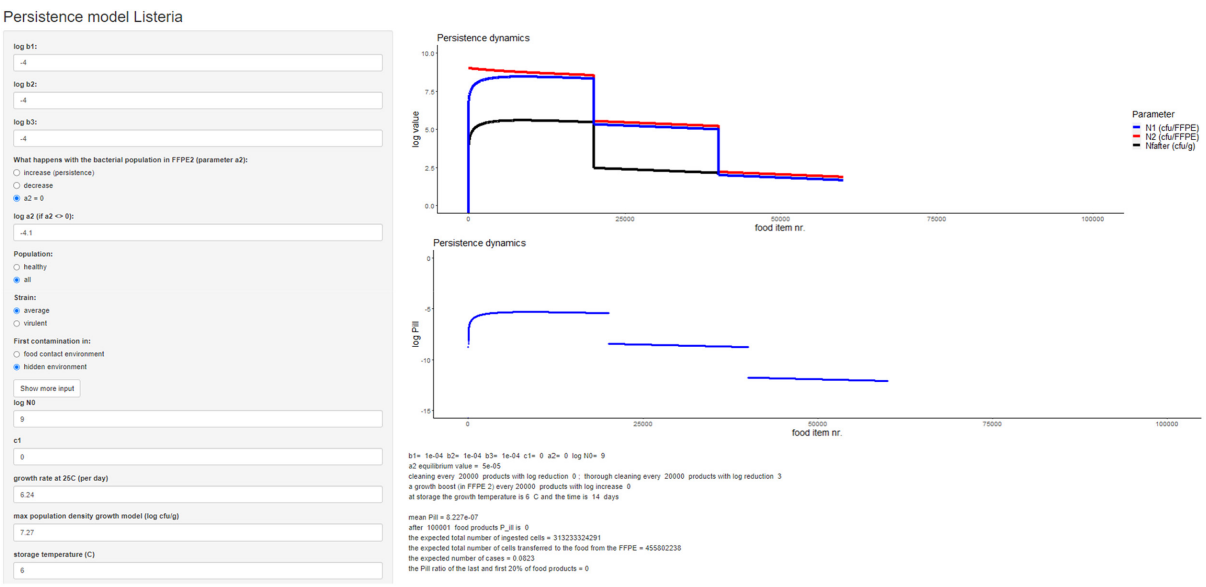

FIGURE E.16 The model of Figure E.13 with a log decrease of 3 log in both FoPE1 and FoPE2 every 20,000 food products shows that C&D is very effective in this case, despite the lower effect of C&D (3 log reduction) per event.

E.5 | Risk assessment: the impact of persistence

E.5.1 | The *L. monocytogenes* QMRA

QMRA models can be used to compare the impact of different processes in the food chain on the PH risk. Here, we explore how the impact of persistence could be compared with the impact of growth during storage and the impact of the DR relationship. The aim is to show the perspectives of using this approach, not to conclude on the actual importance of persistence for PH risk.

For a comparison like this, it is helpful to define a baseline model and then study the relative risk (RR) estimates of different alternative scenarios. The baseline for the persistence model is an arbitrary choice. To allow calculations of the RRs, the baseline should predict some cases, and allow scenarios that result in larger (and smaller) risk estimates. Here we decided to choose the model version shown in Figure E.15 as a baseline. It assumes no growth or inactivation in FoPE1 and FoPE2 ($a_2 = 0; c_1 = 0$) and some C&D effect in FoPE1 (5 log every 20,000 food products). The baseline for the growth model during storage is the one presented in Section E.3.1, with some minor adaptations: a storage time of 14 days, to get a risk estimate > 0 in the baseline; the baseline for the DR model is an average strain in the whole population; as an initial population size in the hidden environment FoPE2, due to a contamination event, we set $N_0 = 10^9$ CFU.

The most relevant risk estimate is the expected number of cases, which is the mean of the probability of illness (P_{ill}) for all food products modelled, multiplied by the number of food products. This expected number of cases for 100,000 products is 0.1237 in the baseline. By dividing the risk estimate of each alternative scenario by the risk estimate of the baseline, the RR of the alternative scenario is derived. Usually, the log of this RR is used in a comparative graphic (Figure E.17) to compare risk increases and risk reductions in a fair way.

It is found that the largest increase in risk, as compared to the baseline, is found in the scenarios where a virulent strain is modelled and where there is much growth in FoPE2 or during storage. Storage time and temperature may also have a high impact on the risk estimate. The effects of changes in the transfer parameters of the persistence model (the *b*-parameters), and the C&D regime are small, unless there is no transfer at all and the food products do not get contaminated. If the inactivation rates in the FoPE (c_1 and negative a_2 values) are similar to the transfer rates, this reduces the risks a little.

These results suggest that, in this analysis, transfer and survival in the FoPE have less impact on the risk than possible growth during storage and the virulence of the strain. However, growth in the FoPE, combined with (indirect) transfer to the food products can have a high impact on the risk.

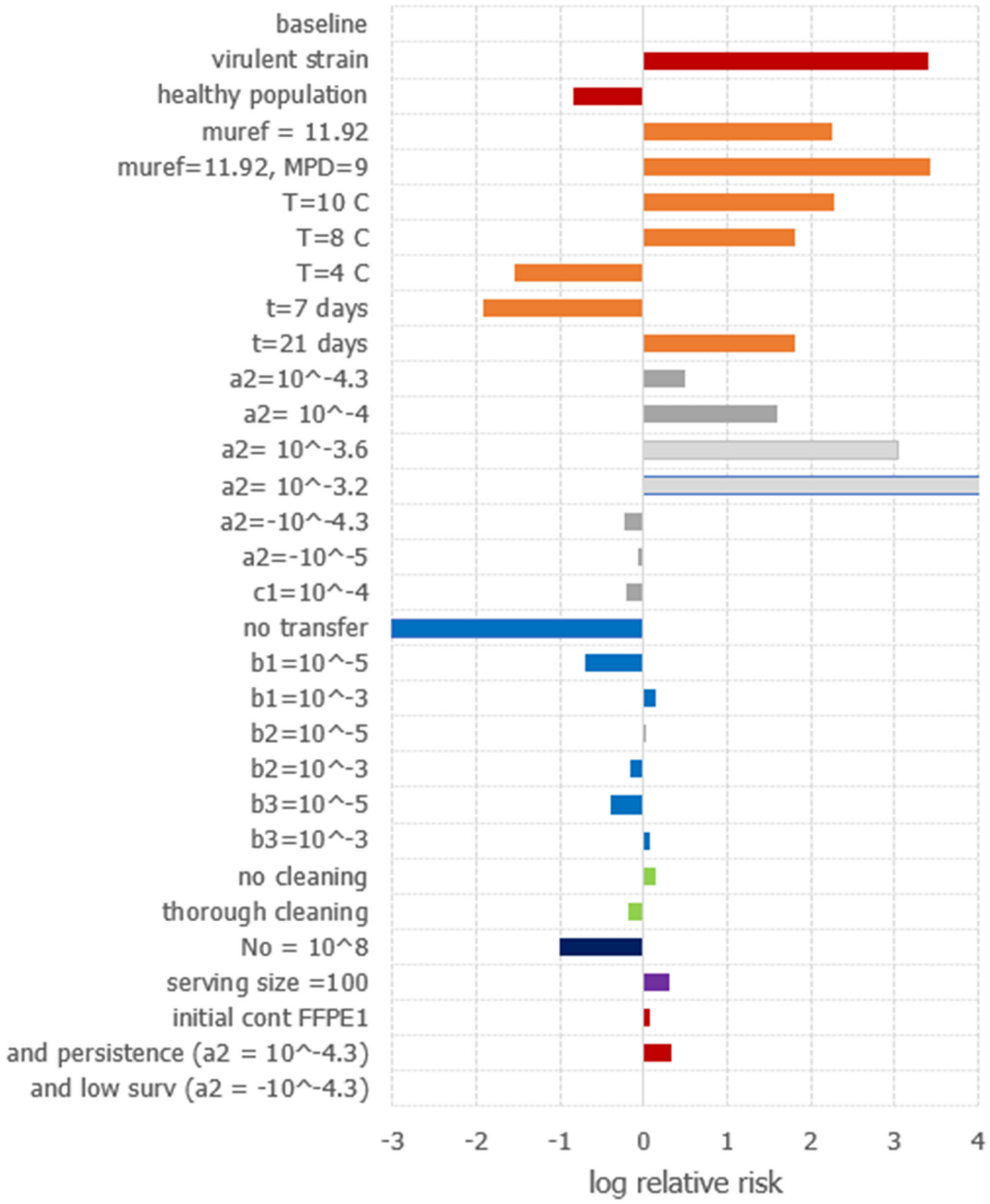

FIGURE E.17 Relative risks of alternative scenarios, with changes in one or more model parameter value. *Note*: Upper red: change in dose–response; orange: change in growth during storage; grey: change in persistence (growth and survival in FoPE); blue: change in transfer parameters; green: change in C&D regime; dark blue: change in initial contamination; purple: change in serving size; lower red: initial contamination in FoPE1 instead of FoPE2. With $a_2 = 10^{-3.2}$, the population size increases beyond realistic limits and the risk as well (>4 log relative risk). In 'thorough C&D', a 5 $\log_{10}$ reduction is obtained in both FoPE1 and FoPE2.

A specific characteristic of persistence is that the risk is maintained over a long time period. By only analysing the mean risk over the whole period, it is unclear whether the risk is equally spread out over all the food products, or it is high at the beginning (or the end) of the process and low at the end (or the beginning). For that reason, the 'case ratio' was evaluated, defined as the ratio of the expected number of cases in the last 20% of the food products and the first 20%. If this ratio equals 1, the risk is the same over the whole period of food processing, which characterises 'full' persistence. A ratio below 1 indicates a decline in the risk, which implies decreasing persistence or absence of persistence with very low case ratios. A ratio above 1 indicates an increasing risk with time which, in the course of time, cannot be sustainable as the risk will get unrealistically high on the long run.

This case ratio for the different scenarios was again compared with the baseline, where the value is 0.0131 (which means that the risk of the last 20,000 products is 1.3% of that of the first 20,000, a decline in the risk). Scenarios with case ratios with higher values than the baseline have higher persistence than the baseline. The results are shown in Figure E.18.

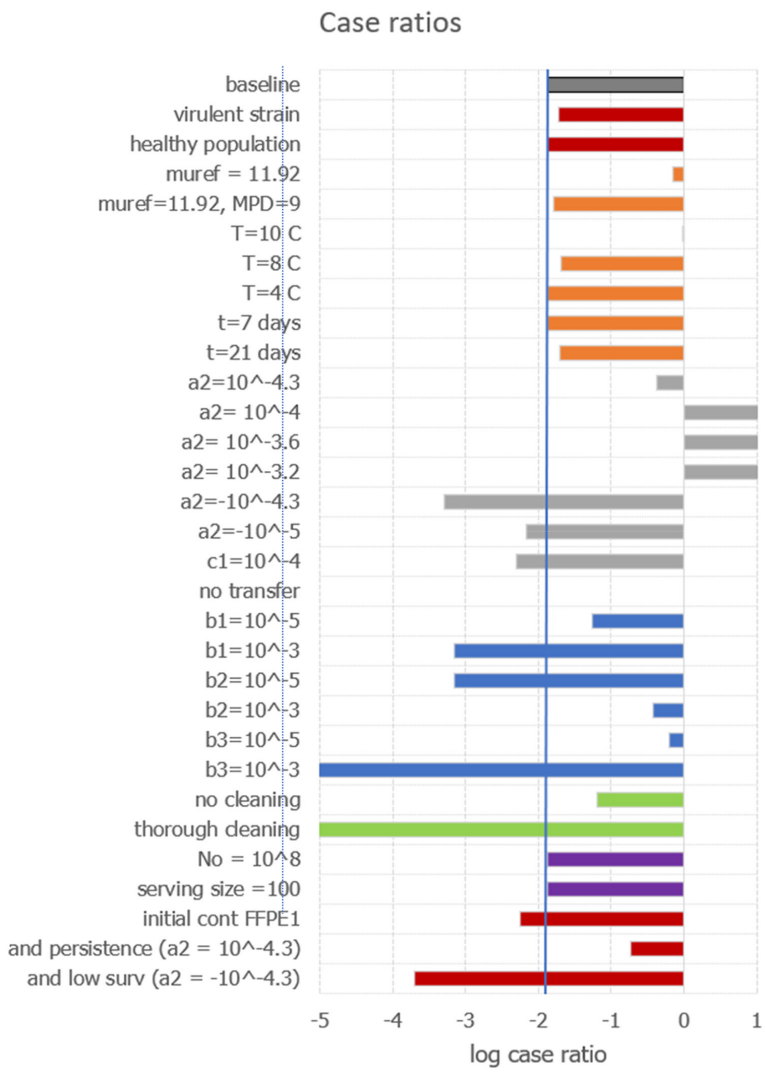

FIGURE E.18 Case ratios (expressed on a log scale) found in alternative scenarios, with changes in one or more model parameter value. *Note*: Colours as in Figure E.17. The vertical blue line indicates the case ratio of the baseline. Scenarios with a case ratio = 1 (log case ratio = 0) do not show any bar. The three scenarios with high case ratios (show as log case ratio = 1) have even higher case ratios, which imply a more than tenfold increase in the risk.

Figure E.18 shows that case ratios close to one can occur due to persistence ($\log a_2$ positive and between −4.3 and −4). If the growth in FoPE2 (a_2) is larger than the equilibrium value,[39] the risk increases during the process. A case ratio close to one is also found in scenarios where the growth during storage reaches the maximum population density (MPD) ($T = 10$°C or a higher maximum specific growth rate). Analyses where the food production is followed for a longer time (500,000 iterations instead of 100,000, data not shown) indicate that the persistence in high temperature scenarios is not maintained on the long run, when the MPD is no longer reached when the contamination of processed food products declines. The case ratio of 1 in the 'no transfer' scenario is an artefact, as there is no risk at all.

Figure E.19 combines Figures E.17 and E.18 and shows the association of the risk estimates of different scenarios and the case ratios. It illustrates that both the risk estimate (log cases) and the case ratio are sensitive for the persistence parameter a_2 (white dots), and less for the transfer parameters. The risk estimate is also sensitive for the growth during storage and the DR (virulence).

Interestingly, the transfer parameters seem to have little impact on the risk and higher impact on case ratios. This relates to what has been discussed in Section E.4.1. The transfer rates have an impact on the observation of persistence, but not so much on the PH risk.

[39]The equilibrium value is derived in Section E.2.4. This is not exactly the equilibrium in the baseline model, as this includes cleaning, which adds an inactivation that is not included in the equilibrium.

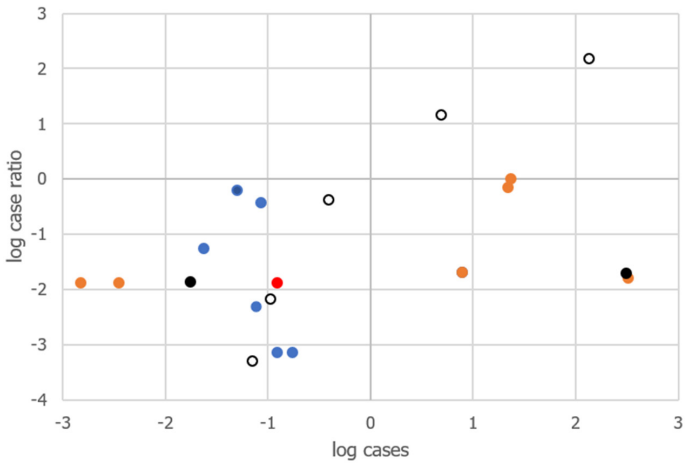

FIGURE E.19 Scatter plot of the risk (the log of the number of cases found with the model, the basis of Figure E.17) and the case ratio (as in Figure E.18) Red dot: baseline; white dots: results for different values of the persistence parameter a_2; orange dots: scenarios with altered growth during storage; blue dots: scenarios with altered transfer parameter values; black dots: scenarios with change in dose–response.

E.5.2 | Comparison of strains

Some of the model parameters can be interpreted as strain characteristics. This can be useful if the PH risk of persistent strains is to be compared with that of strains with other characteristics.

Figure E.20 shows how knowledge on these characteristics translated into model parameter values could be used to compare the relative risks of strains. The baseline is the same as in Section E.5.1. Further, we defined a persistent ($a_2 = 10^{-4.3}$) and strongly persistent ($a_2 = 10^{-4}$) strain, a fast-growing strain ($\mu_{ref} = 11.92$, the 97.5th percentile of the distribution provided by Pouillot and Lubran (2011)), a fast-growing strain with high MPD ($MPD = 9\text{logCFU / g}$), a virulent strain (see Section E.3.2.1) and combinations thereof.

In this particular example, the impact of persistence (characterised by parameter a_2) on the risk is relatively small. Other factors lead to larger risks, and larger values of a_2 would induce too high concentrations of *L. monocytogenes* in the food products (as shown by the grey bar).

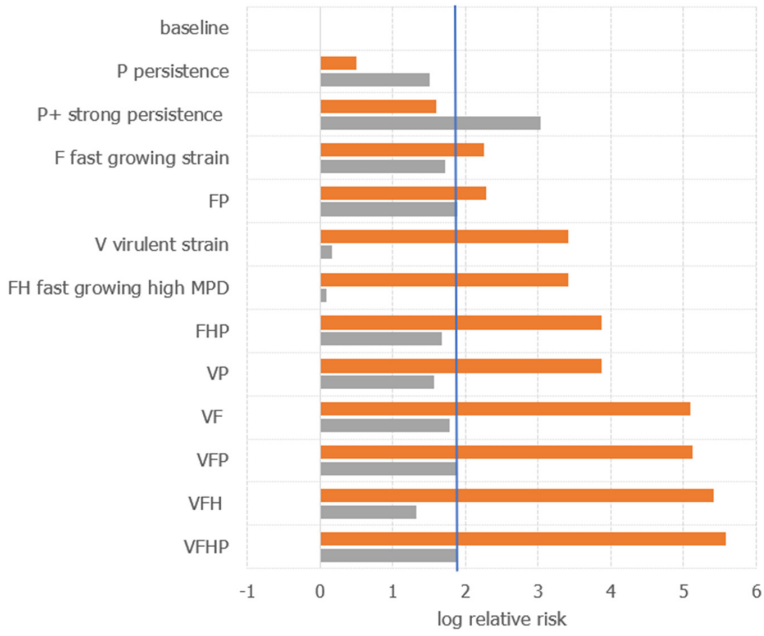

FIGURE E.20 Comparison of risks and case ratios associated with different potential strain types. *Note*: P: $a_2 = 10^{-4.3}$; P+: $a_2 = 10^{-4}$; F: $\mu_{ref} = 11.92$; *V*: virulent strain; *H*: $MPD = 9\text{logCFU / g}$. The orange bars show the relative risks (as in Figure E.17); the grey bar is the relative maintenance of risk of the indicated strain, which is the case ratio (the expected PH risk of the last 20% of food products divided by that of the first 20%) of this strain divided by the case ratio of the baseline strain; the blue vertical line indicates a relative maintenance of risk corresponding to 100% (a case ratio of 1, no change in PH risk associated to the first and last 20% of food products).

Although the figure may suggest that persistence is not a very important factor for the risk, this is not necessarily so. The baseline assumes that there is 100% survival (and not growth) in FoPE2, without an effect of C&D in this environment. This is a form of persistence as well, that is required to get an effect of growth during storage and virulence. A baseline without any persistence is not useful for the model comparison, as it is arbitrary and would give a very low risk. However, it may be realistic in many cases.

E.5.3 | The Salmonella QMRA

For *Salmonella* in a LMF a similar approach was used as for the *Listeria* QMRA. As for *Salmonella* we have no DR model that includes variation in virulence, and growth during storage does not occur in the LMF, the model is simpler. As a baseline, we define a model with $a_2 = 0; b_1 = b_2 = b_3 = 10^{-5}; c_1 = 0; logN_0 = 7$ and C&D of FoPE1 resulting in 5 log reduction every 20.000th food product. This results in an expected number of cases of 173.3 and a case ratio of 0.487. Hence, despite the lower level of initial contamination of FoPE2, and the absence of growth during storage, the risk (number of cases) is higher and the observed persistence (case ratio) is larger than for *L. monocytogenes*. This can be explained by the DR relation.

Results for different scenarios are shown in Figure E.21, as in Figures E.17 and E.18. Next, the association between the number of cases and the case ratios found for different scenarios is shown in Figure E.22, as in Figure E.19.

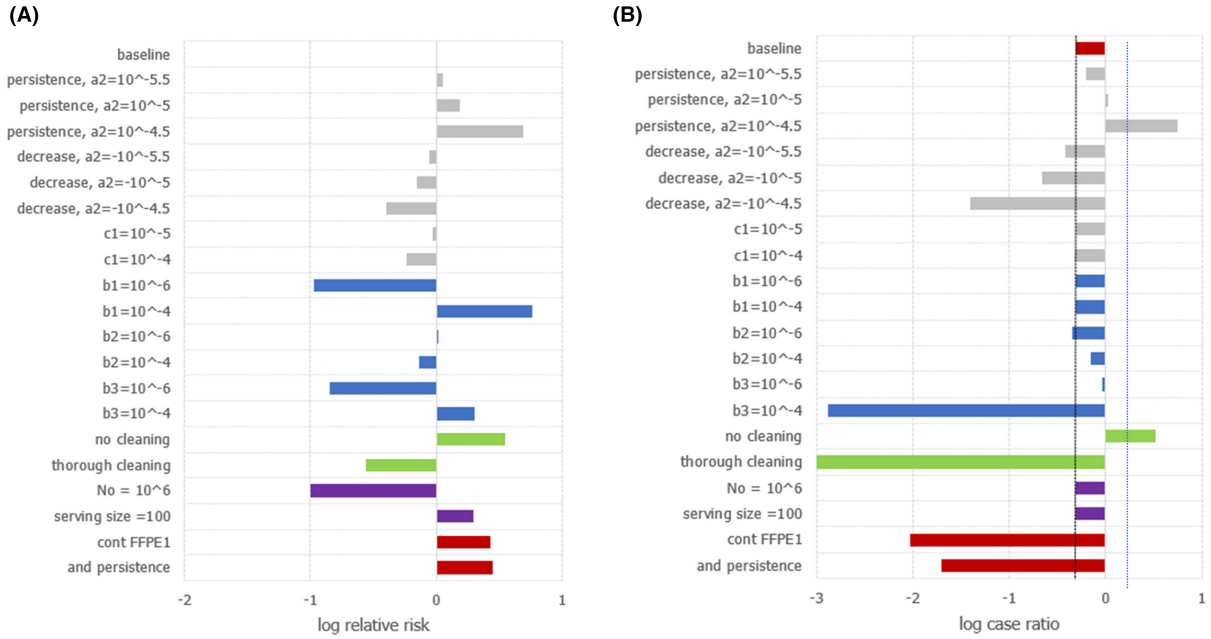

FIGURE E.21 Relative risks (A) and case ratios (B) found for different scenarios in the *Salmonella* model.

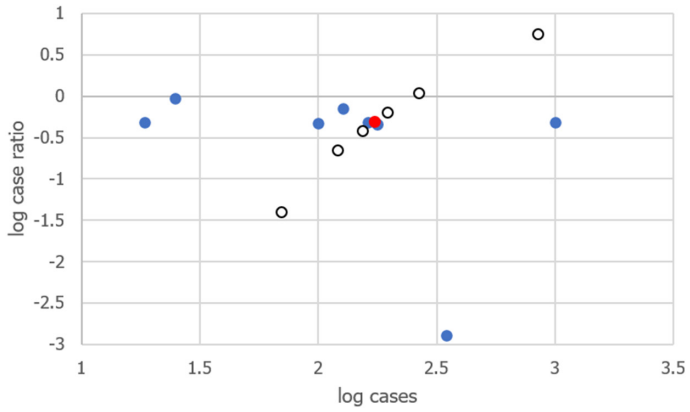

FIGURE E.22 Scatter plot of the risk (the log of the number of cases found with the model) and the case ratio. *Note*: Red dot: baseline; white dots: results for different values of the persistence parameter a_2; blue dots: scenarios with altered transfer parameter values.

As for *Listeria*, results show that both the risk estimate (log cases) and the case ratio are sensitive for the persistence parameter a_2. Interestingly, for *Salmonella* the C&D of FoPE 1 has large effect on the case ratio, as without C&D it reaches a value far above 1. If the initial contamination occurs in FoPE1, the overall risk is larger, but the case ratio is very low, which means that all cases will occur for the first food products, so it will not appear as persistence. This effect is more pronounced for *Salmonella* than for *Listeria*.

E.6 | Discussion

The model analyses above show how the persistence model can be integrated in a QMRA model, and how it can be used to assess the impact of persistence. Although the analyses are still incomplete, they illustrate the perspectives of this type of modelling to increase our understanding of the role of persistence in public health.

In the model analysis, a duality in the definition of persistence appeared. On the one hand, persistence defined as survival and, potentially, growth in the FoPE, is included in the model input by model parameter a_2. At the other hand, persistence is recognised as several model outputs, such as the long-term presence on the FoPEs, long-term contamination of food products and ultimately by means of the case ratio, which shows whether the risk is pertained over a longer time. The modelling results show that, as to be expected, the observed persistence is strongly related to the value of a_2, the parameter defining the persistence (survival and growth potential in the FoPE) in the input. The transfer parameters (*b*) have a less profound effect, but it was found that 'moderate' transfer rates are required to assure that the bacteria are not quickly transferred out of the FPPE, or are not transferred to the food products at all.

It is notable how sensitive the model results are for the precise value of the parameter a_2: for example, in the *L. monocytogenes* model, a case ratio close to 1 (say between 0.9 and 1.1) is only found for values of a_2 between $10^{4.214}$ and $10^{4.194}$, i.e. between 0.0000611 and 0.0000644, a very small range of values. The model predicts that, outside that range, persistence either fades out on the (not so) long run or gives ever increasing concentrations (to unrealistically high levels) on the food product. This small range seems to lack a biological basis, which means the model has difficulty in explaining long-lasting persistence in the FoPE, as frequently observed. Possible explanations for this unrealistic feature of the model can be the stochasticity of the process (see also Section E.2.5) and the fact that bacteria in a FoPE (often in biofilms) do not grow with a constant rate as defined in the model. Too much growth and too high concentrations of the bacteria in a FoPE will be slowed down by a lack of substrate, and parts of a biofilm may loosen from the FoPE in a stochastic manner. These types of processes could be added to a more mechanistic version of the model.

In the analysis above, observed persistence is assessed by means of the case ratio, i.e. as the 'persistence' of the probability of illness. Although persistence can be identified on the basis of long-lasting outbreaks from cases caused by the same bacterial strain and traced back to the same source, over a longer time, it is usually defined on the basis of microbiological samples from the FoPE. The model does not include this type of sampling from the FoPE. Next to the ambition to only do a limited number of analyses, a reason for leaving out this sampling is its stochastic nature. Sampling cannot be included meaningfully in a deterministic model like this, and when realistic stochastic random variation is added for the sampling, but not for the 'persistence dynamics' in the FoPE (see Section E.2.5), the value of the analyses can be debated. Still, in future work, it will be relevant to simulate sampling to identify persistence and compare it with observations.

The comparison of risks of different hypothetical *L. monocytogenes* strains (Section E.5.2) shows that persistent strains (with higher values of a_2) do lead to higher PH risks, but not more than virulent strains or strains that have a higher growth potential at storage. Strains combining these traits ('super bugs') obviously lead to the highest risks. An interesting next step would be to consider specific *L. monocytogenes* strains for which the characteristics in terms of persistence, virulence and growth potential are known, and compare the PH risks that, according to the model, would be associated with them. It may give insight into the actual occurrence of 'super bugs'.

ANNEX A

Protocol for the assessment of the persistence of microbiological hazards in food and feed production and processing environments

Annex A is available under the Supporting Information section on the online version of the scientific output.