International Journal of Food Microbiology 356 (2021) 109351

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Review

Processing environment monitoring in low moisture food production facilities: Are we looking for the right microorganisms?

François Bourdichon [a,b,*], Roy Betts [c], Christophe Dufour [d], Séamus Fanning [e], Jeffrey Farber [f], Peter McClure [g], Despoina Angeliki Stavropoulou [h], Ellen Wemmenhove [i], Marcel H. Zwietering [j], Anett Winkler [k]

[a] Food Safety, Microbiology, Hygiene, 16 Rue Gaston de Caillavet, 75015 Paris, France
[b] Facoltà di Scienze Agrarie, Alimentarie Ambientali, Università Cattolica del Sacro Cuore, Piacenza-Cremona, Italy
[c] Campden BRI, Chipping Campden, Gloucestershire, United Kingdom
[d] Mérieux NutriSciences, 25 Boulevard de la Paix, 95891 Cergy Pontoise, France
[e] UCD – Centre for Food Safety, University College Dublin, Belfield, Dublin D04 N2E5, Ireland
[f] Department of Food Science, University of Guelph, Guelph, Ontario, Canada
[g] Mondelēz International, Bournville Lane, Birmingham B30 2LU, United Kingdom
[h] ILSI Europe, Avenue E. Mounier 83, Box 6, B-1200 Brussels, Belgium
[i] Arla Foods Ingredients, Sønderupvej 26, Videbæk, Denmark
[j] Food Microbiology, Wageningen University, PO Box 17, 6700AA, Wageningen, The Netherlands
[k] Cargill Germany GmbH, Cerestar str. 2, D-47809 Krefeld, Germany

ARTICLE INFO

Keywords:
Recontamination
B. cereus
Listeria monocytogenes
Salmonella spp.,
Cronobacter spp.,
Enterobacteriaceae
Dry foods
Food safety
Processing environment monitoring

ABSTRACT

Processing environment monitoring is gaining increasing importance in the context of food safety management plans/HACCP programs, since past outbreaks have shown the relevance of the environment as contamination pathway, therefore requiring to ensure the safety of products. However, there are still many open questions and a lack of clarity on how to set up a meaningful program, which would provide early warnings of potential product contamination. Therefore, the current paper aims to summarize and evaluate existing scientific information on outbreaks, relevant pathogens in low moisture foods, and knowledge on indicators, including their contribution to a "clean" environment capable of limiting the spread of pathogens in dry production environments. This paper also outlines the essential elements of a processing environment monitoring program thereby supporting the design and implementation of better programs focusing on the relevant microorganisms. This guidance document is intended to help industry and regulators focus and set up targeted processing environment monitoring programs depending on their purpose, and therefore provide the essential elements needed to improve food safety.

1. Introduction

Although the issue is known for more than fifty years, recent events have highlighted again the role of low moisture foods (LMF) in foodborne disease, with several recently published foodborne outbreaks. Foods that decades ago would have been considered "safe" due to their inability to support the growth of microorganisms, have become of concern. It is since currently recognized that it is not necessary for certain foodborne pathogens to be able to grow within a food for them to be considered a risk, simply their presence can be a threat to human health. Additionally, it has become clear that LMF provide an excellent environment for the long-term survival of some foodborne pathogens. These organisms can survive for long periods of time in a dry environment, and be more resistant to various antimicrobial treatments, e.g., heat processes. There is also evidence that the presence of high levels of fats/oils (as is present in nut butters and chocolate) coating microbial cells may protect them from the body's defences, e.g., the high level of acid within the stomach, when consumed.

Foodborne outbreaks have been traced to a wide range of LMF: nuts, chocolate, dried dairy products, dry infant foods, nut butters, seeds, spices, dried meats and flour.

* Corresponding author at: Food Safety, Microbiology, Hygiene, 16 Rue Gaston de Caillavet, 75015 Paris, France.
E-mail addresses: francois.bourdichon@gmail.com, publications@ilsieurope.be (F. Bourdichon).

https://doi.org/10.1016/j.ijfoodmicro.2021.109351
Received 23 February 2021; Received in revised form 16 July 2021; Accepted 28 July 2021
Available online 2 August 2021

1.1. What are LMF and what is an LMF production environment?

LMF are not simply foods that are naturally very low in moisture or have had water removed from them. The term encompasses also foods with a higher moisture content, but that contain agents that prevent the moisture from being available to microorganisms to allow their growth. "Low moisture" should be defined by the use of water activity (a_w) as its key cut off.

Defining references are ILSI Europe (2011) and Codex (2015), both of which define LMF as having an a_w of 0.85 or below. At these a_w values, all common foodborne bacterial pathogens are unable to grow. Xerophilic moulds and some specialised halophilic bacteria are able to grow, but these are outside the scope of this document, which will consider bacteria that are considered human foodborne pathogens.

1.2. The LMF production environment

The production environment covered in this document includes production equipment, production surfaces, floors/walls/ceilings, and the air within the production area. In most cases, dry food production areas will contain/use a minimum amount of water. Processing environment surfaces (food contact and non-food contact) have been previously proposed as critical sampling locations in the microbial assessment scheme to assess the performance of the operational food safety management system (Jacxsens et al., 2009).

As long as an area remains dry, microbial growth is minimized and/or inhibited. As soon as water is introduced, the potential for microbial growth occurs and the risk of product contamination increases. Any water use in dry production areas must be controlled and kept to a minimum.

1.3. Processing environment monitoring (PEM)

Monitoring the environment should be done for two reasons:

1. To provide ongoing monitoring and an ability to trend the level of hygiene within production environments. This type of monitoring will usually require testing of "hygiene indicator" microorganisms or groups and tends to indicate the level of general hygiene, and in many cases can be used to check the efficacy of cleaning within production environments. Microbial tests that are commonly used include aerobic mesophilic counts, *Enterobacteriaceae* counts or yeast and mould counts (Article 5, EU Regulation 2073/2005).
2. To check for the presence of pathogens of concern within production areas. The pathogens being evaluated will depend on the product being produced and its intended use and would reflect the risk of that specific product becoming contaminated by that organism within the production environment. This type of testing is related to food safety and results in a greater assurance that the production environment is not contaminated with the pathogen or pathogens concerned.

The focus of this review is on evaluating the presence of food pathogens for food safety purposes, which is one of the two components of processing environment monitoring (PEM) as described above.

1.4. PEM in guidelines and legislation

PEM is widely recognized as a requirement in the production of many types of food and is mentioned in numerous publications. For example, in the European Commission Regulation on Microbiological Criteria for Foods (European Commission, 2005), as well as highlighting a range of food safety criteria, it is noted in Article 5 that: "Samples shall be taken from processing areas and equipment used in food production, when such sampling is necessary for ensuring that the criteria are met". The regulation goes on to specifically identify ready-to-eat (RTE) foods which may pose a *Listeria monocytogenes* risk, and dried infant formula that may pose a risk from *Salmonella* spp. and *Cronobacter* spp., as two product types where PEM is required. This regulation also gives some guidance on the importance of sampling when undertaking PEM and points to ISO 18593 as a standard for the swabbing examination (ISO, 2018).

Similarly, the British Retail Consortium Global Standard (BRCGS) for Food Safety Issue 8, clause 4.11.8, states that risk-based environmental monitoring programs shall be in place for pathogens or spoilage organisms and they shall, at a minimum, include all production areas with open and RTE products. The standard goes on to define the constituent parts of a PEM programme. Specifically, it defines that it should be based on risk and include as a minimum: i) a sampling protocol; ii) the identification of sample locations; iii) the frequency of microbiological tests; iv) identified target organism(s); v) defined test methods (for example, sedimentation plates, rapid testing and swabs) and vi) the recording and evaluation of results. Furthermore, the PEM programme and its associated procedures should be fully documented.

1.5. Requirements of a PEM programme

When designing any PEM programme, it should first be based on risk to the product from environmental contamination that will ultimately lead to an adverse human health outcome. This assessment will identify the microbial hazards of concern and their origin. The hazards will be specific microorganisms, however, there is often reference made to the use of indicator organisms. It is important to understand that no evidence has ever been produced that would conclusively confirm that one organism type would indicate the presence of another. However, indicators can be useful in unmasking the presence of unhygienic conditions that should be addressed.

Once specific hazards have been identified, there needs to be a scoping of the areas that should be examined, acceptable and unacceptable criteria and the actions to be taken when an unacceptable result is obtained. Any PEM should be well defined and fully documented and reviewed at regular intervals, to ensure that it meets the requirements of the manufacturer.

2. PEM in the context of HACCP

The concept of using process controls instead of relying only on finished product testing results to ensure the food safety of products, was initially established by NASA and the Pillsbury company in the 1950's. It was called HACCP – Hazard Analysis and Critical Control Points – and gradually developed into the most successful concept within the food industry to ensure food safety. The HACCP approach has the big advantage that it considers the whole production chain from beginning to end, is proactive, structured, can be applied for any process and/or product, and can even be used during the design of a product or a process. Using HACCP, it became possible to control processes and establish corrective actions during the process to ensure safe products. In contrast, end product testing (due to potential heterogeneous low-level contamination) can never give sufficient assurance of safety across the production chain. It is only an end-of-process measure that does not provide any insight on where problems might have occurred - and does not allow for corrective actions in the process. In 1974, FDA incorporated the HACCP concept in their low-acid and acidified food regulations, and in the 1980's it was globally taken up by international organizations such as the World Health Organisation (WHO), Codex Alimentarius and ICMSF in their publications (Codex Alimentarius, 2020; ICMSF, 1989). Following that, HACCP principles have been embedded in US legislation (starting with specific food types and requiring HACCP principles for all food businesses within FSMA in 2011) and in European legislation in 1993 (EC 93/43/EEC European Commission, 1993).

Since HACCP covers the whole production chain and its influence on the safety of products, it considers the contamination level of the ingredients, as well as the potential growth and inactivation kinetics.

However, with increasing emphasis, it also recognizes the significance of the environment in food production. Contributors to that were several outbreaks, where environmental contamination was identified to be the major factor (*Salmonella* Agona-cereals, 1998–2008, and in powdered infant formula (PIF), 2005–2017, see Part 3: Past Learnings, *L. monocytogenes* in frozen corn, 2018).

In order to set up a meaningful PEM programme in the framework of HACCP, it is important to perform a thorough hazard analysis to identify the relevant pathogens for the process and product(s) investigated. Furthermore, PEM has been highlighted as "an essential component for *Salmonella* control", and a "tool to verify the effectiveness of the overall *Salmonella* control program" (GMA, 2009a). In this context, *Salmonella* is now recognized as the major pathogen of concern in LMF (GMA, 2009a; ICMSF, 2018).

As far back as 2002, ICMSF published an equation that allows one to easily visualize how microbial hazards, risks and control options are interlinked:

$$H_0 - \sum R + \sum I \leq FSO$$

H_0 = initial level of the hazard
$\sum R$ = total reduction (inactivation or removal)
$\sum I$ = total increase (growth or recontamination)
FSO = food safety objective

In that respect, "recontamination" relates to exposure of products to an environment, where recontamination could occur. Taking that into account (and in the equation above), control of the processing environment is recognized as a significant process control factor that needs to be considered. In addition, the equation helps to establish a risk-based approach in that, for example, for products being treated (ΣR) within their final packaging, contamination by environmental factors would not be significant and no PEM is required in that context for the step before treatment. For products that are more exposed to the environment, more control measures are required to prevent post-processing contamination with foodborne pathogens than for products with less exposure.

Furthermore, growth and inactivation are exponential processes and are often expressed, for example, a 6D reduction or 3D increase. That means that an increase or decrease is expressed as "log" steps, e.g., having an initial level of 1 log or −2 log, a 6D reduction would result in a −5 log or −8 log, or a 3D growth would lead to 4 and 1 log. It is also important to note that recontamination is not an exponential process, but an additive one. For example, if a product with an initial contamination level of 1 CFU/g (0 log CFU/g) is contaminated with 100 CFU/g of product, the increase would be 2 Log, while if the initial contamination level was 1 CFU/100 g (−2 Log), the increase would be 4 Log.

This can be exemplified in an example: during the production of chocolate, cocoa is treated before the final product is mixed/made (i.e., other ingredients like milk powder being added). So, one could use the following formula to examine the effect of recontamination:

$H_0 - \sum R + \sum I \leq FSO$

Applying an effective treatment of cocoa that would result in $H_0 - \Sigma R = 0$, thereby leaving $\Sigma I \leq FSO$.

Due to the high infectivity of some *Salmonella* strains (very few cells in one single serving have been shown sufficient to cause illness) in cocoa, the absence/non-detection of *Salmonella* in higher sample amounts of product (10–60 samples/lot) is analysed for according to different schemes.

Considering that chocolate with a high content of cocoa is on the market (as high as 99%), a serving size estimation could be done to determine the FSO. Based on a New Zealand study, serving sizes of chocolate were determined as follows: "Mean serving sizes for adults and children were 36 and 32 g respectively, with median serving sizes of 25 and 23 g, respectively, and 95th percentiles of 93 and 89 g, respectively, for adults and children." It could therefore be assumed that a serving size of 100 g is a conservative estimate.

Taking into account a maximum serving size of 100 g and not expecting 1 cell in that amount would result in <1 cell/100 g of cocoa/chocolate: <1 CFU/100 g = 0.01 CFU/g = −2.0 Log.

Relating that to a recontamination event would mean that even 1 cell/100 g final product could result in a potentially unsafe product- and therefore, such contamination from the environment and/or from adding products needs to be prevented. Even this level can be considered potentially unsafe since, if the contamination level would be lower than −2 log, for example a factor 10 lower, this would result in 0.1 CFU/100 g, which equates to one cell in every 10 servings of 100 g. We could further assume that one single cell of *Salmonella enterica* has a probability of causing illness of 1 in 400 (WHO/FAO, 2002; Zwietering et al., 2021). Therefore, with 1 out of 10 chocolate bars containing a single cell of *S. enterica*, illness would occur in every 4000 chocolate bars, a value that is certainly not insignificant. However, caution is required as the calculation is only as good as the data input – and where data are scarce and assumptions are being made, results need to be interpreted with caution.

Nevertheless, it can be concluded here that recontamination should be prevented in order to reduce largely [substantially limit] any potential increase in the numbers of *Salmonella* during chocolate production.

The major processing environment controls are part of the prerequisites programs in place, and include hygienic zoning (segregation of production areas), hygienic design of equipment, cleaning and disinfection and utility controls (e.g., water, air, steam). Zoning is seen as an important element in preventing the ingress and spread of pathogens in a production plant (GMA, 2009b). It not only refers to the physical segregation of production areas, but also to traffic-based controls of personnel and materials. PEM provides the opportunity to monitor the effective implementation of these prerequisites.

It is also worthwhile to re-iterate the importance of the hazard analysis: if it is not done correctly and either insignificant or the wrong hazards are identified, or relevant ones are missed, then incorrect target organisms would be used in PEM. This could then result in potentially unsafe food by having not controlled those hazards or wasting a lot of employee and financial resources on non-hazardous microorganism(s) – and even potentially providing a false sense of "security" when doing so. This has also been recognized in FSMA (FSMA, 2015), in which risk-based preventive controls to minimize or prevent the identified hazards are required to be in place. One of those "preventive controls" is PEM.

It is difficult to specifically relate levels of pathogens of concern or hygiene indicators as found in the environment quantitatively towards levels and risks in the food, since generic transfer models cannot be developed. In specific situations, a specific transfer phenomenon could be described quantitatively. Transfer from different zones (product contact, near product, far way zones) is difficult to define, but also the sampling methodology (where to sample, how to sample, how much area, etc.) is difficult to standardise and relates to contamination levels. Nevertheless, it is valuable to try and set parameter values (microbial limits) for environmental monitoring and also to verify, by sampling, if there has been an increase in microbial contaminants along the product line.

In summary, a risk-based PEM is an invaluable tool to verify control programs for the ingress and dissemination of pathogens in a food production facility. Specific sampling schemes, target organisms, methods, sampling areas, frequencies, and adequate corrective actions depend largely on the specific product hazard and local conditions and cannot be generically provided.

3. Past learnings

Resident strains and transient isolates have been described in the mid 1990's in food production areas for *L. monocytogenes* in various food categories (Tompkin, 2002). The same type of event was described for

Salmonella Agona on puffed rice cereal: the first outbreak occurred in 1998, and a second one in 2008 with the same product, same facility and, most importantly, the same *Salmonella* strain. Processing environment sampling in the facility revealed the presence of the outbreak strain, and the authors concluded that the strain was able to survive in that environment for 10 years, being able to contaminate products following construction events (Russo et al., 2013). Two recent infant formulation outbreaks linked to *Salmonella* spp. environmental contamination, highlight the importance of implementing relevant monitoring of the processing environment to identify potential harbourage sites/niches: a *Salmonella* Agona milk powder recall and a *Salmonella* Poona rice-based infant formula recall (Jones et al., 2019; Jourdan-Da Silva et al., 2018). These two contamination incidents were caused by an isolate confirmed by whole genome sequencing (WGS) to be similar to a strain previously implicated in a foodborne outbreak in the same facility several years before (12 and 11 years, respectively; Brouard et al., 2007; Instituto de Salud Carlos III, 2011).

During the *S*. Poona foodborne outbreak in 2019, *S*. Poona was not found in the food products or the processing environment, while the presence in the food was confirmed for the *S*. Agona outbreak. While these events further highlight the importance of PEM, on its own it is still not sufficient to ensure the production of safe food. Despite the inspection done by the competent authority, environmental isolates could not be obtained in any of the outbreaks, even during active investigations (ECDC and EFSA, 2019). This emphasizes the importance of setting up targeted, continuously reviewed and updated PEM, as part of an overall food safety management system.

A selected overview of outbreaks with LMF due to microbial pathogens in the US between 2012 and 2019 is shown in Table 3.1 (CDC, 2020). The majority of the outbreaks with LMF were due to contamination with *Salmonella*. Different food categories were implicated in the outbreaks, i.e., nuts and seeds, herbs and spices, but also dried fruits, dried meat and cereal grains. The US *Escherichia coli* outbreaks due to LMF were solely due to the consumption of flour. No outbreaks due to LMF being contaminated with *L. monocytogenes* and *Bacillus cereus* have been reported by the CDC in their selected overview (Table 3.1). The European Food Safety Authority (EFSA, 2019a) reported that from 2010 to 2017, 10 salmonellosis outbreaks occurred due to cereal products including rice and seeds/pulses (nuts, almonds). Further comparison of the outbreak data on LMF in the EU with that of the US was not possible, because EU data on individual food products were not reported by (EFSA, 2019a).

The recalls, withdrawals and safety alerts for the EU member states and US have been reported by RASFF (2020) and the FDA (2020). Table 3.2 shows that for microbial pathogens and LMF, there were 447 and 51 alerts in the EU and US, respectively, in the period from 2012 to 2017. The majority of the alerts were related to *Salmonella* (91.4% of the cases in EU and 58.8% of the cases in US). This is similar to what was reported in previous years (ILSI Europe, 2011). Most *Salmonella* alerts were related to herbs and spices (26.9% of the total *Salmonella* alerts in EU, 20% in US) as well as nuts and seeds (51.7% of the total *Salmonella* alerts in EU, 33.3% in US). Over the years 2017–2019, the number of *Salmonella* alerts increased sharply in the EU, especially those related to sesame seeds and black pepper (RASFF, 2020). It is unknown whether this is an actual increase, or it is due to increased surveillance.

Some LMF alerts were issued for *B. cereus* (5.1% of total cases in EU and no cases in US), *L. monocytogenes* (2.7% of total cases in EU and 25.5% in US) and *E. coli* (0.22% in EU and 10.6% in US) during 2012–2017 (FDA, 2020; RASFF, 2020; see Table 3.2). The *B. cereus* alerts were related to different food categories, whereas the alerts with *L. monocytogenes* were mainly related to dry sausages. (Schmidt and Fontana Jr., 2020). A very limited number of foodborne outbreaks were observed during 2012–2017 for *Clostridium botulinum* (2 in EU and 2 in US), *Clostridium perfringens* (2 in EU and 0 in US), invasive *Cronobacter* spp. infection (3 in EU and 0 in US) and *Staphylococcus aureus* (2 in EU and 1 in US).

In summary, the majority of outbreaks and recalls due to the consumption of LMF, have been due to the presence of *Salmonella*, with only a few being related to *B. cereus*, *L. monocytogenes* and *E. coli*.

4. Pathogens of concern

Salmonella is recognized as a major pathogen of concern in LMF by many well-recognized food safety organizations/associations (ICMSF, GMA, etc.), and is responsible for most of the foodborne outbreaks linked to LMF. *Cronobacter* spp. constitutes a public health risk for special populations (especially infants), and is known to cause outbreaks, associated with contaminated PIF. Pathogenic *E. coli* have emerged more recently as pathogens involved in outbreaks of LMF like flour and hazelnuts. Although *B. cereus* has been recognized as pathogen of concern in LMF, it is also acknowledged that it is related to reconstitution of LMF and keeping such high moisture foods at conditions allowing for the outgrowth and thereby toxin production of *B. cereus*. Finally, *L. monocytogenes* is included, although the significance of this bacterium as it relates to LMF and public health has not been clearly established.

4.1. *Salmonella* species

Salmonella species are Gram-negative, facultative anaerobes with peritrichous flagella that can invade a broad range of hosts causing both acute and chronic infections by means of their ability to replicate and persist within non-phagocytic epithelial cells, as well as phagocytic dendritic cells and macrophages of the host innate immune system (Richter-Dahlfors et al., 1997; Yrlid et al., 2001).

As a pathogen of importance to food safety, *Salmonella* has an impact on human health and the economy. It causes a foodborne illness (known as non-typhoidal salmonellosis [NTS]) that is of concern globally.

Bacteria in this genus are remarkably resilient, being capable of growth under a wide range of environmental conditions. Growth between 6 and 45 °C is typical, although some strains may be capable of extending beyond these parameters. Generally, *Salmonella* can grow at pH values from 4 to 9, and at a water activity (a_w) value above 0.94. The dose response required to cause salmonellosis is dependent on the

Table 3.1
US outbreaks (selected cases) with pathogens related to low-moisture food products in 2012–2019.

Year	Product	Cases	Pathogen	Reference
2013	Tahini	16	*Salmonella* Montevideo and M'bandaka	(CDC, 2013)
2014	Chia powder	31	*Salmonella* Newport, Hartford and Oranienburg	(CDC, 2014)
2016	Pistachios	11	*Salmonella* Senftenberg	(CDC, 2016a)
2016	Flour	63	*E. coli* STEC O26 and *E. coli* STEC O121	(CDC, 2016b)
2018	Tahini	8	*Salmonella* Concorde	(CDC, 2019a)
2018	Cereals	135	*Salmonella* M'bandaka	(CDC, 2018a)
2018	Dried coconut	14	*Salmonella Typhimurium*	(CDC, 2018b)
2019	Tahini	6	*Salmonella* Concorde	(CDC, 2019b)
2019	Flour	21	*E. coli* STEC O26	(CDC, 2019c)

Table 3.2
Overview of recalls, withdrawals and safety alerts with microbial pathogens in the EU and US in 2012–2017. EU data were extracted from (RASFF, 2020) and US data were extracted from (FDA, 2020).

	B. cereus (EU)	*B. cereus* (US)	*E. coli* (EU)	*E. coli* (US)	*L. monocytogenes* (EU)	*L. monocytogenes* (US)	*Salmonella* (EU)	*Salmonella* (US)
Total	23	0	1	5	12	12	416	30
Cereals and bakery products	1			3	5	2	5	5
Cocoa and cocoa preparations, coffee and tea	1			1			5	1
Confectionary						2	6	4
Dietetic foods, food supplements, fortified foods	3		1	1		1	14	1
Eggs and egg products							5	
Fish and fish products								1
Food additives and flavourings							1	
Fruits and vegetables	4						20	
Herbs and spices	13						112	6
Meat and meat products					6		24	
Milk and milk products							6	1
Nuts, nut products and seeds	1				1	6	215	10
Other food product/mixed						1	3	1

nature of the serovar, the vehicle of infection and the susceptibility of the host. For LMF like nuts, chips or chocolate, outbreak data points to very low cell numbers (<1 CFU/g and much lower) being capable of causing illnesses (Podolak et al., 2010; Beuchat et al., 2013).

4.1.1. Key elements relevant to LMF conditions

It is known that *Salmonella* has the capacity to survive for long periods of time in LMF and environments and remain infectious, with several studies demonstrating its ability to persist in the absence of growth (Beuchat and Mann, 2014; Hokunan et al., 2016). *Salmonella* isolated from contaminated LMF have been shown to exhibit tolerance to a number of other stressors including heat and some sanitizers (Gruzdev et al., 2011). According to the Centers for Disease Control and Prevention (CDC, 2019b), approximately 83% of LMF-associated foodborne outbreaks between 2007 and 2018 involved *Salmonella*.

The molecular mechanisms underpinning these phenotypes remain to be fully elucidated. When entering a desiccated state, such as when dried onto an abiotic surface (e.g., plastic, stainless steel or paper), transcriptomic analysis highlights the complex metabolic and cellular process required to maintain homeostasis. Under these conditions, *Salmonella* triggers a protective mechanism, termed osmoadaptation, by activating compatible solute transporters and thus enabling the accumulation of solutes such as glycine betaine and trehalose (Finn et al., 2013a; Li et al., 2012). Other processes and traits found to be differentially expressed include the up-regulation of potassium efflux mechanisms, activation of stress-induced alternative sigma factors (*rpoE* and *rpoS*); Fe—S clusters, fatty acid and amino acid metabolism, among others (Finn et al., 2013b; Gruzdev et al., 2012; Maserati et al., 2018). Genes involved in the citric acid cycle, propanoate metabolism and LPS biosynthesis appear to be down-regulated in powdered milk, black pepper, milk chocolate and dried pet foods (Crucello et al., 2019). Similarly, formation of thin aggregative fimbriae and cell filamentation are also implicated in the desiccation of *Salmonella*. Whilst these data provide some insights into the bacterial response under desiccation-type conditions, they do not reflect the nature of the bacterial response on LMF.

4.1.2. Control options

The thermal tolerance of *Salmonella* represents an important challenge for validation of pathogen control (Garces-Vega et al., 2019). Carefully modelling this phenotype is complex. When inactivation kinetics of *Salmonella*-inoculated almonds was studied at either of two defined conditions- one at a fixed moisture content (% MC) but variable a_w compared with variable % MC but fixed a_w, the individual effects of either parameter was reported to be inconclusive. Nevertheless, a_w is temperature dependent, and characterized by hysteresis between sorption states as reported earlier. The thermal resistance of *Salmonella* when inoculated onto almonds appears to be unaffected by long-term storage (Limcharoenchat et al., 2019). Similarly, the survival of *Salmonella* in LMF and dry built production environments is enhanced by their ability to develop biofilms. In a study using mini-Tn*10* transposon mutagenesis (Chen and Wang, 2020), mutants were generated that either formed more or less biofilm, when compared with their wild-type isogenic parent. Insertions in several genes, including *cdg*; *trx*; *fabI* and *rxt* were identified. Delineation of their functional roles will aid in the development of strategies to control pathogen attachment and subsequent biofilm formation.

The ability to detect a bacterium contaminating a LMF matrix is an essential step for food safety. Undoubtedly, food safety can benefit from developments reported in various molecular strategies including next generation sequencing (NGS). Recently, a quasi-metagenomic approach involving short-term enrichment and immunomagnetic separation (IMS), combined with amplification and nanopore sequencing, was described and applied to assess its suitability for the detection of two key pathogens in flour, *Salmonella* spp. and *E. coli* (Forghani et al., 2020). Following 1-h of sequencing, all target bacteria were detectable after inoculation at 1 CFU/g. Once a bacterium has contaminated a dry-food production environment, it can be extremely challenging to eliminate it. A significant risk factor for product contamination with *Salmonella* in these environments is the presence of water, which allows re-growth and spread of the organism. Therefore, wet cleaning should only be used when it is considered essential.

Salmonella tolerance to sanitiser is higher when attached to a surface than when in suspension, with only one (based on 70% ethanol) in 9 sanitisers shown to remove *Salmonella* dried onto stainless steel, as opposed to all 9 eliminating *Salmonella* in suspension (Møretrø et al., 2009). A number of different sanitisers containing quaternary ammonium compounds (QACs), sodium chlorite and hypochlorite were tested against *S.* Typhimurium, *S.* Thompson, *S.* Berta, *S.* Hadar and *S.* Johannesburg that had been dried onto surfaces. Disinfectants containing sodium chlorite showed better efficacy (Ramesh et al., 2002). To survive in certain environments, many microorganisms will produce biofilms. It is recognized that biofilm formation increases bacterial survival and resistance to disinfectant treatment, as opposed to their planktonic counterparts (Belessi et al., 2011; Joseph et al., 2001; Møretrø et al., 2012).

The control of pathogens associated with LMF starts with adequate zoning, good manufacturing practice (GMP) and other prerequisites in place, and in the case of the ingress of *Salmonella*, requires in addition to these a combination of cleaning and dedicated decontamination methods (Scott et al., 2009). Salmonellae contaminating LMF exhibit an unusually high tolerance to sanitisers. Preventative measures that are

successful when applied to high-moisture foods, may not be effective when targeting the same pathogen in a LMF matrix. A study by Hasani et al. (2020) evaluated two decontamination methods, a peracetic acid-ethanol combination and an advanced oxidation process, for the removal of *Salmonella* inoculated onto LMF. The results demonstrated that both methods could be applied to reduce *Salmonella*, although the efficacy depends on product type.

4.2. *Cronobacter spp.*

Cronobacter multi-species complex is a Gram-negative rod-shaped bacterium that belongs to the family *Enterobacteriaceae* (Farmer et al., 1980; Nazarowec-White and Farber, 1997). It was first described by Farmer et al. in 1980 and named *Enterobacter sakazakii*. Prior to that, it was referred to as a "yellow pigmented *Enterobacter cloacae*" (Farmer et al., 1980; Nazarowec-White and Farber, 1997). Later in 2007, the current name "*Cronobacter* spp." was adopted by researchers (Iversen et al., 2007, 2008).

Cronobacter species are considered as opportunistic foodborne pathogens capable of causing both intestinal and systemic human disease, and except for *C ronobacter condimenti*, all *Cronobacter* species have been isolated from clinical specimens. However, *Cronobacter sakazakii*, *Cronobacter malonaticus* and *Cronobacter turicensis* are considered the primary human pathogens. The main population groups affected by this organism are infants 0–12 months of age, but especially neonates (<28 days) and infants under 2 months of age, the elderly (>80 years old) and immunocompromised persons (Forsythe, 2018; Lepuschitz et al., 2019; Strysko et al., 2020).

The major ecosystem for *Cronobacter* species appears to be plants (Forsythe, 2018). It has been isolated from a wide variety of foods, including infant foods, beverages and processed foods, breast milk, plants and spices, fresh produce, animal products and the environment, including flies, household vacuum dust and inside infant formula processing plants (Nazarowec-White and Farber, 1997; Beuchat et al., 2009; Jaradat et al., 2014). Many food recalls due to the contamination of PIF with *Cronobacter* species have occurred in various countries over the years (Parra-Flores et al., 2018).

Most of the outbreaks due to invasive *Cronobacter* infections among infants reported over the years have been due to PIF that was either intrinsically or extrinsically contaminated with *C. sakazakii* (Henry and Fouladkhah, 2019). Furthermore, there are several reports of infants being infected through contaminated expressed breast milk and through cross-contamination from improperly sanitized breast milk pumps (Bowen et al., 2017; McMullan et al., 2018; Sundararajan et al., 2018). Unfortunately, because the reporting of *Cronobacter* infections is not mandatory in most countries, the true incidence of invasive infant *Cronobacter* infections as well as adult infections is unknown.

4.2.1. *Key elements relevant to LMF conditions*

One of the major characteristics of *Cronobacter* species, including *C. sakazakii*, is their ability to survive in dry environments (Beuchat et al., 2009), and it appears to have a higher tolerance to osmotic stress and desiccation than many *Enterobacteriaceae* (Lehner et al., 2018; Srikumar et al., 2019). In fact, *C. sakazakii* has been isolated from milk powder and powdered infant formula production environments (including roller dryers, drying towers, and tanker bays) and has been shown to persist in these environments for long periods of time due to its resistance to desiccation and ability to survive spray drying (Caubilla-Barron and Forsythe, 2007; Osaili and Forsythe, 2009; Yan et al., 2013).

Although it is now recognized that *Cronobacter* species can persist and survive under high osmotic stress, exactly how they survive and persist in these low-moisture environments is unknown. However, it was found that the expression of many proteins upregulated in a particular strain of *C. sakazakii* grown under desiccation stress, were either outer membrane proteins (e.g., OmpC and A) or proteins involved in the transport of inorganic ions and energy production, such as ATPases (Riedel and Lehner, 2007). Other groups have identified a number of genes involved in osmotolerance when examining growth under low water activity conditions. It has been hypothesised that the osmotic stress response of *C. sakazakii* is regulated at the transcriptional, translational and post-translational levels, with RpoS potentially functioning as a global transcriptional regulator involved in the osmotolerance response, as well as playing some role in the development of mature biofilms in *C. sakazakii* (Fernández-Gómeza et al., 2020; Jameelah et al., 2018).

The ability of *Cronobacter* species to persist in stressful environments may also involve the transport of a number of substrates, e.g., protein, sugar and heavy metal, by the actions of efflux pumps (Negrete et al., 2019). Trehalose, a compatible solute, also appears to play a significant role in the desiccation survival of *C. sakazakii*, i.e., dried stationary phase cells have been found to have more than a 5-fold increase in trehalose concentration (Breeuwer et al., 2003; Feeney et al., 2014). Besides trehalose, other potentially important solutes helping *Cronobacter* species survive desiccation include proline, ectoine and betaine. Feeney and Sleator (2011) did an extensive *in-silico* analysis of one strain of *C. sakazakii* and found 53 putative osmotolerant loci, including 7 copies of the important *proP* encoding gene. This latter gene is known to help *E. coli* in the uptake of osmoprotectants (Lehner et al., 2018).

4.2.2. *Control Options of Cronobacter species in PIF manufacturing facilities*

The safety of PIF needs to be ensured through the implementation of good hygiene practices (GHPs) and GMPs, as well as a HACCP approach. The preventative measures used to control *Salmonella* species form the basis and are a prerequisite to control *Cronobacter* species. Because the *Enterobacteriaceae* (EB) are so widespread, their entry into processing plants can only be minimized and not completely eliminated. The most important part of this effort to minimize the presence of EB and thus *C. sakazakii*, is through very strict water management in high-hygiene areas, e.g., the use of dry-cleaning.

In the latter, the development of control strategies as outlined in guidance documents produced by both FAO and WHO, seem to have resulted in a decrease in the incidence of *Cronobacter* spp. in PIF, plus an apparent decrease in the number of reported invasive *Cronobacter* infections in infants (see Fig. 4.1; Strysko et al., 2020).

Facilities and equipment should be designed, constructed and installed so as to minimize the entry of *Cronobacter* species into high hygiene areas and to reduce their establishment or growth in harbourage sites. Dry processing areas should be maintained as high hygiene areas. The internal design and layout of establishments manufacturing PIF need to ensure the strict physical separation of wet from dry processing areas where post-process contamination from the environment can occur.

It should be noted, that compliance by PIF manufacturers should be accomplished through the development of an appropriate food safety control system and by verification of the effectiveness of control measures. These activities include, as necessary, well documented microbiological sampling plans, not only for product testing, but also procedures defining a sound and effective environmental sampling program for PIF. The latter should include testing for EB, which should be done for verification of the hygiene status of the facility, but not to assess the safety of a specific lot of product. Tracking the levels of EB in the processing plant environment also allows i) corrective actions to be done in a timely manner, and ii) the plant to establish baseline levels, so that trend analysis of the data can be done, i.e., tracking changes in EB levels with time.

The environmental monitoring program should be used to assess control of the processing plant environment in high hygiene areas (dry areas) where contamination might take place, and, thus, would be an essential food safety management tool. It should therefore be designed to assess whether *Cronobacter* species are increasing and whether the control measures are effective to prevent any potential growth of the

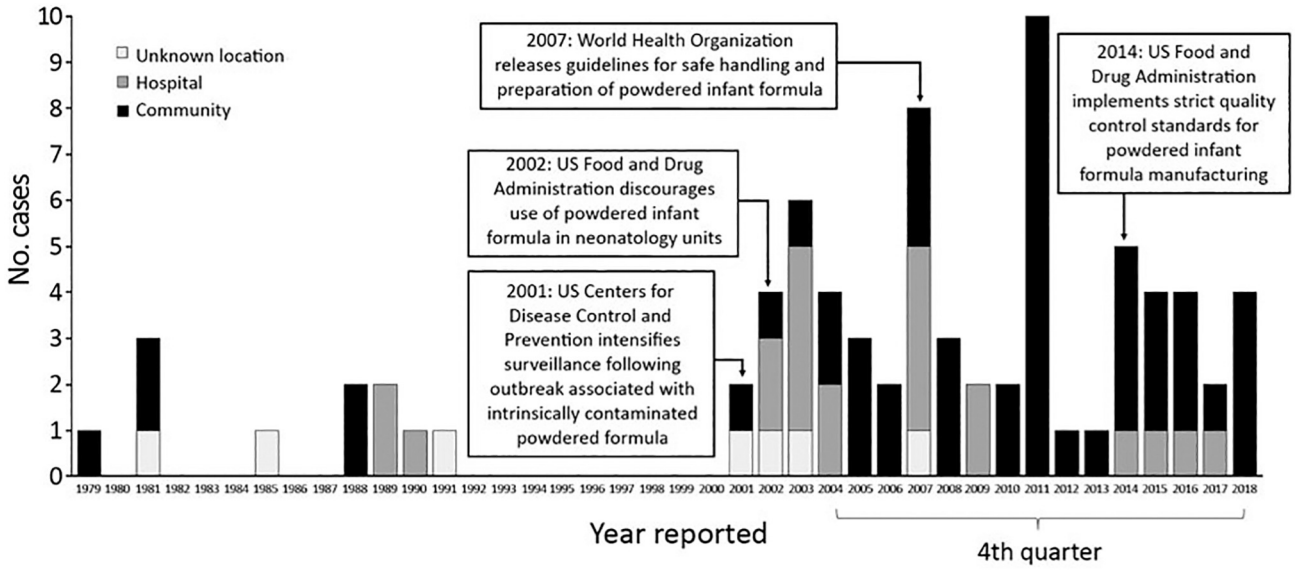

Fig. 4.1. Reported invasive *Cronobacter* spp. infections recorded among infants, U.S. (1979–2018), by location of patient at the time of symptom onset ($n = 79$). From Strysko et al. (2020).

organism.

Increases in the levels or frequency of detection of *Cronobacter* species (or more generally levels of EB) in processing environments can be due to the introduction of bacteria from the exterior environment due to issues, such as in plant construction or, more commonly, due to conditions which allow for the growth of bacteria already present in the environment. Because of the latter, it is critical that the processing environment be kept as dry as possible (Codex, 2008).

The concepts and approach of the hygienic control measures (GHP/GMP and HACCP) needed to control *Cronobacter* species and to produce PIF safely, have previously been described (FAO/WHO, 2004, 2006; Cordier, 2007). The stringency required for a particular processing line will depend on the microbiological criteria for products manufactured on that line. The current Codex criteria for PIF require the absence of *Cronobacter* spp. in each of 30 samples of 10 g of PIF, i.e., a 2-class sampling plan with $n = 30$, m = absence in 10 g, and a $c = 0$. Guidance for the establishment of monitoring programs for *Cronobacter* species and other EB in high hygienic processing areas can be found in Appendix 3 of the FAO/WHO document (FAO/WHO, 2008). In addition, the Codex Code of Hygienic Practice for PIF for infants and young children provides detailed practical guidance and recommendations to industry on the hygienic manufacture of PIF (FAO/WHO, 2008).

Regarding effective ways of inactivating niches/biofilms of *Cronobacter* species in PIF processing facilities, Kim et al. (2007) found that among 13 disinfectants, quaternary ammonium compounds and peroxyacetic acid/hydrogen peroxide-associated disinfectants appeared to be the most effective against *Cronobacter* biofilms formed either in suspension or in biofilms dried on the surface of stainless steel. In contrast, disinfectants routinely used in food industry were found to be insufficient in removing *Cronobacter* biofilms from plastic surfaces. Thus, it is evident that the mechanisms of action of disinfectants on *Cronobacter* biofilms is not fully understood. Because conventional cleaning may not always be adequate for biofilm control, novel control strategies are being investigated, such as nanotechnology-based delivery systems, natural compounds and phages (Ling et al., 2020).

4.3. *Escherichia coli*

E. coli are members of the *Enterobacteriaceae* family, defined as Gram-negative rod-shaped facultative anaerobes. This bacterium is often discharged into the environment through faecal matter and wastewater effluent and is able to grow rapidly under optimal growth conditions, with a replicating time of approximately 20 min.

The majority of *E. coli* are harmless commensals that colonise the gastrointestinal tract of most mammalian animals, including humans. However, when pathogenic, *E. coli* can cause a variety of human diseases which can even occasionally result in death.

4.3.1. *Key elements relevant to LMF conditions*

Like many foodborne pathogens, *E. coli* can survive for lengthy periods of time in LMF, although ultimately survival time is dependent on factors such as temperature and humidity as well as the presence of specific carbohydrates (Hiramatsu et al., 2005). Outbreaks of foodborne illness associated with *E. coli* O157:H7 are traditionally linked to high moisture products such as under-cooked beef (Bell et al., 1994; Ostroff et al., 1990; Wells et al., 1991). However, *E. coli* O157:H7 originating from low-moisture products have also been identified as the causative agent in some cases of foodborne illness. In 1994, a multi-state outbreak occurred in the US after the consumption of dry, fermented salami, with 17 people being affected (Tilden et al., 1996). It is likely that the *E. coli* was present in the raw meat used to produce the salami and was capable of surviving the fermentation and drying process. Another multi-state outbreak of *E. coli* O157:H7 occurred in 2009 which was traced back to raw, refrigerated cookie dough (CDC, 2009) and a total of 72 cases had been identified, 10 of which developed haemolytic uremic syndrome (CDC, 2009). Further outbreaks include 8 cases of *E. coli* O157:H7 connected to in-shell hazelnuts (CDC, 2011), 56 cases of *E. coli* O26 (STEC) linked to a flour production facility (Crowe et al., 2017) and 21 cases of *E. coli* O26, also linked to wheat flour (CDC, 2019c).

4.3.2. *Control options*

The robust ability to survive, as demonstrated by some strains of *E. coli* O157:H7 under a wide variety of conditions, highlights the importance of having appropriate methods in place for disinfection and infection control. To be able to control and limit the spread of contaminating bacteria, it is necessary to apply the principles of the HACCP system combined with GMPs. Decontamination of pathogens in LMF can be a challenge and common methods used in the food industry (e.g. pasteurisation, filtration, preservatives) often cannot be used for some of these foods.

A number of novel control measures have been examined. High Pressure Processing (HPP) and its effect on *E. coli* has been studied in

some non-LMF products. Water activity (a_w) and solutes have been demonstrated to be protective against HPP. At 400 and 600 MPa, inactivation of *E. coli* was determined to be reduced at lower a_w (0.90, 0.95 and 0.99). The protective effect of solutes was shown to differ at different concentrations and by solute, e.g., glycerol, sorbitol, fructose and sodium chloride (Setikaite et al., 2009). Nonthermal plasma has shown to be effective for the decontamination of microorganisms in food. Reductions in the number of *E. coli* O157:H7 on dried figs using microwave-powered nonthermal plasma were shown to be positively correlated with a_w (Lee et al., 2015). Ionising radiation is a well-established alternative method to thermal processing, which is also approved by both the US Food and Drug Administration (FDA) and US Department of Agriculture to remove foodborne pathogens. The application of 3 kGy of electron-beam irradiation to raw cookie dough containing *E. coli* O157:H7 and *S.* Typhimurium resulted in a reduction of bacterial numbers to below the limit of detection (Jeong and Kang, 2017).

A number of chemical treatments have been assessed against *E. coli* O157:H7. Chlorine solutions are regularly used in commercial facilities, as well as free chlorine, chlorine dioxide and a variety of organic acids (del Carmen Velázquez et al., 2009; Park et al., 2009). Propylene oxide (PPO) and its biocidal effects have been studied for many years and have been shown to be effective against *E. coli* in LMF (Beuchat, 1973), however, its use is banned in many countries due to the gas toxicity. Calcium hypochlorite treatment of alfalfa seeds reduced the number of *E. coli* present by approximately 3 logs (Fett and Cooke, 2003).

Triclosan is a biocidal agent in many domestic and industrial formulations. A number of studies have aimed to understand the mechanisms involved in triclosan tolerance of *E. coli*. Using transcriptomics, triclosan-tolerant *E. coli* O157:H19 showed significant differences in the expression of pathways involved in metabolism, transport and chemotaxis, as well as highly upregulating genes for flagellar assembly (Lenahan et al., 2014). Proteomic analysis of *E. coli* O157:H19 also demonstrated in a triclosan-tolerant mutant, changes in protein expression levels of the known triclosan target, Fab1, which encodes enoyl reductase, outer membrane proteins and FliC, the filament structural protein of flagella (Sheridan et al., 2013).

4.4. *Bacillus cereus*

B. cereus is a Gram-positive spore forming bacterium, which is widely distributed in soil. It can also be found in the gut of earthworms. Although soil is recognized as being a main source of contamination of *B. cereus* for foods, further contamination during processing may occur due to i) the strong adhesion properties of *B. cereus* spores; ii) its ability to form biofilms and iii) its persistence on surfaces. In summary, "*B. cereus* is ubiquitous and its presence in most raw foods appears as inevitable" (EFSA, 2005).

Since the spores of *B. cereus* can survive commonly used treatments in food processing (except retort/sterilization processes targeting spores specifically), it is important to prevent conditions along the production chain which would allow for spore germination and outgrowth of *B. cereus*, and thereby toxin production.

B. cereus sensu stricto (commonly referred to as "*B. cereus*") is normally not discriminated from *B. cereus* sensu lato (*B. cereus* group) in diagnostics. It is therefore often not known if food poisoning has been caused by *B. cereus* sensu stricto or another species of the *B. cereus* sensu lato group. Detailed investigations have shown that at least three other species of the *B. cereus* sensu lato have been involved in foodborne outbreaks (Glasset et al., 2016). Organisms in the *B. cereus* group can produce several virulence factors and toxins, which have been associated with two distinct forms of food poisoning: emetic and diarrheal syndrome (EFSA, 2016). The emetic toxin (cereulide) is pre-formed in food when the bacteria have grown to higher numbers (i.e., $>10^5$ cfu/g), but foodborne illnesses with lower numbers have been reported. Once the toxin is formed, it is not destroyed by commonly used treatments due to its acid and heat stability. The diarrheal toxin is potentially formed in the small intestine, when higher numbers of *B. cereus* cells have been ingested (EFSA, 2005).

4.4.1. *Bacillus cereus and LMF*

B. cereus has been recognized as a pathogen of concern in LMF (Codex, 2015). However, it has also been acknowledged that low levels of *B. cereus* (<100 cfu/g) in PIF would not pose a risk to infants when storage and reconstitution conditions would not allow for significant growth (FSANZ, 2004). In that respect, it has been recognized that LMF can contain low levels of *B. cereus* spores, which are of no public health concern- unless the food is reconstituted and those spores are allowed to grow and thereby produce toxins.

Relevant control options for *B. cereus* are therefore focused on avoiding conditions that allow for the outgrowth and proliferation of spores.

With respect to EM, besides equipment, *B. cereus* is normally not sampled for in the food industry, since low levels are expected to be present due its ubiquitous nature - in the food itself, as well as in the environment (Innovation Center for US Dairy, 2019). Due to its ability to form biofilms in pipes, *B. cereus* is considered to be an important spoilage and safety organism in dairy production environments. The hydrophobic properties of *Bacillus* spores allow them to adhere easily to surfaces, and their resistance to disinfectants and heat can lead to long-lasting and hard to remove biofilms in and on equipment. Considerably higher amounts of biofilms have been found on stainless steel surfaces as compared to other materials, which might be due to the more readily availability source of iron. In addition, rougher, more hydrophobic and coated surfaces can more easily support biofilm formation (Ryu and Beuchat, 2005). Cleaning (particularly CIP) systems should therefore be verified to be able to remove potential biofilms. This is usually approached by performing laboratory studies on the efficacy of chemicals used, and not by sampling for *B. cereus* after CIP (Kumari and Sarkar, 2016).

4.5. *Listeria monocytogenes*

L. monocytogenes is a Gram-positive, non-sporulating, facultative anaerobe found throughout the environment as a free-living organism and within mammalian reservoirs such as cattle, pigs and poultry (Gray and Killinger, 1966). Humans are also documented carriers for *L. monocytogenes*, with asymptomatic shedding occurring in an estimated 2 to 10% of the population (Buchanan et al., 2017; Farber and Peterkin, 1991).

Listeriosis is a relatively rare disease with incidence rates in developing countries generally ranging from 0.2 to 0.5 cases per 100,000 population, with trends towards increasing numbers of human listeriosis cases being reported in some regions such as in the EU. Overall, it is generally well-recognized that with regards to pathogenicity, there exists both hypervirulent and hypovirulent clones of *L. monocytogenes* (Buchanan et al., 2017; Lee et al., 2018; Maury et al., 2016; Pouillot et al., 2015).

The main factors that make *L. monocytogenes* unique as a foodborne pathogen include i) the fact that it is widespread in nature; ii) it can grow at refrigeration temperatures; iii) it is associated with a high case-fatality rate (around 20%) and iv) it is hardy and can survive for long periods of time both in nature and in the environment of food processing facilities under favourable conditions. Its persistence in food processing plants is likely due to a number of factors including its ability to form biofilms, tolerate the cold, and harbour resistance to quaternary ammonium compounds (QAC) as well as phages (Buchanan et al., 2017; Kim et al., 2008; Lee et al., 2018).

4.5.1. *Listeria monocytogenes and LMF*

At present, there have not been any reported outbreaks or confirmed cases of listeriosis associated with the consumption of LMF. Based on the

guidelines/policies that exist in the U.S. (which indirectly affects the regulatory position in Canada), several warnings/recalls related to LMF have been initiated in these countries. For example, in Canada, between 2013 and 2020, there were a total of 17 food recall warnings due to the presence of *L. monocytogenes* in LMF and one due to the presence of *Listeria* species. In the USA in 2020, there have been recalls of pumpkin seeds, organic almond, peanut and tahini butters, sunflower butter, plain peanut spread and protein trail mix, arising from the potential to be contaminated with *L. monocytogenes* or *Listeria* spp. Thus, in the U.S., even suspect contamination of LMF with *L. monocytogenes* can result in a voluntary recall, which could have severe economic consequences at all levels of the food production chain. In general, LMF that have been recalled because of potential *L. monocytogenes* contamination include a wide range of commodities including dried fruits, nuts and nut products, baked goods and dried meats (Ly et al., 2019). Although the overall prevalence of *L. monocytogenes* in LMF appears to be low (FAO/WHO, 2004; Ly et al., 2019), there is limited data on the incidence and/or levels of *L. monocytogenes* in LMF (Taylor et al., 2019).

4.5.2. Survival of Listeria monocytogenes on LMF

Several studies have demonstrated the desiccation tolerance of *L. monocytogenes* in which cells survive the drying process and remain viable on the end-product (e.g., tree nuts), for as long as one year (Taylor et al., 2019). Initial inoculum concentrations are generally high (i.e., 5.4 to 6.5 log CFU/g) in these long-term studies, with population reductions ranging from 0 to 5.5 log CFU/g at endpoint. For example, Ly et al. (2020) examined the survival and virulence of *L. monocytogenes* on three low-moisture LMF, namely, chocolate liquor, corn flakes and shelled, dry-roasted pistachios. At 4 °C, *L. monocytogenes* remained stable on the LMF for at least 336 days. At 23 °C, *L. monocytogenes* numbers declined on the chocolate liquor, corn flakes and pistachios at initial rates of 0.84, 0.88 and 0.32 log CFU/g/month, respectively. Decreases in the culturable population of *L. monocytogenes* during storage on the LMF were the result of both cellular inactivation and transition to a viable-but-non-culturable state. The surviving cells, specifically after long-term storage at 4 °C on the chocolate liquor and pistachios, remained infectious and capable of intracellular replication in Caco-2 enterocytes. Storage temperature appears to have a major effect on survival, with higher temperatures leading to a much more rapid inactivation of *L. monocytogenes* on LMF. In addition, Taylor et al. (2018) have shown that water activity (a_w) has a definite effect on the survival of *L. monocytogenes* in LMF, with a lower a_w resulting in less reduction of the organism. Furthermore, as one would expect, limited data suggests that the thermal resistance of *L. monocytogenes* in LMF increases as the a_w decreases (Taylor et al., 2019).

4.5.3. Control

Because the LMF category represents a very low risk for listeriosis, it is generally considered that the use of an environmental monitoring program for *Listeria* species, is not really a good use of food safety resources (GMA, 2014). Rather, the use of the *Enterobacteriaceae* as an indicator of the ingress of water, as well as hygiene and sanitation in the post-processing environment, is recommended. Furthermore, with regards to the control of *L. monocytogenes* in LMF manufacturing facilities, in relation to HACCP, *L. monocytogenes* in LMF would be considered a very low risk because it does not grow in LMF (Beuchat et al., 2013).

It is generally agreed that commonly used disinfectants or sanitizers are effective against *L. monocytogenes* in suspension (Chavant et al., 2004), however, cells attached to surfaces such as in biofilms, appear to be more resistant to sanitizers than cells in suspension (Frank and Koffi, 1990; Mafu et al., 1990; Stopforth et al., 2002). A minimum initial level of *L. monocytogenes* appears to be necessary for bacteria to persist in harbourage sites, with early and effective cleaning and disinfection being the major means to avoid persistence of the organism in the niches/harbourage sites (Carpentier and Cerf, 2011).

In terms of resistance to disinfectants or sanitizers, the major one for which there appears to be reports of resistance to *L. monocytogenes* are the QACs, more specifically benzalkonium chloride (BAC). Although there are several well-characterized *L. monocytogenes* efflux pumps that confer resistance to QACs, it is a generally thought of as a low-level resistance that does not appear to generate resistance to QACs at levels typically used in the food industry (Martínez-Suárez et al., 2016). Furthermore, an association between low-level resistance to QACs and persistence of the pathogen in different food processing environments has only been demonstrated by a few research groups (Martínez-Suárez et al., 2016). In addition, researchers in Spain concluded that although they found a link between *L. monocytogenes* persistence and biofilm formation, there was no correlation between persistence and the resistance of sessile cells to disinfectants such as sodium hypochlorite and BAC (Rodríguez-Campos et al., 2019).

However, exposure of *L. monocytogenes* to sub-inhibitory concentrations of QACs can potentially increase the ability of these strains to form biofilms and thus survive subsequent treatment with high levels of the same disinfectants. In addition, strains carrying the efflux transporter benzalkonium chloride tolerance gene *emrC*, have been associated with an increased incidence of ST6 listerial meningitis in the Netherlands (Kremer et al., 2017). Thus, it may be a good practice to rotate, the disinfectants used in food manufacturing facilities where wet and eventually controlled wet cleaning practices are in place to help maintain the effectiveness of BAC against *L. monocytogenes*.

5. Potential indicators

Microbiological examination of the food manufacturing environment, together with testing of raw ingredients and process intermediates, are important verification activities within an effective food safety and quality system. These activities can only fulfil their purpose when the microorganism chosen for analysis mirror/reflect the safety and quality status of the food items produced. In that respect, pathogens are chosen to evaluate product safety. However, in LMF (as outlined in previous parts of the present review) and their manufacturing environments, relevant pathogens are often only present at very low concentrations and heterogeneously spread. Therefore, often only negative results are obtained, and seemingly small changes in processing environment conditions are not detected until it is too late (see part 3: Past learnings). In order to be able to determine and detect such small and potentially dangerous changes, the concept of monitoring indicator organisms was developed. Today, this monitoring is applied to verify efforts ensuring the microbial quality and safety of foods. The first indicator organisms were established to assess the microbiological status of water (Tortorello, 2003). Even though tests for indicator organisms do not replace specific pathogen detection tests, they can provide useful information about process failures, possible contamination sources, possible toxin formation and the overall hygiene level, including verification of cleaning and disinfection, and they could allow for timely corrective actions before pathogens might emerge.

Singleton and Sainsbury (2001) define an indicator organism as any organism whose presence and/or numbers serve to indicate the conditions or quality of a material or environment. Jaykus and McClure (2010) define a microbial indicator as a single or a group of microorganisms, or alternatively, a metabolic product, whose presence in a food or the environment at a given level is indicative of a potential quality, hygiene, and/or safety problem. Tortorello (2003) discriminates between two categories of indicator organisms: safety indicators to assess a microbial hazard, and quality indicators to assess product acceptability e.g. shelf life, spoilage, etc. According to Mossel and Struijk (1995), the term “index organism” refers to indication of a health risk and the term microbiological “indicator” refers to indication of a process failure. However, no index organisms could be defined, and nowadays the term “indicator” is mainly used.

In more general terms, an indicator is supposed to be a “sign that something exists or is likely to happen” (Cambridge dictionary). As

mentioned above, it is not a replacement for testing for pathogens, where this is required, since pathogens may be present independent of an indicator. For example, the presence of *Enterobacteriaceae* may indicate a higher likelihood of presence of *Salmonella* spp. or other pathogens such as *Cronobacter* spp., but their absence cannot be judged to conclude that the pathogen is absent. Craven et al. (2020) investigated levels of *Enterobacteriaceae* and coliforms as indicators for *Cronobacter* in milk powder-manufacturing environments and reported that enumeration tests for both indicators showed a better association with the prevalence and presence of *Cronobacter* than the presence/absence tests for these organisms. However, when the levels of *Enterobacteriaceae* or coliforms were less than 1 CFU/cm^2, *Cronobacter* was still detected in 2 of 18 samples.

The choice of an indicator is based on the behaviour of the target microorganism (pathogen), the characteristics of the food matrix and manufacturing process, the environment, distribution system and the methodological basis for the assay (Buchanan and Oni, 2012). ICMSF (2018) has listed the factors to be considered when selecting an indicator organism for a particular purpose:

(a) Presence of the indicator suggests a faulty process or practice or a potential for spoilage;
(b) Must be easily detected and/or quantified;
(c) Survival or stability of the indicator should be similar to or greater than the hazard or spoilage organism;
(d) Growth characteristics of the indicator should be similar to or faster than the hazard or spoilage organism;
(e) Identifiable characteristics of the indicator should be stable;
(f) Method for detection and/or quantitation should be easy, rapid, inexpensive, reliable, sensitive, and validated; does not risk analyst health; and is suitable for in-plant use;
(g) Quantitative results should show a correlation between the concentration of the indicator and the level of the hazard or spoilage organism;
(h) Results should be applicable to process control.

Enterobacteriaceae is a useful indicator for hygiene standards in non-raw post heat-process areas. *Enterobacteriaceae* is a family of Gram-negative bacteria, including *Salmonella* spp., *Cronobacter* spp. and (pathogenic) *E. coli* (see Fig. 5.1). An example of monitoring for *Enterobacteriaceae* in a dry goods manufacturing environment is shown in Fig. 5.2 (Cordier, 2007), where it can be seen that the levels of *Enterobacteriaceae* increase following events where water is introduced and eventually, *Salmonella* is detected. Such increases in levels of indicators can provide an early alert to a change in environmental conditions that may lead to an increased risk of pathogens being present, where these may be able to multiply in the environment and pose a threat to the safety of products being manufactured in that environment. The setting of appropriate action limits and an early alert to such conditions allows the relevant functions, e.g., quality manager and team, to respond quickly in isolating the affected areas, identifying and eliminating the source(s) of water leaks and applying appropriate cleaning and disinfection before more serious consequences can develop.

Yeasts and moulds are indicator organisms used in the bakery industry and for dried fruits. They can indicate post-process air quality and plant hygiene issues, as well as provide information on potential condensation, which could provide niches for other microorganisms to grow.

Testing for *Listeria* species can indicate the level of plant hygiene in post-process chilled environments, but is not that relevant for LMF and LMF manufacturing environments, particularly for those foods that are not known to be linked to cases of listeriosis.

In water, *E. coli* was found a reasonable indicator for *Salmonella* (Krometis et al., 2010).

For endospore-forming bacteria such as *B. cereus*, *C. botulinum* and *C. perfringens*, HPLC analysis with fluorescence to determine the dipicolinic acid level could be considered (Lomstein and Jørgensen, 2012). However, it needs to be kept in mind that with this method, it is only possible to determine the presence/level of bacterial spores - it does not provide a determination of the species/genus.

As mentioned previously, action limits or thresholds for acceptable and unacceptable results are often applied to indicators. This allows for warning signs before an actual safety/quality issue could arise. Setting meaningful action limits requires one to establish a "baseline" beforehand to determine, how/what the level of indicators would be under best GMP production conditions, including effective cleaning and sanitation and prerequisite programs in place. Determination of such a "baseline" is commonly performed by multiple sampling rounds of the area(s) and should also include seasonal variations (where applicable). Sampling should be performed after cleaning (best case) and also during production (normal status), as well as before cleaning to determine potential variations. Sampling frequencies should be determined by the

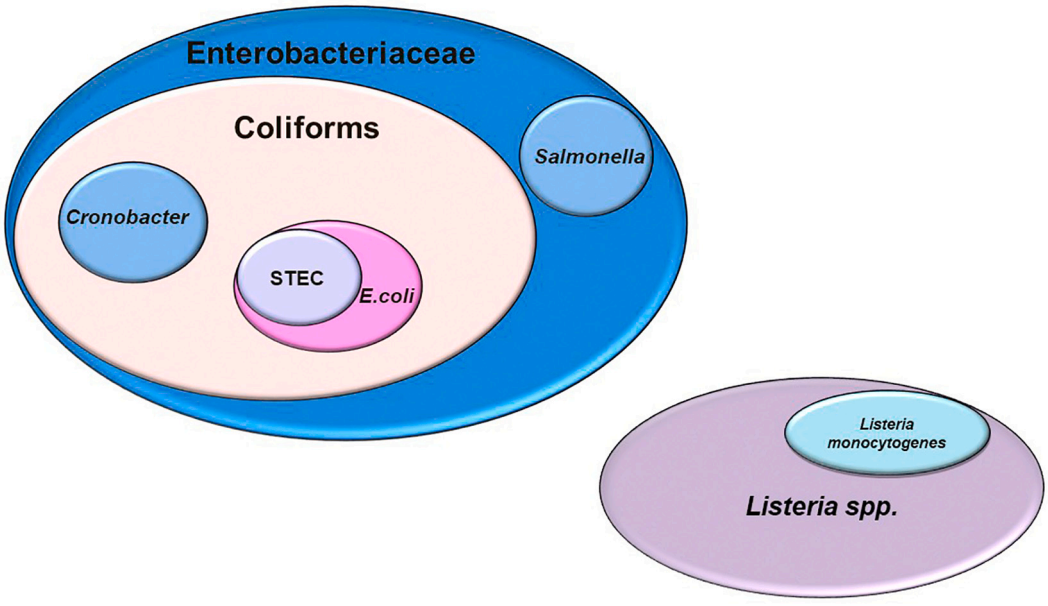

Fig. 5.1. *Enterobacteriaceae* and *Listeria* spp. as hygienic indicators of pathogens of concern.

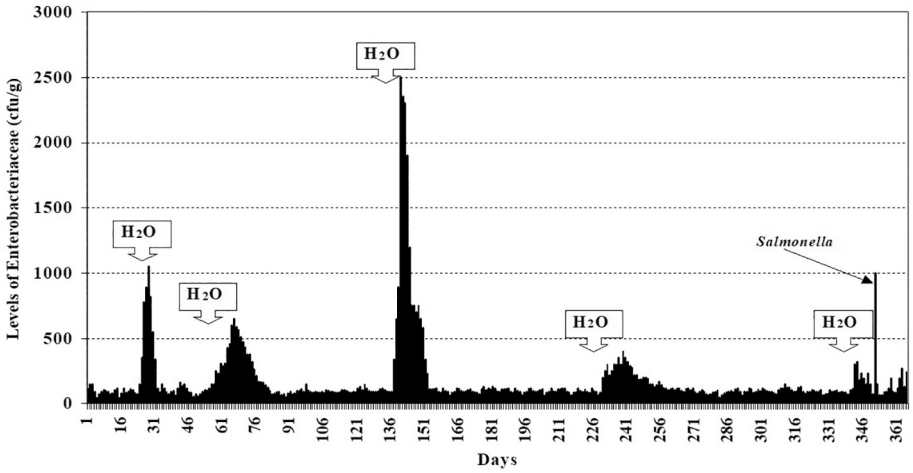

Fig. 5.2. Daily count of *Enterobacteriaceae* on surface swabbing in the processing site. From Cordier (2007).

microbial growth potential (or not) in the sampling area(s): Where there is the potential for growth (like in wet areas), weekly or even daily sampling is recommended, whereas in dry areas not allowing for microbial growth, a monthly sampling frequency could be sufficient. Furthermore, methods of sampling and testing need to be kept the same-otherwise variations could be linked to such changes. Notwithstanding the above, it is clear that establishing a baseline and deriving action limits from that requires considerable time, which can span between 1 and 2 years and should include as a minimum 10 time points. In order to determine significant differences/changes, statistical evaluation of the data and trend analyses are needed, which could then reveal if/where processes will get out of control.

Microbiological testing of the environment plays an important part in verifying the safety/quality of products, if set up adequately. To be able to do that, a good understanding of which microorganisms provide such information and their relationship to the food in question is required. In that respect, it is recommended to i) establish the rationale and record the decisions made (e.g., choice of microorganism(s) or other methods), ii) define the limits for acceptable and unacceptable results, iii) state the frequency of testing and locations to be tested (including rotation of areas to be sampled) and iv) outline follow-up actions in case of unfavourable/unexpected results. These latter actions will commonly include cleaning and disinfection, as well as verification testing of the cleaning and disinfection procedures. There are some methods available for indicators that allow sub-typing of these groups of organisms, and this can be helpful in determining if these are repeat offenders and thus support root cause analysis.

6. Elements in PEM plans

Since 2005 and its first version, the EU Regulation 2073/2005 considers in the whereas #22: "Sampling of the production and processing environment can be a useful tool to identify and prevent the presence of pathogenic micro-organisms in foodstuffs.". The recommended micro-organisms of concern were initially *L. monocytogenes* for RTE Foods (Article 5.2-specific rules for testing and sampling), *Salmonella* spp. and *Cronobacter* spp. for infant formulae, formulae for special medical purposes and follow-on formulae (Whereas #17). How to sample and where to sample nevertheless is not explained in the regulation. The ISO standard 18593 for surface sampling was only recently published, in June 2018 (ISO, 2018), replacing the technical specification that was initially published in June 2004.

The concept of product proximity (Fig. 6.1) with 4 layers, from zone 1 Z1 to zone 4 Z4 was initially proposed in 2002 by ICMSF (ICMSF, 2002) and has been enforced in the regulation by the United States Food and Drug Administration (US-FDA). In Europe, the approach of product

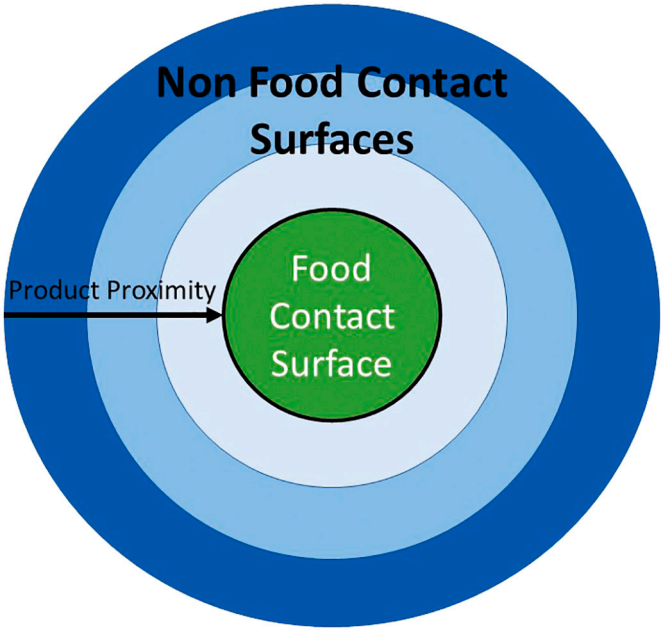

Fig. 6.1. Product proximity, food contact and three non-food contact categories.

proximity is not specifically promoted by the different food safety authorities that use the Codex Alimentarius two layers approach, Food Contact Surface (FCS) or non-Food Contact Surface (nonFCS). The nomenclature of zoning proximity for swabbing is sometimes mistaken with hygienic zoning, leading food handlers unrightfully to think that Zone 1 means high hygiene and Zone 4 low hygiene.

In the various professional guidelines since 2010 from different organizations (Almond Board of California, United Fresh Produce, GMA, Profel), the four levels have been defined as follow:

- Line (L), Product (P) or Zone 1 (Z1): Food contact surfaces in direct contact
- Environment 1 (E1) or Zone 2 (Z2): Non-food contact surfaces in close proximity to food contact surfaces
- Environment 2 (E2) or Zone 3 (Z3): More remote non-food contact surfaces which could eventually lead to contamination
- Environment 3 (E3) or Zone 4 (Z4): Non-food contact surfaces and remote areas from processing environment

In the report on urgent assistance for sampling and *L. monocytogenes* testing in processing plants of frozen vegetables (EFSA, 2018), the recommendation is to identify the critical sampling sites (CSSs) for the processing environment of food facilities (FCS and non FCS, processing water) while considering the potential sources of contamination with the microorganisms of concerns. When sampling the processing environment, 4 different categories should be considered:

1. **Routine sampling points**: they are the gate-keepers, classically non-food contact surfaces with close proximity to the processing lines and the product. Key performance indicators (KPI) and trend analysis can be done on these points, as they are comparable over a time period.
2. **Investigation sampling points**: when performing the sampling in the processing environment, the operator should have the microbiological knowledge to identify abnormal conditions (product on the floor, presence of water in a dry zone, leaking pipes, …) and decide to perform swabbing ad hoc on non-pre-determined points.
3. **In the case of special events**, maintenance or construction, a specific swabbing 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.
4. **Additional samples following a positive sampling**: the FDA refers to these points as vector sampling. When facing a non-compliant result, the food manufacturer should perform additional sampling around the initial positive, considering also different categories of product proximity (nearer to the product to see how far the contamination is spread and further away to identify potential harbourage niches)

The two recent *Salmonella* outbreaks associated with infant formula in Europe (see Part 3: Past learnings) confirm the ability of certain *Salmonella* strains to survive within a processing environment for over a decade. The records of both companies showed the presence of other serotypes of *Salmonella* which, however, were not found over different periods of time or in the finished product, showing a transient presence vs. the resident strains. Little is known precisely of the reason why one strain would have the capacity to survive in the environment while another would not. For years, the process of bacterial adherence to surfaces and resistance to the process (acid resistance, heat resistance) has been considered for the mechanism of survival. Recent publications highlight also for some pathogens of concern, resistance to cleaning agents and cleaning procedures as one of the many potential reasons for survival in the processing environment (Kremer et al., 2017).

Mathematical models to evaluate the number of samples needed to be analysed per factory, as well as the sampling and transfer rates have been proposed recently (Zoellner et al., 2018), but practically, it remains for the time being a case-by-case assessment for each facility, considering the hygienic design of the premises, the PRPs in place and the application of GMP and zoning. When developing the sampling plan, one should carefully consider (Fig. 6.2):

- The hygienic zoning: e.g., high, medium or low hygiene (Codex Alimentarius approach)
- The cleaning regime of the considered room: dry, controlled wet, wet
- The proximity to the product: food contact or non-food contact, and if using the 4 layers approach, specify the category

In order to prioritize the sampling points, a matrix approach considering both zoning and proximity is proposed (Fig. 6.3). A food contact surface in a low hygiene area (e.g., pipe of reception of raw milk) is less relevant than a non-food contact surface in a high hygiene zone. The analytical methods should be chosen appropriately: an alternative proprietary method should be controlled using ISO 16140 Part 2 validation dossier where environment sampling should be in the scope.

Sampling should always take place during production and at least 4 h after cleaning and sanitizing. A safety risk assessment should be done from the sampling activities. For pathogen sampling, priority must be given to E1/Z2 surfaces, where direct contamination of the product could occur (e.g., the external part of equipment), and important E2/Z3 surfaces (e.g., rolling equipment).

As an example, the following ratio for Non-Food Contact Surfaces may be used for routine sampling.

E1/Z2 rating samples: 60–70%.

E2/Z3 rating samples: 30–40%.

E3/Z4 rating samples: 0–10%.

Based on trend analysis of the routine sampling points and the outcome of the investigation sampling points, the PEM plan should be regularly updated and revised. This should also be done in case of modification of the processing environment (e.g., change of equipment, repairs and work, etc.). The latter would be the same approach as expected in the regular revision of any HACCP plan.

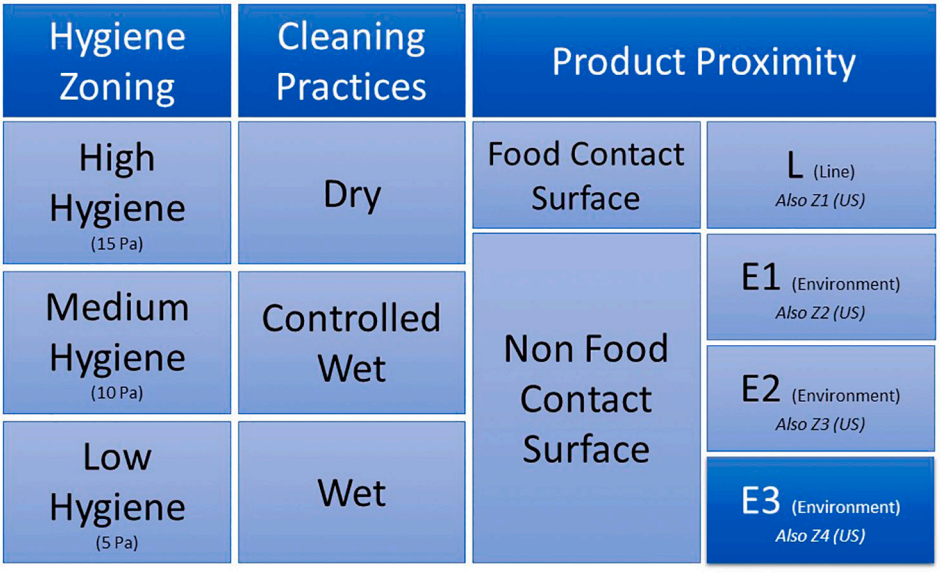

Fig. 6.2. Classification of a sampling point in processing environment monitoring.

Fig. 6.3. Priority of sampling.

7. New tools

Culture-based methods, beginning with growth of a bacterium of interest in a specific broth and/or agar medium designed for the isolation of pure cultures, have been laboratory strategies applied for the identification and subsequent characterisation of bacteria of importance to food safety. These methods have over time benefitted from innovations in the design and formulation of culture media, a feature that has contributed to improvements in reliable detection. Although these approaches are widely used, additional characterisation of various easily detectable bacterial phenotypes relevant to the protection of public health, such as the antimicrobial resistance (AMR) profile, the corresponding phage-type and the biotype, among others, are required. This careful identification of bacteria of interest along with the capability to distinguish between similar and non-related isolates for the purposes of epidemiology is an essential step in surveillance.

Despite the use of these phenotype-based methods, there are recognized limitations particularly in respect of the analytical resolution required, to differentiate between bacterial isolates. Molecular methods (Table 7.1), including the use of amplification-based protocols such as multi-locus variable number of tandem repeats (MLVA) and amplified fragment length polymorphism (AFLP) analysis have contributed to improvements in routine surveillance. Similarly, other sub-typing protocols such as pulsed-field gel electrophoresis (PFGE), that underpin the PulseNet network and genomic multi-locus sequence typing (MLST) are examples of standardised approaches that have been applied extensively to good effect. When used in addition to the phenotype-based methods mentioned earlier, molecular sub-typing methods have improved the ability to perform traceback and source attribution during foodborne outbreaks and have similarly contributed to support routine surveillance in a food production environment. Nevertheless, the development and application of NGS protocols now offer the potential to address some of the technical limitations inherent in traditional culture-based approaches (Allard et al., 2016; Ronholm et al., 2016; Tolar et al., 2019).

Due to the rapidly falling costs associated with NGS, these methods are quickly becoming the preferred tool for surveillance. In food microbiology, DNA sequencing can be used in two ways; [a] to determine the WGS of a biological hazard of interest to food safety, such as a foodborne bacterium, an enteric virus or a protozoan parasite and [b] for

Table 7.1
Some features associated with molecular sub-typing methods used for tracking and tracing.

Protocol	Technical basis	Comments
Pulsed-field gel electrophoresis (PFGE)	DNA digestion with a macrorestriction enzyme, followed by fragment separation by gel electrophoresis	Gold standard sub-typing method, which is now being replaced by WGS-based methods; Not amenable to automation; Band profile interpretation can be subjective and challenging
Multi-locus variable number of tandem repeat analysis (MLVA)	Amplification of variable regions contained in the bacterial chromosome and amplicon separation by gel electrophoresis	Multiple repetitive regions in the bacterial chromosome that are highly variable in terms of numbers of copies [even in related strains and these features can be] used to distinguish between clonal and nonclonal isolates
Amplification fragment length polymorphism (AFLP) analysis	Use of a restriction enzyme to firstly digest template DNA, followed by the addition of primers and PCR-mediated amplification, and then fragment separation by gel electrophoresis	Features of public health interest such as AMR-encoding genes and virulence factors can be incorporated into the typing scheme
Multi-locus sequence typing (MLST)	Amplification and sequencing of a small number of housekeeping genes, to which allelic numbers are assigned by comparison with a database of sequences	MLST is carried out using Sanger sequencing and is typically a low-throughput method; Gene sequences are compared against an established database such as pubMLST/BIGSdb; Alleles correspond typically to phylogeny

Table 7.2
Features associated with commercially available whole genome sequencing (WGS) platforms.

Platform name	Read length	Data yield (Gb)	Advantages	Disadvantages
Illumina MISeq	50–150 bp	1.6–7.5	Low error rate	High cost per Gb
Pacific Biosciences	approx. 20-kbp	1	Very long reads, useful for genome/plasmid closure	High cost per Gb; High error rate
Oxford Nanopore (MinION)	Up to 20-kbp	Up to 10	Low instrument costs and portability	High cost per Gb; High error rate

the complete identification and characterisation of microorganisms isolated in a sample such as a food matrix or an environmental swab. When it comes to establishing corrective and preventive action plans following the isolation of bacteria or other biological hazards of interest, these molecular tools provide valuable information that were not easily accessible until now, such as adherence capability potential, resistance to cleaning agents, heat resistance and biofilm formation.

Several sequencing platforms have now been developed, using different reaction chemistries and which can be applied for the determination of the WGS of bacterial and the genomes of other pathogens (Table 7.2). Following quality control checks of the raw sequence data obtained from one of the platforms above, these data can then be submitted to various bioinformatic pipelines (Fig. 7.1), for mining of particular features, including the identification of the bacterium of interest, its serotype (Zhang et al., 2015), the corresponding sequence type (ST) and/or clonal complex (CC), as determined by *in-silico* MLST, along with genotypes of recognized (acquired) antimicrobial resistance-encoding genes and virulence factors. Typically, MLST relies on the identification of polymorphisms among a few conserved or house-keeping genes and based upon which the ST number is assigned. Using modern sequencing methods this can now be extended to include thousands of additional genes, thereby improving the technical resolution of the method. Core genome MLST (cgMLST) compares all of those genes present in the genome of bacterium of a particular haplotype, thereby facilitating interrogation at the population level.

Similarly, single nucleotide polymorphism (SNP) analysis can be used to explore the genetic relationships between a collection of bacteria of interest, a feature that is useful for tracking and tracing purposes. PulseNet International is now in the process of migrating from PFGE-based sub-typing over to a gene-by-gene approach, including whole genome MLST (wgMLST-which encompasses the cgMLST along with non-core sequences such as those found on plasmids and which is designed to sample between 1500 and 4000 alleles. For SNP analysis, sequence reads obtained after QC checks are analysed at the nucleotide level. These are then mapped against a closely related reference genome, and the nucleotide differences or polymorphisms in both the coding and non-coding regions determined. In order to better quantify the genetic relationship between isolates, consideration must be given to the exclusion of sequence data from mobile genetic elements (MGE) such as plasmids and prophages along with insertion sequence (IS) elements. SNP-based analysis is an accurate way to determine the relationship between two or more bacterial genomes. The choice of method for these types of analyses is dependent on the nature of the question being posed.

Owing to its superior resolution when compared with other available sub-typing methods, WGS offers the possibility of improving the analytical assessment of root cause analysis of pathogen contamination (Harrand et al., 2020) or of a spoilage event. The food industry can also begin to consider refining contamination tracing protocols using these

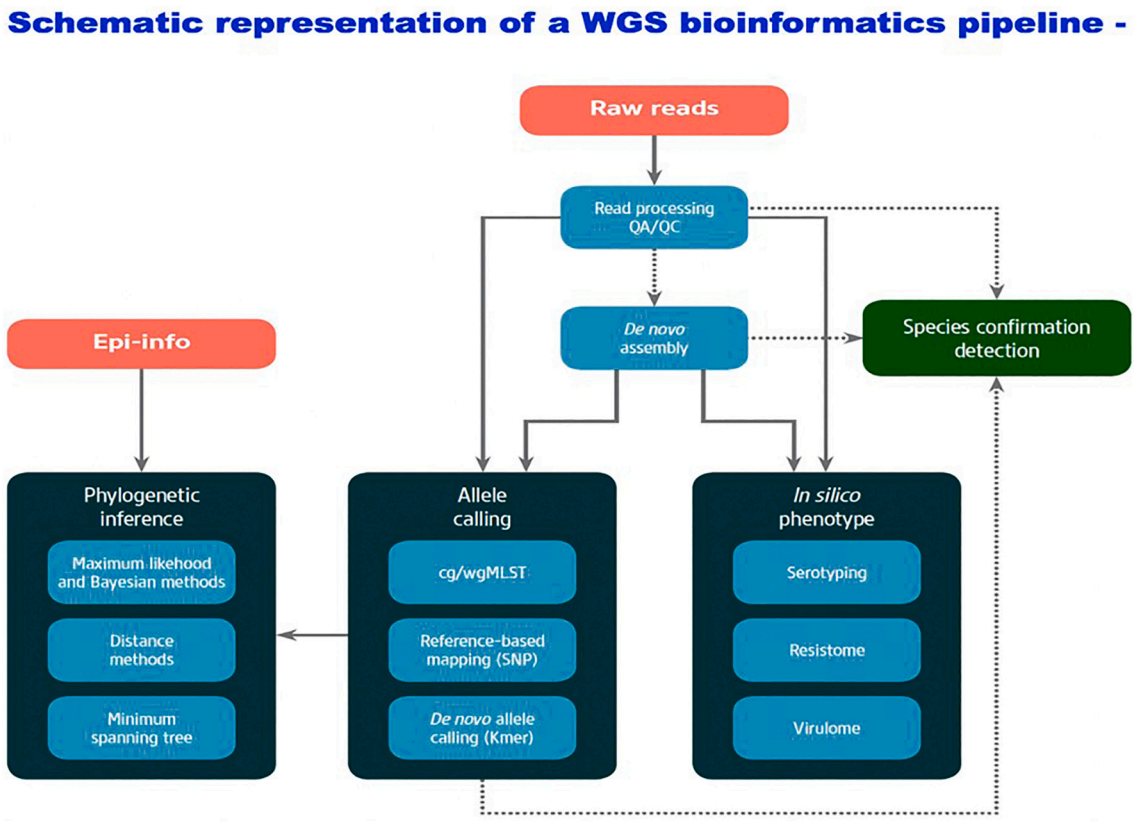

Fig. 7.1. Typical bioinformatics pipeline designed to mine information from bacterial genomes following whole genome sequencing (WGS).

high-resolution tools, as well as exploring the relationship between food stresses and the impact these might have on the colonisation dynamics of the population of microorganisms in a production site. The technology can also be extended, using deep-level RNA sequencing (RNA-seq) to explore the bacterial transcriptome, providing valuable information on the nature of the bacterial adaptive response to a given environmental condition/stress within an ecological niche such as harbourage sites located within the processing environment (Lamas et al., 2019; Srikumar et al., 2019). This approach would further improve our understanding of the genetic basis contributing to bacterial colonisation and persistence of specific isolates over years in a factory, versus other transient isolates as documented in a LMF matrix or a built food production environment (Kovac, 2019; Srikumar et al., 2019). This information could then be translated into more effective food safety control strategies.

Despite the advantages WGS can provide including greater analytical resolution, there are some limitations currently that delay the broad adoption of these modern approaches to monitor the food chain. These relate to the standardisation of nomenclature and the laboratory protocols being applied; use of a validated bioinformatics pipeline; data security, transfer and storage (EFSA et al., 2019b). As an example, there are several tools that can be used for SNP analysis, and it is recommended that users submit their query sequences to previously validated programmes, such as the CFSAN pipeline developed by the US-Food and Drug Administration (FDA) and reviewed recently (Jagadeesan et al., 2019). Furthermore, the manner in which genome data is shared also needs to be addressed. Finally, the way in which WGS data can be used for microbial risk assessment (MRA) needs to be considered (Bortolaia et al., 2020; Njage et al., 2020). Confident prediction of the phenotypic markers as outlined above are important for MRA, since they focus on adverse outcomes that may arise in at-risk populations (Burall et al., 2017; Kuijpers et al., 2018).

Metagenomic analysis can be described as the culture-independent study of all of the genetic material purified from a sample in a defined environment. Whilst the application of next generation sequencing (NGS) protocols, as described above, has the potential to address some of the recognized limitations associated with traditional culture-based approaches, it can also provide insights into the bacterial composition contained therein, using 16S rRNA amplification in addition to the identification of fungal communities by targeting the internal transcribed spacer (ITS) region, followed by DNA sequencing. This strategy could detect low abundance bacterial hazards, independent of the background flora. The proportion of bacterial species can also be determined from these data. Shot-gun metagenomics is another culture-independent method that facilitates the characterisation of the microbiota in a sample of interest. Unlike the former 16S rRNA-based method, this strategy facilitates an untargeted characterisation and provides a richer dataset (Fig. 7.2). In this case, the need for microbiological culture is negated by direct analysis of the total DNA content of a food matrix of interest, environmental swab or other sample type of relevance. Application of this strategy can include the description of those dominant microbes in for example food matrices such as fermented milk products (Shangpliang et al., 2018). The same technical approach can also be applied to sample the processing environment (Anvarian et al., 2016), thereby enabling the description of the microbial population dynamics between different areas of production (Cao et al., 2017). Shot-gun metagenomics not only enables the characterisation of the microbial diversity, but it also provides information on the functionality of individual isolates as well as their ecological interactions within this population (Doyle et al., 2017). This approach can be applied to study food spoilage from the food product itself or from the ecology of the processing environment. Changes in the microbiota (food and/or processing environment) can be determined and thus act as a tool to predict spoilage of a food matrix (Fougy et al., 2016). Standardisation of this technology remains an important challenge (Hoper et al., 2020) and agreed protocols need to be developed and adopted.

8. Conclusion

Low moisture foods ($a_w < 0.85$) have come into focus due to several outbreaks caused by *Salmonella*, pathogenic *E. coli* and in a lesser extent others pathogen of concerns. Interestingly, *L. monocytogenes* has not been implicated in outbreaks related to low moisture foods- unless conditions changed and allowed for its proliferation before consumption (Glass et al., 2015; Ly et al., 2019).

A finished product testing approach is limited when it comes to identifying low prevalence of contamination, which is most commonly the case for low moisture foods. In contrast, processing environment monitoring is recognized as a proactive approach to anticipate finished product contamination. Processing environment monitoring programmes should be designed to both identify critical sampling points to be routinely sampled and search for harbourage niches, as well as seek and destroy pathogens of concern for the respective products/productions. How pathogens can best be monitored is therefore an

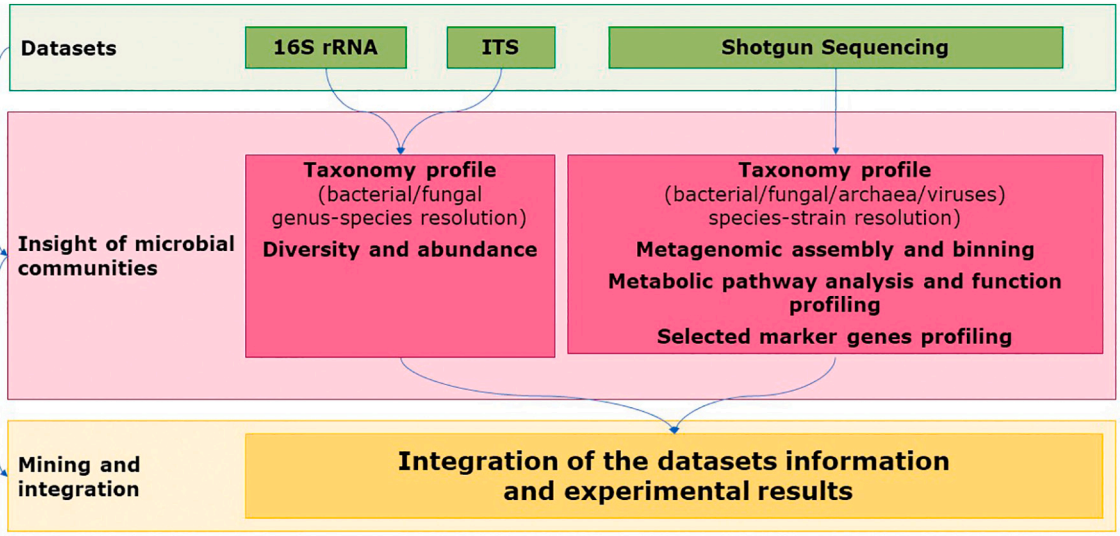

Fig. 7.2. Overview of dataset examples produced by either amplicon-barcoding or shot-gun metagenomic methods and the insights obtained from data analysis. ITS: internal transcribed spacer.

important point, not only for manufacturers, but also for regulators. This also refers to the question of suitable indicator organisms-both for pathogen(s) and for indictors of the hygienic conditions within the plant.

While monitoring the microbial contamination in the processing environment proves to be more proactive and efficient for detecting low level contamination vs. finished product testing, it should be followed by a corrective action and preventive action plans, in order to not only seek and destroy harbourage niches in the production zone, but also ensure efficient application of GHP, proper hygienic zoning and design of the equipment, with adequate cleaning protocols. Required by EU regulation 2073/2005, it is "only" a monitoring action of the situation: however, it provides a clear indication of the efficacy of implementation of the food safety management system. It should be the basis of a global preventative control plan. Like HACCP, it must be dynamically managed and regularly updated.

Funding

Experts are not paid for the time spent on this work; however, the non-industry members within the expert group were offered support for travel and accommodation costs from the Microbiological Food Safety Task Force to attend meetings to discuss the manuscript and a small compensatory sum (honorarium) with the option to decline.

Declaration of competing interest

No conflict of interest declared.

Acknowledgements

This work was conducted by an expert group of the European branch of the International Life Sciences Institute, ILSI Europe. The research question addressed in this publication and potential contributing experts in the field was identified by the Microbiological Food Safety Task Force. Members of this task force are listed on the ILSI Europe website at https://ilsi.eu/task-forces/food-safety/microbiological-food-safety/. According to ILSI Europe policies, the EG is composed by at least 50% of external non-industry members. The complete composition of the EG can be found at https://ilsi.eu/task-forces/food-safety/microbiological-food-safety/. Once the expert group was formed, the research project was handed over to them to independently refine the research question. Consequently, the expert group carried out the work, i.e. collecting/analysing data/information and writing the scientific paper independently of other activities of the task force. The research reported is the result of a scientific evaluation in line with ILSI Europe's framework to provide a precompetitive setting for public-private partnership. The authors would like to thank Dr Guerrino Macori (University College Dublin) for generating certain figures of the manuscript. ILSI Europe facilitated scientific meetings and coordinated the overall project management and administrative tasks relating to the completion of this work. For further information about ILSI Europe, please email info@ilsieurope.be or call +3227710014. The opinions expressed herein and the conclusions of this publication are those of the authors and do not necessarily represent the views of ILSI Europe nor those of its member companies, nor any regulatory authority. Experts are not paid for the time spent on this work; however, the non-industry members within the expert group were offered support for travel and accommodation costs from the Microbiological Food Safety Task Force to attend meetings to discuss the manuscript and a small compensatory sum (honorarium) with the option to decline.

References

Allard, M.W., Strain, E., Melka, D., Bunning, K., Musser, S.M., Brown, E.W., Timme, R., 2016. Practical value of food pathogen traceability through building a whole-genome sequencing network and database. J. Clin. Microbiol. 54, 1975–1983. https://doi.org/10.1128/JCM.00081-16.

Anvarian, A.H., Cao, Y., Srikumar, S., Fanning, S., Jordan, K., 2016. Flow cytometric and 16S sequencing methodologies for monitoring the physiological status of the microbiome in powdered infant formula production. Front. Microbiol. 7, 968. https://doi.org/10.3389/fmicb.2016.00968.

Belessi, C.-E.A., Gounadaki, A.S., Psomas, A.N., Skandamis, P.N., 2011. Efficiency of different sanitation methods on *Listeria monocytogenes* biofilms formed under various environmental conditions. Int. J. Food Microbiol. 145, S46–S52. https://doi.org/10.1016/j.ijfoodmicro.2010.10.020.

Bell, B.P., Goldoft, M., Griffin, P.M., Davis, M.A., Gordon, D.C., Tarr, P.I., Bartleson, C.A., Lewis, J.H., Barrett, T.J., Wells, J.G., 1994. A multistate outbreak of *Escherichia coli* O157: H7-associated bloody diarrheal and haemolytic uremic syndrome from hamburgers. JAMA 272, 1349–1353. https://doi.org/10.1001/jama.1994.03520170059036.

Beuchat, L., 1973. *Escherichia* coli on pecans: survival under various storage conditions and disinfection with propylene oxide. J. Food Sci. 38, 1063–1066. https://doi.org/10.1111/j.1365-2621.1973.tb02149.x.

Beuchat, L.R., Mann, D.A., 2014. Survival of *Salmonella* on dried fruits and in aqueous dried fruit homogenates as affected by temperature. J. Food Prot. 77, 1102–1109. https://doi.org/10.4315/0362-028X.JFP-13-549.

Beuchat, L.R., Kim, H., Gurtler, J.B., Lin, L.C., Ryu, J.H., Richards, G.M., 2009. *Cronobacter sakazakii* in foods and factors affecting its survival, growth, and inactivation. Int. J. Food Microbiol. 136, 204–213. https://doi.org/10.1016/j.ijfoodmicro.2009.02.029.

Beuchat, L.R., Komitopoulou, E., Beckers, H., Betts, R.P., Bourdichon, F., Fanning, S., Joosten, H.M., Ter Kuile, B.H., 2013. Low-water activity foods: increased concern as vehicles of foodborne pathogens. J. Food Prot. 76, 150–172. https://doi.org/10.4315/0362-028X.JFP-12-211.

Bortolaia, V., Kaas, R.S., Ruppe, E., Roberts, M.C., Schwarz, S., Cattoir, V., Philippon, A., Allesoe, R.L., Rebelo, A.R., Florensa, A.F., Fagelhauer, L., Chakraborty, T., Neumann, B., Werner, G., Bender, J.K., Stingl, K., Nguyen, M., Coppens, J., Xavier, B. B., Malhotra-Kumar, S., Westh, H., Pinholt, M., Anjum, M.F., Duggett, N.A., Kempf, I., Nykasenoja, S., Olkkola, S., Wieczorek, K., Amaro, A., Clemente, L., Mossong, J., Losch, S., Ragimbeau, C., Lund, O., Aarestrup, F.M., 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. J. Antimicrob. Chemother. 75, 3491–3500. https://doi.org/10.1093/jac/dkaa345.

Bowen, A., Wiesenfeld, H.C., Kloesz, J.L., Pasculle, A.W., Nowalk, A.J., Brink, L., Elliot, E., Martin, H., Tarr, C.L., 2017. Notes from the field: *Cronobacter sakazakii* infection associated with feeding extrinsically contaminated expressed human milk to a premature infant-Pennsylvania, 2016. Morb. Mortal. Wkly Rep. 66, 761. https://doi.org/10.15585/mmwr.mm6628a5.

Breeuwer, P., Lardeau, A., Peterz, M., Joosten, H.M., 2003. Desiccation and heat tolerance of *Enterobacter sakazakii*. J. Appl. Microbiol. 95, 967–973. https://doi.org/10.1046/j.1365-2672.2003.02067.x.

Brouard, C., Espié, E., Weill, F.X., Kérouanton, A., Brisabois, A., Forgue, A.M., Vaillant, V., de Valk, H., 2007. Two consecutive large outbreaks of *Salmonella enterica* serotype Agona infections in infants linked to the consumption of powdered infant formula. Pediatr. Infect. Dis. J. (2), 148–152. https://doi.org/10.1097/01.inf.0000253219.06258.23. 17259878.

Buchanan, R.L., Oni, R., 2012. Use of microbiological indicators for assessing hygiene controls for the manufacture of powdered infant formula. J. Food Prot. 75 (5), 989–997. https://doi.org/10.4315/0362-028X.JFP-11-532.

Buchanan, R.L., Gorris, L.G.M., Hayman, M.M., Jackson, T.C., Whiting, R.C., 2017. A review of *Listeria monocytogenes*: an update on outbreaks, virulence, dose-response, ecology, and risk assessments. Food Control 75, 1–13. https://doi.org/10.1016/j.foodcont.2016.12.016.

Burall, L.S., Grim, C.J., Datta, A.R., 2017. A clade of *Listeria monocytogenes* serotype 4b variant strains linked to recent listeriosis outbreaks associated with produce from a defined geographic region in the US. PLoS One 12, e0176912. https://doi.org/10.1371/journal.pone.0176912.

Cao, Y., Fanning, S., Proos, S., Jordan, K., Srikumar, S., 2017. A review on the applications of next generation sequencing technologies as applied to food-related microbiome studies. Front. Microbiol. 8, 1829. https://doi.org/10.3389/fmicb.2017.01829.

del Carmen Velázquez, L., Barbini, N.B., Escudero, M.E., Estrada, C.L., de Guzmán, A.M. S., 2009. Evaluation of chlorine, benzalkonium chloride and lactic acid as sanitizers for reducing *Escherichia coli* O157: H7 and *Yersinia enterocolitica* on fresh vegetables. Food Control 20, 262–268. https://doi.org/10.1016/j.foodcont.2008.05.012.

Carpentier, B., Cerf, O., 2011. Review - persistence of *Listeria monocytogenes* in food industry equipment and premises. Int. J. Food Microbiol. 145 (1), 1–8. https://doi.org/10.1016/j.ijfoodmicro.2011.01.005.

Caubilla-Barron, J., Forsythe, S., 2007. Dry stress and survival time of *Enterobacter sakazakii* and other *Enterobacteriaceae*. J. Food Prot. 70, 21110–22117. https://doi.org/10.4315/0362-028X-70.9.2111.

CDC. 2009. Multistate outbreak of *E. coli* O157:H7 infections linked to eating raw refrigerated, prepacked cookie dough.

CDC 2011. Multistate outbreak of *E. coli* O157:H7 infections associated with in-shell hazelnuts.

CDC, 2013. "Multistate outbreak of *Salmonella* Montevideo and *Salmonella* Mbandaka infections linked to tahini sesame paste (final update)".

CDC, 2014. "Multistate outbreak of *Salmonella* infections linked to organic sprouted chia powder (final update)".

CDC, 2016a. "Multistate outbreak of *Salmonella* Montevideo and *Salmonella* Senftenberg infections linked to wonderful pistachios (final update)".

CDC, 2016b. "Multistate outbreak of Shiga toxin-producing *Escherichia coli* infections linked to flour (final update)".

CDC, 2018a. "Multistate outbreak of *Salmonella* M'bandaka infections linked to Kellogg's honey smacks cereal (final update)".
CDC, 2018b. "Multistate outbreak of *Salmonella* Typhimurium infections linked to dried coconut (final update)".
CDC, 2019a. "Outbreak of *Salmonella* infections linked to Karawan brand tahini".
CDC, 2019b. "Outbreak of *Salmonella* infections linked to tahini from Achdut Ltd".
CDC, 2019c. Outbreak of *E. coli* infections linked to flour.
CDC, 2020. List of selected multistate foodborne outbreak investigations. https://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html.
Chavant, P., Gaillard-Martinie, B., Hebraud, M., 2004. Antimicrobial effects of sanitizers against planktonic and sessile *Listeria monocytogenes* cells according to the growth phase. FEMS Microbiol. Lett. 236, 241–248. https://doi.org/10.1111/j.1574-6968.2004.tb09653.x.
Chen, J., Wang, Y., 2020. Genetic determinants of *Salmonella enterica* critical for attachment and biofilm formation. Int. J. Food Microbiol. 320, 108524 https://doi.org/10.1016/j.ijfoodmicro.2020.108524.
Codex Alimentarius, 2008. Code of hygienic practice for powdered infant formulae for infants and young children. CAC/RCP, 66 (2008).
Codex Alimentarius, 2015. Code of hygiene practice for low-moisture foods. CXC 75–2015. Adopted in 2015. Revised in 2016. Amended in 2018.
Codex Alimentarius, 2020. General principles of food hygiene. CXC 1–1969. Adopted in 1969. Amended in 1999. Revised in 1997, 2003, 2020. Editorial corrections in 2011.
Cordier, J.L., 2007. Production of powdered infant formulae and microbiological control measures. Chapter 6. In: Farber, J.M., Forsythe, S.J. (Eds.), Emerging Issues in Food Safety: *Enterobacter sakazakii*. ASM Press, Washington DC, USA.
Craven, H., McAuley, C., Hannah, M., Fegan, N., Forsythe, S., 2020. Applicability of *Enterobacteriaceae* and coliforms tests as indicators for *Cronobacter* in milk powder factory environments. Food Microbiol. 94, 103642 https://doi.org/10.1016/j.fm.2020.103642.
Crowe, S.J., Bottichio, L., Shade, L.N., Whitney, B.M., Corral, N., Melius, B., Arends, K.D., Donovan, D., Stone, J., Allen, K., 2017. Shiga toxin–producing *E. coli* infections associated with flour. N. Engl. J. Med. 377, 2036–2043. https://doi.org/10.1056/NEJMoa1615910.
Crucello, A., Furtado, M.M., Chaves, M.D.R., Crucello, A., Furtado, M.M., Chaves, M.D.R., 2019. Transcriptome sequencing reveals genes and adaptation pathways in Salmonella Typhimurium inoculated in four low water activity foods. Food Microbiol 82, 426–435. https://www.sciencedirect.com/science/article/abs/pii/S0740002018308633?via%3Dihub.
Doyle, C.J., O'Toole, P.W., Cotter, P.D., 2017. Metagenome-based surveillance and diagnostic approaches to studying the microbial ecology of food production and processing environments. Environ. Microbiol. 19, 4382–4391. https://doi.org/10.1111/1462-2920.13859.
ECDC, EFSA, 2019. Joint ECDC–EFSA rapid outbreak assessment: multi-country outbreak of *Salmonella* Poona infections linked to consumption of infant formula. https://www.ecdc.europa.eu/sites/portal/files/documents/12-03-2019_ECDC-EFSA_ROA_UI-537_S_Poona.pdf. (Accessed 12 March 2019).
EFSA, 2005. Opinion of the scientific panel on biological hazards (BIOHAZ) on *Bacillus cereus* and other *Bacillus* spp. in foodstuffs. European Food Safety Authority. EFSA Journal 3 (4), 175. https://doi.org/10.2903/j.efsa.2005.175.
EFSA, 2016. Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs. https://doi.org/10.2903/j.efsa.2016.4524.
EFSA, 2019a. The European Union one health 2018 zoonoses report. EFSA J. 2019;17((12):5926), 1–276. https://doi.org/10.2903/j.efsa.2019.5926.
EFSA, Aerts, M., Battisti, A., Hendriksen, R., Kempf, I., Teale, C., Tenhagen, B.A., Veldman, K., Wasyl, D., Guerra, B., Liebana, E., Thomas-Lopez, D., Beloeil, P.A., 2019b. Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food. EFSA J. 17, e05709 https://doi.org/10.2903/j.efsa.2019.5709.
EFSA (European Food Safety Authority), Allende, A., Barre, L., Jacxsens, L., Liebana, E., Messens, W., Sarno, E., da Silva Felicio, M.T., 2018. Urgent scientific and technical assistance to provide recommendations for sampling and testing in the processing plants of frozen vegetables aiming at detecting *Listeria monocytogenes*. EFSA Supporting Publication 2018 (EN-1445), 41. https://doi.org/10.2903/sp.efsa.2018.EN-1445.
European Commission, 1993. Council directive 93/43/EEC of 14 June 1993 on the hygiene of foodstuffs.
European Commission, 2005. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (Text with EEA relevance).
FAO/WHO, 2002. Risk assessments of *Salmonella* in eggs and broiler chickens. In: Microbiological Risk Assessment Series 1. Available at. https://apps.who.int/iris/bitstream/handle/10665/42618/9291562307.pdf.
FAO/WHO, 2004. *Enterobacter sakazakii* and other micro-organisms in powdered infant formula: meeting report. In: Microbiological Risk Assessment Series, No. 6, p. 59. Rome, Italy. Also available at. ftp://ftp.fao.org/docrep/fao/007/y5502e/y5502e00.pdf.
FAO/WHO, 2006. *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: meeting report. In: Microbiological Risk Assessment Series, No. 10, p. 95. Rome, Italy. Also Available at. ftp://ftp.fao.org/docrep/fao/007/y5502e/y5502e00.pdf.
FAO/WHO, 2008. *Enterobacter sakazakii* (Cronobacter spp.) in powdered follow-up formulae: meeting report. Microbiological Risk Assessment Series 15. 2008. http://www.fao.org/3/a-a0707e.pdf. ISBN 978–92–5-106119-0. Accessed 2021-02-12.
Farber, J., Peterkin, P., 1991. *Listeria monocytogenes*, a food-borne pathogen. American Society of Microbiology 55, 476–511.
Farmer, J.J., Asbury, M.A., Hickman, F.W., Brenner, D.J., 1980. *Enterobacter sakazakii*: a new species of "*Enterobacteriaceae*" isolated from clinical specimens. Int. J. Syst. Evol. Microbiol. 30, 569–584. https://doi.org/10.1099/00207713-30-3-569.
FDA, 2020. Archive for recalls, market withdrawals & safety alerts. https://www.fda.gov/safety/recalls-market-withdrawals-safety-alert.
Feeney, A., Sleator, R.D., 2011. An in-silico analysis of osmotolerance in the emerging gastrointestinal pathogen *Cronobacter sakazakii*. Bioengineering Bugs 2, 260–270. https://doi.org/10.4161/bbug.2.5.17238.
Feeney, A., Kropp, K.A., O'Connor, R., Sleator, R.D., 2014. *Cronobacter sakazakii*: stress survival and virulence potential in an opportunistic foodborne pathogen. Gut Microbes 5 (6), 711–718. https://doi.org/10.4161/19490976.2014.983774.
Fernández-Gómez, P., López, M., Prieto, M., González-Raurich, M., Alvarez-Ordóñez, A., 2020. The role of the general stress response regulator RpoS in Cronobacter sakazakii biofilm formation. Food Res Int 136, 109508. https://doi.org/10.1016/j.foodres.2020.109508. Epub 2020 Jul 8. PMID: 32846586. https://europepmc.org/article/med/32846586.
Fett, W.F., Cooke, P.H., 2003. Reduction of *Escherichia coli* O157: H7 and *Salmonella* on laboratory-inoculated alfalfa seed with commercial citrus-related products. J. Food Prot. 66, 1158–1165. https://doi.org/10.4315/0362-028X-66.7.1158.
Finn, S., Condell, O., McClure, P., Amezquita, A., Fanning, S., 2013a. Mechanisms of survival, responses, and sources of *Salmonella* in low-moisture environments. Front. Microbiol. 4, 331. https://doi.org/10.3389/fmicb.2013.00331.
Finn, S., Handler, K., Condell, O., Colgan, A., Cooney, S., McClure, P., Amezquita, A., Hinton, J.C., Fanning, S., 2013b. ProP is required for the survival of desiccated *Salmonella* enterica serovar Typhimurium cells on a stainless steel surface. Appl. Environ. Microbiol. 79, 4376–4384. https://doi.org/10.1128/AEM.00515-13.
Forghani, F., Li, S., Zhang, S., Mann, D.A., Deng, X., den Bakker, H.C., Diez-Gonzalez, F., 2020. *Salmonella enterica* and *Escherichia coli* in wheat flour: detection and serotyping by a quasimetagenomic approach assisted by magnetic capture, multiple-displacement amplification, and real-time sequencing. Appl. Environ. Microbiol. 86 https://doi.org/10.1128/AEM.00097-20.
Forsythe, S.J., 2018. Updates on the *Cronobacter* genus. Annu. Rev. Food Sci. Technol. 9, 23–44. https://doi.org/10.1146/annurev-food-030117-012246.
Fougy, L., Desmonts, M.H., Coeuret, G., Fassel, C., Hamon, E., Hezard, B., Champomier-Verges, M.C., Chaillou, S., 2016. Reducing salt in raw pork sausages increases spoilage and correlates with reduced bacterial diversity. Appl. Environ. Microbiol. 82, 3928–3939. https://doi.org/10.1128/AEM.00323-16.
Frank, J.F., Koffi, R.A., 1990. Surface-adherent growth of *Listeria monocytogenes* is associated with increased resistance to sanitizers and heat. J. Food Prot. 53, 550–554. https://doi.org/10.4315/0362-028X-53.7.550.
FSANZ, 2004. *Bacillus Cereus* Limits in Infant Formula, Final Assessment Report, Application A454. https://www.foodstandards.gov.au/code/applications/documents/A454_B_cereus_FAR.pdf.
FSMA, 2015. Current good manufacturing practice, hazard analysis, and risk-based preventive controls for human food. https://www.federalregister.gov/documents/2015/09/17/2015-21920/current-good-manufacturing-practice-hazard-analysis-and-risk-based-preventive-controls-for-human.
Garces-Vega, F.J., Ryser, E.T., Marks, B.P., 2019. Relationships of water activity and moisture content to the thermal inactivation kinetics of *Salmonella* in low-moisture foods. J. Food Prot. 82, 963–970. https://doi.org/10.4315/0362-028X.JFP-18-549.
Glasset, B., Herbin, S., Guillier, L., Cadel-Six, S., Vignaud, M., Grout, J., Pairaud, S., Michel, V. Hennekinne, J., Ramarao, N., Brisabois; A., 2016. *Bacillus cereus*-induced food-borne outbreaks in France, 2007-2014: epidemiology and genetic characterization. Euro Surveillance 21 (48): 1–11. doi:https://doi.org/10.2807/1560-7917.ES.2016.21.48.30413.
Glass, K.A., Golden, M.C., Wanless, B.J., Bedale, W., Czuprynski, C., Kaufmann, S.H.E., 2015. Growth of Listeria monocytogenes within a Caramel-Coated Apple Microenvironment. mBio 6 (5), e01232-01215. https://doi.org/10.1128/mBio.01232-15. https://journals.asm.org/doi/full/10.1128/mBio.01232-15.
GMA, 2009a. Control of *Salmonella* in low-moisture foods guidance document. https://forms.consumerbrandsassociation.org/forms/store/ProductFormPublic/SalmonellaControlGuidance.
GMA, 2009b. Annex to control of *Salmonella* in low-moisture foods guidance document. https://forms.consumerbrandsassociation.org/forms/store/ProductFormPublic/SalmonellaControlGuidance-Annex.
GMA, 2014. Listeria monocytogenes Guidance on Environmental Monitoring and Corrective Actions in At-risk Foods. Available at: https://ucfoodsafety.ucdavis.edu/sites/g/files/dgvnsk7366/files/inline-files/208833.pdf.
Gray, M.L., Killinger, A.H., 1966. *Listeria monocytogenes* and listeric infections. Bacteriol. Rev. 30, 309–382.
Gruzdev, N., Pinto, R., Sela, S., 2011. Effect of desiccation on tolerance of *Salmonella enterica* to multiple stresses. Appl. Environ. Microbiol. 77, 1667–1673. https://doi.org/10.1128/AEM.02156-10.
Gruzdev, N., McClelland, M., Porwollik, S., Ofaim, S., Pinto, R., Saldinger-Sela, S., 2012. Global transcriptional analysis of dehydrated *Salmonella enterica* serovar Typhimurium. Appl. Environ. Microbiol. 78, 7866–7875. https://doi.org/10.1128/AEM.01822-12.
Harrand, A.S., Jagadeesan, B., Baert, L., Wiedmann, M., Orsi, R.H., 2020. Evolution of *Listeria monocytogenes* in a food processing plant involves limited single-nucleotide substitutions but considerable diversification by gain and loss of prophages. Appl. Environ. Microbiol. 86 (6), e02493–e02519. https://doi.org/10.1128/AEM.02493-19.
Hasani, M., Wu, F., Hu, K., Farber, J., Warriner, K., 2020. Inactivation of *Salmonella* and *Listeria monocytogenes* on dried fruit, pistachio nuts, cornflakes and chocolate crumb using a peracetic acid-ethanol based sanitizer or advanced oxidation process. Int. J. Food Microbiol. 333, 108789 https://doi.org/10.1016/j.ijfoodmicro.2020.108789.

Henry, M., Fouladkhah, A., 2019. Outbreak history, biofilm formation, and preventive measures for control of *Cronobacter sakazakii* in infant formula and infant care settings. Microorganisms 7, 77. https://doi.org/10.3390/microorganisms7030077.

Hokunan, H., Koyama, K., Hasegawa, M., Kawamura, S., Koseki, S., 2016. Survival Kinetics of Salmonella enterica and Enterohemorrhagic Escherichia coli on a Plastic Surface at Low Relative Humidity and on Low–Water Activity Foods. J Food Prot 79 (10), 1680–1692. https://doi.org/10.4315/0362-028X.JFP-16-081. https://meridian.allenpress.com/jfp/article-abstract/79/10/1680/174551/Survival-Kinetics-of-Salmonella-enterica-and?redirectedFrom=fulltext.

Hiramatsu, R., Matsumoto, M., Sakae, K., Miyazaki, Y., 2005. Ability of Shiga toxin-producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry foods. Appl. Environ. Microbiol. 71, 6657–6663. https://doi.org/10.1128/AEM.71.11.6657-6663.2005.

Hoper, D., Grutzke, J., Brinkmann, A., Mossong, J., Matamoros, S., Ellis, R.J., Deneke, C., Tausch, S.H., Cuesta, I., Monzon, S., Julia, M., Petersen, T.N., Hendriksen, R.S., Pamp, S.J., Leijon, M., Hakhverdyan, M., Walsh, A.M., Cotter, P.D., Chandrasekaran, L., Tay, M.Y.F., Schlundt, J., Sala, C., De Cesare, A., Nitsche, A., Beer, M., Wylezich, C., 2020. Proficiency testing of metagenomics-based detection of food-borne pathogens using a complex artificial sequencing dataset. Front. Microbiol. 11 (575377) https://doi.org/10.3389/fmicb.2020.575377.

ICMSF, 1989. Microorganisms in Foods 4: Application of The Hazard Analysis Critical Control Point (HACCP) System to Ensure Microbiological Safety and Quality. ISBN-13: 978–0632026517.

ICMSF, 2018. Microorganisms in Foods 7: Microbiological Testing in Food Safety Management. https://doi.org/10.1007/978-3-319-68460-4.

ILSI Europe, 2011. Persistence and survival of pathogens in dry foods and dry food processing environments. https://ilsi.eu/wp-content/uploads/sites/3/2016/06/Persistence-and-survival-report.pdf.

Innovation Center for US Dairy, 2019. Controlling pathogens in dairy processing environments, guidance for the US dairy industry. https://www.usdairy.com/getmedia/cdafaa12-e765-4432-820c-de5240e6c7ff/pathogen%20guidance_final.pdf.pdf.

Instituto de Salud Carlos III, 2011. Brote supracomunitario de gastroenteritis por *Salmonella* Poona en 2010-2011. Boletín epidemiológico semanal 19 (13), 176–194.

ISO 18593, 2018. Microbiology of the food chain - horizontal methods for surface sampling. https://www.iso.org/standard/64950.html.

Iversen, C., Lehner, A., Mullane, N., Bidlas, E., Cleenwerck, I., Marugg, J., Fanning, S., Stephan, R., Joosten, H., 2007. The taxonomy of *Enterobacter sakazakii*: proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov. *Cronobacter sakazakii* subsp. *sakazakii*, comb. nov., *Cronobacter sakazakii* subsp. *malonaticus* subsp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter* genomospecies 1. BMC Evol. Biol. 7, 64. https://doi.org/10.1186/1471-2148-7-64.

Iversen, C., Mullane, N., McCardell, B., Tall, B.D., Lehner, A., Fanning, S., Stephan, R., Joosten, H., 2008. *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii*, and proposal of *Cronobacter sakazakii* gen. nov., comb. nov., *Cronobacter malonaticus* sp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov., *Cronobacter* genomospecies 1, and of three subspecies, *Cronobacter dublinensis* subsp. *Dublinensis* subsp. nov., *Cronobacter dublinensis* subsp. *Lausannensis* subsp. nov. and *Cronobacter dublinensis* subsp. *Lactaridi* subsp. nov. Int. J. Syst. Evol. Microbiol. vol. 58 (Pt 6), 1442–1447. https://doi.org/10.1099/ijs.0.65577-0. 18523192.

Jacxsens, L., Kussaga, J., Luning, P.A., Van der Spiegel, M., Devlieghere, F., Uyttendaele, M., 2009. A microbial assessment scheme to measure microbial performance of food safety management systems. Int. J. Food Microbiol. 31 (134 (1–2)), 113–125. https://doi.org/10.1016/j.ijfoodmicro.2009.02.018.

Jagadeesan, B., Baert, L., Wiedmann, M., Orsi, R.H., 2019. Comparative analysis of tools and approaches for source tracking *Listeria monocytogenes* in a food facility using whole-genome sequence data. Front. Microbiol. 10, 947. https://doi.org/10.3389/fmicb.2019.00947.

Jameelah, M., Dewanti-Hariyadi, R., Nurjanah, S., 2018. Expression of rpoS, ompA and hfq genes of *Cronobacter sakazakii* strain Yrt2a during stress and viable but nonculturable state. Food Sci. Biotechnol. (2018) 27 (3), 915–920. https://doi.org/10.1007/s10068-018-0313-5.

Jaradat, Z.W., Al Mousa, W., Elbetieha, A., Al Nabulsi, A., Tall, B.D., 2014. *Cronobacter* spp.–opportunistic food-borne pathogens. A review of their virulence and environmental-adaptive traits. J. Med. Microbiol. 63 (8), 1023–1037. https://doi.org/10.1099/jmm.0.073742-0.

Jaykus, L.A., McClure, P.J., 2010. Introduction to Microbiological Indicators in the Food Industry. BioMérieux Industry Introductory Note book.

Jeong, S.G., Kang, D.H., 2017. Inactivation of *Escherichia coli* O157: H7, *Salmonella typhimurium*, and *Listeria monocytogenes* in ready-to-bake cookie dough by gamma and electron beam irradiation. Food Microbiol. 64, 172–178. https://doi.org/10.1016/j.fm.2016.12.017.

Jones, G., Pardos de la Gandara, M., Herrera-Leon, L., Herrera-Leon, S., Varela Martinez, C., Hureaux-Roy, R., Abdallah, Y., Nisavanh, A., Fabre, L., Renaudat, C., Mossong, J., Mattheus, W., Huard, C., Le Borgne, C., de Valk, H., Weill, F.X., Jourdan-Da Silva, N., 2019. Outbreak of *Salmonella* enterica serotype Poona in infants linked to persistent *Salmonella* contamination in an infant formula manufacturing facility, France, August 2018 to February 2019. Euro Surveillance 24 (13), 1900161. https://doi.org/10.2807/1560-7917.ES.2019.24.13.1900161.

Joseph, B., Otta, S., Karunasagar, I., Karunasagar, I., 2001. Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. Int. J. Food Microbiol. 64, 367–372. https://doi.org/10.1016/S0168-1605(00)00466-9.

Jourdan-da Silva, N., Fabre, L., Robinson, E., Fournet, N., Nisavanh, A., Bruyand, M., Mailles, A., Serre, E., Ravel, M., Guibert, V., Issenhuth-Jeanjean, S., Renaudat, C., Tourdjman, M., Septfons, A., de Valk, H., Le Hello, S., 2018. Ongoing nationwide outbreak of *Salmonella* Agona associated with internationally distributed infant milk products, France, December 2017. Euro Surveillance 23 (2), 17–00852. https://doi.org/10.2807/1560-7917.ES.2018.23.2.17-00852.

Kim, H., Ryu, J.H., Beuchat, L.R., 2007. Effectiveness of disinfectants in killing *Enterobacter sakazakii* in suspension, dried on the surface of stainless steel, and in a biofilm. Appl. Environ. Microbiol. 73 (4), 1256–1265. https://doi.org/10.1128/AEM.01766-06.

Kim, J.W., Siletzky, R.M., Kathariou, S., 2008. Host ranges of *Listeria*-specific bacteriophages from the turkey processing plant environment in the United States. Appl. Environ. Microbiol. 74, 6623–6630. https://doi.org/10.1128/AEM.01282-08.

Kovac, J., 2019. Precision food safety: a paradigm shift in detection and control of foodborne pathogens. mSystems 4 (3), e00164–e00219. https://doi.org/10.1128/mSystems.00164-19.

Kremer, P. H., Lees, J. A., Koopmans, M. M., Ferwerda, B., Arends, A. W., Feller, M. M., Schipper, K., Valls Seron, M., van der Ende, A., Brouwer, M. C., van de Beek, D., Bentley, S. D., 2017. Benzalkonium tolerance genes and outcome in *Listeria monocytogenes* meningitis. Clin. Microbiol. Infect. 23(4) 265.e1–265.e7 doi:https://doi.org/10.1016/j.cmi.2016.12.008.

Krometis, L.A., Characklis, G.W., Drummey, P.N., Sobsey, M.D., 2010. Comparison of the presence and partitioning behavior of indicator organisms and *Salmonella* spp. in an urban watershed. J. Water Health 8 (1), 44–59. https://doi.org/10.2166/wh.2009.032.

Kuijpers, A.F.A., Bonacic Marinovic, A.A., Wijnands, L.M., Delfgou-van Asch, E.H.M., van Hoek, A., Franz, E., Pielaat, A., 2018. Phenotypic prediction: linking in vitro virulence to the genomics of 59 *Salmonella enterica* strains. Front. Microbiol. 9, 3182. https://doi.org/10.3389/fmicb.2018.03182.

Kumari, S., Sarkar, P.K., 2016. *Bacillus cereus* hazard and control in industrial dairy processing environment. Food Control 69, 20–29. https://doi.org/10.1016/j.foodcont.2016.04.012.

Lamas, A., Regal, P., Vazquez, B., Miranda, J.M., Franco, C.M., Cepeda, A., 2019. Transcriptomics: a powerful tool to evaluate the behavior of foodborne pathogens in the food production chain. Food Res. Int. 125, 108543. https://doi.org/10.1016/j.foodres.2019.108543.

Lee, H., Kim, J.E., Chung, M.-S., Min, S.C., 2015. Cold plasma treatment for the microbiological safety of cabbage, lettuce, and dried figs. Food Microbiol. 51, 74–80. https://doi.org/10.1016/j.fm.2015.05.004.

Lee, S., Chen, Y., Gorski, L., Ward, T.J., Osborne, J., Kathariou, S., 2018. *Listeria monocytogenes* source distribution analysis indicates regional heterogeneity and ecological niche preference among serotype 4b clones. mBio 9 (2), 396. https://doi.org/10.1128/mBio.00396-18.

Lehner, A., Tall, B.D., Fanning, S., 2018. *Cronobacter* spp.-opportunistic foodborne pathogens: an update on evolution, osmotic adaptation and pathogenesis. Current Clinical Microbiology Reports 5, 97–105. https://doi.org/10.1007/s40588-018-0089-7.

Lenahan, M., Sheridan, A., Morris, D., Duffy, G., Fanning, S., Burgess, C.M., 2014. Transcriptomic analysis of triclosan-susceptible and-tolerant *Escherichia coli* O157: H19 in response to triclosan exposure. Microb. Drug Resist. 20, 91–103. https://doi.org/10.1089/mdr.2013.0063.

Lepuschitz, S., Ruppitsch, W., Pekard-Amenitsch, S., Forsythe, S.J., Cormican, M., 2019. Multicenter Study of Cronobacter sakazakii Infections in Humans, Europe, 2017. Emerging Infectious Diseases 25 (3), 515–522. https://doi.org/10.3201/eid2503.181652. https://wwwnc.cdc.gov/eid/article/25/3/18-1652_article.

Li, H., Bhaskara, A., Megalis, C., Tortorello, M.L., 2012. Transcriptomic analysis of *Salmonella* desiccation resistance. Foodborne Pathog. Dis. 9, 1143–1151. https://doi.org/10.1089/fpd.2012.1254.

Limcharoenchat, P., James, M.K., Marks, B.P., 2019. Survival and thermal resistance of *Salmonella enteritidis* PT 30 on almonds after long-term storage. J. Food Prot. 82, 194–199. https://doi.org/10.4315/0362-028X.JFP-18-152.

Ling, N., Forsythe, S., Wua, Q., Ding, Y., Zhang, J., Zeng, H., 2020. Insights into *Cronobacter sakazakii* biofilm formation and control strategies in the food industry. Engineering 6, 393–405. https://doi.org/10.1016/j.eng.2020.02.007.

Lomstein, B.A., Jørgensen, B.B., 2012. Pre-column liquid chromatographic determination of dipicolinic acid from bacterial endospores. Limnol. Oceanogr. Methods 10, 227–233. https://doi.org/10.4319/lom.2012.10.227.

Ly, V., Parreira, V.R., Farber, J.M., 2019. Current understanding and perspectives on *Listeria monocytogenes* in low-moisture foods. Curr. Opin. Food Sci. 26, 18–24. https://doi.org/10.1016/j.cofs.2019.02.012.

Ly, V., Parreira, V.R., Sanchez-Madonado, A.F., Farber, J.M., 2020. Survival and Virulence of Listeria monocytogenes during Storage on Chocolate Liquor, Corn Flakes, and Dry-Roasted Shelled Pistachios at 4 and 23°C. J Food Prot 83 (11), 1852–1862. https://doi.org/10.4315/JFP-20-129. https://meridian.allenpress.com/jfp/article/83/11/1852/438399/Survival-and-Virulence-of-Listeria-monocytogenes.

Mafu, A.A., Roy, D., Goulet, J., Savoie, L., Magny, P., 1990. Efficiency of sanitizing agents for destroying *Listeria monocytogenes* on contaminated surfaces. J. Dairy Sci. 73, 3428–3432. https://doi.org/10.3168/jds.S0022-0302(90)79040-6.

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

Maserati, A., Lourenco, A., Diez-Gonzalez, F., Fink, R.C., 2018. iTRAQ-based global proteomic analysis of *Salmonella enterica* serovar Typhimurium in response to desiccation, low water activity, and thermal treatment. Appl. Environ. Microbiol. 84, e00393–e00418. https://doi.org/10.1128/AEM.00393-18.

Maury, M.M., Tsai, Y.H., Charlier, C., Touchon, M., Chenal-Francisque, V., Leclercq, A., Lecuit, M., 2016. Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. Nat. Genet. 48 (3), 308–313. https://doi.org/10.1038/ng.3501.

McMullan, R., Menon, V., Beukers, A.G., Jensen, S.O., van Hal, S.J., Davis, R., 2018. *Cronobacter sakazakii* infection from expressed breast milk, Australia. Emerg. Infect. Dis. 24, 393–394. https://doi.org/10.3201/eid2402.171411.

Møretrø, T., Vestby, L., Nesse, L., Storheim, S., Kotlarz, K., Langsrud, S., 2009. Evaluation of efficacy of disinfectants against *Salmonella* from the feed industry. J. Appl. Microbiol. 106, 1005–1012. https://doi.org/10.1111/j.1365-2672.2008.04067.x.

Møretrø, T., Heir, E., Nesse, L.L., Vestby, L.K., Langsrud, S., 2012. Control of *Salmonella* in food related environments by chemical disinfection. Food Res. Int. 45, 532–544. https://doi.org/10.1016/j.foodres.2011.02.002.

Mossel D.A., Struijk, C.B., 1995. *Escherichia coli*, otras *Enterobacteriaceae* e indicadores adicionales como marcadores de la calidad microbiológica de los alimentos: ventajas y limitaciones [*Escherichia coli*, other *Enterobacteriaceae* and additional indicators as markers of microbiologic quality of food: advantages and limitations]. Microbiologia. 11(1):75–90. PMID: 7546448.

Nazarowec-White, M., Farber, J.M., 1997. *Enterobacter sakazakii*: a review. Int. J. Food Microbiol. 34 (2), 103–113. https://doi.org/10.1016/S0168-1605(96)01172-5.

Negrete, F., Jang, H., Gangiredla, J., Woo, J., Lee, Y.Y., Patel, I.R., Chase, H.R., Finkelstein, S., Wang, C.Z., Srikumar, S., Nguyen, S., Eshwar, A., Stephan, R., Lehner, A., Fanning, S., Tall, B.D., Gopinath, G.R., 2019. Genome-wide survey of efflux pump-coding genes associated with *Cronobacter* survival, osmotic adaptation, and persistence. Curr. Opin. Food Sci. 30, 32–42. https://doi.org/10.1016/j.cofs.2018.11.005.

Njage, P.M.K., Leekitcharoenphon, P., Hansen, L.T., Hendriksen, R.S., Faes, C., Aerts, M., Hald, T., 2020. Quantitative microbial risk assessment based on whole genome sequencing data: case of *Listeria monocytogenes*. Microorganisms 8 (11), 1772. https://doi.org/10.3390/microorganisms8111772.

Osaili, T., Forsythe, S., 2009. Desiccation resistance and persistence of *Cronobacter* species in infant formula. Int. J. Food Microbiol. 136, 214–220. https://doi.org/10.1016/j.ijfoodmicro.2009.08.006.

Ostroff, S.M., Griffin, P.M., Tauxe, R.V., Shipman, L.D., Greene, K.D., Welles, J.O.Y.G., Lewis, J.A.Y.H., Blake, P.A., Kobayashi, J.M., 1990. A state-wide outbreak of *Escherichia coli* 0157: H7 infections in Washington state. Am. J. Epidemiol. 132, 239–247. https://doi.org/10.1093/oxfordjournals.aje.a115653.

Park, E.J., Alexander, E., Taylor, G.A., Costa, R., Kang, D.H., 2009. The decontaminative effects of acidic electrolyzed water for *Escherichia coli* O157: H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on green onions and tomatoes with differing organic demands. Food Microbiol. 26, 386–390. https://doi.org/10.1016/j.fm.2008.10.013.

Parra-Flores, J., Cerda-Leal, F., Contreras, A., Valenzuela-Riffo, N., Rodríguez, A., Aguirre, J., 2018. *Cronobacter sakazakii* and microbiological parameters in dairy formulas associated with a food alert in Chile. Front. Microbiol. https://doi.org/10.3389/fmicb.2018.01708.

Podolak, R., Enache, E., Stone, W., Black, D.G., Elliott, P.H., 2010. Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. J. Food Prot. 73 (10), 1919–1936. https://doi.org/10.4315/0362-028x-73.10.1919.

Pouillot, R., Hoelzer, K., Chen, Y., Dennis, S., 2015. *Listeria monocytogenes* dose response revisited-incorporating adjustments for variability in strain virulence and host susceptibility. Risk Anal. 35, 90–108. https://doi.org/10.1111/risa.12235.

Ramesh, N., Joseph, S., Carr, L., Douglass, L., Wheaton, F., 2002. Evaluation of chemical disinfectants for the elimination of *Salmonella* biofilms from poultry transport containers. Poult. Sci. 81, 904–910. https://doi.org/10.1093/ps/81.6.904.

RASFF, 2020. Notifications list, 2012–2020. https://webgate.ec.europa.eu/rasff-window/portal/?event=notificationsList&StartRow=1.

Richter-Dahlfors, A., Buchan, A.M., Finlay, B.B., 1997. Murine salmonellosis studied by confocal microscopy: *Salmonella* typhimurium resides intracellularly inside macrophages and exerts a cytotoxic effect on phagocytes in vivo. J. Exp. Med. 186, 569–580. https://doi.org/10.1084/jem.186.4.569.

Riedel, K., Lehner, A., 2007. Identification of proteins involved in osmotic stress response in *Enterobacter sakazakii* by proteomics. Proteomics 7, 1217–1231. https://doi.org/10.1002/pmic.200600536.

Rodríguez-Campos, D., Rodríguez-Melcón, C., Alonso-Calleja, C., Capita, R., 2019. Persistent Listeria monocytogenes Isolates from a Poultry-Processing Facility Form more Biofilm but Do Not Have a Greater Resistance to Disinfectants Than Sporadic Strains. Pathogens 8 (4). https://doi.org/10.3390/pathogens8040250. PMID: 31756896; PMCID: PMC6963312. https://www.mdpi.com/2076-0817/8/4/250.

Ronholm, J., Nasheri, N., Petronella, N., Pagotto, F., 2016. Navigating microbiological food safety in the era of whole-genome sequencing. Clin. Microbiol. Rev. 29 (4), 837–857. https://doi.org/10.1128/CMR.00056-16.

Russo, E.T., Biggerstaff, G., Hoekstra, R.M., Meyer, S., Patel, N., Miller, B., Quick, R., Salmonella Agona Outbreak Investigation Team, 2013. A recurrent, multistate outbreak of *Salmonella* serotype Agona infections associated with dry, unsweetened cereal consumption, United States, 2008. J. Food Prot. 76 (2), 227–230. https://doi.org/10.4315/0362-028X.JFP-12-209.

Ryu, J.H., Beuchat, L.R., 2005. Biofilm formation and sporulation by *Bacillus cereus* on a stainless-steel surface and subsequent resistance of vegetative cells and spores to chlorine, chlorine dioxide, and a peroxyacetic acid-based sanitizer. J. Food Prot. 68 (12), 2614–2622. https://doi.org/10.4315/0362-028x-68.12.2614. 16355833.

Schmidt, S. J. and Fontana Jr., A. J., 2020. Water activity values of select food ingredients and products. Appendix E. Water Activity in Foods: Fundamentals and Applications, Second Edition. https://onlinelibrary.wiley.com/doi/book/10.1002/9781118765982, John Wiley & Sons, Inc.

Scott, V.N., Chen, Y., Freier, T.A., Kuehm, J., Moorman, M., Meyer, J., Morille-Hinds, T., Post, L., Smoot, L., Hood, S., 2009. Control of *Salmonella* in low-moisture foods I: minimizing entry of Salmonella into a processing facility. Food Prot. Trends 29, 342–353.

Setikaite, I., Koutchma, T., Patazca, E., Parisi, B., 2009. Effects of water activity in model systems on high-pressure inactivation of *Escherichia coli*. Food Bioprocess Technol. 2, 213–221. https://doi.org/10.1007/s11947-008-0069-7.

Shangpliang, H.N.J., Rai, R., Keisam, S., Jeyaram, K., Tamang, J.P., 2018. Bacterial community in naturally fermented milk products of Arunachal Pradesh and Sikkim of India analysed by high-throughput amplicon sequencing. Sci. Rep. 8 (1), 1532 https://doi.org/10.1038/s41598-018-19524-6.

Sheridan, A., Lenahan, M., Condell, O., Bonilla-Santiago, R., Sergeant, K., Renaut, J., Duffy, G., Fanning, S., Nally, J., Burgess, C., 2013. Proteomic and phenotypic analysis of triclosan tolerant verocytotoxigenic *Escherichia coli* O157: H19. J. Proteome 80, 78–90. https://doi.org/10.1016/j.jprot.2012.12.025.

Singleton, P. and Sainsbury, D., 2001.Dictionary Of Microbiology & Molecular Biology (3rd ed.), Wiley, New York, p. 392.

Srikumar, S., Cao, Y., Yan, Q., Van Hoorde, K., Nguyen, S., Cooney, S., Gopinath, G.R., Tall, B.D., Sivasankaran, S.K., Lehner, A., Stephan, R., Fanning, S., 2019. RNA sequencing-based transcriptional overview of xerotolerance in *Cronobacter sakazakii* SP291. Appl. Environ. Microbiol. 85 (3), e01993–e02018. https://doi.org/10.1128/AEM.01993-18.

Stopforth, J.D., Samelis, J., Sofos, J.N., Kendall, P.A., Smith, G.C., 2002. Biofilm formation by acid-adapted and non-adapted *Listeria monocytogenes* in fresh beef decontamination washings and its subsequent inactivation with sanitizers. J. Food Prot. 65, 1717–1727. https://doi.org/10.4315/0362-028X-65.11.1717.

Strysko, J., Cope, J.R., Martin, H., Tarr, C., Hise, K., Collier, S., Bowen, A., 2020. Food safety and invasive *Cronobacter* infections during early infancy, 1961-2018. Emerg. Infect. Dis. 26, 857–865. https://doi.org/10.3201/eid2605.190858.

Sundararajan, M., Enane, L.A., Kidwell, L.A., et al., 2018. Notes from the Field: Cronobacter sakazakii Meningitis in a Full-Term Neonate Fed Exclusively with Breast Milk — Indiana, 2018. MMWR Morb Mortal Wkly Rep 67, 1248–1249. https://doi.org/10.15585/mmwr.mm6744a7. https://www.cdc.gov/mmwr/volumes/67/wr/mm6744a7.htm#suggestedcitation.

Taylor, M.H., Tsai, H., Rasco, B., Tang, J., Zhu, M., 2018. Stability of *Listeria monocytogenes* in wheat flour during extended storage and isothermal treatment. Food Control 91, 434–439. https://doi.org/10.1016/j.foodcont.2018.04.008.

Taylor, M.H., Quinna, A.R., Kataokab, A., 2019. *Listeria monocytogenes* in low-moisture foods and ingredients. Food Control 103, 153–160. https://doi.org/10.1016/j.foodcont.2019.04.011.

Tilden Jr., J., Young, W., McNamara, A.M., Custer, C., Boesel, B., Lambert-Fair, M.A., Majkowski, J., Vugia, D., Werner, S., Hollingsworth, J., 1996. A new route of transmission for *Escherichia coli*: infection from dry fermented salami. Am. J. Public Health 86, 1142–1145. https://doi.org/10.2105/AJPH.86.8_Pt_1.1142.

Tolar, B., Joseph, L.A., Schroeder, M.N., Stroika, S., Ribot, E.M., Hise, K.B., Gerner-Smidt, P., 2019. An overview of PulseNet USA databases. Foodborne Pathog. Dis. 16, 457–462. https://doi.org/10.1089/fpd.2019.2637.

Tompkin, R.B., 2002. Control of *Listeria monocytogenes* in the food-processing environment. J. Food Prot. 65 (4), 709–725. https://doi.org/10.4315/0362-028x-65.4.709.

Tortorello, M.L., 2003. Indicator organisms for safety and quality-uses and methods for detection: minireview. J. AOAC Int. 86, 1208–1217. https://doi.org/10.1093/jaoac/86.6.1208.

Wells, J., Shipman, L., Greene, K., Sowers, E., Green, J., Cameron, D., Downes, F., Martin, M., Griffin, P., Ostroff, S., 1991. Isolation of *Escherichia coli* serotype O157: H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J. Clin. Microbiol. 29, 985–989. https://doi.org/10.1128/JCM.29.5.985-989.1991.

Yan, Q., Power, K.A., Cooney, S., Fox, E., Gopinath, G.R., Grim, C.J., Tall, B.D., McCusker, M.P., Fanning, S., 2013. Complete genome sequence and phenotype microarray analysis of *Cronobacter sakazakii* SP291: a persistent isolate cultured from a powdered infant formula production facility. Front. Microbiol. 4 (256) https://doi.org/10.3389/fmicb.2013.00256.

Yrlid, U., Svensson, M., Hakansson, A., Chambers, B.J., Ljunggren, H.G., Wick, M.J., 2001. In vivo activation of dendritic cells and T cells during *Salmonella enterica* serovar Typhimurium infection. Infect. Immun. 69, 5726–5735. https://doi.org/10.1128/IAI.69.9.5726-5735.2001.

Zhang, S., Yin, Y., Jones, M.B., Zhang, Z., Deatherage Kaiser, B.L., Dinsmore, B.A., Fitzgerald, C., Fields, P.I., Deng, X., 2015. *Salmonella* serotype determination utilizing high-throughput genome sequencing data. J. Clin. Microbiol. 53, 1685–1692. https://doi.org/10.1128/JCM.00323-15.

Zoellner, C., Ceres, K., Ghezzi-Kopel, K., Wiedmann, M., Ivanek, R., 2018. Design elements of *Listeria* environmental monitoring programs in food processing facilities: a scoping review of research and guidance materials. Compr. Rev. Food Sci. Food Saf. 17, 1157–1171. https://doi.org/10.1111/1541-4337.12366.

Zwietering, M.H., Garre, A., Wiedmann, M., Buchanan, R.L., 2021. All food processes have a residual risk, some are small, some very small and some are extremely small: zero risk does not exist. Curr. Opin. Food Sci. (39), 83–92. https://doi.org/10.1016/j.cofs.2020.12.017.