Environmental monitoring program to support food microbiological safety and quality in food industries: A scoping review of the research and guidelines

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ABSTRACT

Background: Food safety and quality can be compromised by microbiological contamination caused by a variety of pathogenic and spoilage microorganisms present in the production environment. The combination of monitoring both food products and the production environment is a lever to increase food safety and quality. Environmental monitoring programs (EMPs) implemented in food industries allow evaluating the clout of the microbial controls in food processing plants.

Scope and approach: The aim of the present review is to systematically assess, using the Scoping review and PRISMA method, available information and strategies to build efficient EMP in the food industry.

Key findings and conclusions: Despite the available literature on the implementation of EMPs, there is to date no ready-to-use method and its application strongly depends on the characteristics of the processing plant. A common three-step approach has been proposed for the construction of EMP, whatever the food sector including a pre-analytical, an analytical and a post-analytical step. The pre-analytical step aims to design strategies for the implementation of efficient EMP, considering the hazards and the risk associated with food product and food plant. The analytical step consists of sampling stages using cultural or molecular approaches. Finally, the post-analytical step, concerns the management of data collected. EMPs are dynamic programs that undergo change over time and must be updated on a regular basis in order to guarantee their fit for purpose.

1. Introduction

Each year worldwide, unsafe food causes 600 million cases of foodborne diseases and 420 000 deaths, as estimated in 2015 study. Thirty-one foodborne hazards caused 32 diseases, including 11 diarrheal disease agents, 7 invasive infectious disease agents, 10 helminths and 3 chemicals (WHO, 2015). In addition, food contaminated by pathogenic and spoilage bacteria are a source of major economic impacts due to recall, loss of product, investigation to identify the source of contamination, costs of increased insurance and loss of consumer confidence in product and brand (Zacharski, Southern, Ryan, & Adley, 2018). The microbial contamination of the product can occur at each step of the food chain. Processing environments must be considered as a serious source of contamination, either due to ineffective cleaning and disinfection procedures or due to contamination during production.

Developing environmental monitoring programs (EMPs) is a strategy to improve food safety. It is defined as a monitoring program to check cleaning-sanitation procedures, and other environmental pathogen control programs with a range of sampling analysis, in order to prevent environmental contamination of the finished product (3M & Cornell University, 2019). EMP may be considered as a pre-requisite under the food safety programs (Zacharski et al., 2018). This proactive approach (FIL/IDF, 2020) can be used as an early warning indicator, combined with end-product controls, to prevent food contamination. Indeed, testing only end-product might not be sufficient to guarantee its safety, because a negative result of a microorganism presence does not mean its absence in the whole production and can rarely detect sporadic environmental contaminations (ANSES, 2020; FIL/IDF, 2020). Therefore, an

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efficient EMP, with repeated environmental sampling, and a controlled and validated process will be more trustworthy than an end-product control alone (Muhterem-Uyar et al., 2015; UFPA, 2013).

The importance of food processing environment as a reservoir of microbial contamination have been highlighted by foodborne outbreaks from the past. In Finland, a listeriosis outbreak associated with butter consumption, between 1998 and 1999, was attributed to contamination by *Listeria monocytogenes* from the processing plant environment and more precisely from screw conveyor in the butter wagon after pasteurization (Lyytikäinen et al., 2000). In Canada, in 2008, an outbreak due to ready-to-eat (RTE) deli meat, was associated to contamination from the slicer (Simmons & Wiedmann, 2018). More recently, powdered infant formula contamination by *Salmonella* Agona in France was suspected to be associated with a drying tower (ANSES, 2020). The largest listeriosis outbreak to date was described in South Africa in 2017. An RTE, processed meat product called polony, was identified as the source of the outbreak. *L. monocytogenes* strain was found in the production environment from a single factory (Thomas et al., 2020). Consequently, outbreaks due to *L. monocytogenes* and also product recalls were often associated with the environment and equipment from food processing facilities (Zoellner, Ceres, Ghezzi-Kopel, Wiedmann, &), mainly due to poor hygiene (Zacharski et al., 2018). A study from French foodborne illnesses outbreak in 1998 has reported that 40% of the contamination cases were associated with equipment contamination (Cappitelli, Polo, & Villa, 2014). However, the attributable fraction of food contamination by environmental conditions remains difficult to estimate, although studies are emerging to estimate the sources of attribution of foodborne infectious diseases (ANSES, 2017, 2018).

Regulations in matters of food safety have been reinforced by including EMP in the food safety and hygiene programs, in Canada in 2004, European Union in 2005, New Zealand in 2006 and 2020 and in the United States of America in 2011 (FIL/IDF, 2020). As an example, in Europe, regulation 2073/2005 (Commission of the European Communities, 2005), requires the monitoring of the presence of pathogenic bacteria in a processing environment. This concerns *L. monocytogenes* in RTE foods and the presence of *Cronobacter* spp. in powdered infant formulae or powdered foods for special medical purposes intended for infants under six months of age. However, the thresholds for the microbiological criteria are only specified for foodstuffs but not for food processing environment (Commission of the European Communities, 2005). The importance of the EMPs is now considered by risk managers and regulators as some countries are setting laws to regulate it, as reflected for example by the French law No. 2018/938 for balanced trade relations in the agricultural and food sector and healthy, sustainable and accessible food for all. It requires food business manufacturers to report to the competent authority any auto-control positive contamination result of the production environment that could lead to potentially harmful health effect associated with food consumption (Journal Officiel de la République Française, 2018).

In order to underpin the implementation of EMPs, some guidelines are available (3M & Cornell University, 2019; Almond Board of California, unknown; FDA, 2017; FSPCA, 2015; GMA, 2009, 2018; National Fisheries Institute, 2018; UFPA, 2013) as well as standards (e.g. EN 17141:2020, 18593:2018). However, the information about method is scattered and its application strongly depends on the characteristics of the processing plant. As a result, there is a need of information about common method applicable to several food sectors in order to give leads for building the EMP. A scoping review was performed to design elements of monitoring programs in food processing facilities, by mapping the key concepts underpinning EMPs. This review allowed to identify, analyses and synthesize the information available in the literature and to help to elaborate common strategies on EMP to support food manufacturing industries.

2. Methods

2.1. Search question and definitions

The review was conducted largely inspired on from the scoping study framework (Arksey & O'Malley, 2005) and PRISMA (Moher, Liberati, Tetzlaff, Altman, & The, 2009). The scoping review aims to answer the following question "How to support microbial food safety and quality in food industries using environmental monitoring, considering current knowledge and practices?"

Food processing facilities and food plants corresponded to structures where food products are processed, packaged, or stored. Food Processing Environment (FPE) is defined here as any element of food plants which could be into contact with the food product or being likely to represent a source of microbial contamination or recontamination. For example, equipment, walls, premises or operators (NF EN ISO 18593:2018). Monitoring is defined as the implementation of a programmed series of observations or measurements to assess whether the food safety control measures are working as intended (NF V01–002:2005). This study focusses on the monitoring of solid surfaces in the FPE. Therefore, microbiological monitoring of fluids (e.g. ambient air, utility water, etc.) and food products has been excluded. Sampling programs are plans designed to assess the levels of microbial contamination of FPE surfaces in order to implement corrective measures to reduce the risks of contamination of food by microorganisms. Sampling area designed the specific sampling point localization where a surface sample is collected by specific tools (e.g. swab, wipes, etc.) to give information about the components present on the surface.

2.2. Database sources and keys words

The literature search was conducted on 10 July 2020 to collect exhaustively available research and reviews dealing with environmental monitoring plans and strategies used by food processing industries. Legislation and guidelines and additional articles were identified from 4 May 2020 to April 2021. The search was performed on different databases: Scopus, Web of Science and Pubmed. For each database, the following search queries were used:

2.2.1. *Scopus*

TITLE ((control* OR monitor* OR surveillance*)) AND TITLE (facilit* OR factor* OR

plant* OR industr* OR process* OR manufact*) AND ALL (food) AND.

TITLE-ABS-KEY (microbi* OR biolog* OR patho* OR bacter*) AND TITLE (environment* OR surface*)).

2.2.2. *Web of science*

TI=(control* OR monitor* OR surveillance*) AND.

TI=(facilit* OR factor* OR plant* OR industr* OR process* OR manufact*) AND ALL=(food)

AND (TI=(microbi* OR biolog* OR patho* OR bacter*) OR.

AB=(microbi* OR biolog* OR patho* OR bacter*) OR.

AK=(microbi* OR biolog* OR patho* OR bacter*) OR.

KP=(microbi* OR biolog* OR patho* OR bacter*)) AND TI=(environment* OR surface*)

2.2.3. *Pubmed*

((((control* [Title] OR monitor* [Title] OR surveillance*[Title]) AND (facilit* [Title] OR factor* [Title] OR plant* [Title] OR industr* [Title] OR process* [Title] OR manufact*[Title])) AND (food)) AND (microbi* [Title/Abstract] OR biolog* [Title/Abstract] OR patho* [Title/Abstract] OR bacter*[Title/Abstract])) AND (environment* [Title] OR surface*[Title])

The grey literature was also searched in governmental and industrial websites, Google and Google scholar search. Citations retrieved were

imported and filtered with Endnote software (version X8.2).

2.3. Inclusion and exclusion criteria

The term "environmental" refers to everything that surrounds the product during its production. Therefore, articles on ecology, sustainable development and other fields were excluded. All fluids such as air and water as well as any technological performance testing and surfaces of the food products were excluded as they were out of scope (only solid surfaces). Only biological hazards, including viruses, parasites, bacteria, yeast and moulds, were considered. Therefore chemical, physical and allergenic contaminations were excluded. Field of agri-food, and more specifically food processing industries were included whereas fields like, livestock, agriculture, pharmaceutical industry were excluded. Only food processing environments were included while studies conducted at laboratory, home or collective kitchen, farm were excluded. Finally, articles in English, French, Portuguese or Spanish were included.

2.4. Screening

The different screening steps were performed by a single examiner. First, duplicates from different sources of the search were removed, by title and authors name screening. Secondly, the selection considering the titles and the abstracts were carried out considering the inclusion and exclusion criteria indicated above. References without full text available were excluded. Finally, full text was screened considering the same inclusion and exclusion criteria (Fig. 1).

2.5. Data charting and thematic categorization

In order to extract pertinent the information of each the articles in a structured way, a standard template was developed. This later included several sections such as the year of publication, the country, and the type of document (e.g. research paper, review, etc.), the goal of the study, the food sector, the biological organism, the sampling technique, the sampling area, the frequency of the sampling, the analytical method and so on.

3. Results and discussion

3.1. Literature research characteristics

A total of 290 references were identified from 3 databases and other sources. 205 records were listed after de-duplicates removal and 69 relevant studies were selected considering exclusion and inclusion criteria, relevance and text availability (Fig. 1). All the records selected in the study are listed in the references (69 papers plus 2 articles about scoping review (Arksey & O'Malley, 2005) and PRISMA (Moher et al., 2009) methods). The majority of publications considered in this review were recent (Fig. 2), with 40 publications from 2015 to 2021. Most of the publications came from research papers (38%), reviews (22%) and guidelines (19%), followed by book or book chapters (10%), legal rule or regulation (4%), reports (4%), web pages (1%) and symposiums (1%). Most of the publications selected in the critical review were not specific to a single biological hazard (n = 33). *Listeria* spp. was cited in 28 publications followed by *Salmonella* spp. (n = 7) and *Cronobacter* spp. (n = 3) (Fig. 3). The majority of publications were not dedicated to a specific type of food (n = 36) and when mentioned, it mainly concerned dairy products (n = 12), RTE products (n = 11) and meat (n = 10), followed by fish and seafood products (n = 6) (Fig. 4).

After analysis of the documents and the background knowledge, we propose to divide EMP in three stages:

(1) Pre-analytical step: design and programming,
(2) Analytical step: detection and quantification analysis and
(3) Post analytical step: data processing and decision-making support (Fig. 5).

3.2. Pre-analytical step: designing and programming an EMP

3.2.1. Purpose of EMPs

It is essential to first define the goal of the EMP as an early warning

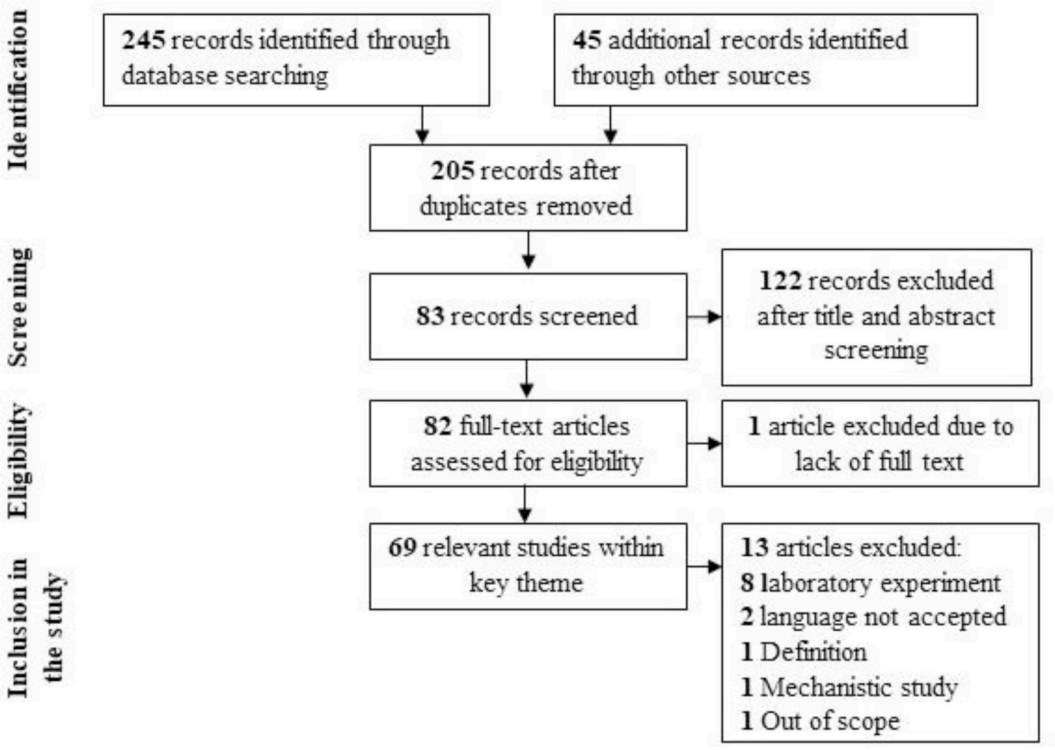

Fig. 1. Framework for searching relevant publications for the comprehensive review.

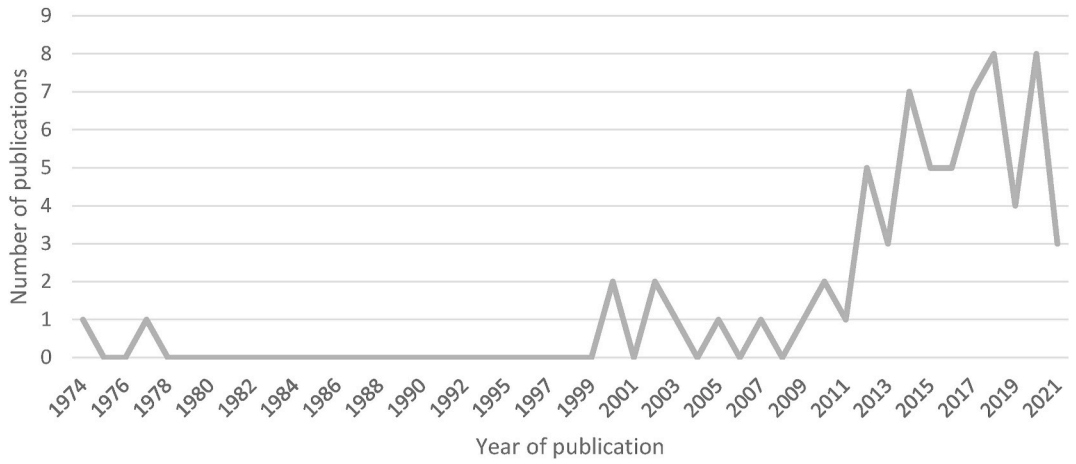

Fig. 2. Number of publications per year included in the review. One publication has not indicate the date of publication.

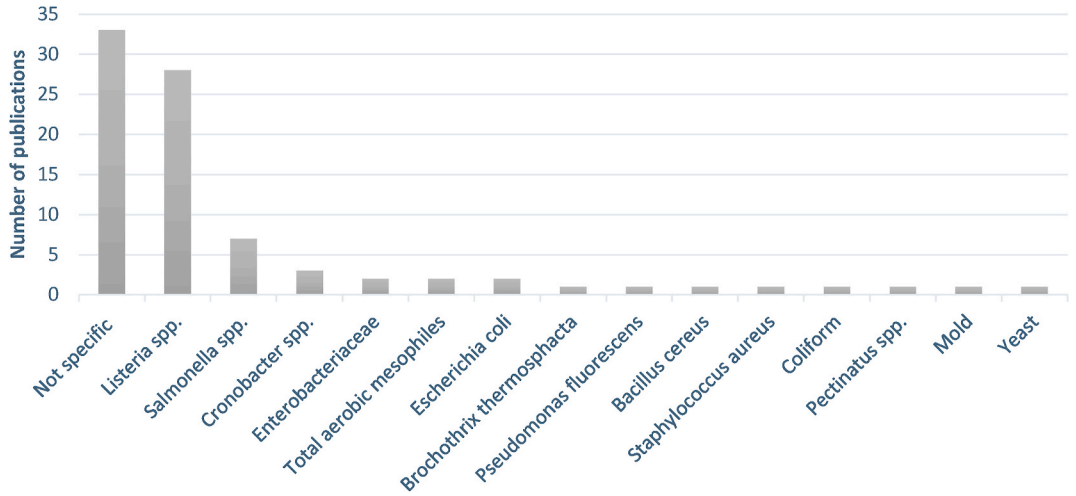

Fig. 3. Number of biohazards identified in the publications included in the review. The sum of the count is over the number the number of the articles included in the reviews because some articles included more than one biohazard.

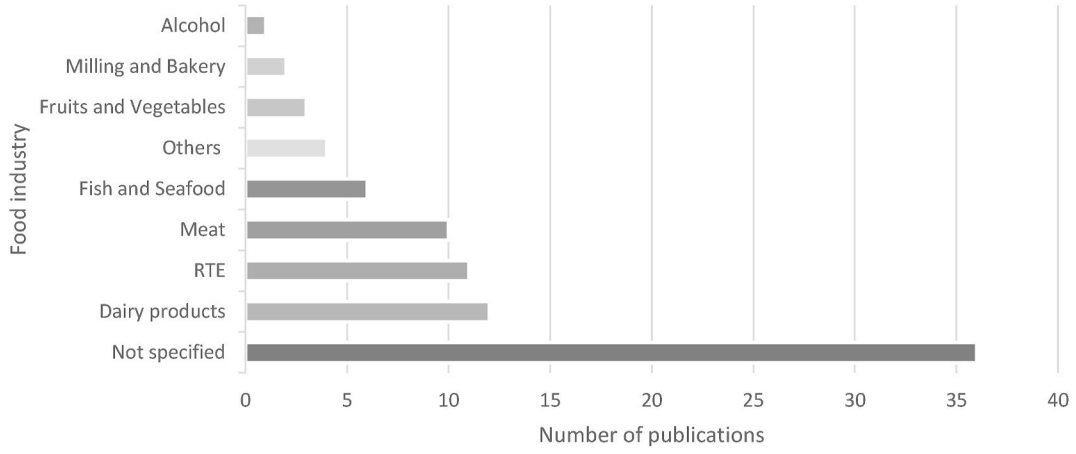

Fig. 4. Types of food industries identified from the publications included in the review expressed in numbers. The sum of the count is over the number the number of the articles included in the reviews because some articles included more than one type of food.

system for the prevention of foodborne microbiological risks (Zacharski et al., 2018). Four goals of an efficient EMP are to (1) determine the efficiency of the cleaning and disinfection procedures, (2) to identify and monitor the presence of specific pathogens, whether persistent or transient, (3) to increase knowledge about the microbial ecology in food plants and (4) to identify potential sources of contaminations (DeVault, 2018; Spanu & Jordan, 2020).

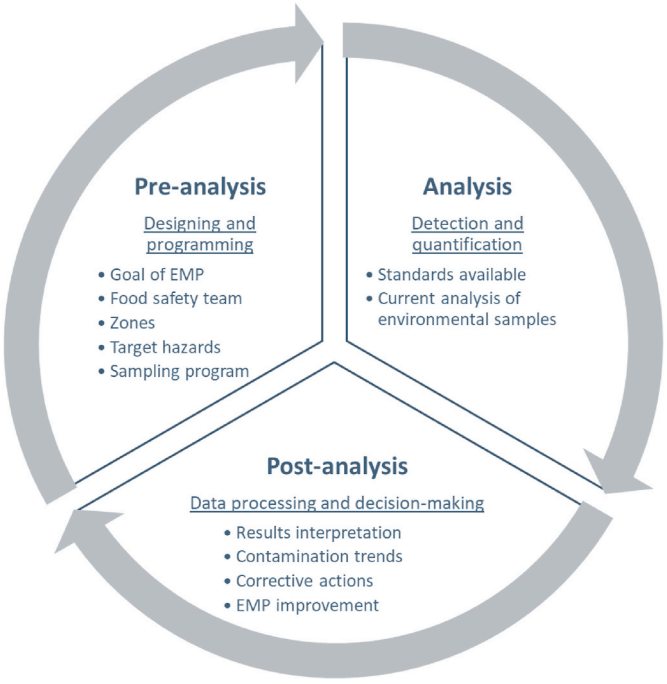

Fig. 5. Identified steps of Environmental Monitoring Programs.

3.2.2. Cross-functional food safety team

To establish an effective EMP, there is a need to set up a cross-functional team trained in food safety. This team must be familiar with the operational procedure and able to identify relevant microorganisms likely to be present in facilities and locations with a high risk of food contamination (Channaiah, 2013; Spanu & Jordan, 2020). This food safety team can include the plant quality manager, the line supervisor or operators, sanitation supervisors and microbiologist experts (Channaiah, 2013; Spanu & Jordan, 2020). The food safety team must be able to estimate the risk level according to the area in the facility, the potential microbiological hazards of interest, where to sample, how to sample, the frequency, the samples analysis and how to manage the results.

Finally, adequate financial and human resources must be provided to design an efficient and adapted EMP.

3.2.3. Relevant microorganisms to be monitored

Targeted microorganisms can be classified in five groups: pathogens, index, surrogates, indicators, and spoilers. *L. monocytogenes* is the most cited pathogen in the literature related to environmental monitoring, mainly in ready to eat foods. This psychrotrophic microorganism is able to grow and survive at low temperatures, and it was shown that the contamination source is more frequently from food processing environment than raw material (Jones, Ricke, Keith Roper, & Gibson, 2020; Norton, McCamey, Boor, & Wiedmann, 2000). Another pathogen of high importance is *Salmonella* spp. that has been shown to persist for at least 10 years in dry food processing facilities (Beno et al., 2016). This microorganism and *Cronobacter* spp. are pathogens of concern for milk powder products (ANSES, 2020; Craven, McAuley, Duffy, & Fegan, 2010; Jacobs, Braun, & Hammer, 2011).

Indicator microorganisms correspond to organisms that reflects the microbiological flora condition of a food or the environment of the processing plant (Chapin, Nightingale, Worobo, Wiedmann, & Strawn, 2014). These microorganisms are routinely tested and are representative of the overall food quality and hygienic conditions in food processing facilities. To a lesser extent, indicator organisms may suggest the potential presence of pathogens (Jones et al., 2020). Indicator organism term is often used to define indicator, surrogate and index organisms interchangeably (Chapin et al., 2014). The term indicator organism includes a variety of microorganisms present in the environment (Chapin et al., 2014), such as aerobic plate count, *Enterococcus* spp. and total coliforms (Channaiah, 2013). Surrogate organisms are non-pathogenic organisms with similar properties to pathogenic organisms (Chapin et al., 2014), such as *Geobacillus stearothermophilus* for spoilage organism (Sinclair, Rose, Hashsham, Gerba, & Haas, 2012). Index organisms are used as markers to detect the possible presence of pathogenic microorganisms with similar ecological characteristics. For example, *Listeria* spp. is often searched as an indicator of the potential presence of *L. monocytogenes* (Chapin et al., 2014; Spanu & Jordan, 2020) and it is considered as a useful indicator of post-harvest and processing hygiene and cleaning effectiveness (UFPA, 2013). Indeed, if the ground is favorable for the development of *Listeria* spp. then it is also favorable for *L. monocytogenes* growth (Spanu & Jordan, 2020; UFPA, 2013; USDA FSIS, 2014). If a positive result occurs for *Listeria* spp., the corrective action is carried out as if the presence of *L. monocytogenes* had been established (UFPA, 2013; USDA FSIS, 2014). Confirmation of *L. monocytogenes* can be applied if the unsatisfactory results are observed in spite of corrective actions or if this pathogen is detected on food contact surfaces or in the product (UFPA, 2013).

Microbiological spoilage organisms make a product unacceptable for consumption. Indeed, they affect either the taste, odor, color or/and appearance as well as texture (Remenant, Jaffrès,), due to their development on foods, leading to the production of metabolites such as amines, sulphides or aldehydes. Spoilage organisms include bacteria such as *Brochothrix thermosphacta* (Illikoud et al., 2019) and *Pseudomonas fluorescens* (Brauge et al., 2020).

It was shown that some microorganisms (e.g. *L. monocytogenes, Pectinatus* spp.) can be present in food plants in two groups: transient strains and persistent strains. The transient strains remain in the plant only for a limited time because it can be removed by cleaning and disinfection procedures. Persistent strains are able to withstand these sanitation treatments and have been isolated and identified by molecular methods such as pulsed-field gel electrophoresis (PFGE) or by whole genome sequence (WGS) profile in the plant for at least 6 months (Rodríguez-Saavedra, González de Llano, Beltran, Torija, & Moreno-Arribas, 2021; Spanu & Jordan, 2020).

Detection of targeted microorganisms can be misleading due to the presence of biofilms, corresponding to aggregated and adhered bacteria to a surface. Most of the time, it is formed of different species with an extracellular matrix composed of polymeric substances (Phillips, 2016). Biofilms are difficult to remove, even after mechanical and chemical procedures (den Besten, Ding, Abee, & Yang, 2016). Therefore, biofilms can become a reservoir of microorganisms constantly released in food and be responsible for illnesses (den Besten et al., 2016; Lungu, Ricke, & Johnson, 2010) or food waste. In addition, it is now recognized that pathogenic agents and spoilage organisms of meat, develop predominantly in the form of biofilm rather than in their planktonic form (Giaouris et al., 2014).

3.2.4. The zoning concepts

A recommended method to organize the EMP is the zoning concept, where the facility is divided into several zones (Jackson, 2014). This allows adapting a sampling and testing strategy to each zone individually. Zones can be defined according to specific criteria, such as the level of risk contamination, processing steps or the nature of the food. Different zoning definition can be found which increases the difficulty to establish a standardized EMP framework. In Australia, the New South Wales Government, defined 2 zones: food contact surfaces (FCS) and non-food contact surfaces (non-FCS) (NSW Government, 2016). Most of the time, four zones are identified. Zone 1 is a FCS, defined as a location where the risk is higher because the surfaces are in contact with the product, such as slicers and the filling machines. Zone 2 is a non-FCS with the risk of transferring contamination into Zone 1 because directly adjacent. It can include equipment framework and maintenance

tools. Zone 3 is a non-FCS adjacent to zone 2 and located in the food processing area (e.g. floors, walls, drains). Finally, Zone 4 is the surface with the lower risk for food in the facility because it is located outside of the processing area, such as the office and employee break areas (Channaiah, 2013; FIL/IDF, 2020; Spanu & Jordan, 2020; Zoellner et al., 2018).

Generally, in EMP sampling program, indicators and spoilage microorganisms are sampled in zone 1 to 4, while pathogens are sample in zones 2 to 4 (Almond Board of California, unknown; Channaiah, 2013). Indeed, indicator organisms are present at a higher level so can be easily enumerated, and do not need Bio Safety Level-2 equipment (Channaiah, 2013). In addition, if pathogens were monitored in a routine procedure in Zone 1, the product would have to be held until results are received (Almond Board of California, unknown). Therefore, pathogens monitoring in zone 1 should be done only in specific situations (e.g. risk of contamination detected from previous sampling).

3.2.5. Environmental sampling program

The environmental sampling program is the core of the EMP. This includes the identification of the surfaces that must be sampled, based on the zone's delimitation identified previously, the sampling method (e.g. material, procedure), when to sample, the number of samples and the frequency.

- **Choice of surfaces to be sampled**

Surfaces of all zones can be sampled, but with particular attention to surfaces where the level of microbial population and the probability of its transfers is higher (Jones et al., 2020; USDA FSIS, 2014), based on previous microbiological results (Ripolles-Avila, Hascoet, Martinez-Suarez, Capita, & Rodriguez-Jerez, 2019; USDA FSIS, 2014). For instance, the FSIS compliance guidance to control *L. monocytogenes* in RTE and poultry products identifies two types of sampling programs. The first is random sampling with an equal probability of sampling that will cover all the sampling areas over a period of time, in order to ensure that the control system is effective. The second type is discretionary sampling (i.e., non-probability sampling) based on risk after a previous unsatisfactory result such a positive result of contamination, suspicion of conditions that could support the development, the harboring or the contamination in the surfaces after treatment of the product or to verify if the corrective actions are effective (USDA FSIS, 2014). It has also been recommended that the sampling programs should include areas that are always sampled, areas frequently sampled, with a rotation system, and areas that are sampled randomly and not provided in the sampling plans, giving the sampler the freedom to choose (Zoellner et al., 2018).

Drain, floors, cutting areas, brine, walls, storage racks are often cited as surfaces to be sampled (Beno et al., 2016; Leong, Alvarez-Ordóñez, & Jordan, 2014; Ripolles-Avila et al., 2019; Silva, Almeida, Alves, & Almeida, 2003). A mapping of the processing plant completed with additional information such as zones, the processes, the machines and the tools used, is essential in order to draw up a list of locations to be considered and to establish the number and the frequency of sampling (3M & Cornell University, 2019). Usually, operator's hands are also considered in the sampling program (Silva et al., 2003).

In addition to the classification of several zones according to the vulnerability of the product, niches or potential transfer points can also be identified in the sampling program. The first terms, niches, correspond to areas where microorganisms can grow and survive after sanitation procedures, being continuously contaminated, such as in a hose. The second terms, transfer points, are locations where a transfer of microorganism from a site to another is probable, such as a door handle, but is not continuously contaminated (T. J. Malley et al., 2013; T. J. V. Malley, Butts, & Wiedmann, 2015; Simmons & Wiedmann, 2018). Another possible classification of the sampling areas can be defined as verification and indicator sites. Verification sites correspond to areas where it is important to check the effectiveness of the environmental pathogen control program to prevent product contamination. This verification site essentially includes food contact surfaces in Zone 1. Verification site can also include Zone 2 and 3 sites, because of the risk of microorganism transfer. Indicator sites are generally transfer points located near niches or near hurdles and barriers. These later are mainly located in Zone2, 3 or 4 (T. J. V. Malley et al., 2015; Simmons & Wiedmann, 2018).

- **Available tools for sampling**

Several tools are available to the teams in charge of sampling. The International Organization for Standardization (ISO) 18593:2018 provides information about horizontal methods for surface sampling. The over-mentioned European standard gives guidelines to sampling procedure in terms of sampling techniques - contact plates stick swabs and sponge/cloth methods – the location, the area, the time and frequency of sampling. According to this standard, the sampling area must be identified and cover an area of 1 000 to 3 000 cm^2 for the detection of specific microorganisms and 100 cm^2 for microorganisms counts. The United States Department of Agriculture Food Safety and Inspection Service indicates a sampling covering 30 cm × 30 cm or less if the surface is lower (USDA FSIS, 2014).

Due to the heterogeneity of each processing plant characteristics, the recommendations remain generic. Recently, a European survey was conducted to collect surface sampling information to detect or enumerate *L. monocytogenes* biofilms, and to evaluate the effectiveness of the sampling methods for *L. monocytogenes* and *Pseudomonas fluorescens* according to sampling methods recommended by the standard EN ISO 18593:2018. The team in charge of the survey concluded to a preference of friction methods of sampling by food producers, but no significate differences were detected between the effectiveness of friction methods and contact plate methods to collect these viable and culturable microorganims (Brauge et al., 2020).

Several reviews and research papers summarized existing tools and methods of sampling and their efficiency (Baldock, 1973; Gómez, Ariño, Carramiñana, Rota, & Yangüela, 2012; Jones et al., 2020; Maillet et al., 2021; Moore & Griffith, 2002). The varying degrees of recovery of microorganisms were shown to depend on the nature of the surfaces, the accessibility of the sampled area, the time of recover and the biohazard sought (Jones et al., 2020). In food industries, stainless steel, plastics and rubber surfaces are usually found. For some microorganisms, such as enteric virus, the stainless steel, which is a negatively charged surface, may enhance an irreversible attachment (Jones et al., 2020). The time and the handling of recovery have an impact on the efficiency of the sampling. A greater recovery was observed when the elution buffer covered the surface for 15 min, followed by scraping and aspiration (Jones et al., 2020).

The type of biohazard also influences the efficiency of the recovery, such as bacterial Gram-type. Indeed, Gram-positive bacteria, such as *Listeria* spp., have a thick peptidoglycan layer compared to Gram-negative such as *Salmonella* spp., *E. coli* and *Campylobacter* spp. (Jones et al., 2020). Gram-negative bacteria have been reported to be more resistant to environmental stress (Keeratipibul et al., 2017), and a study revealed that the efficiency of recovery of Gram-positive bacteria from dry surfaces were higher than those of Gram-negative bacteria (Keeratipibul et al., 2017).

Another challenge is the recovery of microorganisms from biofilms. Therefore, before recovery, it is necessary to identify the presence of biofilms. In the market, several options exist to detect them by visual inspection through the appearance of microbubbles on the surface, or by staining the surface with the biofilm detection kit such as BioFinder® (Ripolles-Avila et al., 2019) or Realco®.

- **Number of environmental samples and frequency**

The number of samples to be taken in a time period is a key and

challenging decision in the EMP. It depends on food plant characteristics, contamination records, availability of resources, and risk-based approaches. Zoellner et al. (2018) identified three calculations to estimate the number of samples to be taken according to the objective of the environmental surveillance. The first was based on previously prevalence data to estimate the real proportion of samples with the desired precision. The second formula estimated the number of samples needed to confirm the absence of a microorganism with a confidence level and assumed a minimum level of contamination. The third formula compared two independent sampling results of the production environment and tested if the resulting prevalence differed. However, these formulas based on statistical methods for sampling need pre-required information that are complex to be estimated. Moreover, the number of estimated surfaces to be sampled does not consider the period of time to sample, the type of microorganism and the risk. Generally, the number of samples is not based on calculation because it is not easy to determine a common calculation, but is more often determined with an individual expertise (Zoellner et al., 2018). For *Listeria* spp. surveillance, guidance documents recommend sampling at least 3 to 5 areas on food contact surfaces (USDA FSIS, 2014), collected per line with a frequency depending on the level of risk contamination or the production volume per day. Pathogen environmental monitoring program from the Almond Board of California to prevent *Salmonella* spp. recontamination recommends a weekly (zone 1 to 3) to monthly (zone 4) surveillance with 5–10 samples in zone 4 and 10 to 15 in zones 2 and 3. No typical number of samples were given for zone 1 because it is line dependent (Almond Board of California, unknown).

Some authors suggest that FCS in Zone 1 should be tested weekly if the risk of food product contamination by a pathogen is high (T. J. V. Malley et al., 2015). For plants where a low risk of *Listeria* spp. contamination was demonstrated, the sampling can be reduced monthly (3M & Cornell University, 2019).

The frequency must be increased if a non-compliance occurs, such as a presence of *Listeria* spp. with additional investigation to find the root cause (UFPA, 2013). It can also be reduced if the food safety team considers the results of an appropriate risk assessment that shows production environment remains to be well monitored despite a reduction of the number of control sessions (Jackson, 2014; Motarjemi, 2016).

More generally, sampling frequency and number of samples for pathogens, indicators and spoilage organisms in the EMP depends on the plant specificities, the process, sanitation frequency and the type of product (3M & Cornell University, 2019). It also depends on the risk of harmful effect level of the microorganism considered, the probability of transfer to the food, the amount of food product produced and the facility history (UFPA, 2013).

The sampling can be done pre-shift, mid-shift or post-shift of food process according to the goal of the EMP. If the aim is to verify the sanitation efficacy, then sampling must take place after sanitation cycle and before production. If there is a suspicion of contamination over time, from an equipment in the food processing plan, the sampling can be performed when the equipment is operating. After a sanitation procedure, it is recommended by ISO 18593: 2018 to wait a certain time before sample, to limit the effect of the sanitizer's residues on bacteria and consequently to avoid sampling viable but not cultivable cells. It is also recommended to start up the machinery before the sampling. Indeed, it will make accessible microorganisms that were not removed because they were protected by the equipment (e.g. located between the conveyor belt and a wheel) (3M & Cornell University, 2019; ANSES, 2020; Spanu & Jordan, 2020). If the objective is not to verify the efficiency of the cleaning and disinfection procedures, it is recommended to sample during the production (Spanu & Jordan, 2020; Tompkin, 2002). The standard ISO 18593:2018 and scientific documents recommend to wait at least 2 h of production before sampling, or to do it at the end of the production cycle and before cleaning and disinfection procedures (ANSES, 2020; Carpentier & Barre, 2012; Spanu & Jordan, 2020). In order to do an effective and representative EMP, it is recommended to rotate the sampling days and shift (Spanu & Jordan, 2020). As example of food manufacturers practices, the responses to the survey of Magdovitz, Gummalla, Thippareddi, and Harrison (2020), concerning environmental monitoring practices among 150 frozen food contact studies, showed that aerobic plate counts and coliforms were tested in Zones 1 and 2 in pre-shift, mid-shift and post-shift production time, with a reduced frequency respectively, and mainly in pre-shift and in Zone 1. For *Listeria* spp., sample testing was mainly done in mid-shift period (zones 2 and 3 for the most part), followed by pre-shift. *L. monocytogenes* monitoring frequency was similarly tested in pre-shift, mid-shift and post-shift and in all zones (1–4) (Magdovitz et al., 2020).

3.3. Analytical step: detection and quantification analysis in EMPs strategies

Each surface sample must be labelled with relevant information such as sampling date, sampling time, area, zone, and other type of useful information in compliance with defined EMP requirements. Samples must be stored and transported under IS0 18593 guidance. A neutralizer broth might be used as a transport solution to neutralize the residual action of the disinfectants. The analytical microbiology can be performed internally food testing laboratory (e.g. if the food plant has an analysis laboratory, for current analysis) or in an external laboratory (e.g. analytical methods not performed by the internal laboratory).

3.3.1. Standards guiding analysis of environmental samples

Analyses must be done using food safety recognized methods by food safety administrations such as by FDA in the United States and EFSA in Europe (Spanu & Jordan, 2020). FDA's Bacteriological Analytical Manual (BAM) indicates the preferred laboratory procedures for the microbiological analysis of foods and cosmetics. In Europe, standard ISO 7218:2007 gives general requirements and recommendations for food microbiology. Reference methods to analyze samples for specific microorganisms and type of food are listed in the annex I of the European Regulation N°2073/2005. These methods are generally cultural methods that require several days to provide the result and not always appropriate in case of urgent need.

Alternative methods can be used when certified by authorized independent third parties, such as AFNOR (the French Standards Association), MicroVal and NordVal after comparison with reference methods, according to ISO 16140–2:2016 standard. Inter-laboratory test validation organizations such as the Association of Official Analytical Chemists (AOAC) can also be used to validate alternative methods (Baur & Ensminger, 1977).

As an example of analysis after sampling procedure, swab and sponges tested for *Salmonella* spp. must follow method from BAM chapter 5, ISO 6579–1:2017 or alternative methods certified and validated according to ISO 16140–2:2016. For *L. monocytogenes* and *Listeria* spp. environmental samples must follow BAM chapter 10, ISO 11290–1:2017 or alternative methods certified and validated according to ISO 16140–2:2016. For aerobic plate count, analysis follows BAM chapter 3 procedures or ISO 4833–2:2013 which details the horizontal method for the enumeration of microorganisms by surface plating technique.

3.3.2. Current analysis of environmental samples

Four steps are commonly used to analyze samples: detection, identification, confirmation and characterization (Rao & Arora, 2020).

Detection, according to reference or alternative methods, is usually done to determine the presence of a presumptive pathogen. Identification and confirmation must follow this first step to ensure the occurrence of a specific microorganism in the samples. Identification can be assessed through biochemical testing, 16S rRNA gene sequencing, flux cytometry, Matrix-Assisted Laser Desorption/Ionization - Time-Of-Flight (MALDI-TOF) mass spectrometry. MALDI-TOF has been validated by AOAC and MicroVal regarding ISO 16140-6 to be used for the

confirmation step.

The confirmation is performed by selective culture media or by molecular approaches following ISO reference methods or alternative methods, biochemical test or serological tests. Bacteriophage, defined as viruses that infect bacteria, are a promising method to detect and control food pathogens contamination such as *Salmonella* spp. or *L. monocytogenes,* or food spoilers (Brovko, Anany, & Griffiths, 2012; Vongkamjan, Benjakul, Kim Vu, & Vuddhakul, 2017; Wei, Rubab, Oh, & Ahn, 2019). These phages have a high specificity to the bacteria host, harmless to humans – even immunocompromised - are able to differentiate alive and dead cells and are active against antibiotic-resistant bacteria (Brovko et al., 2012; Wei et al., 2019). Bacteriophages can be combined to rapid methods such real-time PCR for detection, ELISAs or MALDI-TOF MS for rapid results (Wei et al., 2019). However, some limitations have to be considered with the use of bacteriophages, such as the limited host range, the possibility of the development of resistant strains of mutants or virulent trait transduction from one bacterial strain to another (Brovko et al., 2012).

To characterize molecular profiles of microorganisms, Pulsed Field Gel Electrophoresis (PFGE) was applied in the past to give information on strain genotype and to identify the transfer of the food pathogen between food to environment (Snehal Jadhav, Bhave, & Palombo, 2012; S. Jadhav, Gulati, Bhave, & Palombo, 2014; Leong et al., 2014). Other techniques can be used to subtype isolates, such as Ribotyping, Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD) or Multilocus Sequence Typing (MLST). Jadhav et al. (2012) listed these different subtyping techniques and highlighted the advantages and the disadvantages of each.

The contribution of Whole Genome Sequencing (WGS) combined with the development of bioinformatics has considerably opened the way to better control of food safety (Doyle, O'Toole, & Cotter, 2017). In particular, this approach has made possible to develop the most accurate bacterial typing schemes or methods to date, which are used in epidemiology to attribute sources of contamination, for example. In addition, the use of whole genome multi locus sequence typing (wgMLST) or single nucleotide polymorphisms (SNPs) approaches to obtain an optimal level of discrimination between bacteria of the same species, facilitate auto-control within production facilities (Doyle et al., 2017; Jagadeesan et al., 2019). With these tools, industrials are able to source track foodborne pathogens within their processing environment for an accurate root cause analysis or to determinate whether or not, an isolate is a persistent or a transient strain.

3.4. Post-analytical step: data processing and decision-making support in the EMP

Data processing step consist to manage the results from environmental samples analysis (traceability, detection of non-compliance), apply corrective actions and to improve the EMP over time.

It is important to keep in mind that the main purpose of EMP is not to demonstrate compliance of the end-product with food safety criteria, but to be aware of the status of contamination of the food plant environment. Therefore, food safety team might be able to decrease the risk of contamination of the product and *in fine* to ensure an increased level of food safety (Spanu & Jordan, 2020).

3.4.1. EMP analysis results interpretation

Results of environmental analysis must be analyzed by the teams in charge of environmental monitoring. Interpretation depends on the tools used for the sampling, the method and the goal of the analysis (Zoellner et al., 2018). Generally, results of swabbing from environmental are reported as positive or negative in particular because these analyses are usually carried out after enrichment steps (Spanu & Jordan, 2020). When the objective is to count the microorganisms from a specific surface, standard ISO 7218:2017 indicates the methods of reference for enumeration on solid medium after specific incubation time and temperature per targeted organism. In the case of enumeration, results are transcribed as a number of colonies forming unit per cm^2 surface (CFU/cm^2). It is important to note that a negative result or an absence of a colony is not equal to an absence of the microorganism. It also may be due to a quantity sampled that is below the detection limit of the method (Spanu & Jordan, 2020) or the sampling program that is not enough robust to detect all the sources of environmental contamination (Spanu & Jordan, 2020; UFPA, 2013).

3.4.2. Following trends of microbial contamination of surfaces to detect non-compliance

Guidance documents recommend to record results and to follow trends of contamination in food processing plant as well as information about previous non-compliant results. This follow-up allows to increase knowledge about environmental conditions over the time and to prevent the risk of contamination (NSW Government, 2016; Spanu & Jordan, 2020). By following the trend of environmental condition, a baseline of a specific microorganism or group of microorganism presence expressed in a predetermined unit (e.g. CFU, CFU/cm^2), per area and equipment can be determined, with previous data from 6 to 12 months of sampling program (Almond Board of California, unknown; Channaiah, 2013). To follow the trends of microbial contamination, a facility design diagram including control charts per area sampled can be used. It can additionally indicate if the sampling result was negative or positive, or providing information on the number of CFU per targeted organism or group of organisms.

Food safety team in charge of the EMP should be able to detect any deviation of surface contamination by establishing thresholds with associated corrective actions (Channaiah, 2013).

There are few references available on environmental microbial criteria. In France, process hygiene indicators were defined for monitoring of surface equipment in slaughterhouses and cutting plants for slaughtered animals and poultry (Ministère de l'Agriculture et de la Pêche, 2007). For the evaluation of the cleaning and disinfection process, satisfactory and unsatisfactory categories of results were recommended for total aerobic and *Enterobacteriaceae* counts. For example, unsatisfactory result for total aerobic was over 10 CFU/cm^2, and over 1 CFU/cm^2 for *Enterobacteriaceae* (Ministère de l'Agriculture et de la Pêche, 2007). Even if this document is out of date, it can serve as a reference for manufacturers in the over mentioned field. In 2017, Giovinazzo et al. (2017) summarized the benchmark values for several microorganisms found in the literature for Healthcare, Pharmaceutical and Food work environments. In food processing environment, from 6 publications, benchmark guidance value was found for *Pseudomonas fluorescens*, *E. coli*, *Staphylococcus aureus*, *Salmonella* spp., *L. monocytogenes* and total coliforms, sampled by contact plates or by swabs (Giovinazzo et al., 2017). However, these values do not consider the type of food and food plant specificities, but they can be used as a starting point for setting microbiological criteria by food manufacturers.

3.4.3. Decision-making and corrective actions

If an anomaly is detected, corrective action plans must be predetermined, in order to act as quickly as possible to ensure food safety. The action plans are linked to the zones and consequently, to the level of risk. These action plans must include immediate corrective actions, verification that the non-compliance has been removed and root cause analysis of the irregularity to prevent it in the future (Almond Board of California, unknown). All actions and results of the action plans must be documented with dates and people involved (3M & Cornell University, 2019).

A draft of guidelines for the control of *L. monocytogenes* in RTE foods established corrective actions recommendations in case of positive results. This later included actions up to 4 environmental sampling procedures found to be non-compliant. If a routine sampling detects an irregularity, all areas concerned must be cleaned, sanitized and retested during the next production cycle. In FCS, a comprehensive investigation

must also be carried out. If the follow up sampling remains non-compliant, intensified cleaning and sanitizing actions must be performed, with disassembly of the equipment if necessary, then more regular sampling and testing must be performed. In addition, if food product supports the growth of the bacteria in the FCS, it should be held, tested and reprocessed if necessary, followed by a comprehensive investigation. After a third non-compliant sampling in a non-FCS, a root cause analysis must be done (FDA, 2017) to detect if the isolate is transient or resident (e.g. by subtyping or WGS) (UFPA, 2013). For FCS, in addition to the corrective action performed previously, if a third sample is positive, the production must be stopped, and experts must be consulted to perform a comprehensive investigation. If the food supports the growth of *L. monocytogenes*, the product must be held and 3 consecutive days of testing must be negative. If the product does not support the growth of the pathogen, it can be reprocessed or destroyed (FDA, 2017) (Fig. 6). Some guidelines, intended for other microorganism, are more drastic as soon as a non-conformity detected since the first sampling. These latter recommend holding the product if a pathogen is detected in FCS (e.g. *Salmonella* spp. in almonds production plan), to place the area concerned in quarantine and to stop the

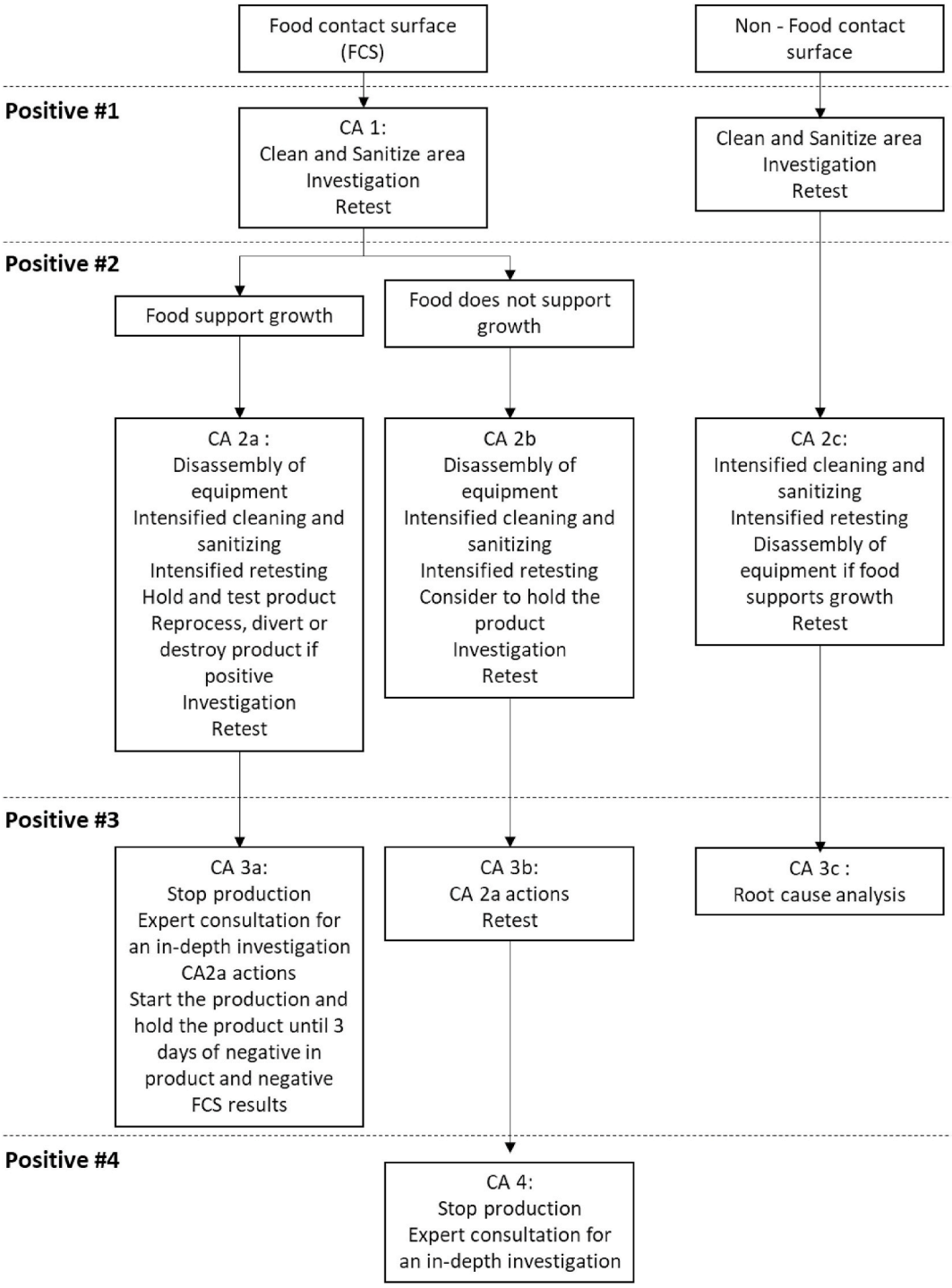

Fig. 6. Example of corrective actions after consecutive positive samples, extracted from FDA draft (2017) for environmental *Listeria* spp. monitoring. "Positive" result corresponded to a non-conformity detected after surface sampling analysis and "CA" corresponded to corrective actions.

production lines in order to carry out visual checks and enhanced sampling. The number and the frequency of the sampling procedures must be increased and extended to the areas surrounding the location concerned by the non-compliance (Almond Board of California, unknown).

Considering the goal and the requirements for food safety, each food plant must build its own plan of corrective actions with a risk-based approach.

3.4.4. Improving environmental monitoring programs

EMPs must be reviewed regularly as environment condition changes over time, due to the deterioration of the equipment, the hiring of new staff, the introduction of new tools and equipment, plant renovations and maintenance, production of new food products and even changing seasons. Guidance documents and scientific articles recommend to check the efficiency of the EMP at least every 6 months (Spanu & Jordan, 2020).

3.5. Limitations of the EMP implementation

The difficulty of developing and implementing an EMP is the lack of a common procedure (Spanu & Jordan, 2020; Zacharski et al., 2018). Indeed, it is highly dependent on the specific characteristics of each food plant, such as the type of food products, the architecture of the facility, its size, the number of operators and the degree of implementation of automated manufacturing processes as well as the type of sterilization procedures (Spanu & Jordan, 2020; Zacharski et al., 2018). Fortunately, regulations and guidelines have begun to emerge over the last ten years to provide a framework for environmental monitoring.

Most of guidelines are intended to support the surveillance of pathogens in the environment, especially for *Listeria* and *Salmonella* species. Few focus on indicator organisms and even less on spoilage organisms.

Another limitation identified is related to the biases associated with the tools and practices used for monitoring. Indeed, the transfer efficiency between the sampling tool and the surfaces is very variable. For example, the recovery percentage of *L. monocytogenes* was found to vary between 0.17% and 5.83%, with a higher efficiency of a mini-roller compared to the sponge on stainless steel surfaces, while the sponge was better for polyethylene cutting board (Gómez et al., 2012). Indeed, the percentage of recovery depends on the tool used, the type of surface sampled, the targeted organism and the procedure used by the operator. It is therefore difficult to compare the results between different factories. Moreover, the monitored area is quite small, with a surface area ranging from 10 cm^2 to 100 cm^2 for enumeration and up to 3000 cm^2 for detection (ANSES, 2020). As a result, there is a possibility to miss a niche or a transient contamination area.

In addition, there is a need for greater communication in practices to increase knowledge about EMPs and to help food processors to build and apply them in their food processing environment. Indeed, there are guidelines for conducting of EMP, but without concrete indicators (e.g. number of samples, CFU limits, etc.). Few studies on actual practices, such as the one carried out by Magdovitz et al. (2020) exist for different types of food.

4. Conclusion

EMPs are essential to ensure food safety and quality. The information on the design and application of these programs remain rather blurred because there is no common method. Therefore, communication on ongoing practices by the food industries and its associated results would be beneficial to help food manufacturers to compare their practices and to improve their EMPs own. This identified gap can be filled by future surveys and studies on EMP.

The present document has highlighted that EMPs are more and more seen as essential for food safety, in conjunction with food product control. In addition, EMP should be supported by preventive actions (e.g. good hygiene practice) and corrective actions (e.g. improvement of cleaning and disinfection procedures) to ensure the safety and quality of food products (Cinar & Onbaşı, 2021). Guidance documents are increasingly being developed in several regions of the world as well as research projects on the subject. The synthesis of the existing documents in the literature has allowed to give more leads for a harmonized approach of the EMPs practices. Indeed, a three-step method is proposed to develop one's own EMP with the aim of harmonizing practices between food plants. Therefore, this document can be used as a synthesis of basics for the construction of EMPs plan in food facilities.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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