

Microbiological Factors of Food Quality for Risk Assessments in Europe: A Systematic Review

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Abstract

Public authorities and consumers worldwide are increasingly concern about food quality and safety. Europe has established and suggested management systems in food industry that control hazards in food products. However, outbreak incidents by foodborne pathogens existing until now. European studies that present microbiological risk factors or studies which have conducted microbiological risk assessments about a variety of food products, which represent increased risk of harboring pathogens, are included. By using PRISMA guideline, we searched for the most recent publications from 2017-2021 referring microbiological risk assessments from online databases Scopus, PubMed and Science Direct. From 505 articles initially captured, data was extracted from 84 studies regarding microbiological risk factors in terms of food quality and safety, that are evidenced in European studies. Moreover, information about country of origin, food type, production phase and technology used for detection of pathogens, are also presented. Our results, indicate that quality systems should be further developed in order to control all possible routes of contamination in the supply chain. This work provides information to managers in food industry and scientists for further research regarding microbiological risk assessments.

Keywords: microbiological risk assessment, risk factors, food quality, food safety, foodborne pathogens

DOI: 10.7176/FSQM/113-03

Publication date: February 28th 2022

1. Introduction

Food quality can be described as a plethora of essential requirements, which are targeted at “consumer’s satisfaction” (Peri, 2006). Consumers requirements for food quality, include food safety, nutritional needs, appearance and sensory attributes, packaging, food traceability, availability, price etc.(Savelli et al., 2019). Since food safety has become mandatory for food quality, public authorities are insisting that comprehensive quality management systems should be developed and utilized in food industry (Panghal et al., 2018).

A main challenge of food safety includes microbiological safety (Fung et al., 2018). Numerous pathogens are transmitted via food and according to a recent survey, there are millions of incidents recorded in Europe in the last decade (Sarno et al., 2021). More specific, foodborne pathogens such as bacteria, fungi, parasites and viruses can be detected in different stages of production or distribution of a food product (Martinović et al., 2016). *Listeria monocytogenes*, *Salmonella spp.*, *Vibrio spp.*, *Campylobacter spp.*, *E. coli*, and adenovirus are well-known pathogens responsible for foodborne illnesses in all regions of the world. Examples of predominant symptoms caused by these foodborne pathogens are nausea, vomiting, diarrhea, cramps, fever, headache, cough etc. (Bintsis, 2017). Therefore, it is essential need to detect, manage and prevent of foodborne pathogens “from farm to fork” regarding consumers health risks (Alegbeleye et al., 2018).

According to FAO, risk analysis is proposed as suitable tool for quality management systems in food industry (FAO, 2020). By monitoring food control procedures efficiently and facing up foodborne pathogens in terms of food safety and quality, risk analysis approaches are essential for consumers protection (Suhendra et al., 2020). Nowadays, Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Point (HACCP) are utilized in the majority of sectors involved in food industry. Additionally, quality systems include risk analysis, which target to the entire food supply chain and therefore, address issues related to microbiological hazards (Ramos et al., 2021).

Microbiological Risk Assessment, Risk Management and Risk Communication considered to be the sections which constitute a risk analysis (European Parliament and Council, 2018). In terms of a Microbiological Risk Assessment, the most important step is to identify risk factors, which are responsible for the occurrence of foodborne pathogens (FAO/WHO, 2021). Importantly, by a microbiological risk assessment, a better understanding of the behavior of microorganisms in the food matrix, while evaluation of procedures and hygiene conditions in supply chain can be achieved (den Besten et al., 2018). Hence, the last decade, omics- technologies have been widely recruited, in order to obtain information about the ecology and physiology of microorganisms (Rantsiou et al., 2018). Metagenomics, metatranscriptomics, metaproteomics and meta-metabolomics

approaches have been adapted in microbiological risk assessments and have been demonstrated as key solution for food safety concerns (Cocolin et al., 2018).

The aim of this systematic review is to identify risk factors related to food quality and safety issues, in correlation to foodborne pathogens, food type and production phase. This work will also provide to managers in food industry and scientists an overview of current publications regarding microbiological risk factors and technology used for pathogens detection, with an insight on gaps identified.

2. Materials and Methods

2.1 Protocols

The present systematic review has been conducted, based on PRISMA guidelines: Literature search, Study selection and Analysis process.

2.2 Eligibility Criteria

All microbiological risk assessments were included in the first step, respective of their publication date. The literature search was conducted with no language or publication status restrictions except the condition that an abstract in English existed, referring the information of interest. The inclusion and exclusion criteria were set as follows:

Inclusion criteria:

- Studies conducted in Europe
- Studies about foodstuff
- Foodborne pathogens
- At least one of the following information is referred: microbiological risk assessment, microbiological risk factor

Exclusion criteria:

- No factors reported
- Studies about drugs/medicine/water type
- Non-EU-based study and outbreaks

2.3 Information Sources and Literature search

All empirical studies were identified by systematical searching of the following electronic databases: Science Direct (Elsevier), Scopus (Elsevier) and PubMed (NCBI). Studies published over the past 4 years i.e. 2017 onwards were included in this comprehensive literature review by using these three online databases. The study was performed in September 2021. We used the following search terms (adapted for each database): (Microbiological) AND (Risk assessment) AND (Food safety) AND (Food quality) AND (foodborne pathogens). For the comprehensive literature search, the search strings used are as follow: TITLE-ABS-KEY (microbiological AND risk AND assessment AND food AND quality AND foodborne AND pathogens AND food AND safety) AND (LIMIT-TO (PUBYEAR, 2020) OR LIMIT-TO (PUBYEAR, 2019) OR LIMIT-TO (PUBYEAR, 2018) OR LIMIT-TO (PUBYEAR, 2017))

2.4 Study selection

Two reviewers (MD and AV) assessed all records for inclusion basically upon the article title and the abstract provided. Mendeley was used to identify duplicated articles. For all publications included, the two reviewers (MD and AV) extracted data separately using standardized data extraction form. All abstracts that did not meet our criteria were excluded, while the remaining articles and those whose abstracts did not clearly provide proper information, were further assessed via their full-text evaluation. The authors reviewed the potentially relevant studies according to the eligibility criteria to define which studies would finally be selected for this systematic review.

2.5 Data collection process and items

Two authors (MD and AV) independently analyzed and extracted the following data: first author name, country of risk assessment conducted, food type involved, production phase of food product examined, foodborne pathogen detected, technology used in the article and finally risk factors reported.

3. Results

3.1 Study selection

Only original research papers published with an abstract in English were included. Initially, a total of 505 articles were identified. Afterwards, the number of publications was reduced to 498 by removing duplicates. The following stage involved the elimination of articles in which titles and abstracts either did not refer to microbiological risk assessments or did not constitute a part of it. After assessing the full text of the remaining

papers (n =290) in terms of food quality and safety, relevant research papers were identified. From those, 206 papers were discarded since they were non-Europe studies or there was not reported a case study of microbiological risk assessment. Papers in the final set (n = 84) mainly concerned microbiological risk assessments in Europe. The systematic literature Review approaches are depicted in Fig. 1.

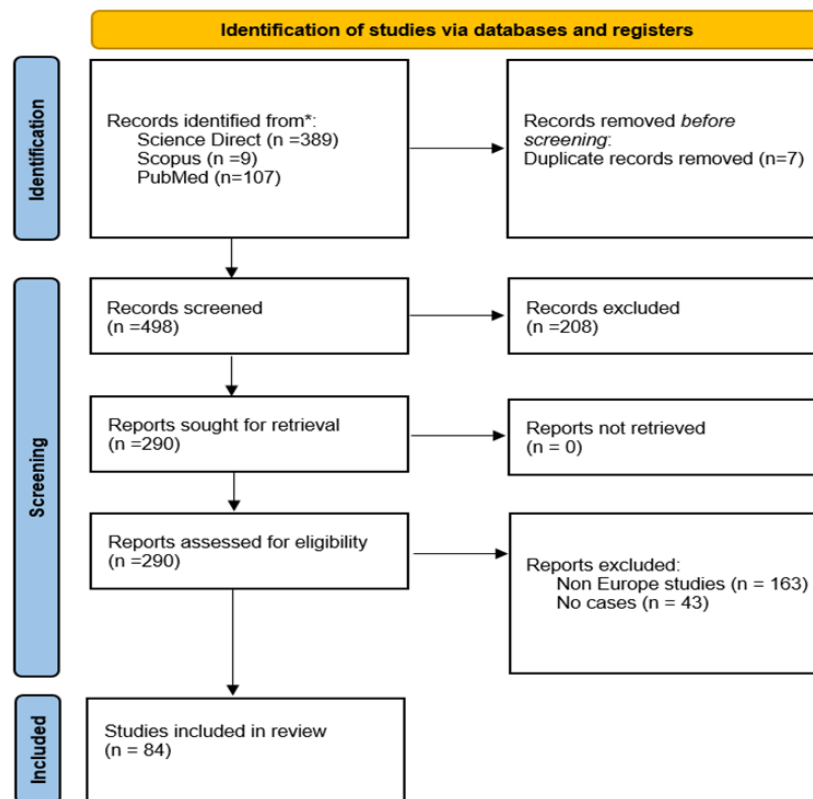


Figure 1. Prisma flow diagram

3.2 Study characteristics

Details of the included studies are presented in Table 1. using the following parameters: first name of author, food product, production phase, foodborne pathogen, country, risk factor and technology used.

Table 1. Characteristics of all articles included in this work (matrix, production phase, foodborne pathogen, country, risk factor, technology)

	Matrix	Production Phase	Pathogen	Country	Risk Factor	Technology
(Loukiadis et al., 2017)	Beef	Grinding	<i>E.coli</i>	France	Sampling process	Microbial cultures
(Fernandez-Cassi et al., 2017)	Parsley	Production	Virus	Spain	Irrigation	Next Generation Sequencing (NGS)
(Bolivar et al., 2018)	Seabream/Seabass	Retail market	<i>Listeria monocytogenes</i>	Spain	Storage conditions	Microbial cultures
(Pérez-Rodríguez et al., 2019)	Lettuce	Process	Norovirus	Spain	Food handling practices	Not mentioned
(Duqué et al., 2021)	Poultry	Slaughter process	<i>Campylobacter jejuni</i>	France	Storage conditions	Microbial cultures
(Mataragas et al., 2018)	Cheese	Manufacture	<i>Lactobacillus spp.</i>	Greece	Ripening	Next Generation Sequencing (NGS)
(Filipello et al., 2020)	Dairy	Distribution	<i>Listeria monocytogenes</i>	Italy	Reservoir level	Whole genome sequencing (WGS)
(Guillén et al., 2020)	N.M	Supply chain	<i>Salmonella spp.</i>	Spain	Preservation	Microbial cultures
(Zampieri et al., 2020)	Mollusk	Depuration	<i>Vibrio spp.</i>	Italy	Ozone treatments and density	Microbial cultures/ Next Generation Sequencing (NGS)
(Lüth et al., 2020)	Meat	Production	<i>Listeria monocytogenes</i>	Germany	Niches in the production line	Whole genome sequencing (WGS)
(Merget, B., Forbes, K. J., Brennan, F., McAteer, S., Shepherd, T., Strachan, N. J., & Holden, 2019)	Seeds/Veg etables	Production	<i>E.coli</i>	UK	plant species, tissue type, Temperature	Microbial cultures

	Matrix	Production Phase	Pathogen	Country	Risk Factor	Technology
(Merget et al., 2020)	Seeds/Veg etables	Production	<i>E.coli</i>	UK	metabolic flexibility	Microbial cultures
(Rahman et al., 2017)	Mollusk	Breeding	<i>Vibrio spp.</i>	Italy	Temperature	Multi-locus Sequence Analysis (MLSA)
(Lyto et al., 2020)	Marinated chicken	Retail market	<i>Campylobacter spp.</i> , <i>Salmonella spp.</i> , <i>Listeria monocytogenes</i>	Greece	Shelf-life/ hygiene requirements	M. cultures/multiplex Polymerase Chain Reaction
(Díaz-Jiménez et al., 2020)	Chicken and turkey meat	Retail market	<i>Enterobacteriaceae</i>	Spain	exposition to antibiotics	M. cultures/ Multi-locus sequence typing / Whole genome sequencing
(Gonzales-Barron et al., 2020)	Cheese	Ripening	<i>Listeria monocytogenes</i>	Portugal	starter culture/ environmental conditions	Microbial cultures
(Lianou et al., 2018)	Vanilla cream pudding	Manufacture	<i>Listeria monocytogenes</i>	Greece	storage temperature	Microbial cultures
(Devleeschauwer et al., 2017)	Tomato	pre-harvest production	<i>Salmonella spp.</i>	Belgium	humidity	Microbial cultures
(Duqué et al., 2018)	Chicken	Slaughter process	<i>Campylobacter</i>	France	season effect/ skin area	Microbial cultures
(Ripolles-Avila et al., 2019)	Fish	Packaging	<i>S. aureus</i>	Spain	plastic Materials	Microbial cultures
(Jansen et al., 2019)	Cheese	Ethnic markets	<i>Brucella spp.</i>	Belgium	raw milk /short ripening period	Microbial cultures/ qPCR
(Dinh Thanh et al., 2017)	Paprika, Pepper, and Oregano	Process	<i>S. aureus</i>	Germany	long term-storage	Microbial cultures
(Kapetanakou et al., 2019)	Bakery products	Manufacture	<i>Salmonella spp.</i>	Greece	storage temperature	Microbial cultures
(Kapetanakou et al., 2017)	Cheese	Retail market	<i>Listeria monocytogenes</i>	Greece	Shelf-life	Microbial cultures
(Schill et al., 2017)	pork meat	Process	<i>Enterobacteriaceae</i>	Germany	livestock, farm level, environment of facilities, workers	Microbial cultures / PCR/MLST
(Wright & Holden, 2018)	Microgreens	Harvest	<i>E.coli</i>	UK	humidity	Microbial cultures
(Osimani et al., 2017)	Mealworms	Retail market	Antibiotic resistance strains	Italy	rearing/ antibiotics	Microbial cultures / PCR
(Matteo Crotta et al., 2019)	Pork meat	Slaughter process	gastrointestinal pathogens	UK	belly opening/removal of gastrointestinal tract stage	Not mentioned
(Osimani et al., 2018)	Edible insects	Retail market	Bacteria	Italy	manufacturing practices during insect process	PCR-DGGE, metagenomics sequencing, qPCR
(Cadavez et al., 2019)	Cheese	Production	<i>Listeria monocytogenes</i>	Portugal	starter cultures/ storage temperature	Microbial cultures
(Evert-Arriagada et al., 2018)	Cheese	Manufacture	<i>Listeria monocytogenes</i>	Spain	High pressure processing/ sanitization	Microbial cultures
(Akineden et al., 2017)	Powdered infant formula	Retail market	<i>Cronobacter spp.</i>	Germany	Manufacturing process	Microbial cultures / PCR/MLST
(Garre et al., 2019)	Milk	Retail market	<i>Listeria monocytogenes</i>	Spain	Thermal treatment	Microbial cultures
(Alvseike et al., 2018)	Pork meat	Slaughter process	Bacteria+Parasite	Norway	belly opening/removal of gastrointestinal tract stage	Not mentioned
(Heir et al., 2019)	Salmon	Filleting	<i>Listeria monocytogenes</i>	Norway	dry-salting process	Microbial cultures / Next Generation Sequencing
(Papadopoulou et al., 2018)	Cheese	Manufacture	<i>Listeria monocytogenes</i>	Greece	starter cultures/ storage temperature	Microbial cultures
(Santarelli et al., 2018)	leafy green vegetables	Retail market	Bacteria +Virus	Italy	washing step	Microbial cultures / PCR
(Valero et al., 2018)	Cheese	Retail market	<i>Listeria monocytogenes</i>	Spain	refrigeration temperature	Microbial cultures / PCR
(Imran et al., 2019)	Cheese	Retail market	Gram-Negative Bacteria	France	starter cultures	Microbial cultures
(Rodríguez-López et al., 2020)	Fish+Meat	Process	<i>Listeria monocytogenes</i>	Spain	disinfection regimes	Microbial cultures / PCR
(Juliana Lane Paixão dos Santos et al., 2020)	Strawberry purees	Process	<i>Aspergillus Fischeri</i>	Belgium	storage temperature /pasteurization	Microbial cultures
(Xylia et al., 2019)	Salad	Retail market	Bacteria	Cyprus	season/ processing practices	Microbial cultures / PCR
(El-Hajjaji et al., 2020a)	Milk butter	Manufacture/ Retail market	<i>Listeria monocytogenes</i>	Belgium	starter cultures	Microbial cultures
(Fritsch et al., 2019)	Multiple	Multiple	<i>Listeria monocytogenes</i>	France	cold conditions	Genome-wide Association study(GWAS)

	Matrix	Production Phase	Pathogen	Country	Risk Factor	Technology
(Bogdanovičová et al., 2017)	Milk Powder	Retail market	<i>S. aureus</i>	Czech Republic	storage temperature/ long-term storage	Microbial cultures
(Allende et al., 2017)	Spinach	Harvest	<i>E.coli</i>	Spain	season/irrigation water/weather	Microbial cultures
(Gérard et al., 2020a)	Cheese	Manufacture	<i>Listeria monocytogenes</i>	Belgium	starter cultures	Microbial cultures
(Possas et al., 2019)	Sausage	Retail market	<i>Listeria monocytogenes</i>	Spain	shelf-life	Microbial cultures
(Tirloni et al., 2020)	Shrimp	Manufacture	<i>Listeria monocytogenes</i>	Italy	contaminated ingredients/ production practices	Microbial cultures
(Beneduce et al., 2017)	Tomato/ Broccoli	Harvest	Bacteria	Italy	irrigation	Microbial cultures / PCR
(Reich et al., 2018)	Meat	Slaughter process	Campylobacter	Germany	sampling process	Microbial cultures
(Correia Peres Costa et al., 2020)	Seabream /Seabass	Filleting	Bacteria	Spain	location/ shelf-life	Microbial cultures / PCR
(Li et al., 2018)	Berries	Retail market	Hepatitis A/Norovirus	Belgium	virucidal treatments	qPCR
(Ssemenda et al., 2018)	Vegetables	Manufacture	Bacteria	Netherlands	irrigation/ compost manure	Microbial cultures / PCR
(Márcia Oliveira et al., 2019)	Berries	Manufacture	Pathogens	Portugal	surface structure/ worker's hands	Microbial cultures / PCR
(Petruzzelli et al., 2018)	Meals	Catering	Bacteria	Italy	washing /refrigeration /temperatures/ contact surface/worker's hands	Microbial cultures
(Lourenco et al., 2020)	Cheese/Milk	Production/ Ripening	Bacteria	Ireland	surface biofilm	Microbial cultures
(López-Gálvez et al., 2020)	Peppers	Harvest	Bacteria	Spain	washing Line	Microbial cultures
(Iannetti et al., 2020)	Poultry	Slaughter process	Campylobacter/ Salmonella	Italy	animal welfare/neck skin	M. cultures/ multiplex PCR
(Garayoa et al., 2017)	Prepared foods	Catering	Bacteria	Spain	surfaces/ temperatures	Microbial cultures
(Nastasijevic et al., 2017)	Meat	Process	<i>Listeria monocytogenes</i>	Serbia	air flow/surface biofilms/slaughter line	Microbial cultures / WGS
(Rajkovic et al., 2017)	Raspberries	Manufacture	Pathogens	Serbia	storage/water control/hygiene requirements	Not mentioned
(Jofré et al., 2019)	Meat	Retail market	Bacteria	Spain	storage temperature	Microbial cultures
(Gkogka et al., 2020)	Meat pies	Production	Clostridium perfringens	Netherlands	Shelf-life/handling of raw ingredients	Not mentioned
(Soon, 2019)	Meals	Food vendors	Pathogens	UK	hygiene facilities and infrastructure	Not mentioned
(Szczeczek et al., 2018)	Vegetables	Production	Bacteria	Poland	composts/ fertilization practices	Microbial cultures
(M. Crotta et al., 2017)	pork meat	Supply chain	<i>Toxoplasma gondii</i>	UK	infected animals/interaction of parasite with host	Modified agglutination test
(Valero et al., 2017)	Meals	Catering	Bacteria	Spain	food handlers and contact utensils	Microbial cultures
(Madden et al., 2018)	Meals	Process	<i>Listeria monocytogenes</i>	UK	refrigeration temperature/ sanitizers	Microbial cultures / Whole genome sequencing
(Araújo et al., 2017)	Vegetables	Retail market	<i>E.coli</i>	Spain	irrigation water	Microbial cultures / PCR
(Rubini et al., 2018)	Mollusk	Production	<i>E. coli/ Salmonella enterica</i>	Italy	shellfish-growing waters/environmental conditions	Microbial cultures
(Costa et al., 2020)	N.M	Production	<i>Listeria monocytogenes</i>	Spain	starter cultures/ temperature	Microbial cultures
(Papadopoulos et al., 2019)	Dairy	Production	<i>S. aureus</i>	Greece	facilities biofilm/ sanitizing procedures	Microbial cultures / PCR
(Bailey et al., 2018)	Poultry	Production	<i>Campylobacter</i>	UK	partial depopulation /season	Microbial cultures
(Banach et al., 2018)	Lettuce	Process	<i>E.coli</i>	Netherlands	washing tank	Microbial cultures
(Juliana L.P. Santos et al., 2018)	Strawberry puree	Process	<i>Byssochlamys fulva/nivea</i>	Belgium	heat process, stored temperature, °Brix and oxygen concentration	Microbial cultures
(Castro et al., 2017)	Milk	Manufacture	<i>Listeria monocytogenes</i>	Finland	refrigeration temperature/ handling	Microbial cultures / PCR
(Utaaker et al., 2017)	Vegetables	Retail market/Street vendors	<i>Cryptosporidium m/ Giardia</i>	Norway	animals/ consumers handling	PCR
(Carvalho Santos et al., 2017)	Sheep butter	Production	Bacteria	Portugal	manufacturing process	Microbial cultures
(Benítez-Cabello et al., 2019)	Table olives	Production	Bacteria	Spain	seasoning and salt material in packaging	Next Generation Sequencing

	Matrix	Production Phase	Pathogen	Country	Risk Factor	Technology
(Juliana Lane Paixão dos Santos et al., 2018)	Fruits	Process	Moulds	Belgium	pasteurization	Microbial cultures / PCR
(Caradonna et al., 2017)	Salads	Manufacture/ Retail market	Parasites	Italy	Agricultural practices	qPCR/NGS
(González-Tejedor et al., 2018)	Smoothies	Retail market	<i>Listeria monocytogenes</i>	Spain	storage temperature,pH	Microbial cultures
(Zuber et al., 2019)	pork meat	Process	<i>Listeria monocytogenes</i>	Montenegro	infected animals	Microbial cultures / Whole genome sequencing

3.3 Food Type

Publications included in this systematic review are categorized by food product examined. According to Fig.2 the highest percentage of studies, presented microbiological risk assessments targeted to crops (fruits and vegetables). More in detail, 19 articles examined a variety of vegetables such as peppers, lettuce, spinach, while 7 works examined fruits such as strawberries and raspberries. In addition, 20 articles referred to microbiological risk factors of dairy products, such as milk and cheese. The aim of 13 works was to investigate microbiological factors associated to meat industry such as pork meat and sausages. Furthermore, the main subject of 9 publications was about seafood's pathogens and factors. Finally, 6 articles presented microbiological risk factors regarding poultry. Additionally, 5 articles included in this work, used prepared meals as matrix.

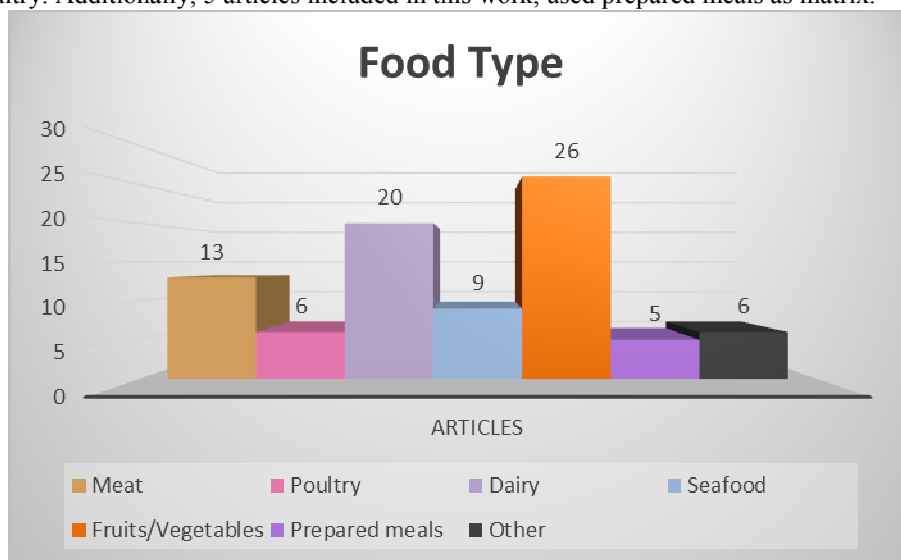


Figure 2. Number of publications according to the food type examined

3.4 Pathogens

The reports described in this systematic review, pinpoint one specific foodborne pathogen to investigate, while some articles are focused on a variety of microorganisms (Fig.3). For instance, most articles included, deal with information about *Listeria monocytogenes* occurrence. Risk factors for *E. coli* occurrence was the main subject of 8 publications. Furthermore, 6 out of 84 articles, focused on factors related to *Campylobacter* growth (Bailey et al., 2018; Duqué et al., 2018, 2021; Iannetti et al., 2020; Lytoug et al., 2020; Reich et al., 2018). Similarly, 6 articles explored risk factors about *Salmonella spp.* contamination (Devleesschauwer et al., 2017; Guillén et al., 2020; Iannetti et al., 2020; Kapetanakou et al., 2019; Lytoug et al., 2020; Rubini et al., 2018). Finally, 4 works were studied *S. aureus*, while 2 works studied *Vibrio spp.* (Bogdanovičová et al., 2017; Dinh Thanh et al., 2017; Papadopoulos et al., 2019; Rahman et al., 2017; Ripolles-Avila et al., 2019; Zampieri et al., 2020). As far as viruses concerns, articles aimed to examine factors associated to Hepatitis A, Hepatitis E and Norovirus presence in food products (Fernandez-Cassi et al., 2017; Li et al., 2018; Pérez-Rodríguez et al., 2019; Santarelli et al., 2018). Regarding parasites, 4 articles demonstrated factors responsible for the occurrence of *Toxoplasma gondii*, *Trichinella spp.* *Cryptosporidium spp.* and *Giardia duodenalis* (Alvseike et al., 2018; Caradonna et al., 2017; M. Crotta et al., 2017; Utaaker et al., 2017). Last but not least, growth and occurrence of *Aspergillus fischeri* and *Byssoschlamys fulva/nivea* were presented in 3 articles (Juliana Lane Paixão dos Santos et al., 2018, 2020; Juliana L.P. Santos et al., 2018).

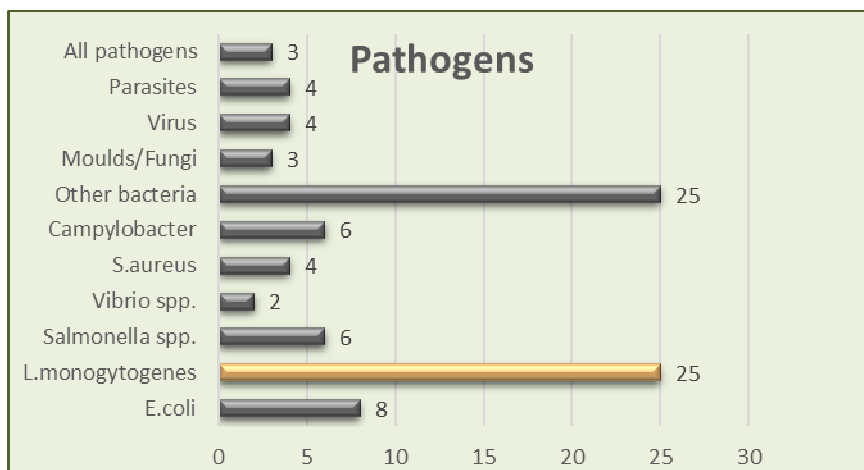


Figure 3. Number of publications according to foodborne pathogen examined

3.5 Country

According to Fig. 4, from studies included in this review, Spain holds the lead, following by Italy. Spain was the first country in publications related to microbiological risk assessments with total 21 studies. For example, studies about the presence of *E. coli* in baby spinach or viral contamination on fresh parsley are published (Allende et al., 2017; Fernandez-Cassi et al., 2017). On the other hand, Italy has also contributed to issues related to microbiological risk factors with 12 publications. Articles focused on *Listeria monocytogenes* in shrimp cocktails or *Salmonella enterica* in mollusks were included in this review (Rubini et al., 2018; Tirloni et al., 2020). Belgium, UK, Greece, France and Germany are countries with approximately 8 studies regarding microbiological risk factors of food products. Netherlands, Portugal and Norway have published 3 works, during this period. Only one study took place in countries such as Finland, Poland, Montenegro, Ireland, Cyprus, Chest Republic.

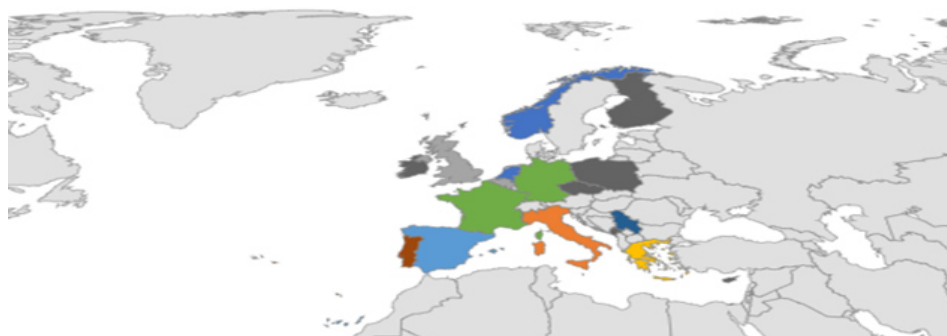


Figure 4. Origin countries of publications on microbiological risk assessment

3.6 Technology

The techniques applied for detection of foodborne pathogens are presented in Fig.5. Most of the studies have conducted microbiological cultures for detection of foodborne pathogens. More in detail, for this purpose, 66 articles used culture dependent technique as gold standard methods. More specific, 42 studies selected only gold standard methods for their experiments, while the other used a combination of culture-dependent and culture-independent techniques. Microbial cultures were performed for assessment of gram-negative bacteria associated with traditional French cheeses or for spoilage risk assessment of pasteurized strawberry purees by *Aspergillus fischeri* (Imran et al., 2019; Juliana Lane Paixão dos Santos et al., 2020). Moreover, 22 out of all articles included, used Next Generation Sequencing approaches for identification of microorganisms such as *Listeria monocytogenes* in cold-smoked salmon or *Vibrio spp.* in Manila clams (Heir et al., 2019; Zampieri et al., 2020). Polymerase chain reaction and similar methods (multiplex PCR, qPCR digital PCR) were applied in 17 studies,

as tools for detection and verification of pathogens. For example, PCR was performed for detection of *Cryptosporidium* and *Giardia* in vegetables, while qPCR for foodborne viruses in berries (Li et al., 2018; Utaaker et al., 2017). Finally, in 7 studies included either do not mentioned applied technology or an alternative methodology was used such as PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE), Multilocus sequence analysis (MLSA) and Multilocus sequence typing (MLST).

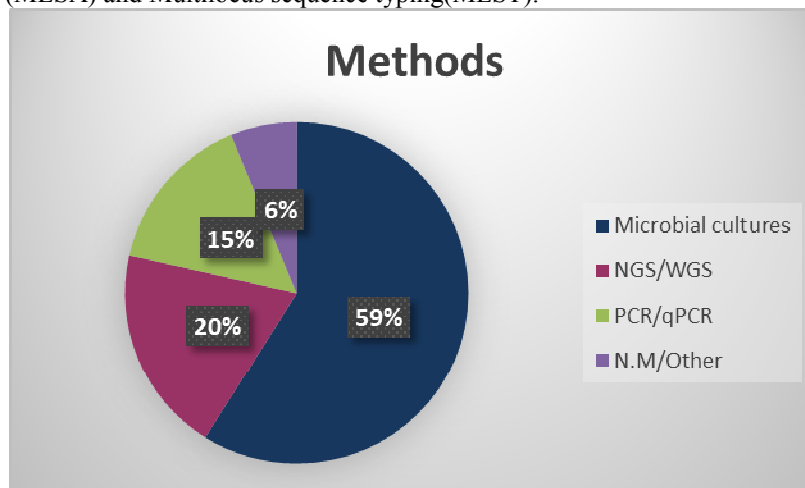


Figure 5. Number of publications according to methodology applied for detection of foodborne pathogens

3.7 Risk factors

Depending on the type of food sample, a variety of risk factors were highlighted for foodborne pathogens contamination. Figure 6. depicts microbiological risk factors reported in correlation to food type. As far as fruits and vegetables concerns, most common risk factors mentioned, were irrigation water, processing practices and storage temperature, following by fertilizations practices and compost, washing, and food handling regarding hygiene (Banach et al., 2018; Fernandez-Cassi et al., 2017; López-Gálvez et al., 2020). Some studies pointed out the importance of plant species and tissue type for risk contamination or how humidity in farms can affect the growth of foodborne pathogens (Devleeschauwer et al., 2017; Merget, B., Forbes, K. J., Brennan, F., McAteer, S., Shepherd, T., Strachan, N. J., & Holden, 2019; Wright & Holden, 2018). For meat products such as beef and pork, infected animals were indicated as the highest microbiological risk factor. Regarding poultry, infected animals was the most referred risk factor, as well. Also, slaughter line, storage temperature and shelf life considered to be potential risk factors for meat products contamination. More in detail, niches in production of meat products, were responsible for *Listeria monocytogenes* contamination (Lüth et al., 2020). Storage temperature and selection of starter cultures for dairy products, were reported as risk factors. Furthermore, ripening stage, manufacturing processes and surface biofilms are presented in the articles included. Storage conditions proved to affect microbiological quality of seafood, poultry and prepared meals. Although, washing step was the most common risk factor reported, regarding prepared meals.

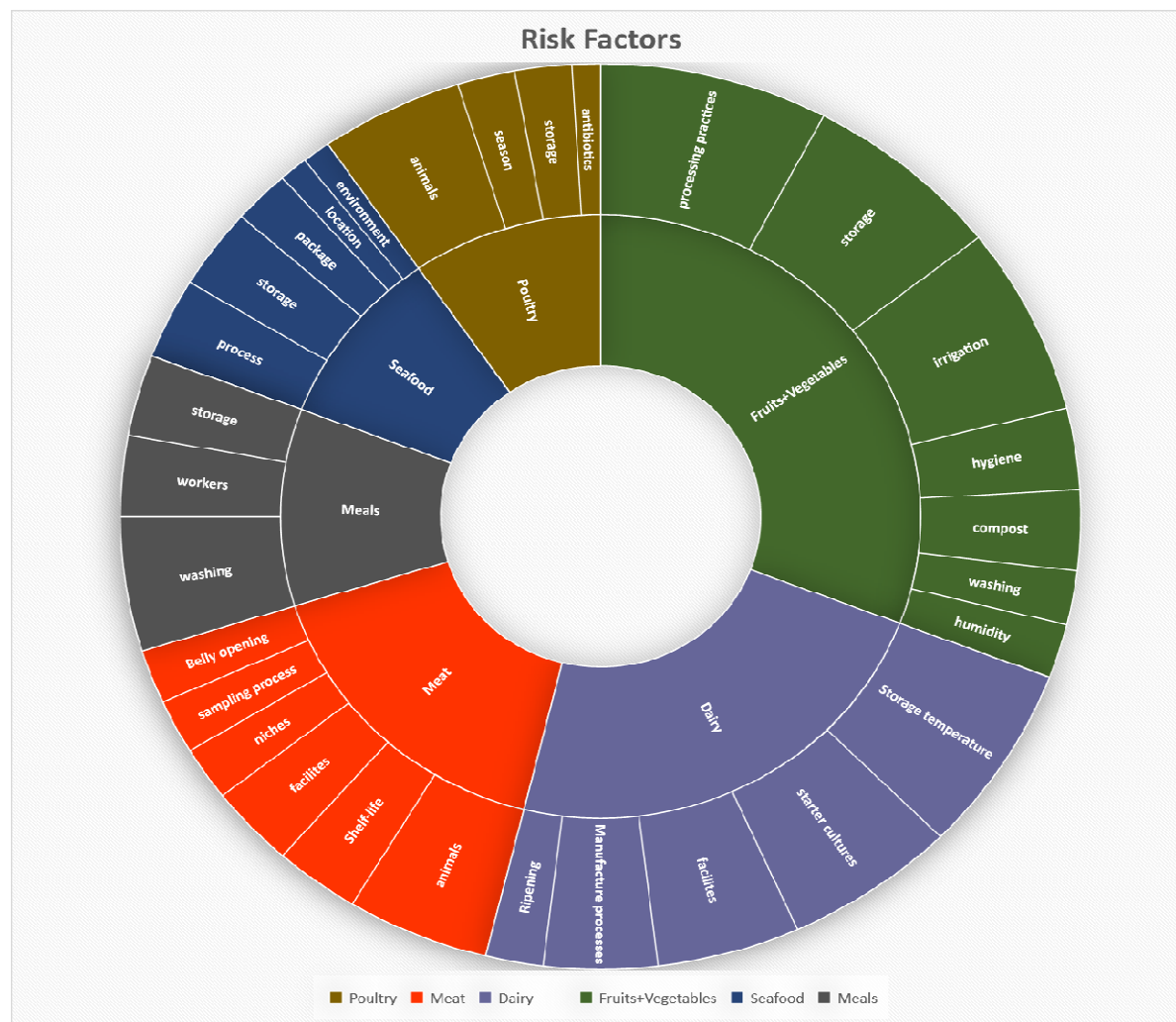


Figure 6. Microbiological Risk factors of each food type

4. Discussion

The current systematic review sought to synthesize quantitative evidence regarding microbiological risk factors in food industry in Europe. Food type, foodborne pathogen, country and technology are presented as well.

Most of the studies included, examined microbiological risk factors, about fruits and vegetables. This can be explained by the fact, that fruits and vegetables are often consumed raw (Balali et al., 2020). The last decade, fruits and vegetables consumption increased as a result of healthier lifestyle (Machado-Moreira et al., 2019). Furthermore, some procedures in production may cause damage in tissue plants and therefore, these food products can be easily contaminated by microorganisms (Kaczmarek et al., 2019). Thus, it is essential need for scientists to focus on this food type and examine all the factors involved.

The majority of the studies focused on contamination of food products by *Listeria monocytogenes*. *Listeria monocytogenes* considered to be an important food associated-pathogen due to the fact that it can adapt, survive and multiple in a variety of environments such as low pH, refrigeration temperatures and high salt concentration (Ballom et al., 2020; Marik et al., 2020; Sheng et al., 2017; Smith et al., 2018). There are numerous works that studied contamination routes or ecology and physiology of *Listeria monocytogenes* in a great range of food products. Seafood and aquatic products, fresh produce, milk, meat, cheese are some food types already examined (Castro et al., 2018; Honjoh et al., 2018; Selvaganapathi et al., 2018).

Spain is the leader country regarding microbiological risk factors in foodstuff, with 21 publications. There are many surveys which indicated that further prevention strategies against foodborne pathogens, should be developed in Spain (Arnedo-Pena et al., 2016; Ballesté-Delpierre & Vila Estapé, 2016; Herrador et al., 2019; Letchumanan et al., 2019; Romalde et al., 2018). Additionally, authors highlighted that targeted prevention such as food safety education or upgraded industrial measures should be prioritized. Studies included in this systematic review revealed that Spain has the highest number of reports, followed by Italy with 12 publications about microbiological risk factors. A great variety of food products, which hold quality schemes from European

Union, take place in Italy. Therefore, last decade, Italy has emerged in the field of food safety and quality (Dimitrakopoulou & Vantarakis, 2021).

Culture-dependent techniques are traditionally used for pathogens detection in food samples. However, culture-independent techniques have been developed in order to face up existing limitations and challenges (Dimitrakopoulou et al., 2021). DNA based and immunological methods are alternative and validated tools that have already utilized in food industry (Rohde et al., 2017). For example, Next Generation Sequencing approaches provide more information about microbial profiling of a food product (Fanning et al., 2017; Ronholm, 2018). Therefore, technology used will influence pathogens detection. A key solution for this issue, would be the application of both culture-dependent and independent techniques (Dissanayake et al., 2018; Fenske et al., 2020; Zampieri et al., 2021).

In terms of risk factors, our results are in agreement to a study conducted in 2018 about sources and contamination routes of microorganisms to fresh produce, which evidenced irrigation water and soil, as well (Alegbeye et al., 2018). Disinfection treatments of water as intervention strategy could be applied and positively affect total microbiota (Dandie et al., 2020; Truchado et al., 2018). Furthermore, there are several studies which highlighted, that biological soil amendments are as also a potential source for pathogens in fresh produce (Iwu & Okoh, 2019; Sharma & Reynnells, 2018). Implementation of bacteriophages technology in pre and post-harvest processes could be an alternative solution to control pathogens in fresh produce (López-Cuevas et al., 2021).

In contrast, infected animals, facilities and storage conditions are risk factors for meat contamination. These findings are similar to studies, which were conducted in order to present microbiological risk factors of the meat chain and underlined contamination sources (Fasanmi et al., 2018; Rosette et al., 2019; Zwirzitz et al., 2020). Infected animals can be source for poultry contamination, as well (Heredia & García, 2018). Antimicrobial use in animal husbandries is proposed as prevention strategy for animal origin foods (Pérez-Rodríguez & Mercanoglu Taban, 2019). Moreover, risk factors for poultry contamination can be storage temperature and long-term food storages (Duqué et al., 2021; Lytjou et al., 2020). Storage temperature considered to be a high risk for pathogens growth in dairy products, too (Lianou et al., 2018; Papadopoulou et al., 2018). Intelligent packages which contain time-temperature indicators could be utilized in food industry and provide information about storage stability of a food product throughout the supply chain (Alfei et al., 2020; Ndraha et al., 2018). Interestingly, starter cultures used for dairy products production are associated to quality and safety (El-Hajjaji et al., 2020b; Gérard et al., 2020b; Imran et al., 2019). The role of microbial starters for products such as milk, cheese and yoghurt in terms of safety and quality, considered to be crucial (Russo et al., 2017). Therefore, special attention should be given to starter culture selected for dairy products (Motawee & Neveen, 2016). Finally, our results are in agreement with a study about microbiological risk factors in dairy facilities, that pointed out storage temperature and hygiene of environment and equipment are crucial. In general, better knowledge and understanding of food safety issues are suggested as an additional preventative measure (Phiri et al., 2021).

Storage conditions, process line, packaging and environment resulted as responsible factors for sea products contamination. Naturally occurring pathogens in aquatic environments are reported in several studies as risk factor for seafood (Afreen & Ucak, 2021; Ali et al., 2020; Pumipuntu & Indrawattana, 2017). Furthermore, seafood contamination during processing, storage and packaging is also a challenge, that managers are trying to face up by utilizing Good Manufacturing Practice (GMP), Good Hygiene Practice (GHP) and Hazard Analysis and Critical Control Point (HACCP) systems (Elbashir et al., 2018). For instance, refrigerated storage can affect growth of *Listeria monocytogenes* in seafood (Dumen et al., 2020). Continued surveillance system could be important for future prevention efforts.

Finally, microbiological risk factors for prepared meals considered to be poor hygiene of workers, storage conditions and washing, especially for foods that not subjected in any heat treatment (Valero et al., 2016).

Therefore, it is essential need to evaluate all available information about risk factors, microorganisms and food products and finally, fulfil gaps regarding food safety and food quality. It is evidenced, that all data for risk factors of contamination each food type by potential pathogens should be further examined, so that the already existing managements systems could be upgraded by effective control measures. Definitely, by culture-independent techniques, extra information about foodborne pathogens and routes of contamination are obtained. Thus, utilization of advanced methods in food industry could be considered as an added-value under the risk-based food safety management framework.

5. Conclusion

Based on this systematic review of the literature, potential microbiological risk factors in terms of food quality and safety, were summarized and presented. In conclusion, our analysis can provide risk managers and public authorities, an overview of microbiological risk factors, from “farm to fork”. Implementation of effective risk management systems in food industry could contribute to identify and eliminate potential risks and thus,

consumer's health and food quality could be reassured. Therefore, our findings could provide managers in food industry either to build up more effective management systems or even help scientists to better understand ecology of pathogens regarding food matrix and environmental conditions. Moreover, omics technologies such as WGS or NGS, considered to be very promising, addressing issues regarding foodborne pathogens in supply chain and should be incorporated into risk assessments strategies. These tools could open up new perspectives in microbiological hazard identification, such as better understanding of foodborne pathogen dynamics or pathogens variability. Further studies regarding the development and practical application of improved measures in managements systems and survival of pathogens in complex matrices are recommended.

Author Contributions: Conceptualization, M.D.; methodology, M.D.; validation, M.D. and A.V.; formal analysis, M.D.; investigation, M.D and A.V.; resources, M.D.; data curation, M.D and A.V.; writing—original draft preparation, M.D.; writing—review and editing, A.V; visualization, M.D.; supervision, A.V; project administration, A.V.; funding acquisition, A.V. All authors have read and agreed to the published version of the manuscript.

Funding: The present research received fund from the Single State Action Aid for Research, Technological Development & Innovation «INVESTIGATE - CREATE – INNOVATE" project "Trust Trace" T1EDK-04028.

Conflicts of Interest: The authors declare no conflict of interest.

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