Novel strategies and tools for microbial risk assessment of foods of animal origin

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Abstract. Risk assessment aims at providing structured information for decision making, public health improvement, regulatory actions and research initiatives. The four very distinct steps in the risk assessment process enable risk management and risk communication, and thereby, a functional food safety system. Identification, characterization and assessment of risks demand the application of science-based, accurate and reliable methodologies. Nowadays, several different widely recognized approaches to risk assessment are applied worldwide. Novel omics technologies are benchmarking a new era of pathogen testing, providing much more than just accurate identification. These technologies have now opened the door for a more integrated approach that can enlighten transmission patterns and predictions of the transmission routes. Merging data on virulence, interaction of pathogens with different food matrices and the host, multiple data processing is resulting in reliable and science-based responses to the forthcoming challenges.

1. Introduction
Food culture is a universal language, a primary form of cultural expression that joins people together. Food research should always something for more than just profit, since food has a significant impact on human health, economic growth and world sustainability. Being a strong pillar of the global economy, the food production sector still needs significant improvements through increasing the efficiency and competitiveness of food production companies, primarily through application of new science-based practices and contemporary achievements in this area that would boost innovations required for economic growth and prosperity.

Food safety, being an ultimate priority in the field of food production, demands accurate and reliable models for the assessment and analysis of foodborne risks of hazards to human health and effective control system in place. Following Regulation EC No. 178/2002 of the European Parliament [17], it is clear that food safety risk analysis should be based on the following principles: risk management, risk assessment and risk communication.

Microbiological hazards can cause outbreaks of foodborne illness. On a large scale, these food incidents can cause numerous illnesses, hospitalizations, and chronic medical conditions and can have significant mortality rates. Microbiological risk assessment (MRA) minimises the risks of foodborne hazards that could adverse events among the population. This complex area, as defined by the Codex Alimentarius Commission [12] can be properly monitored if the following activities are undertaken: hazard identification, hazard characterization, exposure assessment and risk characterization. As evidenced so far in numerous epidemiological surveys and foodborne illness outbreak reports, the foodborne disease burden is constantly growing. Therefore, it is necessary to develop comprehensive tools for assessment of the hazards, their proper identification and quantification, determination of their occurrence probability and prioritisation of microbiological risks on the basis of country-specific uncertainties.
The EU and other developed countries worldwide apply MRA strategies to enable comprehensive evaluation of risks that influence the shaping of the food safety strategies globally. Due to the high genetic diversity of microorganisms, it is still a challenge to encompass all the niches of their occurrence, transmission patterns along the food chain and impact on human health. MRA, being a scientific and analytical tool, encompasses the analysis of all the steps in the food production chain (from collection of raw materials, through processing, to the different food consumption patterns, retail outlets, restaurants and private homes). MRA is necessary, and to be effective, it demands science-based data collection, analysis and processing which then lead to valid decision making.

Traditional microbiological methods have long been applied in food safety control, investigation of food-related illnesses and scientific research in this field [11, 41, 44]. However, new technologies are increasingly available that ensure rapid or early detection of precise signals indicating the presence of microbiological hazards in foods [11, 10, 28, 41]. In the digital era, the implementation of new reporting models is mandatory for improving the detection of food-related hazards (and evaluating their risks). For this reason, analysis of population-based studies is needed to provide science-based knowledge of foodborne pathogens.

Rapid detection of foodborne pathogens, understanding their transmission routes and their relation to environmental conditions, monitoring trends, and understanding antimicrobial resistance and prioritisation are of the utmost importance for food safety. Therefore, health authorities worldwide have established comprehensive programs for the purposes of surveillance and management of effective food safety systems.

Nowadays, whole genome sequencing (WGS), a relatively new technique, provides the most reliable tool for typing of foodborne pathogens. Not yet widely applied, its potential indicates it will be a primary choice for accurate and reliable implementation of MRA.

2. **Risk analysis paradigm**

A broader paradigm of risk analysis includes three important components [12] as depicted in Figure 1.

![Figure 1. The components of risk analysis](image)

*Risk management* is the process of weighing policy alternatives in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair-trade practices, and, if needed, selecting appropriate prevention and control options.

*Risk communication* is the interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk
managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk assessment, recognized as a reliable tool for providing information necessary for setting up regulations, programs and research in the area of food safety, according to the Codex Alimentarius Commission [12,13] has the following steps: (a) hazard identification; (b) hazard characterization; (c) exposure assessment and (d) risk characterisation. MRA is a science-based approach applied to estimate the probability of exposure to a microbial hazard [12, 13, 18, 19, 21, 22, 23]. It also encompasses scoping, planning, decision making within the frame of risk management, communication with relevant stakeholders, etc. (Fig. 2).

**Figure 2.** Risk assessment framework and its relationship with other components of risk analysis (adapted and modified from [37])

The widely used hazard-based approach, when adequately applied, has the purpose of preventing or reducing pathogenic microorganisms in the food production chain. However, since the zero-risk approach cannot be applied to microbial agents, the regulatory microbiological criteria define criteria for the acceptability of food products or food production processes.

The Codex Alimentarius Procedural Manual, 25th edition [13] states that the Hazard identification is the “qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food”. Hazard identification is a qualitative process in identifying chemical, biological or physical hazards, their description, impact on human health and mechanisms of action [33].

Hazard characterization is defined in the as “the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food” [13]. When applied to MRA, hazard characterisation is the qualitative or quantitative defining of the different effects that a microorganism or its toxin in food can cause. For such definition, different sources of information are used, such are clinical or epidemiological information, mathematical modelling, dose-response models, etc. [13]

Exposure assessment is defined as “the qualitative and/or quantitative evaluation of the likely intake of biological, chemical and physical agents via food as well as exposures from other sources if relevant” [13]. This is actual or anticipated exposure to microbial pathogens or their toxins, and the most useful tools for exposure assessment are predictive models. Exposure assessment must include frequency of food contamination by the hazard, ecology of the specific food, possible contamination of the raw material, processing, packaging, and storage and distribution pathways, and the final preparation of the given food.

Risk characterisation is defined as “the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation and
exposure assessment” [13]. Risk characterisation is actually merged information obtained through previous steps. It estimates the degree of the risk of a hazard in a specified food for a population group.

MRA can be classified in two general categories, quantitative and qualitative. Qualitative risk assessment is mainly descriptive and estimates the risk and factors affecting the risk. This type of risk assessment covers an overview of the literature and available scientific knowledge and uses a systematic approach even though it does not include numerical parameters [4, 18, 19, 24]. It is usually implemented before quantitative assessment [24, 35].

Quantitative microbiological risk assessment generally uses two types of models: deterministic or stochastic. Unlike deterministic models, stochastic models can use estimation of randomness (when defined) and therefore might be a better choice for natural systems. For deterministic assessments, all variables are assigned a certain fixed value, which could be a mean or maximum value or a “worst-case scenario” from a variable data set, for example [4, 18, 19].

Numerous adverse incidents connected to meat production and processing (Bovine Spongiform Encephalopathy (BSE), avian flu, foot and mouth disease, some emerging and/or evolving pathogenic bacteria such as *Escherichia coli* O157:H7 and *Listeria monocytogenes*) have been in the focus of all the stakeholders [27, 42, 43]. Even given existing global regulations and awareness concerning animal production, there are still many arguments confirming that this chain still poses a big threat to public health. Microbiological risks are of the biggest concern in the meat production sector. The possible sources of contamination occur initially at the animal husbandry level, but at all other stages too, including raw meat, through all the steps of production, biofilm formation, storage and distribution. The hazards in food of animal origin can be intrinsic to the live animal or are introduced during handling and processing. These hazards can be classified into zoonoses or environmental hazards. Therefore, application of efficient and accurate methodologies, enabling fast predictive matrices will be in the focus of the attention when MRA for food of animal origin is conducted.

3. **Novel methodologies used for microbial risk assessment**

In the last five decades, a significant improvement has been evidenced in the field of food safety through application of novel, accurate and reliable techniques [10, 7, 11, 28, 44]. Novel technology has induced the inevitable shift from classical microbiological testing to rapid detection methods based on PCR and the most recent omics technologies.

The application of novel omics techniques in MRA addresses the challenges. Recent initiatives in this area were discussed by different stakeholders at the European Symposium of Food Safety, Cardiff, April 20–22, 2015, organized by the International Association for Food Protection (IAFP), while the IAFP European Symposium in Athens, May 11–13, a workshop on Next Generation MRA (Microbiological Risk Assessment) - Integration of Omics Data into Assessment was co-organized by ILSI Europe, IAFP and ICFMH [10, 11].

![Diagram](image)

**Figure 3.** Different applications of omics techniques in the analysis of food pathogens
The application of new technological developments (known as omics) significantly improved general understanding of microbial patterns, physiological triggers and behaviour of microorganisms in different food matrices (Figure 3). Among these techniques, the most important ones are genome sequencing, protein analyses and assessment of the metabolic profiles of microorganisms [11, 48]. However, until recently, these technologies were applied solely for scientific research purposes, and not for risk assessments in the area of food safety. Recent studies showed they could be powerful tools in this area [14, 30, 42].

Structuring and analysis of omics data is rather complex, making it difficult to successfully incorporate them into the current MRA paradigm. Rather than focusing on genotypic characterization, the solution to this problem might be the proper analysis of gene expression [6, 10].

Identification of the transmission patterns and origin of diseases can be revealed by the application of Next Generation Sequencing (NGS) techniques, also termed WGS [10, 31, 41, 45]. The technique can immediately offer completely accurate strain-level identification of pathogens, and information on virulence potential and antibiotic resistance that is now competitive with several current methods that must be coupled together to produce the same result [8, 15, 16, 27]. Furthermore, WGS provides information on genes, mobile genetic elements, and horizontal gene transfer [34].

WGS is applied to detection of food pathogens in the United Kingdom, Denmark, France and the United States [2, 3, 27, 32, 35]. In the US, WGS techniques to detect listeriosis outbreaks can more efficiently identify the causative agent at smaller concentrations than traditional microbiological approaches, showing the good potential of WGS for further larger-scale applications [32]. The potential of WGS lies in the fact that, if properly designed and applied, it can replace numerous time-consuming techniques such as serotyping, virulence profiling, antimicrobial resistance, sequencing, etc. [25].

Detection of the presence of foodborne pathogens in a food matrix can be impaired by their low numbers in comparison to the remainder of the microbiota present in the food matrix [9, 20]. However, another possible option for successful employment of the WGS techniques is to focus on identification of pathogens’ virulence genes or bacterial interactions and their impact on food safety [9, 10, 20, 26, 47].

Predictive Metagenomic Profiling (PMP) can reveal the functional genes in microbial communities, resulting in prediction of their contribution to risk [11, 46], which can be applied to support MRA studies. Genome Scale Metabolic Models (GSMMs) can be appropriate for detection of specific pathogens [5]. This technique can reveal important information about gene and protein patterns and metabolism of microorganisms [39] and, therefore, can be useful as a predictive tool. This relates especially to the specific components of MRA such as hazard identification and exposure assessment [1, 5, 36, 38].

These novel techniques have limitations, such as their validation for purposes of quantitative risk assessment as well as the lack of standardization of these methods that can impair reproducibility result [40].

4. Conclusion
There is a constant need for improvement of the accuracy and reliability of the current food safety system and the tools for MRA, a strong pillar of food safety. Following on from implementing methodologies and strategies for MRA, novel omics techniques, especially WGS, are a promising area for collection and analysis of vast data on pathogens, their characteristics, and their relation to food matrices and the human population. These technologies should open a new era of fast, reliable, science-based information on foodborne pathogens, validated protocols and improvement of fundamental science. Food safety and microbial modelling should both advance.

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