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Microbial food safety in the 21st century: emerging challenges and foodborne pathogenic bacteria

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The challenge of foodborne disease

With billions to feed worldwide, the need to produce adequate amounts of safe food, unadulterated by bacterial, viral and protozoan pathogens, as well as harmful residues, pesticides and allergens, remains one of the major challenges in modern times.

According to the World Health Organisation, unsafe food containing harmful bacteria, viruses, parasites or chemical substances, causes more than 200 diseases – ranging from diarrhoea to cancers. An estimated 600 million, i.e. almost 1 of 10 people in the world, fall ill after eating contaminated food and 420,000 die every year, resulting in the loss of 33 million healthy life years (DALY's = disability adjusted life years) (WHO, 2015a, b). Diarrhoeal diseases are the most common illnesses resulting from the consumption of contaminated food (WHO, 2015a, b). A data synthesis (Kirk et al., 2015) on the global and regional disease burden of 22 foodborne diseases in 2010 estimated that these caused 580 million foodborne illnesses in 2010. Norovirus alone was responsible for 125 million foodborne illnesses, the largest number for any pathogen. Other pathogens resulting in high numbers of foodborne cases were *Campylobacter* spp., non-typhoidal *Salmonella* spp., Enterotoxinogenic *E. coli*, Enteropathogenic *E. coli*, STEC and *Shigella* spp. (Table 1) (Kirk et al., 2015).

Looking at the European situation, zoonoses monitoring activities carried out in 2016 in 37 European countries found campylobacteriosis the most commonly reported zoonosis, followed by salmonellosis, yersiniosis, Shiga Toxin-producing *Escherichia coli* (STEC) infections and listeriosis (Table 1). However, while the increasing EU trend for human campylobacteriosis cases since 2008 stabilised during 2012-2016, within the same period the decreasing EU trend for confirmed human salmonellosis cases ended, due to the recent *Salmonella* Enteritidis outbreaks, accounting for 59% of all salmonellosis cases in EU (EFSA and ECDC, 2017). On the other hand, the number of confirmed STEC infections in humans remained stable whereas the decreasing EU trend of confirmed cases of yersiniosis since 2008 stabilised during 2012-2016. Moreover, a further increased number of confirmed human listeriosis cases was registered in 2016 (EFSA and ECDC, 2017). Of the 4,786 weak- and strong-evidence foodborne and waterborne outbreaks reported in 2016 by 27 member states, bacteria were the most commonly detected causative agents of zoonoses (33.9%), followed by bacterial toxins (17.7%), viruses (9.8%), other causative agents (2.2%) and parasites (0.4%). Hereby *Salmonella* was accounting for 65% of the outbreaks caused by bacterial agents. The main foods involved in the strong-evidence outbreaks were from foods of animal origin and these were from ‘eggs’ (23.0%), ‘poultry meat’ (18.5%), ‘fish and fisheries’ including ‘crustaceans, shellfish, molluscs and its products’ (22.4%), ‘meat and meat products other than poultry’ (21.7%), and ‘milk and milk products’ (14.4%), while one-third of all strong-evidence outbreaks involved ‘buffet meals’, ‘mixed food’ and ‘other foods’ including ‘unspecified foods’.

Rising to the challenge

Acknowledging that we have made considerable progress in taking action for increasing food safety in the last 15 years, we still have considerably high numbers of illnesses and hence risks associated with the consumption of food, and the disease burden still is high (Kirk et al., 2015). Moreover, because about 20% of the population of the United States and the United Kingdom belong to the so-called “vulnerable people” (Lund and O’Brien, 2011; Lund, 2015) (especially the very young, the elderly and immunocompromised), we are more pressed for finding solutions for increasing food safety. A “from farm-to-fork” approach of food safety along the whole food chain has been adopted by many countries already a number of years ago (EU, 2014). Recognizing that the farm-to-fork approach may not be sufficient, in the last years the “one-health-initiative” emerged, stating that we have to start at the farm level, with pathogen-controlled feed and with healthy livestock to assure food safety, as well as with a healthy environment (Kahn, 2017). This came with the realisation that the health of livestock affects human health, especially in connection with antibiotic resistant bacteria (including foodborne pathogens). Use and misuse of antibiotics in both humans and animals are responsible for the development of resistant bacteria (WHO, 2018) and antibiotic resistance is therefore an important topic within the One-Health initiatives. Especially misuse or overuse of antibiotics in animal husbandry could finally result in resistant bacteria occurring in the food chain. Thus, although progress towards safe food production has been made, new emerging challenges arise at the consumer, microorganisms or food processor levels (Fig. 1), which require us to re-think food safety and keep a constant vigil for emerging threats.

Emerging challenges

Having considered food safety from the 'one health' and the 'farm-to-fork' approaches, one challenge remains at the level of the consumer, particularly the ***vulnerable consumer***. The very young may be particularly at risk, because of the immaturity of their immune and physiologic systems (IUFoST, 2015). For the elderly (25% of the European population in 2017) and whose number is projected to further increase worldwide from the estimated 962 million in 2017, to 1.4 billion in 2030 and 2.1 billion in 2050 (United Nations, 2017), weakness of the immune system also increases vulnerability. The vulnerable are also those having poor nutritional status, existing health problems, and drug therapies which suppress the immune system (IUFoST, 2015; Lund and O'Brien, 2011; Newman et al., 2015). Such persons are more likely to acquire foodborne illness and are prone to more severe disease outcomes, including higher mortality rates (IUFoST, 2015). The challenge will be to produce foods with low microbial risks, to define and exclude high risk foods and to disseminate clear advice about food safety.

The 'one health' initiative quite rightly connects environmental and animal health with human health. Changes in the agri-food chain, social changes and advances in the detection and reporting systems, coupled with bacterial adaptation and evolution, may lead to certain microorganisms becoming new or ***emerging zoonotic pathogens***. Examples of such include shigatoxigenic/ enterohaemorrhagic *E. coli* (STEC/EHEC) and *Campylobacter* spp. in the meat chain, *Listeria monocytogenes* in vegetable, meat or milk products, *Cronobacter* spp. in infant milk formula, *Arcobacter* spp., *Yersinia enterocolitica* serobiotype O3/4, parasites such as *Cyclospora* on fruit and *Cryptosporidium* and *Giarda* in water, as well as hepatitis E virus in pork and boar meat (Duffy et al., 2008; Batzilla et al., 2011; Park et al., 2016; Ramees et al., 2017). Recognising these current zoonotic pathogens and their potential for foodborne transmission will be essential for identifying emerging foodborne pathogens.

Viruses (adeno-, calici- and enterioviruses) are important pathogens which in many countries are the most numerous causes (norovirus) for foodborne infection. For adenoviruses or caliciviruses no standardized methods for cultivation or detection exist. While standardized procedures for cultivation of some enteroviruses exist, these methods are not capable of distinguishing between virus types and are not applicable for all enteroviruses (Hartmann and Halden, 2012). Detection is also challenging because viruses have a high mutation rate and many have a high probability of infection even at 10 virions (Hartmann and Halden 2012). This has obvious implications regarding the difficulty for the ***detection and monitoring of foodborne viruses***. Here, methods for virion concentration, as well as sensitive molecular biological or serological methods, or even mass spectrometry, need to be developed for an accurate and specific detection at low contamination levels.

Decreasing the excess use of antibiotics in animal husbandry and in human medicine is especially important to decrease the occurrence and spread of ***antibiotic resistant bacteria***. Yet antibiotic use cannot be decreased to zero in the interest of human and animal health. It will be important, therefore, to define points of pathogen entry, trace transmission routes along the food chain, to determine the evolution of transferable antibiotic genes and more importantly to find control measures which prevent or diminish the entry and spread of resistant microorganisms or resistance genes. Here, not only true foodborne pathogens are of importance, but also opportunistic pathogens such as *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp. and *Serratia* spp. These are well known to occur in various foods (e.g. meats, vegetables, milk) and to cause hospital infections (Nordmann et al., 2012; Fusco et al., 2018). Additionally, even non-pathogenic bacteria may become antibiotic resistant and can be relevant in spread. The challenge here is to monitor the spread and evolution of such bacteria to prove an animal/environment/human connection. One approach may be a syst-OMICS approach, as was recently reported to be adopted for salmonellosis to ensure food safety and reduce the economic burden. The study by Emold-Rheault et al. (2017) sets out to sequence the genomes of 4500 *Salmonella* genomes and to build an analysis pipeline for the study of *Salmonella* genome evolution, antibiotic resistance and virulence genes. This way, the study aims to draw potential links between strains found in fresh produce, humans, animals and the environment (Emold-Rheault et al., 2017). A similar approach would be worthwhile for adopting for other bacterial pathogens such as *Campylobacter, Listeria monocytogenes*, pathogenic *E. coli* strains, or the opportunistic pathogens mentioned above.

Climate change may well be important for microbial food safety in the 21st century. There is reasonable evidence that the environment and weather play a role in the transmission of e.g. *Salmonella* and *Campylobacter* spp. to humans, even though there is uncertainty about the mechanisms behind this (Justus et al., 2017; Lake, 2017; Nichols et al., 2018). Possibly global warming may have such an effect on increased transmission also with other pathogens, or may even become a key factor in selecting for other emerging pathogens. Food will also be produced in altered climatic conditions in modified surrounding ecosystems, and the interactions between these changes and the food production systems are complex and uncertain (Lake and Barker, 2018). For example, increased indoor animal husbandry to counteract heat stress may elevate the potential for animal to animal transmission of zoonotic pathogens. Increased growing seasons may lead to greater use or outdoor pastures and increase the probability of transmission of pathogens from the environment. Flooding or drought may favour the spread of pathogens to produce, or have consequences on water quality and pathogen transmission (Lake and Barker, 2018). Another important aspect concerns the increasing water shortage and the worldwide demand for fresh water. As a result, an increase in the use of waste water for irrigation and sewage sludge could be expected, accompanied by increasing risks of contamination of agricultural land and plants with pathogens.

Research into ***novel food preservation methods*** (or technologies) remains a challenge, particularly when considering the production of foods with low microbial diets for vulnerable people. Against this background we need to discuss whether all food needs to be made suitable and available to the vulnerable, or whether specific safe diets need to be formulated or especially produced? Specific preservation technologies that have been researched and to some extent applied in the last years include high hydrostatic pressure, pulsed electric fields, high voltage arc discharge and cold plasma (Stoica et al., 2013), as well as pulsed light or UV-C treatments. One promising biocontrol tool would also be the use of lytic bacteriophages to specifically control pathogens or antibiotic resistant opportunistic pathogens. Due to their host specificity, lytic bacteriophages would act very target specific (Jordan et al., 2014). This would be of obvious advantage also for use in specific foods suitable for this technology, in which a pathogen of concern needs to be inactivated to improve its safety for the vulnerable people group.

Interestingly, the Executive Summary of Food Safety by the EFSA (2009), reporting on a survey of consumer risk perception showed that the consumer is more likely to worry about risks caused by external factors, over which they have no control, e.g. consumers expressed concern regarding contamination of food by bacteria and unhygienic conditions outside home. On the other hand, they seemed less concerned about factors linked to their own behaviour (e.g. food preparation, food hygiene at home). Apart from such optimistic bias and illusion of control, other reasons for unsafe food preparation by the consumer were shown to include habits and lack of knowledge concerning food safety during domestic food preparation, as well as disagreement with some recommendations for safe food handling (Al-Sakkaf, 2012; Young and Waddell, 2016). Regarding the latter for example, a study by Kosa et al. (2015) on consumer-reported handling of poultry products at home showed that there was low adherence to current recommended food safety practices by the consumers regarding that they should not wash raw poultry before cooking, proper refrigerator storage of raw poultry, use of a food thermometer to determine doneness, and proper thawing of raw poultry in cold water. Clearly, therefore, risk assessment agencies or communicators should in future spend more effort in gathering and utilizing such information to develop and update science-based education materials.

Microbial Risk Assessment

Risk assessment is a science-based process consisting of hazard identification, hazard characterization, exposure assessment, and risk characterization (CAC, 2014). Microbial risk assessment (MRA) can largely help to understand the behaviour of pathogens over a food chain, to predict health risks and the expected public health effects of interventions and standards (Havelaar et al. 2010). For risk assessment studies, many quantitative data are needed, like prevalences of foodborne pathogens, characteristics of organisms, food products and processes, virulence of organisms and susceptibility of humans, as well as public health and epidemiological data. In the last decades, more and more of these data became available, not always perfect, but the quantity and the availability of data has increased largely. This information is even in certain cases overwhelming (big data), not only regarding microorganisms characteristics (genomics) and behaviour (transcriptomics and metabolomics), but also the tenacity (survival or dying) or growth with regard to products, processes, intrinsic and extrinsic factors of foods, and even human behaviour. The exact meaning of data and defining its quality and applicability can then become problematic. Difficulty apart from searching, collecting, defining, interpreting and valuing the data sources is also how to make use of it, since large variability and uncertainty exist (Zwietering, 2015, Koutsoumanis & Aspridou, 2016, Membré & Guillou, 2016). Adaptation and evolution of microorganisms within a changing environment might affect the genotypes or lineages of pathogens which become problematic. Thus genotype-specific risk assessment (Carlin et al., 2013) and individual cell-based modelling (Koutsoumanis, 2008; Metselaar et al., 2016) are becoming increasingly important. These approaches might contribute to fine-tune the hazard identification, hazard characterization and exposure assessment elements of microbiological risk assessments and thereby reducing the uncertainty in risk characterizations (see for a more in-depth discussion e.g. Cocolin et al., 2018; Den Besten et al., 2018; Haddad et al., 2018; Membré & Guillou, 2016; Pielaat et al., 2015; Rantsiou et al., 2018). Reports with data, databases (e.g. Combase, http://www.combase.cc), and many tools are developed (e.g. PMM-Lab, https://foodrisklabs.bfr.bund.de/pmm-lab/, Baseline, http://www.baselineapp.com/) that can make implementation of risk assessments more available for more people. Ultimately the integration of genotypic data that can be obtained with omics technologies and quantitative phenotypic data (i.e. quantitative descriptors for growth, survival and inactivation for genotypes and heterogeneity between individual cells) and simulation tools and experimental challenge tests make it possible to get better grip on magnitudes and sources of risks. This is needed to evaluate various ways to effectively control the microbial risks with technical solutions, behavioural changes, changes in product formulations and in standards and legislation, for a balanced control of hazards in our foods.

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Table 1: Estimated global cases and reported European cases of food borne illness

Bacterial zoonotic pathogen	Estimated global cases of foodborne illnesses in 2010 (Kirk et al., 2015)	Reported European cases of foodborne illnesses in 2016 (EFSA and ECDC, 2017)
Campylobacter spp.	95 613 970	246 307
Non-typhoidal *Salmonella* spp.	78 439 785	94 530
Enterotoxinogenic *E. coli*	86 502 735	n.r.*
Enteropathogenic *E. coli*	23 797 284	n.r.*
STEC	1 176 854	6 378
Shigella spp.	51 014 050	n.r.*
Listeria monocytogenes	14 169	2 536
Yersinia	n.r.*	6 861

*n.r.: not reported

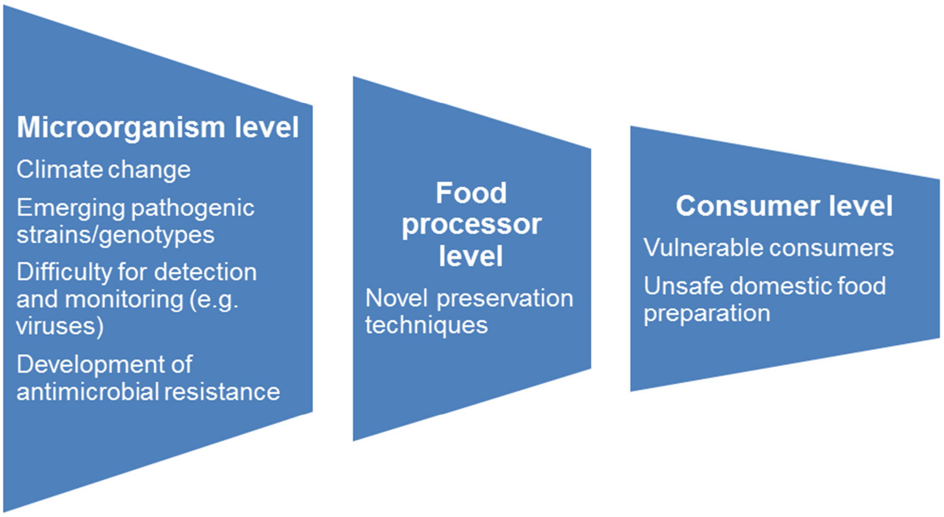

Figure Legend

Figure 1: Challenges for food safety emerging at the consumer, microorganisms or food processor levels

Highlights

High number of infection are still caused by foodborne microorganisms

Increasing number of vulnerable people needs safer food

emergence and spread of antibiotic resistant bacteria should be controlled

new methods for effective food preservation are needed

magnitudes and sources of risks and ways to effectively control these are needed