CONFERENCE ARTICLE

Advancing biological hazards risk assessment

Winy Messens[1], Marta Hugas[1], Ana Afonso[1], Jaime Aguilera[1], Thomas U Berendonk[2], Alessandra Carattoli[3], Sofie Dhollander[1], Peter Gerner-Smidt[4], Nikolaus Kriz[1], Ernesto Liebana[1], Jolyon Medlock[5], Tobin Robinson[1], Pietro Stella[1], David Waltner-Toews[6] and Mike Catchpole[7]

[1]European Food Safety Authority (EFSA), Parma, IT; [2]Technical University of Dresden, Institute for Hydrobiology, DE; [3]National Institute of Health, IT; [4]Centers for Disease Control and Prevention, USA; [5]Public Health England, UK; [6]University of Guelph, CA; [7]European Centre for Disease Prevention and Control (ECDC), SE

Abstract

This paper focusses on biological hazards at the global level and considers the challenges to risk assessment (RA) from a One Health perspective. Two topics – vector-borne diseases (VBD) and antimicrobial resistance (AMR) – are used to illustrate the challenges ahead and to explore the opportunities that new methodologies such as next-generation sequencing can offer. Globalisation brings complexity and introduces drivers for infectious diseases. Cooperation and the application of an integrated RA approach – one that takes into consideration food farming and production systems including social and environmental factors – are recommended. Also needed are methodologies to identify emerging risks at a global level and propose prevention strategies. AMR is one of the biggest threats to human health in the infectious disease environment. Whereas new genomic typing techniques such as whole genome sequencing (WGS) provide further insights into the mechanisms of spread of resistance, the role of the environment is not fully elucidated, nor is the role of plants as potential vehicles for spread of resistance. Historical trends and recent experience indicate that (re)-emergence and/or further spread of VBD within the EU is a matter of when rather than if. Standardised and validated vector monitoring programs are required to be implemented at an international level for continuous surveillance and assessment of potential threats. There are benefits to using WGS – such as a quicker and better response to outbreaks and additional evidence for source attribution. However, significant challenges need to be addressed, including method standardisation and validation to fully realise these benefits; barriers to data sharing; and establishing epidemiological capacity for cluster triage and response.

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1.   Introduction

Globalisation encompasses the intentional movement of people, animals, plants, food and feed around the planet, and the unintentional co-transport of infectious agents, vectors and invasive species. Advances in transport, trade and communication technology have intensified the rate of globalisation in the last decade. Further integration of regional and national economies, societies and cultures is expected as a growing number of countries sign up to free-trade agreements and emerging economies' share of global trade increases. This increasingly globalised trade in food and feed is also associated with the 'trade' of hazards and risks: e.g. food-borne diseases (FBD) and antimicrobial resistance (AMR). Tracing these threats will become even more challenging because of the increasing complexity of food supply webs. Indeed, biological hazards do not respect any borders: the source of an infection may be in a different continent than where the patient becomes ill.

Vector-borne diseases (VBD), owing to their potential impact on animal and public health, trade and environment, justify efforts to assess their risks and improve preparedness. Assessing the risks of VBD, however, with their complex epidemiology being determined by a delicate interaction between vectors, hosts and pathogens, is challenging. Besides globalisation, climate change, political instability, and changes in landscape use or management can be potential drivers for the introduction and establishment of vector-borne pathogens in previously unaffected regions. Big data are generated from trade and the movement of animal and human populations, but information concerning essential epidemiological parameters is often scarce. This disparity needs to be addressed.

Antimicrobial drugs play a critical role in the treatment of bacterial diseases, and their effectiveness is essential to protect both human and animal health. Owing to the rise of antimicrobial-resistant bacteria, it is estimated that globally at least 700,000 people die every year of resistant infections, and this number is expected to increase. Humans can acquire antimicrobial-resistant infections from many different sources, including other humans, animals, and the environment. Food of both animal and non-animal origin is one possible route of transmission. Most of the antimicrobial use in agriculture originates from animal production. The use of antimicrobials, especially if excessive or inadequate, for treatment and prevention of diseases in the livestock sector, aquaculture and crop production, is associated with the risk of emergence and spread of antimicrobial-resistant microorganisms. Contamination of the agricultural environment with antimicrobial-resistant bacteria can also originate from the agricultural use of recycled water or urban wastewater.

In this context, next-generation sequencing (NGS) technologies have entered the field, offering opportunities to characterise food-borne pathogens in much more detail and giving access to the genetic information of pathogens at the highest resolution. They are considered as a rapid and cost-efficient way for the whole genome sequencing (WGS) analysis of a microorganism and hence offer a powerful tool to provide detailed knowledge about its identity and genetic characteristics. This enables tracing of the origin of outbreaks of pathogens and mapping their spread and prevalence. WGS analysis has also an important predictive value in detecting undesirable properties of microorganisms such as their potential for spreading AMR determinants or being toxigenic or pathogenic. This methodology, along with other supporting data, will assist with effective and timely responses to rising threats as well as preventing further ones.

This publication focusses on biological hazards/threats at a global scale and considers the challenges to risk assessment (RA), from a One Health perspective. Two topics, – VBD and AMR – are used to illustrate the challenges ahead and the opportunities that new methodologies, such as WGS, can offer. Currently, only a few organisations have invested in WGS approaches for real-time analysis of pathogens and for the RA of microorganisms in the food chain. It aims:

- to understand the impact of globalisation on assessing and managing biological hazards and threats;
- to illustrate the need for ongoing vector surveillance to detect and respond to changes in distribution, the emergence of new vector-related issues and the importance of non-native species;
- to illustrate the epidemiology of AMR in bacteria causing food-borne human infections, also considering the role of the environment in the spread of AMR;
- to investigate and disseminate the opportunities and challenges that WGS can offer to investigate and eventually prevent human illness.

It will inform EFSA on current challenges and opportunities in the area of biological hazards/threats in the context of globalisation and help EFSA to better define its strategic objectives and adapt its

approaches and methods to future needs in terms of providing scientific advice in the area of biological hazards.

This publication builds upon presentations made and discussions held during the break-out session 'Advancing risk assessment science – biological hazards' at EFSA's third Scientific Conference 'Science, Food and Society' (Parma, Italy, 18–21 September 2018).[1]

2. Summary of presentations, discussions and outcomes

2.1. Key messages of the speakers' presentations

2.1.1. Spam's Labyrinth: impact of globalisation on food-borne disease

The biggest impact of globalisation on FBD is that it has obscured the already-labyrinthine causal webs of those diseases. It made the challenges of elucidating those pathways more complex and discouraged prospects for assessing and managing those impacts. Since the 1960s, global trade in food and feed has increased exponentially, and a number of countries at least partially reliant on this trade have formed International Agri-Food Trade Networks (IATN[2]). Reported FBD have increased in parallel with this, but cases associated with imported foods are uncommon and often surprising. Causal relationships are unclear or only useful retrospectively in specific cases (King et al., 2017). Complex systems approaches have produced stunning diagrams of agri-food fluxes (Ercsey-Ravasz et al., 2012). However, these are difficult to relate to food safety and still more difficult to translate into policies and regulations. Risk analyses have been useful for understanding and managing the impacts of individual hazards in specific foods. Even in RA, the entanglement of science with value judgments is challenging. For example, the relevant outcomes to measure and the extrapolation across species are non-scientific, value-laden choices (Brunk et al., 1995). When risk-based approaches are applied to longer time frames and globally heterogeneous, rapidly changing political and agricultural landscapes, the problems multiply exponentially. The challenge of dealing with globalisation and FBD is therefore less like a scientific research problem and more akin to a professional practice, bringing together data from various sources (case histories, outbreaks, laboratory tests, physical exams, and socio-economic studies) and making clinical judgements. In the context of globalisation, this is a collective judgement, embedded in contested power arrangements, with variable consequences for stakeholders in different parts of the world. Given this situation, there are many possible responses, and there is no global consensus on prioritising desirable outcomes. Some are likely to have larger negative social and ecological impacts than others. The task for risk analysts is to work at different temporal and spatial scales with people who have conflicting goals and unequal power, and where outcomes and processes need to be continually renegotiated in the context of dynamically changing technological, political and ecological landscapes. More information can be retrieved in the invited article 'The Impact of Globalisation on Foodborne Disease' (Waltner-Toews, this issue).

2.1.2. Molecular epidemiology of antimicrobial resistance in bacteria causing food-borne human infections: what do we know, what should we find out?

The relatively few bacterial species that cause food-borne human infections represent a subset of bacteria involved in AMR transmission and dissemination. To link AMR in bacteria from humans to microbial populations from food and animals, a detailed, quantitative understanding of the dynamics of bacteria is needed. Mobile genetic elements allow the horizontal transfer of resistance determinants between different bacterial populations that may be harboured by animals, food and humans (Woolhouse et al., 2015). The ability to trace the circulation of resistance determinants located on such mobile genetic elements within different bacterial populations helps clarifying all the routes by which AMR bacteria and their related genes can be transmitted to humans and can spread around the world. The development of new molecular approaches can help to identify and describe how food can be a vehicle for AMR bacteria or a source of AMR genes, and plasmid databases can facilitate sharing of information and investigating the geographical spread and relatedness of mobile genetic elements (Carattoli et al., 2018). For example, phylogenetic analysis of bacterial genomes combined with profiling of mobile genetic elements (plasmids) and epidemiological data can clarify the impact of

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[1] All conference materials are available at https://www.efsa.europa.eu/en/events/event/180918
[2] https://agrifood.net/

specific antimicrobial drugs used in farm animals on the selection of resistance determinants also identified in strains from human clinical cases (Hansen et al., 2016). Three types of resistance linked to possible transmission by food are particularly important: resistance to third- and fourth-generation cephalosporins, resistance to colistin and resistance to carbapenems (Leverstein-van Hall et al., 2011; Dierikx et al., 2013). The dynamics of plasmid-mediated transmission of AMR genes between food-producing animals and humans appear useful to trace the spread of common AMR features between the two sources. For example, data allow inference on the route of transmission and dissemination of extended-spectrum beta-lactamases (ESBLs) between bacteria from animals and humans and the circulation of mobile colistin resistance in bacteria that may cause food-borne human infections (Rhouma et al., 2016; Dandachi et al., 2018).

2.1.3. Wastewater treatment plant effluents and their implications for antimicrobial resistance in surface water and water reuse

Water shortage is of great concern today in Europe and therefore water reuse is a relevant opportunity to conserve valuable water resources. In particular, wastewater can be used for irrigation in agriculture. This has not only benefits, but also poses some concerns, such as the presence of pathogens and of AMR determinants and their possible spread in the environment through irrigation. Because of the worldwide health impacts of antibiotic resistant pathogens, scientists are increasingly interested in the role of wastewater treatment plants (WWTPs) as a sink and source for antimicrobial resistant bacteria and their genes. To date, the dynamics of resistant bacteria and associated genes in municipal WWTPs has just begun to be explored, but there is clear evidence that antimicrobial resistant organisms and genes are released with WWTP effluents into receiving environments (Berendonk et al., 2015). Studies have demonstrated that the absolute quantity of AMR gene copies is reduced during conventional wastewater treatment, but it is also apparent that the relative abundances of key resistance genes normalised by 16S rRNA copy numbers frequently show no significant reduction and sometimes even increase. These results and their implications for the spread of resistance genes within the context of water reuse were discussed, together with results of the COST action NEREUS focusing on the fate of antibiotic resistance when wastewater is used for irrigation (Fatta-Kassinos et al., 2015; Gatica et al., 2016), in particular the uptake and translocation of organic microcontaminants and antimicrobial resistant bacteria and genes in crops. Prioritised biological agents with potential for uptake by crops include, for example, *Escherichia coli* and other Gram-negative bacteria and a number of mobile genetic elements conferring clinically important AMR. Other projects investigate the fate of antimicrobial resistant bacteria and genes within urban wastewater, soil, ground and surface water, and crops. Some recent research results on the reuse of wastewater for irrigation showed persistence of resistance in bacteria in soil at different depths following irrigation. Other recent research is providing indications on the uptake of antimicrobial resistant bacteria and genes by vegetables.

2.1.4. Vector-borne diseases in Europe: expectations and preparedness

Emerging infectious diseases (EID) are now tackled using a One-Health approach, bringing together experts in human and veterinary health as well as those in bacteriology, virology, entomology and wildlife diseases. As a large proportion of EIDs are zoonoses (pathogens transferred between animals and humans), this multidisciplinary approach is of paramount importance for RA and for mounting a coordinated response. Failure to consider all aspects of disease ecology and epidemiology hinders successful control and a proportionate response. In the case of VBD (infectious diseases transmitted by arthropods), knowledge of arthropod vectors and their relative roles in pathogen transmission is an important first step. To inform RA and provide accurate and timely advice to risk managers, ongoing vector surveillance to detect and respond to changes in distribution are needed to recognise the emergence of new vector-related issues or of non-native vector species. Routine surveillance can trigger alerts to further enhance targeted surveillance and set research priorities. It facilitates risk management, mitigation of disease implications and ensures that governments have the empirical evidence to be well-informed and better placed to prevent and control any emerging vector-borne disease (Medlock et al., 2018). This is illustrated by examples relating to surveillance of invasive mosquitoes whilst addressing concerns about local transmission of Zika and chikungunya virus. Furthermore, routine surveillance of native mosquitoes is important in relation to West Nile virus risks, and knowledge of vector distribution needs to be incorporated into ongoing clinical virology diagnostics. In addition, possible introduction of ticks needs to be monitored to develop evidence for

policy changes on tick controls on travelling pets (Hansford et al., 2017). Tick monitoring will also inform RA on emerging tick-borne arboviruses such as Crimean-Congo haemorrhagic fever virus and tick-borne encephalitis virus. Data generated during 12 years of tick surveillance (Cull et al., 2018) have led to better public awareness, discovery of new foci of disease, and better-informed governments on both public and veterinary VBD issues.

2.1.5. Challenges and opportunities in next-generation sequencing as a tool in food-borne disease surveillance and outbreak investigation

With ever-increasing international travel and globalisation of trade in animals, food and feed, food-borne infections are no longer regional: the source of an infection may be in a different continent from the one where the patient becomes ill. A One-Health approach involving stakeholders from the whole food chain and health sectors is needed to efficiently address the problem. Analysis and comparison of pathogens isolated from patients and throughout the food chain is critical to investigate outbreaks and determine their sources, which is essential to control and prevent them. In the last decade, NGS has emerged as a cost-efficient way to determine the whole genome sequence of a microorganism, thus providing authorities, academia and industry with detailed knowledge of the pathogens and their routes of transmission that was previously unattainable. Because of WGS analysis, it is now possible to detect more outbreaks and trace and control them faster. The technology also allows the identification of the pathogen species and predict characteristics such as serotype, virulence and AMR, thereby complementing and sometimes replacing a multitude of different microbiological workflows with just one analysis (Carleton and Gerner-Smidt, 2016; Nadon et al., 2017). However, sequences generated on different NGS platforms and analysed with different applications ('pipelines') do not provide the same results, and there are no internationally agreed standards for sequence quality. This poses a challenge when comparing WGS data generated in different laboratories both nationally and globally. Some NGS platforms are more accurate than others; some tend to produce specific errors. When should single nucleotide polymorphism (SNP) analysis be used for subtyping and when is whole genome or core genome multilocus sequence typing (wg/cgMLST) more appropriate? Are the genotypic data analysis pipelines used to predict the same phenotypic characteristics (e.g. serotype, virulence profile and AMR) equally reliable and accurate? How much do food safety decision makers need to know about NGS and WGS? In order to avoid having to consider these issues, PulseNet International[3] and its partners work towards creating international standards for WGS analysis and validating different sequencing platforms and workflows to ensure accurate comparisons of data generated in any laboratory. Finally, it should be remembered that WGS data will always need to be interpreted along with other supporting data in any given context to ensure correct decision-making in food safety.

2.2. Discussion

The use of predictive models for vector distribution and efficient surveillance tools for early detection of (new) VBD outbreaks increase the possibility of their control and need to be considered in the context of projected changes in climate, land use, environment, and temperature. This should be kept high on the agenda of risk managers, and sufficient resources should be allocated to preparedness. Views were expressed that resources are currently allocated based on immediate crises rather than planning for the long run. Another point of discussion was the need to integrate vector control in environmental RA. Highlighted was the need for a multidisciplinary approach to vector control involving all relevant RA bodies and stakeholders in proposing the most appropriate tools for vector control available, particularly in cases of VBD outbreaks with potentially important health consequences.

The environment is regarded as a possibly important conduit in the spread of AMR between animals, food and humans. However, monitoring data on AMR bacteria and genes in the environment are currently lacking. This, together with the numerous possible AMR transmission pathways through the environment, hampers the possibility of carrying out RA. The collection of such data is therefore of crucial importance, as well as, for example, the investigation of the role played by the reuse of wastewater in agriculture and the uptake of resistance determinants by food of non-animal origin. Data are also lacking on how AMR is being spread within the global food commodities market.

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[3] http://www.pulsenetinternational.org/

WGS might be applied in epidemiological studies of AMR-carrying plasmids to define threshold numbers of SNPs to consider plasmids from two bacterial isolates as being the same. A strategy to target AMR-carrying plasmids directly to control AMR spread is not available, and the current approach is to kill the bacteria hosting resistance determinants. A plasmid-based strategy could be a tool for the medicine of the future.

The platforms for WGS data generation and analysis are evolving, presenting challenges to the implementation of this technology for routine use. Validation is needed to compare datasets obtained in different places and at different times. It was noted that the economic cost of analysis is still considerable although, with increasing competition in the market, costs are expected to be further reduced, leading to a hoped-for wider application of WGS. Another challenge, especially for plasmid and AMR determinant databases, is the curation of databases for WGS analysis, as different databases require different levels of curation, and curators do not always follow the same criteria. Adequate curation requires considerable work, and currently, the maintenance and curation of databases depend on the availability of resources within the institutions that keep them.

Solving food safety problems at the source of raw materials and products may be the desired option. However, EU policy tends to support import restrictions at the border thus not solving food safety problems at the global level. For example, AMR is certainly a global concern and needs to be tackled as such because it is difficult to control at borders. For AMR, there are no measures in legislation that could prevent food carrying AMR bacteria or genes from entering the EU, and there is no information on resistance genes or bacteria carried by imported food. Data collection is needed, and food safety systems and policies should be agreed and implemented at a global – not local – level. This requires capacity building and involvement of all stakeholders. Scientist can provide data and advice, but solutions cannot come from science alone. Expertise on rare diseases needs to be derived from outside Europe. Therefore, a collaborative environment is key.

3. Conclusions

- Globalisation brings complexity and introduces drivers for infectious diseases, and the control of those drivers may be very difficult (e.g. because the source of contamination is outside the sphere of direct influence of the risk managers). To be sustainable and scientifically and ethically defensible, RA in this context will need to be reframed as a multiperspective approach and in a broader context (going beyond the RA concept as in the Codex Alimentarius Commission paradigm (CAC, 1999), with risks and benefits characterised by and for producers, traders and consumers throughout the global food webs.
- AMR is one of the biggest threats to human health in the infectious disease environment. Whereas new genomic typing techniques provide further insights into the mechanisms of spread of resistance, the role of the environment is not fully elucidated, nor is the role of plants as potential vehicles for spread of resistance through uptake of resistant bacteria and resistance genes from contaminated waste water used for agricultural irrigation.
- Historical trends and recent experience indicate that the (re)-emergence and/or further spread of VBD within the EU is a matter of when rather than if. Anticipation of threats is important, as is active and continuous surveillance. Inclusion of vector control in environmental RA is needed.
- WGS has benefits such as enabling a quicker and better response to outbreaks and giving more evidence for source attribution and the spread of AMR determinants. There are also significant challenges, however, that need to be addressed to fully realise these benefits. These include method standardisation, barriers to data sharing, and establishing epidemiological capacity for cluster triage and response.

4. Recommendations

- Apply an integrated RA approach to biological hazards based on a 'One Health' approach and cooperation that takes in consideration food farming and production systems and includes social and environmental factors.
- Develop methodologies to identify emerging risks at the global level and propose corresponding prevention strategies.
- Investigate the role of the environment and crops as potential vehicles for the emergence and spread of AMR through uptake of residues and antimicrobial resistant bacteria/genes deriving

from reuse of wastewater in agriculture, and develop monitoring systems to collect data on the distribution of AMR determinants in the aquatic and terrestrial environment.

- Develop standardised and validated vector monitoring programs and implement them at international level to allow continuous surveillance and assessment of potential threats. Guide the decisions for vector control by knowledge of disease pathogenesis and epidemiological conditions. Integrate education and prevention of VBD into animal and public health policies.
- Develop international WGS quality standards, standardised and validated methodologies, and tailored tools to use WGS analysis for RA purposes. This should be focused on pathogen epidemiology (better tracking and response to outbreaks) and assessment of microorganisms intentionally introduced into the food chain (e.g. probiotics, biopesticides, productions strains of fermented foods).

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Abbreviations

AMR	antimicrobial resistance
CAC	Codex Alimentarius Commission
cgMLST	core genome multilocus sequence typing
EID	Emerging infectious diseases
ESBLs	extended-spectrum beta-lactamases
FBD	food-borne diseases
IATN	International Agri-Food Trade Networks
NGS	next-generation sequencing
RA	risk assessment
SNP	single nucleotide polymorphism
VBD	vector-borne diseases
wgMLST	whole genome multilocus sequence typing
WGS	whole genome sequencing
WWTP	wastewater treatment plant