Omics Methods For the Detection of Foodborne Pathogens

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Foodborne Disease, a Global Burden

Over millennia our food supply has been constantly affected by multiple issues and serious challenges. Many of these challenges would and continue to be the result of key human developments such as agriculture, urbanisation, industrialisation, and leaps in technological innovation. More recently this includes globalisation, whereby advances in worldwide transportation and rapid digital communication technologies have led to food supply chains/networks becoming increasingly more complex, and fragmented; that is to say, the supply chain is rarely horizontal but is a multidimensional network where many participants within that network only have knowledge of their immediate supplier and who they supply. These more recent challenges have included issues such as the intentional adulteration or deliberate misrepresentation of food products for economic gain, so-called food fraud; an issue first highlighted in the scientific literature during the industrial revolution and now again affecting many countries worldwide (Ellis et al., 2012). Of course food fraud falls within the scope of one of the major contemporary challenges of food supply, namely, food security, where serious nutritional disparities exist between developed and developing countries (as well as communities within all countries dependent on socio-economic status), when people do not have constant and sufficient access to a safe and nutritious food supply (FAO, 1996). However, despite these challenges within our food supply system changing over time, one constant and major challenge throughout human history and still affecting all communities worldwide today is the impact and burden of foodborne disease.

Foodborne disease is a serious and largely preventable public health challenge, specifically described as a global burden in the World Health Organisation's (WHO's) first global estimate of the effects of eating contaminated food published in 2015 (WHO, 2015). Illness and deaths caused by foodborne disease have also been described as a constant threat not only to public health but also as a significant impediment to socio-economic development worldwide (Havelaar et al., 2015). Results from the most recent WHO global estimate showed that every year, almost 1 in 10 people across the world become ill following the ingestion of contaminated food, and these numbers are likely to be under reported. Results also showed that 420,000 deaths result from foodborne disease annually, with significant regional variation worldwide, and with children below 5 years of age accounting for 125,000 deaths, almost one-third of the total (Kirk et al., 2015). Some 31 agents are known to cause foodborne disease including bacteria, parasites and viruses, as well as chemicals and toxins. Over half of the fatalities, 230,000, are the result of diarrheal disease, with diarrhoea caused by the ingestion of raw or undercooked fresh produce contaminated either by norovirus, or a range of bacterial pathogens mainly including *Salmonella* spp., *Campylobacter* spp., and *Escherichia coli* 0157, amongst many others (**Table 1**). Interestingly, foodborne bacterial pathogens have also been observed to have regional variation and different impacts related to socio-economic status: with Africa and South-East Asia having the highest overall incidence and death rates; whilst pathogenic strains of *E. coli* are more prevalent in low-income countries; *Campylobacter* is said to be an important pathogen in high-income countries. By contrast, non-typhoidal strains of *Salmonella* are a major public health concern across *all* regions and *all* incomes.

It is these types of information which allows for the targeting of resources for the detection and reduction of these important foodborne pathogens, as well as increasing our knowledge in several areas such as 'pathogenomics' (Hain et al., 2007), food safety, risk monitoring and reduction (Brul et al., 2012), epidemiology, and antibiotic resistance in food chains (Ramos et al., 2016). It is also apparent that there is a constant need to develop new methods, as well as refine existing approaches, for the detection and analysis of foodborne bacterial pathogens and food disease outbreaks, especially so with the emergence and global threat of antibiotic resistant strains (Ramos et al., 2016), in order to reduce the burden of foodborne bacteria. With the largest ever recorded *Listeria monocytogenes* outbreak in South Africa, multi-State foodborne outbreaks of *Salmonella* in the USA, *Salmonella* found in baby formula in France, and continued concerns over *Campylobacter* in Europe, reinforcing this view. Here, in this short overview we offer a brief background introduction to the potential role of omics approaches and look at some of the recent research undertaken using these methods for the analysis of some of the most important foodborne bacterial pathogens.

Omics, a Short Introduction

We define the neologism omics as a suffix to a molecular target in order to suggest that many of the potential molecules within that molecular target class are collectively characterized or measured. The development of various omics approaches and advancements in their technologies and potential applications (Ellis and Goodacre, 2016; Ellis et al., 2016) has enabled the high-throughput detection and quantification of various bio-molecules at different cellular levels, which has provided us with a deeper understanding of different biosystems and bioprocesses, including of course those directly related to and involving pathogenic foodborne bacteria (Bergholz et al., 2014) and food microbiology in general (Walsh et al., 2017). These can generally be divided into four main areas, namely: genomics, proteomics, metabolomics, and lipidomics. It is perhaps also worth noting at the onset, that no single omics analysis alone can fully reveal and characterize the complexity of a biological system. Thus, application of

Table 1 A range of important foodborne bacterial pathogens and their known symptoms and potential impact

Bacteria	*Symptoms and potential impacts*
Campylobacter jejuni	Responsible for most campylobacter-related infections. Common symptoms include inflammatory watery diarrhoea which maybe bloody sometimes, abdominal pains, nausea and vomiting and fever. Symptoms usually manifest between 2 and 7 days following ingestion of food contaminated with *C. jejuni* and may last for a week. In immunocompromised people (e.g., those living with HIV/AIDS), the pathogen may cause fatal bacteremic conditions.
Clostridium botulinum	The spores of this soil bacterium produce a highly toxic biological chemical called botulinum which is usually transferred to vegetables and intestines of fish, birds and other herbivores which feed on vegetation. Symptoms may appear between 6 and 36 hours, but may sometimes delay until 10 days after eating contaminated food. Botulinum affects the nervous system leading to neurological complications such as slurred speech, dry mouth, blurred vision and muscle weakness. May paralyse muscles and even lead to death if no therapeutic strategies (antitoxins) are administered in the early stages of the symptoms.
Clostridium perfringens	Within 6–24 hours of clostridium infection nausea, diarrhoea and strong abdominal cramps appear. Immunocompromised people are at higher risk of developing serious health complications following infection which may even lead to death if it is left untreated.
Cryptosporidium	Nausea, mild to severe watery diarrhoea leading to body dehydration and weight loss, vomiting and stomach cramps appear within 10 days of infection and may persist for 14 days. Usually children under 5 years old and adults with weak immune system are at higher risk of serious illnesses.
Escherichia coli O157:H7	Most people infected with this pathogen start feeling sick after 7 days following ingestion of contaminated food or drinks. Early symptoms may appear in 3–4 days which include severe watery diarrhoea (often bloody), vomiting, weight loss and abdominal cramps. Prolonged exposure causes potentially life-threatening health complications including haemolytic uremic syndrome (HUS). HUS may cause kidney failure if no treatment is administered on time.
Listeria monocytogenes	Common symptoms include fever, diarrhoea; muscle ache and nausea are reported from 1 to 4 weeks after swallowing infectious dose of the pathogen. Pregnant women, elderly and people with weak immune system are at higher risk. In addition to common symptoms pregnant women experience fever, fatigue and if the pathogen spreads beyond the gut it may lead to premature birth, miscarriage or stillbirth. People other than pregnant women suffer severe convulsions, headache, stiff neck and loss of balance. Ultimately may lead to death if no timely therapeutic intervention is made.
Salmonella (over 2300 types)	Diarrhoea, stomach cramps, fever, and life-threatening body dehydration. Symptoms develop between 12 and 72 hours after eating contaminated food. People with healthy immune system recover within 4 to 7 days without any treatment. The elderly and immunocompromised (e.g., cancer, AIDS) people may develop life-threatening illnesses.
Shigella (over 30 types)	Watery diarrhoea, abdominal pain, fever and tenesmus appear in 1–2 days after becoming infected and last for 5–7 days in people with healthy immune system. If the pathogen enters the digestive system, it would lead to HUS which is usually accompanied by bloody diarrhoea.
Staphylococcus aureus	Early symptoms include diarrhoea, severe nausea, vomiting and abdominal pains. Symptoms appear as early as 30–60 minutes after swallowing food infected with *S. aureus* but disappear after 1–3 days in healthy immune system. People with weak immune system and those who have had severe skin trauma are at higher risk for invasive staphylococcal infections. If left untreated it may lead to severe and life-threatening illnesses including septic arthritis, pneumonia and sepsis.
Vibrio vulnificus	Symptoms vary depending on the immune system; healthy individuals experience diarrhoea, vomiting and abdominal pains whilst people with weak immune system have fatal illnesses including dangerously low blood pressure, blistering skin lesions, sudden chills and fever which may lead to death within 2 days of infection on average.

Adapted from the USDA, Food Safety and Inspection Service (USDA, 2013).

multi-omics approaches (Burnum-Johnson et al., 2017) may provide a clearer picture, and allow for a more precise conclusion to be made. A typical omics workflow is depicted in **Fig. 1**.

Genomics can be described as the study and assessment of variability and function of DNA sequences (Aebersold and Mann, 2016; Stefanovic et al., 2017). During the past two decades, the unprecedented advancements in DNA sequencing technologies and computational-based annotation (bioinformatics) techniques has revolutionised the fields of genomics and transcriptomics (Andjelkovic et al., 2017; Valdes et al., 2013). These fast evolving technologies provide detailed information on genome structure, gene function, and various metabolic networks and pathways. However, unlike the genome which is considered more static, and does not show significant response to short-term external changes, the transcriptome allows for the detection and quantification in gene expression levels in a dynamic manner (Zhang et al., 2010).

Proteomics (Martinovic et al., 2016) is the study of the structure, function and abundance of different proteins and peptides (or complexes) in a system. Proteins are important parts of cells and are involved in various processes and regulatory mechanisms. The proteome is also considered highly dynamic, which under different environmental or physiological conditions will result in changes in its properties, such as: protein abundance, structure, localization, synthesis, degradation and modification (Larance and Lomond,

Figure 1 A typical omics workflow from experimental design to biological interpretation.

2015). Therefore, understanding the changes in the proteome requires the identification and detection of changes in protein abundance, interactions, structure and properties. Subdivisions of proteomics include the fields of peptidomics (Giacometti and Buretic-Tomljanovic, 2017; Giacometti et al., 2013; Korte and Brockmeyer, 2017) and secretomics (He et al., 2015; Sibbald et al., 2006). Many proteins are also modified by post-translational modifications (PTM) including (for example) glycosylation (50% of the proteins in man contain glycans) and phosphorylation which are known to change the function of the protein. It is therefore also important to have information about the protein as well as its PTM.

Metabolomics (Dunn and Ellis, 2005; Ellis et al., 2007) is the comprehensive and systematic study of low molecular weight compounds (metabolites) in a system. The metabolome generally refers to the complete set of small molecules involved in metabolism (metabolites) present in a cell that contributes to metabolic reactions which are required for the maintenance, growth and normal function of a cell in a particular physiological or developmental stage (Fiehn, 2002). The metabolome is considered one of the main components within systems biology (Patti et al., 2012), linked to other cellular processes such as: gene expression, post-transcriptional and translational events and physiological changes. This field encompasses a range of approaches such metabolic profiling (Li and Zhu, 2017), metabolic fingerprinting (Ellis et al, 2007, 2012; Muhamadali et al., 2016a), metabolic footprinting (Kaderbhai et al., 2003; Kell et al., 2005) and multiple technologies such as a range of mass spectrometry (MS) (Singhal et al., 2015; Ouyang and Cooks, 2009), NMR (Garcia-Perez et al., 2017; Wright et al., 2009), and vibrational spectroscopy (Ellis et al., 2017; Black et al., 2016). The field of fluxomics (Brul et al., 2012; Yadav et al., 2018), which uses isotope tracing to monitor fluxes, is a subdivision of metabolomics.

Lipidomics (German et al., 2007; Jain et al., 2007) is a growing area of study, also originally considered a subdivision of metabolomics, and can be described as the study of pathways and networks of different lipid species in a system. Lipids are considered highly complex and crucial metabolites with important and diverse functions in biological systems, such as extremely important components of cellular membrane and barriers, signalling, and interconnecting various metabolic pathways (Rolim et al., 2015). However, detection and quantification of such a diverse and complex class of compounds requires robust analytical tools. So-called hyphenated methods, where MS (usually tandem and MS^n) is/are coupled to chromatographic separation-based techniques for example, such as liquid chromatography (LC), and are considered the dominating analytical platforms in the field of lipidomics (Cajka and Fiehn, 2016).

Recent Omics Studies of Foodborne Pathogens

At the time of writing, the largest recorded outbreak of foodborne bacterial disease is underway in southern Africa with a total of 973 victims and 183 deaths to date (March 2018). Caused by *L. monocytogenes*, this record outbreak is thought to involve ready-to-eat meats such as polony, a regionally very popular form of low-cost sausage typically made from highly processed meat. The ST6 sequence strain has been confirmed at production facilities in South Africa, and the outbreak is beginning to spread to neighbouring countries such as Namibia (reported this month in (Heiberg, 2018; Rosa, 2018)). It is large-scale and lethal foodborne disease outbreaks such as these, that bring the potential effects of pathogenic bacteria present within highly industrial modern food supply chains sharply into focus. Here we very briefly describe a selected range of recent omics studies involving some of the most well-known foodborne bacteria and also direct readers to several reviews of interest in this important area of food safety.

So-called pathegenomic studies of *Listeria* spp. have been undertaken for some considerable time in order to explore data from multiple omics approaches (genomics, transcriptomics, proteomics) to understand better the genomic diversity and evolution, and

the physiological aspects related to the metabolomics, of this deadly genus when these bacteria are exposed to and grow in diverse environments (Hain et al., 2007). Furthermore, this particular genus has recently gained its own omics suffix with the publication of listeriomics (Becavin et al., 2017), as well as already being a distinct area of study within microbiology termed listeriology (Lebreton et al., 2016). In addition, one could state that a commonality between many, if not all, of the omics approaches is that they are data rich, generating and subsequently requiring the analysis of large and very highly complex datasets (Gromski et al., 2015), with them once being described as "producing bounteous data floods" (Goodacre et al., 2003) (more on this below).

The listeriomics project appears to be no exception, with this very recent study comprising an eponymous web-based platform integrating the complete genomes (>80), transcriptomes (~350) and proteomes (25) published to date, with various tools for omics data analyses (Becavin et al., 2017). These multiple tools are said to allow for an integrative systems biology (Kell, 2004) approach, which the interested reader can find here: https://listeriomics.pasteur.fr/Listeriomics/. This platform includes an interactive genome viewer allowing for the display of gene expression arrays, tiling arrays, sequencing, proteomics, and genomics data sets; a protein and expression atlas to connect genes, RNA, or proteins with the most relevant omics data; a protein conservation exploration tool; and a co-expression network tool. Allowing for what the authors state to be the ability to study and browse multiple mechanisms in this important pathogen such as host–pathogen interactions, RNA regulation and this organisms adaptions to stress (Becavin et al., 2017). Another computational-based and related, if less comprehensive, approach is termed the *Salmonella* foodborne syst-omics database (SalFoS), https://salfos.ibis.ulaval.ca/. This consortium-led database hopes to improve food safety via the reduction of salmonellosis. With an analytical pipeline of genomic and phenotypic metadata on genome evolution, antibiotic resistance and virulence, its aims are to improve the accuracy of diagnostic methods, identify prognostic markers to aid surveillance and epidemiology, as well as develop in-field control methods (Emond-Rheault et al., 2017).

Any further development of in-field control methods is very encouraging, as large-scale outbreaks of foodborne disease involving pathogenic bacteria, such as the devastating South African Listeriosis outbreak, always have the potential to remain undetected for some considerable time. Whilst the specific source(s) of a foodborne outbreak remain undetected, this can obviously result in an increase in the incidence and spread of disease through communities and increased mortality rates. Therefore there is an obvious and urgent requirement to be able to attribute the source of foodborne disease rapidly and on-site/in-field, which in itself has been said to be leading towards a trend for more so-called culture-independent diagnostic tests (CIDTs) (Forbes et al., 2017), which can link clinical cases both to each other and of course directly to food products. These can include metagenomics approaches (also referred to as environmental genomics) such as next-generation sequencing (NGS) which is increasingly being used for whole genome sequencing (WGS), as well as other omics methods and single cell technologies which have been stated to have the potential to revolutionize the fields of food safety and public health (Forbes et al., 2017).

Some envision that data from genomics and related omics tools could lead to a paradigm shift in contemporary and future food safety similar to those already underway in human medicine (Kambouris et al., 2018; Chen et al., 2012; Wishart, 2016) and the veterinary field (Van Borm et al., 2015). With precise pathogen detection, characterization and identification, leading to highly accurate risk assessments and the foundation for evidence-based food safety surveillance and monitoring decision making, a truly systems level approach (Capra and Luisi, 2014) which Kovac and co-workers term "precision food safety" (Kovac et al., 2017). Others agree, and also that a transition is underway at the molecular level, from traditional molecular biology-based methods currently seen as gold standard, to omics technologies, such as WGS, NGS tracking, as well as high resolution technologies such as CRISPR-based typing methods which constitute practical and powerful alternatives and will provide valuable insights into problematic food-associated bacterial pathogens (Barrangou et al., 2016).

Others have investigated the role of multiple omics methods and in particular the growing role of proteomics for the detection of specific microbial toxins (Martinovic et al., 2016; Josic et al., 2017). Microbial toxins can be placed in foods deliberately of course as a form of bioterrorism (Wein and Liu, 2005), and one recent study investigated the potential of proteomics methods in detecting toxins predominantly isolated from known bacterial food pathogens, namely *Clostridium perfringens*, *Staphylococcus aureus*, *Shigella dysenteriae*, entero-hemorrhagic *E. coli* strains, and cytolethal distending toxin from *Campylobacter jejuni*. With the goal being to develop an antibody-free proteomics assay to improve the multiplexing capacity of foodborne toxins. Antibody-free sample preparation was followed by LC-MS/MS, a targeted proteomics approach also known as LC-SRM (selective reaction monitoring), with highly specific detection and quantification enabled through the use of isotopically labelled protein references spiked into food matrices. The sensitivity of the assay for multiple toxins was lower than the oral LD_{50} likely to be used to contaminate the food supply, and whilst initially developed to improve food defense (Manning et al., 2005; Manning and Soon, 2016), this antibody-free proteomic assay could also be applied to food quality control and public health monitoring (Gilquin et al., 2017). For a recent review of sensors used to detect specific pathogens and identify toxin-contaminated foods and beverages the reader is directed to Alahi and Mukhopadhyay (Alahi and Mukhopadhyay, 2017).

Metabolomics has grown exponentially as a field over the last decade with what has been said to be exciting applications across a wide range of biosciences (Putri et al., 2013), including multiple applications to food (Ellis et al., 2012; Cevallos-Cevallos et al., 2009; Nychas et al., 2008; Scalbert et al., 2014; Vrhovsek et al., 2012) and foodborne bacterial pathogens (Pinu, 2016; Cevallos-Cevallos et al., 2011; Xu et al., 2010; Ellis et al., 2002). With far less lipidomics studies relating to food (Hyotylainen et al., 2013; Murphy and Nicolaou, 2013) and even less so to foodborne bacterial pathogens (Dubois-Brissonnet et al., 2016), it is not surprising to find very recent food-related research which integrates both metabolomics and lipidomics approaches (Trivedi et al., 2016).

Interestingly, one of these integrated approaches specifically involved species of bacteria belonging to the well-known foodborne pathogen *Campylobacter*. This study primarily used metabolic fingerprinting approaches utilizing Raman and high-throughput

Fourier-transform infrared (FT-IR) spectroscopies, and matrix-assisted laser desorption ionisation-time of flight-mass spectrometry (MALDI-TOF-MS), with confirmation by 16S rRNA gene sequencing, for the differentiation of 11 strains of *Campylobacter* from six species. These six species included the most common foodborne pathogens from this genus, *C. jejuni* and *C. coli*, as well as *C. lari*, *C. hyointestinalis*, *C. fetus*, and *C. consisus*. Analysis of data from these three methods not only showed successful differentiation of all isolates, but also discrimination of two phylogenetically very closely related subspecies of *C. fetus* by FT-IR and MALDI-TOF-MS, which was found to be partly as a result of information from the lipid region of each rapid method. Subsequently, targeted lipidomics using LC-MS was used to further explore this and confirmed the findings from both fingerprinting methods, revealing major differences in the intensities of several classes of lipids between the two subspecies of *C. fetus* including phosphatidylcholine, phosphatidylethanolamine and phosphatidic acids. These results were said to show the potential of these rapid high-throughput techniques for the simultaneous detection and differentiation of different *Campylobacter* spp. down to subspecies levels (Muhamadali et al., 2016b).

Furthermore, as studies such as those by Muhamadali et al. (2016b) involve rapid methods; they also have the potential to be undertaken with field deployable instrumentation, or on/at-line within food production facilities. Field deployable instrumentation has much promise for the future. With methods such as handheld Raman (Ellis et al., 2017) and other forms of optics and spectroscopy (Crocombe, 2018), as well as developments in miniature MS (Ouyang and Cooks, 2009; Gao et al., 2006) and other sensors (Seo et al., 2016), which can be deployed on-site and/or coupled with smartphone technology (Gallegos et al., 2013) for rapid detection of bacteria on food, as recently demonstrated by Pearson et al. (Pearson et al, 2017, 2018).

Outlook

Here, we have very briefly discussed the global burden of foodborne disease, provided a short background to the main omics approaches, and showed the potential of these approaches applied to the study of a range of common bacterial food pathogens. The reader will note that the final applied section began with two large data analytical studies, on *Listeria* and *Salmonella* respectively, using web-based platforms, followed by examples from genomic, proteomic, metabolomic and lipidomics approaches and their respective technologies. As already inferred above and by many others previously, it is important to highlight the data rich nature of all omics approaches and therefore the pivotal and indeed crucial role of a range of computational methods employed to analyse highly complex datasets generated by the omics.

What Kell once termed "data floods" over a decade and a half ago (Goodacre et al., 2003) could now quite accurately be described as data reservoirs. Data reservoirs which can not only store but are also able to analyse the massive volumes of data captured from multiple omics sources, with a range of built-in tools and functionality (as per listeriomics above). With these data reservoirs populated with primary data generated by omics, now having the additional potential to be accessed anywhere in the world to be utilised, analysed, or augmented by researchers in the field during and following food disease outbreaks. In order to influence decision-making, reduce the potential risk and impacts, or aid the rapid, accurate, on-site analysis of foodborne pathogens within food production facilities as well as in communities.

Omics can be said to have much potential for the detection of foodborne pathogens, as well as monitoring contamination and disease outbreaks, particularly when integrated with each other but especially so when incorporated within emerging and vitally important current and future technologies and resources such as the Internet of Things (IoT) (Seo et al., 2016; Badia-Melis et al., 2015) and Cloud-based computing (Gorelick et al., 2017; Tzounis et al., 2017).

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