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# Omics approaches in food safety: fulfilling the promise?

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## Abstract

Genomics, transcriptomics, and proteomics are rapidly transforming our approaches to detection, prevention and treatment of foodborne pathogens. Microbial genome sequencing in particular has evolved from a research tool into an approach that can be used to characterize foodborne pathogen isolates as part of routine surveillance systems. Genome sequencing efforts will not only improve outbreak detection and source tracking, but will also create large amounts of foodborne pathogen genome sequence data, which will be available for data mining efforts that could facilitate better source attribution and provide new insights into foodborne pathogen biology and transmission. While practical uses and application of metagenomics, transcriptomics, and proteomics data and associated tools are less prominent, these tools are also starting to yield practical food safety solutions.

### Keywords

Genomics; transcriptomics; proteomics; synthetic biology; foodborne pathogens; food safety

## Food safety challenges are constantly changing and require new approaches and tools

Foodborne diseases caused by bacteria, viruses and parasites cause considerable disease burden worldwide. For example, it has been estimated by the Centers for Disease Control and Prevention (CDC) that in the US about 1 million foodborne illnesses caused by known pathogens or unspecified agents occur every week [1,2]; about 50 – 100 deaths per week are associated with these cases. With a US population of about 300 million, this translates into a 1 in 300 chance for a given individual to experience a foodborne illness episode in a given week. While the US data are typically cited as the US CDC has been a leader in providing estimates of total foodborne illness rates, on a population basis, foodborne illness rates and burdens are probably similar in many developed countries in North America and Europe.



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Data on foodborne illness burdens in many parts of the world, including many developing countries, are difficult to come by. A World Health Organization (WHO) publication suggests that a total of 2.2 million deaths due to diarrheal illnesses occur annually worldwide [3]; many of these illnesses are likely caused by microbes transmitted via food and/or water.

While some advances in reducing specific foodborne illnesses have been made in parts of the world (e.g., listeriosis in the US [4]), overall progress in reducing foodborne illnesses has been slow. Importantly, use of molecular surveillance systems and in particular PulseNet [5] has had a major impact by improving the ability to (i) rapidly detect foodborne disease outbreaks, leading to smaller outbreaks with fewer cases (e.g., [6]), and (ii) identify outbreak sources, providing information on foodborne disease transmission routes that can be used to target industry and government efforts to control foodborne pathogen transmission. Use of molecular tools also has raised concerns though, such as the potential for rapid detection methods to lead to a decrease in pathogen isolation, which subsequently reduces the ability to perform subtyping (due to a lack of pure isolates from human specimens or food samples); this issue could at least be partially addressed through (omics) methods that allow for rapid detection and subtyping without a need for a bacterial isolation. As detailed below, many omics tools have the potential to considerably improve our ability to prevent foodborne illness cases and outbreaks (Table 1). Full consideration of both benefits and challenges associated with these tools is essential to assure that use of these tools realize their full potential (Box 1).

## Whole genome sequencing allows for improved outbreak detection and has the potential to rapidly replace currently used subtyping methods

Use of whole genome sequencing (WGS) as a tool for subtyping of foodborne pathogen isolates represents considerable potential for improving our ability to rapidly detect foodborne disease outbreaks. While currently used subtyping methods, in particularly pulsed-field gel electrophoresis (PFGE) and multiple-locus variable number tandem repeat analysis (MLVA), have provided valuable tools for surveillance and detection of foodborne disease outbreaks [5,7], these methods have some shortcomings that can be overcome by WGS. For instance, for highly clonal pathogens, both PFGE and MLVA may provide limited discriminatory power [8]. WGS can overcome this issue as it provides substantially increased discrimination. Examples of bacterial pathogens where PFGE may not provide sufficient discriminatory power includes *Bacillus anthracis* and *Yersinia pestis* [9–11]. An early example of the increased discriminatory power of WGS is provided by the use of this tool to characterize *B. anthracis* isolates, including isolates linked to the 2001 bioterrorism incident in the US [12]. More importantly, PFGE and MLVA often fail to provide appropriate discriminatory power for specific subtypes within a given pathogen species; such as for certain *Salmonella* serovars. For example, it has been well documented that PFGE shows limited discrimination among highly clonal serovars such as Enteritidis or Montevideo [13,14]. In addition, DNA-based subtyping methods (e.g., multilocus sequence typing [MLST]), may not always discriminate between closely related *Salmonella* serovars (e.g., Typhimurium and 4,5,12:i:- [15]). WGS, on the other hand, will be able to

differentiate closely related *Salmonella* serovars, and further experimental work is needed to develop approaches that allow for reliable serovar prediction by WGS. Recent publications have specifically shown that WGS can provide substantially increased discriminatory power which can group isolates into epidemiologically relevant groups and can help with outbreak investigations. In two independent retrospective studies, a whole genome single nucleotide polymorphism (SNP)-approach successfully discriminated *S.* Montevideo isolates linked to the 2009 outbreak associated with spices from non-outbreak strains with identical pulsotypes (based on PFGE with several enzymes, e.g., *Xba*I, *Bln*I, *Spe*I, *Sfi*I, and *Pac*I) [14,16]. Based on the rapid improvements of both sequencing technologies and bioinformatics pipelines, routine application of WGS for foodborne disease surveillance is highly feasible and will provide for improved outbreak detection. The US CDC, for example, has implemented routine WGS of human *Listeria monocytogenes* isolates starting in 2013 (http://www.cdc.gov/media/releases/2013/p0604-listeria-poisoning.html). WGS will not only provide improved discriminatory power over PFGE, but also will provide the data needed to determine whether strains that differ by 3 or less bands in the PFGE pattern are closely related and share a recent common ancestor, suggesting a common source. For example, retrospective WGS of *L. monocytogenes* isolates that differed by 3 bands and were linked to a large human listeriosis outbreak in Canada in 2008 indicated that these isolates were closely related and likely both were part of the outbreak [17].

In addition to providing improved subtyping, next generation sequencing methods also provide an opportunity for rapid generation of whole genome sequence data that can be used to develop assays to detect specific outbreak strains or new and emerging organisms for which no detection methods are available, as illustrated by the *Escherichia coli* O104:H4 outbreak in Europe in 2011. Whole genome sequences for multiple isolates of the highly virulent O104:H4 strain responsible for this outbreak were generated within weeks from outbreak onset and genomes were publically deposited [18,19]. Availability of these genome sequences was followed by rapid development of real time PCR assays that specifically detect the outbreak strain [20–22]. Software that allows for rapid identification of molecular targets without the need for genome annotation is available [21] and will facilitate similar applications with other organisms in the future.

Next generation sequencing methods have also been used for subtyping and detection of foodborne viruses. A number of published studies [23–25] show how these tools can allow for improved detection of virus-related outbreaks and improved ability to track virus transmission routes. For example, WGS of viral RNA from stool samples of patients implicated in a norovirus outbreak in a children hospital provided for improved subtype discrimination over sequencing of the capsid gene (region D), which represents the standard scheme for subtyping of noroviruses as implemented in CaliciNet; WGS data also facilitated implementation of successful control strategies in this outbreak [26]. For foodborne parasites, whole genome sequencing has not yet been used as extensively as for bacterial and viral foodborne pathogens. While genome sequences have been generated for a number of strains representing different parasites that can be transmitted via food and water (e.g., *Giardia lamblia*, *Cryptosporidium parvum*, and *Cryptosporidium hominis* [27–29]), application of WGS to support outbreak investigations has been limited. For both viral and

parasitic foodborne disease outbreak investigation, next generation sequencing methods are often used in a metagenomics approach where DNA or RNA extracted from patients or foods is sequenced to detect pathogen signatures as discussed in the next section.

Examples of next generation sequencing technologies that have been used to sequence foodborne pathogens include Ion Torrent [18], 454 pyrosequencing [14], PacBio [30], and Illumina [31]; in addition, combination of technologies have also been used. WGS of a bacterial isolate can be performed for less than $100, which is competitive with other currently used subtyping methods (as detailed in http://www.cdc.gov/pulsenet/next-generation.html). While WGS data will allow for improved outbreak investigations, they cannot replace epidemiological investigations. Even when WGS approaches are used in outbreak investigations, concordance of subtyping and epidemiological data is essential for reliable identification of outbreaks and outbreak sources. Importantly, definition of a clear SNP cut-off that determines when two isolates are ‘unrelated’ (and thus not involved in a transmission event) does not seem feasible. Rather, micro-evolutionary analyses can be used to determine the most likely time of a recent common ancestor of closely related isolates and epidemiological data can then be used to help define meaningful clusters of closely related isolates (as detailed in [32]). Recent identification of a hypermutator phenotype in isolates of methicillin-resistant *Staphylococcus aureus* involved in an outbreak [33] also shows a specific mechanism that may complicate interpretation of SNP differences, along with the fact that the mutation rates in natural populations of foodborne pathogens are typically unknown. In addition to development of improved approaches for interpretation of SNP differences, further development of whole genome sequence databases for foodborne pathogen isolates (including associated metadata) is also needed. Efforts developing these databases are underway at the Center for Genomic Epidemiology in Denmark (http://www.genomicepidemiology.org/index.html) and include projects such as the 100K Foodborne Pathogen Genome Project (http://100kgenome.vetmed.ucdavis.edu/index.cfm), along with projects in other labs and organizations around the world.

## Metagenomics tools provide a powerful approach to disease diagnostics and food safety testing, but will require a cautious approach to data analyses and communication

The term metagenomics refers to a culture-independent analysis on the genetic material of microbial communities in a given environment [34]. The ability of next generation sequencing to generate large amounts of DNA sequence data has considerably facilitated metagenomics studies, including of food-associated and intestinal microbes [35–38]. Specific applications of metagenomics in food safety include, among others, (i) identification, from clinical specimens, of novel and non-culturable agents that cause foodborne disease [39]; (ii) characterization of microbial communities (including pathogens and indicator organisms) in foods and food associated environments (e.g., processing plants); and (iii) characterization of animal and human intestinal microbiomes to allow for identification of microbiota that may protect against infection with foodborne pathogens.

Use of metagenomics approaches has been well documented to provide a valuable approach to detect and identify the causative agent of foodborne disease cases from clinical specimens. For example, Nakamura *et al.* (2008) [40] used metagenomics approaches to identify *Campylobacter jejuni* as the causative agent for a foodborne illness case that was not diagnosable by conventional microbiological culture. Briefly, metagenomic analyses showed that the DNA of *C. jejuni* was present in a fecal sample collected from a patient that experienced campylobacteriosis-like symptoms, but was not present in a fecal sample collected from the same patient three months following clearance of the infection, suggesting *C. jejuni* as the causative agent [40]. Viral metagenomics studies can similarly be used to identify novel or known viruses in humans [41]. Interestingly, a recent study in Japan illustrates how metagenomic approaches can identify novel parasitic pathogens that cause foodborne illness [42]. In this specific study, a combination of epidemiological investigations, metagenomic studies, and animal studies helped identify the myxosporean parasite *Kudoa septempunctata* as the likely etiological agent responsible for a number of foodborne illness outbreaks associated with consumption of a specific fish species (olive flounder; *Paralichthys olivaceus*).

While there are a number of opportunities for using metagenomics tools to support detection of foodborne pathogens from foods and food associated environments, most metagenomics studies on detection of microbes in foods have focused on characterizing the microbial ecology and microbial successions during fermentations [43], e.g., of kimchi [44,45]. The opportunities for metagenomics approaches to improve foodborne pathogen detection are illustrated in a study that used metagenomics approaches to characterize the species composition associated with the tomato phyllosphere both on the native plant and in pre-enrichment and enrichment media used to isolate *Salmonella* [46]. This study was conducted as isolation of *Salmonella* from the tomato phyllosphere has previously proven challenging despite the fact that tomatoes have been implicated as the source of a number of human salmonellosis outbreaks. Interestingly, this metagenomic study identified considerable growth of *Paenibacillus* spp. during enrichment, which is important as this organism may outcompete or even kill *Salmonella* during enrichment. Also, sequences matching different *Salmonella* serovars were identified from both the uncultured samples as well as different enrichments suggesting the presence of *Salmonella*, despite the fact that these samples were negative by both bacteriological analytical manual (BAM) methods and real time PCR. While these findings do support the possibility that *Paenibacillus* may have outcompeted *Salmonella* during enrichment, it is also possible that the detection of *Salmonella* DNA sequences is due to presence of dead *Salmonella* cells. While a number of publications thus support the potential for metagenomics applications in food safety, use of metagenomics as a tool for detection of foodborne pathogens in food associated environments and foods still faces a number of challenges. For one, metagenome sequencing will detect DNA from both dead and alive organisms. While DNA from dead cells may degrade over time, this still represents a challenge as samples may be classified as positive for a foodborne pathogen due to dead cells (e.g., for food samples tested after pasteurization or environmental samples tested after sanitation). This challenge could potentially be overcome by using metatranscriptomics approaches [47], but even there extended persistence of some mRNA species, after cell death, cannot always be excluded. An additional challenge is that both

metagenomics and metatranscriptomics approaches will create massive sequence data sets linked to a given food or food associated facility (e.g., processing facility or farm), which are likely to contain at least some sequence data that can easily be misconstrued as indicating a food safety hazard (e.g., presence of antimicrobial resistance genes or virulence genes). As, at least in some countries, food safety testing data, may have to be released, under certain circumstances, to lawyers or regulatory agencies, some facilities may be reluctant to use these tools out of fear that the data created could inadvertently (and incorrectly) implicate a facility as having evidence of pathogen presence in a food or environment. In addition, data from metagenomic studies of human specimens could potentially be linked to individuals as the data generated may also contain host sequence data that could potentially identify a patient. Both of these potential issues may be addressed through initial filtering and removal of sequence data (e.g., human sequences). Future development of guidelines on the proper and ethical use of metagenomics data in food safety may be necessary though to encourage and facilitate use of these potentially powerful tools.

## Transcriptomics, proteomics, and metabolomics provide future opportunities to develop improved approaches to control foodborne pathogens from farm-to-table

With the aim of developing rational control strategies for foodborne pathogens in the food supply, there is a need to determine the physiological state of pathogens when present on foods. Modulation of gene and protein expression in response to stress indicates activation or repression of a specific physiological response, and can be used to determine the physiological state of the pathogen under different conditions. A number of studies over the past 10 years have assessed transcriptomes and/or proteomes of bacteria under conditions simulating those a pathogen may experience on a food, such as the low water activity and low temperature that *E. coli* O157:H7 could encounter during beef carcass chilling [48]. Other recent studies have evaluated changes in gene expression of pathogens inoculated onto actual food products, such as *Salmonella* on cilantro and lettuce [49], *E. coli* O157:H7 on lettuce [50], and *L. monocytogenes* in milk [51]. While all of these studies are assessing different pathogens in different food matrices, all of these studies show an increase in expression of stress response genes, including the general stress response and cell envelope stress response [50], and the oxidative stress response [51]. Activation of these stress responses while on foods has the potential to impact resistance or sensitivity of the pathogen to subsequent processing treatments, for example, the significant upregulation of genes involved in *E. coli* O157:H7 cell envelope stress response on lettuce could lead to increased resistance of the pathogen to decontamination treatments that damage the cell envelope.

In addition to understanding the physiological state of pathogens on foods, transcriptomics can be used to assess how microbes respond to physical, chemical, or biological food preservation treatments. In many cases, it is known that a specific compound has an antimicrobial effect, but there is limited information about the effects of that antimicrobial at the molecular level. A characterization of the transcriptional response of *E. coli* O157:H7 to the antimicrobial cinnamaldehyde highlighted that the initial response of the pathogen was to activate the oxidative stress response, and after a relatively short period of time, was able

to overcome the antimicrobial stress by converting cinnamaldehyde to cinnamic alcohol [52]. A characterization of the proteome of *S.* Enteriditis exposed to propionate determined that the DNA binding protein Dps and the cell envelope stress response regulator CpxR played significant roles in ability of the pathogen to survive the stress [53]. Typically food preservation treatments are used in combination, referred to as hurdle technology. Hurdle technology combines different preservation methods to inhibit microbial growth. Ideally, the hurdle components will exhibit synergy, meaning that the level of inhibition achieved by the combination of the growth inhibitors is greater than the sum of the levels of inhibition achieved by each inhibitor alone. Conversely, multiple hurdles could have antagonistic effects and lead to cross protection, where bacterial adaptation to one hurdle reduces the effectiveness of sequential or concurrent hurdles. An understanding of the modes of action of these growth inhibitors enables us to understand how synergy works at a mechanistic level. For example, an investigation of changes in the transcriptome of *L. monocytogenes* exposed to commonly used growth inhibitors lactate and diacetate, both singly and in combination, suggests that the synergistic effect of these two organic acid salts is because the pathogen must shift fermentation pathways to produce acetoin instead of lactate or acetate [54]. Production of acetoin prevents further acidification of the cytoplasm, but also results in less energy produced, which contributes to the reduced rate of growth of *L. monocytogenes* in the presence of these acids. In addition to providing mechanistic information on how bacteria are responding to control strategies, the data suggest that additional treatments that interfere with energy generation processes could be used to further reduce the ability of *L. monocytogenes* to grow.

There is tremendous potential for transcriptomics and proteomics data to be utilized for rational development of new control strategies for foodborne pathogens. One promising approach is to use the information from these studies to identify new compounds that specifically interfere with pathways important for survival in foods. As an example, a recent study identified that the small molecule fluoro-phenyl-styrene-sulfonamide (FPSS) specifically inhibits activation of the general stress response sigma factor, SigB, in *L. monocytogenes* [55]. If the general stress response is induced by *L. monocytogenes* in foods, compounds such as FPSS may be useful as an additional control measure to inhibit the general stress response, and reduce survival of the pathogen. Transcriptomics data from foodborne pathogens under different environmental stresses has also been used to identify biomarkers related to specific resistance characteristics of the pathogen [56]. This data is proposed to be integrated into mathematical models to predict microbial behavior [57], also with the potential to improve control measures.

## Combination of synthetic biology and omics approaches provides new opportunities to solve old food safety problems

While synthetic biology may not be considered an ‘omics’ technique per se, the tools associated with this emerging discipline may have some important applications in food safety. Synthetic biology is often defined as the application of engineering design principles to biology [58]. Synthetic biology can be used to design organisms or systems to effectively produce biological compounds, including for use in food [59] and thus represents a

promising platform for the development and synthesis of new antimicrobial compounds, including compounds that could be used in foods, such as bacteriocins. For example, synthetic biology could be used to facilitate synthesis of novel types of leaderless bacteriocins [60].

While the generation of a completely synthetic microorganisms in 2010 [61] illustrates the potential for synthetic biology to break new ground, one does not need to look that far to see potential opportunities for use of custom-designed bacteria in food safety. For example, pathogen strains that have been constructed to contain unique deletions or signature sequences (e.g., strains expressing green fluorescence protein [GFP]) can be used as control strains in testing laboratories [62]; use of these types of control strains facilitates detection of false positive laboratory results due to contamination with a laboratory control strain. Similarly, Murphy *et al.* [63] reported *S. enterica* and *L. monocytogenes* strains constructed to express GFP and associated real time PCR assays for GFP detection, which, in combination, could be used as positive process internal controls for food samples.

In the future, one could also envision use of specifically constructed bacterial mutant strains (or even fully synthetic bacteria) as challenge strains for in plant process validation. Currently, validation of bacterial kill steps has to be performed either in Biosafety Level 2 (BSL-2) laboratories or processing facilities with pilot scale equipment or under experimental conditions (which does not reflect the in-plant conditions) or in plants, but using surrogate organisms (which do not necessarily have the same characteristics as the pathogenic target organisms) [64]. While use of attenuated strains for validation studies has already been reported [65], in the future pathogen strains could potentially be constructed for validation studies to (i) be virulence attenuated, and (ii) have deletions in appropriate genes to assure that these strains are not detected with conventional or molecular methods. In addition, these strains could be fully characterized by both genome sequencing and transcriptomics studies to assure absence of potential genes of concerns (e.g., antibiotic resistance genes) and stress response systems equivalent to the parent strains. While use of these types of strains of course would have to be discussed and potentially approved by regulatory agencies before efforts like this are undertaken, this provides an example of the type of new approaches to food safety a combination of synthetic biology and genomics approaches could facilitate.

Synthetic biology, along with genomics, can also be used to design new phages that can be used for either pathogen detection or biocontrol. Genetically engineered phages have been constructed for pathogen detection (e.g., for detection of *B. anthracis* or *E. coli* O157:H7 [66–68]). Appropriate reporter phages (e.g., phages expressing luciferase or GFP) permit rapid detection for the target organisms from different matrices (e.g., clinical specimens or food samples), even though a short enrichment or resuscitation step may still be required. Phages have also been engineered to affect their virulence; for example, a lysozyme-inactivated GFP-labeled phage was constructed to detect viable and viable but non-culturable *E. coli* [68]. In addition, for biocontrol of *E. coli* O157:H7, non-lytic phages have been engineered to encode proteins that are lethal for the host cell (lethal transcriptional regulator); this engineered phage can kill *E. coli* without releasing a phage progeny, and therefore, without potential ecological disturbance [69]. As viable synthetic phages have

already been constructed [70,71], it is likely that we will see use of synthetic phages to control and detect foodborne pathogens in the not too distant future. The potential of combining omics methods and synthetic biology is also illustrated by new approaches that have been taken to address food contamination by aflatoxin, a cancer-inducing mycotoxin produced by *Aspergillus flavus* [72–75]. As of December 2013, approximately 20 genomes of *Aspergillus* strains have been sequenced and deposited in a database that contains these genomes and associated RNA-seq data (http://www.aspgd.org). These omics data have led to the identification of genes involved in aflatoxin production and to the development and application of engineered atoxigenic *A. flavus* strains; these atoxigenic strains compete with toxigenic strains in the field, and thus reduce aflatoxin in food [74]. While the examples provided here illustrate that synthetic biology represent considerable opportunities for improving food safety, there also is a need to further define potential risks (e.g., through horizontal gene transfer) that may be associated with release and use of synthetic bacterial or fungal strains and phages in foods.

## Concluding remarks

While omics approaches are on the verge of potentially making major impacts in some areas of microbial food safety, there are a number of areas where use of these approaches is still in its early stages. Adoption of next generation sequencing as a common tool for outbreak detection and research in food microbiology will not only require investment in equipment, but also in trained personnel (Box 1). To facilitate further use of these approaches efforts are needed to train scientists that can bridge food and public health microbiology, omics tools, and bioinformatics. Development of faster, less computationally intensive, and easier to use bioinformatics tools will also play a critical role in facilitating further use of omics tools and may be more important than the almost inevitable further improvements in sequencing technology. In addition, development of appropriate legal frameworks on use of omics data and results will also be important to facilitate industry and government use of these tools.

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**Box 1**

## Outstanding questions

**Outstanding questions on omics use**

- How long will it take to globally apply next generation sequencing in foodborne outbreak investigations?
- Will developing countries be able to widely adopt next generations sequencing for outbreak investigations?
- How will the application of omics in food safety affect the international food trade?
- Will industry stakeholders be willing to use omics methods to detect and control foodborne pathogens?
- Will industry and regulatory agencies approve use of challenge strains, constructed using synthetic biology, in commercial processing facilities and in fields?

**Outstanding research questions**

- Is it possible to genetically engineer phages to have the host specificity needed to allow for highly specific detection of target organisms?
- Is it possible to develop fully synthetic phages for biocontrol and pathogen detection?
- Do foodborne pathogen populations show enough structure to allow for reliable source tracking (i.e., are there distinct pathogen subtypes, as defined by WGS, in different regions)?
- Is it possible to develop appropriate sequencing techniques and bioinformatics tools to allow for prediction of PFGE patterns from WGS data?
- Is it possible to predict effective chemical hurdles that control pathogen growth from transcriptomics and metabolomics data?

## Highlights

- Whole genome sequencing can be useful in foodborne disease outbreak detection.
- Metagenomics can characterize the diversity in food systems and detect new pathogens.
- Transcriptomics and proteomics have provided data to develop new control strategies.
- Synthetic biology provides new opportunities to detect and control foodborne pathogens.

## Table 1

Selected omics techniques and examples of their application in food safety

| Technique[a] | Examples of uses and applications in food safety |
|---|---|
| Genomics (genome sequencing) | Subtyping; characterization of new and emerging pathogens; identification of assay and therapeutic targets; characterization of transmission routes |
| Transcriptomics | Characterization of pathogen response to stress and antimicrobial treatments; new antimicrobial discovery |
| Proteomics | Characterization of pathogen response to stress and antimicrobial treatments; new antimicrobial discovery; characterization of host response to pathogens; identification of protein-based assay targets |
| Metabolomics | Characterization of pathogen response to stress, antimicrobial treatments, and different environments (including competitive microorganisms); characterization of host response to pathogens |
| Metagenomics | Detection of pathogens in mixed cultures, identification of transmission routes; identification of new non-culturable pathogens; characterization of bacterial diversity in the food chain and effect on pathogen diversity and presence |
| Synthetic biology | Construction of control strains for detection and validation studies; construction of antimicrobial producer strains to be used for biocontrol; construction of highly virulent and wide host range bacteriophages for detection and biocontrol |

[a] Techniques discussed here are not necessarily all covered in the text of this review.