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Translating 'big data': better understanding of host-pathogen interactions to control bacterial foodborne pathogens in poultry

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Review

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Abstract

Recent technological advances has led to the generation, storage, and sharing of colossal sets of information ('big data'), and the expansion of 'omics' in science. To date, genomics/metagenomics, transcriptomics, proteomics, and metabolomics are arguably the most ground breaking approaches in food and public safety. Here we review some of the recent studies of foodborne pathogens (*Campylobacter* spp., *Salmonella* spp., and *Escherichia coli*) in poultry using big data. Genomic/metagenomic approaches have reveal the importance of the gut microbiota in health and disease. They have also been used to identify, monitor, and understand the epidemiology of antibiotic-resistance mechanisms and provide concrete evidence about the role of poultry in human infections. Transcriptomics studies have increased our understanding of the pathophysiology and immunopathology of foodborne pathogens in poultry and have led to the identification of host-resistance mechanisms. Proteomic/metabolomic approaches have aided in identifying biomarkers and the rapid detection of low levels of foodborne pathogens. Overall, 'omics' approaches complement each other and may provide, at least in part, a solution to our current food-safety issues by facilitating the development of new rapid diagnostics, therapeutic drugs, and vaccines to control foodborne pathogens in poultry. However, at this time most 'omics' approaches still remain underutilized due to their high cost and the high level of technical skills required.

Introduction

People are eating approximately 3000 kcal of food daily to produce energy (FAO, 2017; Roser and Ritchie, 2017); however, every single bite of food increases the risk of food illnesses. Foodborne illnesses may result in mild illnesses (diarrhea, abdominal cramps, fever, and vomiting) lasting anywhere from few hours to several days, and in some cases, these symptoms can become severe and persist longer, resulting in hospitalizations and sometime deaths (Lawrence *et al.*, 2007). It is estimated that one in ten people fall ill after ingesting contaminated food and/or water, and over 420,000 die annually worldwide (Havelaar *et al.*, 2015; WHO, 2015; Hoffmann and Scallan, 2017). Accordingly, foodborne illnesses represent an important economic and public health concern that needs to be addressed.

Bacterial foodborne pathogens in poultry: an endless story

Over 250 foodborne diseases are caused by the consumption of food products contaminated with infectious agents (bacteria, fungi, viruses, or parasites) or, occasionally, harmful toxins and chemicals (Lawrence *et al.*, 2007; Linscott, 2011). All food products are potential sources of foodborne illnesses; however, poultry and egg products are among the most frequent and recurrent causes of bacterial gastroenteritis worldwide (Scallan *et al.*, 2011; Behravesh *et al.*, 2012; Basler *et al.*, 2016; Chai *et al.*, 2017; Rouger *et al.*, 2017; Gonçalves-Tenório *et al.*, 2018; Heredia and García, 2018). Two major reasons are the high prevalence and transmission of *Salmonella* spp. and *Campylobacter* spp. from poultry products and poultry farm environment to humans (EFSA Panel on Biological Hazards (BIOHAZ), 2011; Jørgensen *et al.*, 2002; Pires *et al.*, 2011; Sahin *et al.*, 2015; Wales *et al.*, 2007). Previous studies conducted in the USA and in Europe reported that up to 80 and 100% of poultry flocks could harbor *Salmonella* spp. and *Campylobacter* spp., respectively (Stern *et al.*, 2001; Newell and Fearnley, 2003; Luangtongkum *et al.*, 2006). Furthermore, these two bacteria can extensively colonize the poultry intestinal tract (up to 10^9 colony forming unit (CFU) per g of cecal content) while causing little or no clinical disease, presenting high risks of carcass contamination during post-slaughter processes (Newell and Fearnley, 2003; Johnson *et al.*, 2017). It is estimated that up to 50 and 33% of poultry products at the retail harbor *Salmonella* spp. and *Campylobacter* spp., respectively in the USA

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(Naugle *et al*., 2006; Andrews, 2013; Gonçalves-Tenório *et al*., 2018); and 20% of salmonellosis and 80% of campylobacteriosis cases in humans are associated with poultry (EFSA Panel on Biological Hazards (BIOHAZ), 2011; European Food Safety Authority (EFSA), 2004; Hoffmann and Scallan, 2017). *Listeria* spp., *Staphylococcus aureus*, *Escherichia coli*, and *Clostridium perfringens* have also been associated with foodborne illnesses related to poultry; however, the occurrence of these pathogens remain isolated and sporadic in poultry compared with *Salmonella* and *Campylobacter* and/or are caused by post-harvest cross contaminations with other animal carcasses (Van Immerseel *et al*., 2004; Ali *et al*., 2017; Rothrock *et al*., 2017; Rouger *et al*., 2017; Udhayavel *et al*., 2017; Gonçalves-Tenório *et al*., 2018; Heredia and García, 2018; Liu *et al*., 2018*a*). In the USA, from 1998 to 2012, a total of 1,114 foodborne disease outbreaks were reported and among them, poultry was associated with 279 outbreaks (25%), accounting for the highest number of outbreaks, illnesses, hospitalizations, and the second highest number of deaths (Chai *et al*., 2017). Similarly, a surveillance reports of foodborne disease outbreaks in USA, from 2009 to 2015, also indicated that poultry was associated with the highest number of foodborne illnesses (4860 illnesses out of 26,550 illnesses; 18%) and the second highest number of outbreaks (179 outbreaks out of 1281 outbreaks; 14%) (Dewey-Mattia *et al*., 2018).

To remedy these food-safety threats, the poultry industry is constantly upgrading its management practices (foams, formaldehyde fumigant, heat, high-pressure treatments, antibiotics, vaccines, feed additives, and antagonistic organisms) to prevent and control foodborne pathogens in the flock and the farm environment (Dawkins, 2017; Mehdi *et al*., 2018). However, foodborne pathogens can persist for an extended period of time within hosts and agricultural environments despite decontamination efforts and therapeutic measures (Winfield and Groisman, 2003; Dinçer and Baysal, 2004; Foley *et al*., 2011, 2013). Further, evidence of the association between antimicrobial use in animal production and the emergence of cross resistance, multi-drug resistant (MDR) isolates, and other evolutionary adaptations have been reported (Kassem *et al*., 2017; Nisar *et al*., 2017*a*; Deblais *et al*., 2018*b*). Based on the National Outbreaks Reporting System (NORS) database, 54.7% of the total enteric zoonotic disease outbreaks recorded in the USA between 1996 and 2017 were associated with poultry; therefore, novel control and management practices must be established to assure food and public safety. To resolve the recurrent and expanding safety issues, current and future scientific and governmental efforts need to focus on (1) disease surveillance, monitoring, and forecasting and (2) understanding the complex interactions between the foodborne pathogens, their hosts, the environment, and management practices at the system and molecular level. Yet, such approaches need extensive manpower, data storage capacities, and technological tools.

Big problems need big solutions

Since 1980, the beginning of the digital age, technological advances tremendously expanded our capacities to store, communicate, and compute colossal sets of information, also called 'big data'. More precisely, big data is defined by the World Health Organization (WHO) as 'the emerging use of rapidly collected, complex data in such unprecedented quantities that zettabytes of storage may be required' (Ward and Barker, 2013). To date, 42 criteria have been designated to describe big data (the concept of the 42 Vs) (The 42 Vs of Big Data and Data Science, n.d.). In science, big data is also obtained with high-throughput methods (new generation sequencing, microarrays, mass spectrometry (MS), etc.), which allow the generation of detailed and complete information about a specific type of molecule (DNA, RNA, proteins, hormone, lipids, etc.) within a studied sample. The discoveries made using these techniques over the past few decades have improved our society in many ways. More precisely, in food and public safety, big data facilitates the discovery of new drug/vaccines/biocontrol candidates, and the understanding of the complex interaction between hosts and pathogens, and the implication of specific genetic and environmental factors within complex ecosystems. In this review, we describe how big data is helping in the fight against bacterial foodborne pathogens in poultry by elucidating the complex interactions between bacterial foodborne pathogens and their hosts/environment and the development of novel prevention/control strategies.

Genomics and metagenomics

Genomics is the study of an organism's whole genome at the nucleic acid level; whereas, metagenomics is the study of genomes in a microbial community or population as a whole (microbiome). Tools developed for this discipline allow in-depth and comprehensive studies of genomes (epigenomics and structural and functional genomics) and the microbial diversity within a specific environment (environmental genomics, ecogenomics, or community genomics). Over the past few decades, genomics and metagenomics have proven to be great tools to (1) understand association between health/disease and microbial communities (microbiome), (2) study the evolution of microorganisms, (3) understand the exchange of antibiotic-resistance genes (ARGs) and mobile genetic elements (MGEs) within and between species, and (4) analyze the microbial populations and their genetic background in complex environments. These discoveries led to epidemiologic breakthroughs, the designing of mitigation strategies, development of novel sanitary and therapeutic/preventive strategies, and the more judicious use of antibiotics in animals and humans. Further, unlike conventional microbiology approaches, genomics and metagenomics allow the study of difficult or unculturable organisms and do not require enrichment or isolation steps, enhanceing the quality and veracity of the results (Ranjan *et al*., 2016). Genomics and metagenomics also represent key technologies for the success of surveillance systems and for the understanding of microbial communities and their interactions in an environment (Andersen *et al*., 2017). Details of major genomics and metagenomics studies presented in this review are displayed in Table 1.

Importance of genomics and metagenomics in predicting disease and health in poultry production

The power of genomics in improving avian breeding efforts

Since the recent restrictions on prophylactic use of antimicrobials in poultry production in the USA (Guidance for Industry, #213, Food and Drug Administration) and the interdiction in the European Union (Maron *et al*., 2013), there is an increasing need for the development of alternative control methods to protect animal health. There is a thin frontier between health and sickness; however, it can be predetermined based on the host and pathogen genotypes and be reinforced by the presence of specific gut microorganisms. For example, genomics has facilitated the identification of quantitative trait loci (QTL) in chickens involved in the resistance to specific foodborne pathogens using

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Table 1. Genomics and metagenomics studies

Organism	Objective	Method	Major finding	Reference
C. jejuni	Identify QTL in chickens involved in the resistance to *C. jejuni* using high-density genome wide SNP mapping	580K high-density whole genome SNP array; ENSEMBL; Illumina GEMMA; ASREML; GenABEL	Chromosomes 4, 7, 11, 14, and 16 harbored QTL involved in the resistance to *Campylobacter*. Two of these QTL on chromosome 14 (*TRAP1* and *AXIN1*) were co-located with *Salmonella* resistance loci.	Psifidi *et al.* (2016)
C. jejuni	Characterize impact of *C. jejuni* on the chicken cecal microbiome	454 Genome Sequencer GS20; SEED and MG-RAST; PHACCS	Chicken ceca colonized with *C. jejuni* NCTC11168 were characterized with higher microbial diversity and evenness, and a higher *Firmicutes*: *Bacteroidetes* ratio compared with the non-challenged chickens. Mobile DNA elements and metavirulome might play important roles in shaping the functional evolution of the cecal microbiome.	Qu *et al.* (2008)
E. coli	Determine the genomic relatedness of ESBL *E. coli* isolates from chickens and humans in China from 2008 to 2014 and study the co-occurrence of *bla*CTX and *mcr*-1	Hiseq2500; ResFinder and PubMLST	Close relationship between chicken and human isolates were observed. The co-existence and transmission of ESBL and *mcr*-1 in both clinical medicine and veterinary medicine is increasing in China.	Wu *et al.* (2018)
E. coli	Determine the molecular epidemiology of ESBL-producing *E. coli* isolates from the intestinal tract of humans and chicken collected in rural Ghana in 2015 to assess potential transmission routes	MiSeq; Resfinder; and BLAST	High level (10%) of closely related isolates were detected between chicken and humans (with both chromosomal and plasmid-mediated ESBL genes). Poultry farms or meat products might be an important source for ESBL-producing bacteria in rural Ghana leading to difficult-to-treat infections in humans.	Falgenhauer *et al.* (2019)
E. coli	Develop bioinformatic tool to determine the resistome relatedness of selected pathogens collected from pig, chicken, and human feces in China in 2012	HiSeq2000; SNC-ARDB, CARD	The resistome is influenced by both the host and environment. The probable outflow and horizontal gene transfer of *E. coli* O25b: H4-ST131 from chicken feces to the environment may increase the risk of infection for humans and other animals.	Ma *et al.* (2016)
E. coli	Investigate the occurrence of ESBL-/AmpC-producing Enterobacteriaceae during the course of the broiler fattening period with the special focus on horizontal transmission routes	MiSeq	Proper hygiene measures are important to reduce the horizontal transmission of ESBL-/AmpC-producing Enterobacteriaceae between two flocks consecutively fattened in the same farm.	Daehre *et al.* (2017)
E. coli	Determine the genomic relatedness of CMY-2-producing *E. coli* isolates collected at different levels of the food production chain in Germany between 2008 and 2016	MiSeq	Clonal transmission of bla_{CMY-2} is a rare while the horizontal transfer of temporally stable bla_{CMY-2}-carrying IncK2 and IncI1 plasmids is more likely the dominant way of transmission between humans and animals.	Pietsch *et al.* (2018)
E. coli	Understand the propagation of MDR *E. coli* isolates from diseased chickens in Northeast China using comparative genomics	HiSep; RAST, BLAST, Resfinder, CARD	The dissemination of *mcr*-1-carrying pathogens throughout China might be related to the food chain.	Ding *et al.* (2018)
E. coli	Determine the genomic relatedness of CMY-2-producing clinical and commensal *E. coli* isolates collected from different hosts in Denmark between 2006 and 2012	MiSeq; Artemis v8	The spread of bla_{CMY-2} in Denmark is mainly associated with IncK and IncI1-Iγ plasmids, with limited similarity of plasmid subtypes across different host species.	Hansen *et al.* (2016)

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Table 1. (*Continued.*)

Organism	Objective	Method	Major finding	Reference
E. coli	To estimate the frequency of extraintestinal human infections arising from foodborne ST131 strains	HiSeq	Human clinical isolates harbored ColV plasmids suggesting foodborne transmission of ST131 strains from poultry products.	Liu *et al.* (2018*a*)
Salmonella spp.; *E. coli*	Determine the prevalence of *mcr*-mediated colistin resistance genes in food-animals in Italy between 2014 and 2015	MiSeq and HiSeq2000; RAST, BLAST, ISfinder	High risk of *mcr* horizontal transfer between commensal bacteria and major food-borne pathogens (*Salmonella*) within the same type of productions.	Alba *et al.* (2018)
S. Enteritidis	Identify QTL responsible for the resistance to chicken diseases	60K high-density SNP array; GenomeStudio GT module; PLINK 1.07	*CD1b*, *IL4L1*, *TRIM27*, *GNB2L1*, and *ZNF692*, located on chromosome 16 are associated with the total serum IgY level and are likely to play an important role in the immune response against *S.* Enteritidis.	Zhang *et al.* (2015)
S. Enteritidis	Determine the impact of *S.* Enteritidis infection on the chicken cecal microbiota	MiSep; FLASH; UCLUST; MEGA; R 3.4	*Salmonella*-challenged chickens harbored a higher *Firmicutes*: *Bacteroidetes* ratio compared with the non-challenged chickens. The age of the chicken had an impact of the cecal microbiota composition.	Liu et al. (2018*b*)
S. Heidelberg	Determine the genomic relatedness of *S.* Heidelberg isolates ($n = 31$) collected from chicken- and turkey-farm environment	MiSeq; SPAdes; RAST; SEED; CARD; ARDB	The genetic materials involved in conjugative and phage horizontal transfer were differentially represented in chicken and turkey farm environmental isolates, which might contribute to *Salmonella* genome plasticity, and thereby emergence of antibiotic resistance, survival and virulence abilities.	Deblais *et al.* (2018*b*)
S. Typhimurium	Identify QTL in chicken involved in the resistance to *S.* Typhimurium using high-density genome wide SNP mapping	ENSEMBL; Illumina BeadStation genotyping platform; BEADSTUDIO v3.2	Chromosomes 2, 3, 12, and 25 harbored QTL involved in the resistance to *S.* Typhimurium F98 NaI^R.	Fife *et al.* (2011)
S. Typhimurium	Identification of novel narrow spectrum growth inhibitors small molecules effective against *S.* Typhimurium in chicken	MiSeq; QIIME 1.9; GreenGene	*In silica* microbiota data highlighted a potential combined anti-*Salmonella* effect between compound #2848076 and *B. pullicaecorum*.	Deblais *et al.* (2018*a*)
S. Typhimurium	Determine the genomic relatedness of MDR *S.* Typhimurium isolates from human and poultry feces collected in Burkina Faso in 2010	MiSeq	The poultry and human isolates were genetically similar showing a potential food-safety risk for consumers.	Kagambèga *et al.* (2018)
Salmonella spp.	Characterize the blaTEM-52 carrying plasmid of ESBL-producing *Salmonella* from chicken meat in Japan between 2004 and 2012	MiSeq	The plasmid responsible for the ESBL resistance in Japan is identical than the one identified in Denmark.	Matsumoto *et al.* (2014)
All bacteria	Study the resistome of pig and chicken farms from nine European countries between 2014 and 2015	HiSeq3000 and HiSep2500; Resfinder	The total acquired AMR level was associated with the overall country-specific antimicrobial usage in livestock and that countries with comparable usage patterns had similar resistome. However, functionally determined AMR genes were not associated with total drug use.	Munk *et al.* (2018)
All bacteria	Investigate the changes in bacterial community, variations in ARGs, and their bacterial hosts in the feces of broiler chickens over a full-treatment course of chlortetracycline at low and therapeutic dose levels	MiSep; QIIME and HiSeq4000; ARGs-OAP, CARD, Resfinder, BLAST	The effects of chlortetracycline on the fecal resistome were dependent on the specific ARG subtypes and not simply overall ARGs at the community level. The changes in the structure of the antibiotic-induced fecal microbial community accompany changes in the	Xiong *et al.* (2018)

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Table 1. (*Continued.*)

Organism	Objective	Method	Major finding	Reference
			abundance of bacterial hosts carrying specific ARGs.	
All bacteria	Understand the epidemiology of *mcr*-1-carrying *Enterobacteriaceae* and carbapenem-resistant *E. coli* across the Chinese farming sectors	HiSEq2500; BLAST	First published study linking flies to the spread of carbapenem resistance.	Wang et al. (2017*b*)
All bacteria	Study the resistome of production and household chickens' feces collected in Ecuador in 2015	Miseq; QIIME 1.9.1	Free range household chickens harbored higher microbial diversity and lower levels of ARG and MGEs (up to 157-fold) compared with the production chickens; however, small-scale farms (more abundant in Ecuador) harbored higher levels of ARGs and MGEs compared with the larger farms.	Guo *et al.* (2018)
All bacteria	Investigate the effect of clay on the diversity of antibiotic-resistant bacteria	MiSeq	Clay additions significantly reduce the abundance of antibiotic-resistant bacteria.	Awasthi *et al.* (2019)
All bacteria	Investigate the overview of ARGs in Chinese live poultry markets	HiSeq	59.63% of samples from live poultry markets contain *mcr*-1 gene responsible for resistance to colistin.	Wang *et al.* (2019)

genome wide single-nucleotide polymorphism (SNP) mapping (Hasenstein *et al.*, 2008; Fife *et al.*, 2011; Zhang *et al.*, 2015; Psifidi *et al.*, 2016). The genotypic analysis (1255 SNPs) of a backcross ($F_1 \times N$) population ($n = 288$) identified four independent QTL on chromosomes 2, 3, 12, and 25, involved in the resistance to *S.* Typhimurium F98 Nal^R (Fife *et al.*, 2011). A similar study using 1243 SNPs was performed to identify QTL involved in the resistance to *Campylobacter jejuni* in White Leghorn chicken lines (Psifidi *et al.*, 2016). The authors identified QTL on chromosomes 4, 7, 11, 14, and 16 involved in the resistance to *Campylobacter*. Interestingly, two of these QTL on chromosome 14 (*TRAP1* and *AXIN1*) were co-located with *Salmonella* resistance loci, which might facilitate the development of new breeds resistant to both foodborne pathogens (Chiang *et al.*, 2008; Psifidi *et al.*, 2016). Another study, using a chicken 60K high-density SNP array, identified five candidate genes (*CD1b*, *IL4L1*, *TRIM27*, *GNB2L1*, and *ZNF692*) on chromosome 16 associated with the total serum immunoglobulin Y (IgY) levels which are likely to play an important role in the immune response against *S.* Enteritidis (Zhang *et al.*, 2015). These results were further validated using quantitative reverse transcription polymerase chain reaction (RT-qPCR) to confirm the expression of the candidate genes. However, it is important to note that whole genome sequencing (WGS) data only enable the identification of potential genes involved in the resistance to specific foodborne pathogens and more studies are needed to identify specific roles. Overall, genomics revealed important information about molecular mechanisms of resistance to foodborne pathogens in chickens. This knowledge will improve the safety of poultry products by facilitating future breeding efforts for the development of novel breeds with higher resistance to foodborne pathogens.

Metagenomics to understand the importance of the gut microbiota for resistance to foodborne pathogens in poultry

As previously stated, the host genome content has a major role in regulating host resistance toward specific foodborne pathogens. In addition, recent metagenomics studies have emphasized the importance of the gut microbiota in health and disease through identification of unique gut microbial alterations caused by specific foodborne pathogens and treatments (Oakley *et al.*, 2014; Choi *et al.*, 2015; Wang *et al.*, 2017*a*; Borda-Molina *et al.*, 2018; Shang *et al.*, 2018). By studying the chicken cecal microbiome ($n = 45$), Qu *et al.* discovered that chicken ceca colonized with *C. jejuni* NCTC11168 were characterized by higher microbial diversity and evenness, and a higher *Firmicutes*:*Bacteroidetes* ratio compared with the non-challenged chickens (Qu *et al.*, 2008). Despite these microbiota changes, the functional gene content was similar in both microbiomes. Interestingly, a similar increase of *Firmicutes* population in *S.* Enteritidis CVCC3377 challenged chickens compared with the non-challenged group was observed (Liu *et al.*, 2018*b*). Further, Qu *et al.* showed that metavirulomes of the microbiomes seemed to be affected by the host species (Qu *et al.*, 2008), which supports the metagenomic data reported by Deblais *et al.*, where distinct prophage profiles were observed in *S.* Heidelberg isolates collected from chicken- and turkey-farm environments (Deblais *et al.*, 2018*b*).

Several metagenomics studies also analyzed the impact of specific treatments such as a novel anti-*Salmonella* growth inhibitor (PubChem ID: 2834410, 2847561, 2848076, and 9586485) (Deblais *et al.*, 2018*a*), *S.* Typhimurium attenuated vaccines (ΔΔmetRmetD and $P_{BAD\text{-}mviN}$) (Park *et al.*, 2017), and antibiotic growth promoters (bacitracin, dimethyl salicylate, or enrofloxacin) (Li *et al.*, 2017; Kumar *et al.*, 2018) on the gut microbiota and the persistence of *Salmonella* spp. and *Campylobacter* spp. in chicken. These studies have provided detailed information about the microbiota alteration caused by the treatments. For example, Deblais *et al.* observed that the antimicrobial efficacy of four novel anti-*Salmonella* growth inhibitors tested on chickens infected with *S.* Typhimurium LT2 was associated with the limited impact of the compounds on the cecal microbiota (Deblais *et al.*, 2018*a*). Further, the authors showed that chickens treated with #2848076 harbored a higher relative abundance of *Butyricicoccus pullicaecorum* in cecal contents compared with the

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other groups. *B. pullicaecorum* was previously described as a probiotic that is potentially effective against *Salmonella*, *Campylobacter*, and *C. perfringens* in chickens (Geirnaert *et al.*, 2014; Eeckhaut *et al.*, 2016). Overall, discoveries related to metagenomics studies provide important information on the impact of specific pathogens and treatments on the gut microflora. This information will enhance our understanding of the contribution of gut microflora and the mechanisms associated with health and disease. Further, it will aid in improving the rational use of antimicrobial agents and the discovery of novel narrow spectrum agents.

Importance of genomics and metagenomics in the detection of antimicrobial resistance

The use of antibiotics in poultry production has facilitated the control of numerous avian bacterial diseases. The majority of the antimicrobials are almost exclusively administrated to livestock through feed or water (Mehdi *et al.*, 2018). Bacitracin, tylosin, salinomycin, bambermycin, virginiamycin, and especially tetracycline represent the antibiotics most currently used on poultry farms in North America (Diarra and Malouin, 2014; Gonzalez Ronquillo and Angeles Hernandez, 2017; Mehdi *et al.*, 2018). However, recent studies showed that the massive use of antibiotics has consequences such as the accumulation of antibiotics in the environment, untargeted effects of the antibiotics on human, animal and environmental microbiota, and the emergence of antibiotic-resistance mechanisms (Furtula *et al.*, 2010; Forgetta *et al.*, 2012; Gonzalez Ronquillo and Angeles Hernandez, 2017; Agyare *et al.*, 2018; Mehdi *et al.*, 2018).

Poultry, a source of extended spectrum beta-lactamase- and mcr*-carrying bacteria*

As previously stated, MDR bacteria have been, and continue to be, one of the major food and public safety concerns of this century. One of the emblematic examples of current antimicrobial resistance (AMR) concerns is the high prevalence of extended spectrum beta-lactamase (ESBL)/AmpC-producing *Enterobacteriaceae* in livestock (especially poultry) worldwide (Smet *et al.*, 2008; Leverstein-van Hall *et al.*, 2011; Laube *et al.*, 2013; Blaak *et al.*, 2014; Huijbers *et al.*, 2014; Casella *et al.*, 2017; Saliu *et al.*, 2017; Wu *et al.*, 2018). ESBL-producing bacteria are resistant to beta-lactams (eg, penicillin, cephalosporins, monobactams, and carbapenems) commonly used as therapeutics against several bacterial infections (Kong *et al.*, 2010; Shaikh *et al.*, 2015). The resistance to beta-lactams is principally conferred via the acquisition of plasmids. Previous studies showed that in few cases similar ESBL genes could be shared between poultry and humans (Leverstein-van Hall *et al.*, 2011; Overdevest *et al.*, 2011; Wu *et al.*, 2013). Therefore, WGS was used to elucidate the horizontal mechanisms of transmission of these ARGs at the farm level (Agersø *et al.*, 2012; Dierikx *et al.*, 2013; Blaak *et al.*, 2014; Hansen *et al.*, 2016; Daehre *et al.*, 2017; Pietsch *et al.*, 2018). Pietsch *et al.* monitored the occurrence of ESBL population in seven broiler flocks during the course of the fattening period at three time points (beginning, middle, and end) in a contaminated environment (Pietsch *et al.*, 2018). By the end of the fattening period, all the flocks were positive for ESBL/AmpC-producing bacteria and $bla_{\text{CMY-2}}$ was the most frequent gene detected. The whole genome analysis confirmed that contaminated environment is a major source of transmission to the flock. Therefore, the authors reinforced the importance of proper decontamination procedures between flocks to reduce the horizontal transmission of bacterial pathogens. Another study focused on the transmission of $bla_{\text{CMY-2}}$ throughout the food chain by studying the whole genome of isolates ($n = 164$) from livestock and food products (especially poultry), and humans collected in Germany between 2008 and 2016 (Pietsch *et al.*, 2018). The authors showed that a clonal relationship between the animal/food and human isolates was rare. However, poultry represented a very common reservoir of $bla_{\text{CMY-2}}$-carrying plasmids, and could be an upcoming threat of CMY-2-producing *E. coli* isolates causing infections in both humans and animals. Hansen *et al.* investigated the possible transmission of CMY-2 across poultry, dogs, and humans in Denmark by sequencing the $bla_{\text{CMY-2}}$-carrying plasmids from *E. coli* isolates ($n = 93$) collected between 2006 and 2012 (Hansen *et al.*, 2016). The authors showed that horizontal transfer of plasmids (IncK and IncI1-Iγ) was the principal route of transmission of CMY-2-mediated cephalosporin resistance in *E. coli* between animals and humans in Denmark compared with clonal dissemination. However, their findings did not support the transmission of *E. coli* carrying $bla_{\text{CMY-2}}$ from poultry meat to humans, as previously reported by Bonten and Mevius (2015).

On the other hand, a close genomic relationship has been observed with *E. coli* isolates from chickens and humans ($n = 341$) collected in China between 2008 and 2014 (Wu *et al.*, 2018) and in the USA in 2012 (Liu *et al.*, 2018*a*). A recent study investigated the occurrence and phylogenetic relatedness of *E. coli* isolated from meat products (chicken, turkey, and pork; $n = 1923$) and humans with urinary tract infections ($n = 1188$; urine and blood samples) using multilocus sequence typing, *fimH* typing, and WGS. Among all the *E. coli* ST131-H22 isolates studied, 24 isolates from humans harbored the ColV plasmids, which is associated with *E. coli* fitness and virulence in avian hosts, and displayed the same high-resolution phylogenetic clades as the ones from the meat products. From these studies, the authors concluded that ST131-H22 is well established in poultry production worldwide. Furthermore, a study from China observed that 13% of the bla_{CTX}-carrying isolates (both human and chickens) also harbored *mcr*-1 on the Incl2 plasmid, conferring the resistance to colistin (Wu *et al.*, 2018). The co-occurrence of both bla_{CTX} and *mcr*-1 in *E. coli* isolates seems to be an increasing trend (McGann *et al.*, 2016; Sun *et al.*, 2017*b*; Ding *et al.*, 2018). Therefore, *E. coli* might represent an emerging foodborne antibiotic-resistant bacterial threat in the next decades. Colistin is considered as the last resort solution against Gram-negative bacterial infections (Osei Sekyere, 2016); however, this might not be the case. The resistance to colistin can be conferred by mobilizable *mcr* genes, which were first detected in *E. coli* from poultry meat and humans in Denmark (Hasman *et al.*, 2015). It is now detected in various foodborne pathogens from a multitude of sources worldwide (Cannatelli *et al.*, 2016; Liu *et al.*, 2016; Xavier *et al.*, 2016; Borowiak *et al.*, 2017; Carattoli *et al.*, 2017; Chiou *et al.*, 2017; Wise *et al.*, 2018). So far, over 21 variants of the *mcr* genes have been characterized, which clearly show the rapid evolution of the resistance (Kluytmans, 2017; Alba *et al.*, 2018). Further, the lack of molecular data limits the epidemiologic monitoring of colistin resistance (Alba *et al.*, 2018; Schrijver *et al.*, 2018). Therefore, genomics can be used as a tool to fill this gap and mitigate the risk of *mcr* transfer to human in a 'One Health' perspective (Alba *et al.*, 2018). A study from Italy analyzed the whole genome of 42 *mcr*-positive isolates (*E. coli* and *Salmonella enterica*) from various livestock (turkeys, pigs, and cattle) collected between 2014 and 2015 (Alba *et al.*, 2018). The isolates harbored a high diversity of *mcr* genes (*mcr*-1.1 was the most frequently detected) within and across the animal sources. By

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combining WGS data with a conjugation approach, they confirmed that *E. coli* and *Salmonella* isolates from turkey harbored *mcr*-1.1 and *mcr*-1.2 on the IncX4 plasmid. IncX4 plasmid was first described in a human *Klebsiella pneumoniae* isolate (Poyart *et al.*, 1998; Di Pilato *et al.*, 2016), suggesting that the IncX4 plasmid might be a source of colistin resistance transmissible between bacteria of different species and a significant cause of foodborne MDR infections (Sun *et al.*, 2017*a*; Alba *et al.*, 2018), as previously suggested with *E. coli* ST101 in Brazil (Fernandes *et al.*, 2016). In addition, they observed the co-occurrence of *mcr*-3.2 and the ESBL $bla_{\text{CTX-M-55}}$ genes in cattle isolates (Hernández *et al.*, 2017; Roer *et al.*, 2017; Alba *et al.*, 2018). Based on these findings, the authors cautioned about the ability of the *mcr* cassette to jump between plasmids, which would represent a high risk for the creation of new mega-plasmids and the co-selection of the colistin resistance during the use of other antimicrobials (Alba *et al.*, 2018). This study also reinforced the possibility that poultry could be a reservoir of human extra-intestinal pathogenic *E. coli* (ExPEC) in Europe given at least 25% of the isolates belonged to the same sequence type and displayed similar AMR profiles to human ExPEC isolates.

The increasing importance of poultry in drug-resistant infections in human

Despite the various geographic locations, hosts, and pathogens studied, the final message of these studies remain the same; the intensive and continuous use of antimicrobials in modern farming could favor the horizontal transfer of plasmidi carrying ARG organisms from animals, especially poultry, and increase the potential transmission of MDR isolates to humans. We acknowledge that the majority of the studies described above were conducted in countries (the USA, Europe, and China) with intensive poultry production systems, which might not be representative of the rest of the world; however, similar trends have been observed in countries in Africa (Kagambèga *et al.*, 2018; Falgenhauer *et al.*, 2019). One such study analyzed *S.* Typhimurium isolates from human ($n = 2$) or poultry ($n = 2$) collected in Burkina Faso in 2015 (Kagambèga *et al.*, 2013, 2018). Despite the small population studied, the isolates were very similar to each other independent of their source. In addition, all the isolates matched with the invasive sub-Saharan MDR *S.* Typhimurium ST313 strain (Kingsley *et al.*, 2009; Leekitcharoenphon *et al.*, 2013; Ley *et al.*, 2014; Kariuki *et al.*, 2015). ST313 was believed to be transmitted mostly person to person (Wain *et al.*, 2013); however, recent genomic studies identified poultry as a leading cause of ST313 infections in sub-Saharan countries (Parsons *et al.*, 2013; Kagambèga *et al.*, 2018). Therefore, these observations raise concerns about chickens co-existing in households with people who have a poor understanding of hygiene, and with the uncontrolled use of antimicrobials (Alonso *et al.*, 2017). These points were emphasized by Falgenhauer *et al.* in 2019, where they analyzed the genomic relatedness of *E. coli* isolates from human ($n = 33$) and poultry ($n = 41$) feces collected in rural Ghana in 2015 (Falgenhauer *et al.*, 2019). They concluded that a high percentage (10%) of the poultry ESBL isolates were closely related to the human ESBL isolates. In addition, the whole genome analysis showed that the $bla_{\text{CTX-M-15}}$ gene was predominant in both poultry (96%) and human (97%) isolates. The study from Burkina Faso also observed that all the isolates were co-resistant to four additional antibiotics (chloramphenicol, streptomycin, sulfonamide, and trimethoprim) in addition to carrying ESBL genes, which is an alarming threat for the future of human medicine in Africa (Kagambèga *et al.*, 2018). A study similar to the Burkina Faso one was conducted in Ecuador, where they studied the resistome between production and free-ranged household chickens ($n = 32$) (Guo *et al.*, 2018). As expected, household chickens raised in a more diverse environment harbored a higher microbial diversity and lower levels of ARGs and MGEs (up to 157-fold) compared with the production chickens; however, small-scale farms (more abundant in Ecuador) harbored higher levels of ARG and MGEs compared with the larger farms. Therefore, small-scale farms might play an important role in the proliferation of AMR. Another study from Asia showed that poultry manure collected from breeding farms harbored higher levels (up to three times) of ARGs compared with poultry manure collected from domestic farms (Awasthi *et al.*, 2019). Further, the authors showed, using metagenomics, that addition of clay (6%) into the manure was an effective method to reduce the relative abundance of ARGs.

Dissemination of antimicrobial resistance worldwide revealed through genomic/metagenomic studies

To date, AMR is an increasing threat worldwide (Prestinaci *et al.*, 2015; Jasovský *et al.*, 2016). Nevertheless, the high similarities between the isolates and/or the MGEs carrying ARGs between countries support the intercontinental dissemination of these elements despite the surveillance monitoring system efforts put in place by national and international organizations. For example, all the sequenced *Salmonella* isolates collected from retail chicken meat ($n = 10$) in Japan between 2005 and 2012 harbored IncX-type plasmids, which were identical to the pDKX1 plasmid first identified in *E. coli* in Denmark in 2011 (Johnson *et al.*, 2012; Matsumoto *et al.*, 2014). Also, the majority of the MDR *Salmonella* isolated from Burkina Faso (60%) belong to the sequence type ST19, which is a frequent sequence type detected in Europe and America (Kagambèga *et al.*, 2018). Therefore, to understand the factors contributing to the dissemination of MDR bacteria, Wang *et al.* used WGS to study the resistome (focusing especially on carbapenem and colistin resistance) from poultry, dogs, flies, sewage, and wild birds samples in China (Wang *et al.*, 2017*b*). In this study, the authors provided concrete evidence about the transmission of bla_{NDM}-carrying *E. coli* between poultry farms and the environment and proposed flies as major source of close environmental contamination while migratory birds could be a risk for long distance environmental contamination. A similar observation was reported by Mather *et al.* (2016). *S.* Typhimurium isolates ($n = 11$) collected from wild Passerines in England and Wales between 2001 and 2014 were closely genetically related to other non-Passerines isolates ($n = 13$), based on whole genome SNP profiling. In addition, Wang *et al.* introduced the fact that the level of ARGs in the environment is underestimated due to the 'phantom resistome' (non-pathogenic bacteria harboring ARGs), which limits the understanding of the antimicrobial element exchanges between microorganisms (Wang *et al.*, 2017*b*). Therefore, more metagenomic studies should be carried out to better estimate the 'phantom resistome' of a specific environment, which would enhance the quality and precision of epidemiological studies and improve the understanding of specific biotic and abiotic factors. To this end, Xiong *et al.* provided metagenomic data about the impact of antibiotic treatments (chlortetracycline) on the microbiome and associated resistome of chicken feces ($n = 27$) (Xiong *et al.*, 2018). As expected, chlortetracycline altered the fecal microbial population of treated chickens (especially *E. coli* and *Shigella*); however, it did not modify the relative abundance of total

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ARGs nor did it alter the three predominant ARG types (aminoglycoside, tetracycline, and multidrug). The authors also acknowledged that the effect of chlortetracycline on the fecal microbiota may vary depending on the host (age, genotype, and health), the diet, and the environment. *Escherichia*, *Klebsiella*, *Shigella*, and *Salmonella* were the major bacteria of the fecal resistome and harbored very similar ARG profiles. *Enterococcus* was the only genera with vancomycin resistance genes. In the future, the acquisition of these types of data would significantly help in reducing non-judicious use of antibiotic and in targeting the best antimicrobial treatment in both animal and human medicine. Following the same strategy, Munk *et al.* performed a metagenomic study on the fecal resistome of pigs and poultry ($n = 359$) collected from nine European countries (Spain, Belgium, Bulgaria, Germany, Denmark, France, Italy, The Netherlands, and Poland) (Munk *et al.*, 2018). To date, this study represents the largest metagenomic AMR monitoring study. The authors found that the geographic location had a significant impact on the resistome, which might be explained by differences in antimicrobial usage. The pig resistome was richer (higher total abundance of ARGs) while the poultry resistome was more diverse. The poultry resistome was characterized by the detection of numerous *mcr*-1-carrying flocks (especially Bulgaria, Italy, and Spain), which is consistent with the intensive use of colistin (ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals, 2015). In addition, this study allowed estimation of the propagation of a new antibiotic-resistant gene *optrA*, (which confers resistance to oxazolidinones and phenicols in several countries, thus helping surveillance monitoring systems in the future (Wang *et al.*, 2015). Similar trends were observed by a metagenomic study from China, which showed that the poultry fecal resistome was higher in both diversity and abundance than pigs or humans, and 59.6% of the samples studied harbored colistin resistance genes (Wang *et al.*, 2019). Accordingly, these findings accentuate the impact of poultry production in the emergence, persistence, and propagation of ARGs worldwide.

Tremendous technological advances were made in relation to genomics (speed and cost of sequencing) over the past decade, which significantly increased our understanding of the CELLMAP (a design for a system to organize biological information), the importance of the gut microflora in health and disease, identification of emerging and re-emerging pathogens, and development of novel therapeutics (Brenner, 2010; Relman, 2011; Pang and Weatherall, 2012; Borda-Molina *et al.*, 2018). Nevertheless, genomics and metagenomics are interdisciplinary fields requiring extensive knowledge and advanced technological tools related to DNA sequencing (PCR-based approaches, new generation, or capillary sequencing) and bioinformatics (quality control, cleaning, assembly, annotation, comparison, and statistical analyses of the reads); therefore, currently only few countries can afford to use them. However, the use of third-generation/long-read sequencing methods and online tools/servers (Galaxy/Hutlab, MG-RAST, and IMG/M) could potentially facilitate genomics and metagenomics studies in developing countries (Pal *et al.*, 2018; van Dijk *et al.*, 2018). For example, MinION is a less expensive and portable RNA and DNA sequencing device able to generate 10–30 Gb of DNA sequence data. That said, the detection of specific genes in a given organism does not always means that it will be functional and/or expressed (pseudogene, located in a non-coding region, truncated gene, etc.); and so, can mislead phenotypic interpretation. Therefore, despite the high accuracy of the *in silica* AMR profiling tools, conventional microbiology and molecular approaches are still required to confirm the phenotypic antibiotic-resistance profile of suspected organisms (Nisar *et al.*, 2017; Deblais *et al.*, 2018*b*).

Transcriptomics

Transcriptomics is the study of all RNA molecules, especially messenger RNA, expressed at a given circumstances within a cell (Lowe *et al.*, 2017). Transcriptomic techniques (microarrays and RNA-Seq) allow study of expression of organism's genes in different tissues, conditions, or time points (Lowe *et al.*, 2017). Transcriptomics studies have led to better understanding of the pathophysiology and immunopathology of foodborne pathogens in poultry by analyzing the host cell's/tissue's responses, and local and systemic immune responses to the pathogen during infection (van Hemert *et al.*, 2006; Chiang *et al.*, 2008; Sandford *et al.*, 2011; Schokker *et al.*, 2011*a*, 2011*b*; te Pas *et al.*, 2012; Huang *et al.*, 2016; Sun *et al.*, 2017*a*). Further, it allows elucidation of key features of the complex interactions between poultry and foodborne pathogens (Crépin *et al.*, 2008; Palyada *et al.*, 2009; Van Immerseel, 2010), and have led to the identification of host-resistance mechanisms (Hiett *et al.*, 2008; Schokker *et al.*, 2012; Xu *et al.*, 2014) and the development of novel therapeutics such as microRNAs to control foodborne pathogens in poultry (Jia *et al.*, 2016; Wu *et al.*, 2017). Details of major transcriptomics studies presented in this review are displayed in Table 2.

Transcriptomics to understand the pathophysiology of foodborne pathogens

Infections by pathogens induce characteristic pathological and physiological changes, the understanidng of which, might help in the design of counter measures to modulate and prevent these pathophysiological changes; ameliorating the prognosis of the infection (Casadevall and Pirofski, 2000; Ashida *et al.*, 2011). For example, several studies investigated the chicken systemic (spleen and cecal tonsil) and local (intestine) responses when challenged with *S.* Typhimurium using RNA microarray (20.6K chicken oligo arrays and Agilent 4 × 44K chicken arrays) (Schokker *et al.*, 2010, 2011*a*; te Pas *et al.*, 2012). The authors showed that the protein phosphorylation (especially on serine, threonine, or tyrosine), protein acetylation (in the cytoplasm and lumen of other cellular components), and adenosine triphosphate (ATP) consuming processes were the major chicken systemic responses during *S.* Typhimurium infection (te Pas *et al.*, 2012). Whereas, in jejunum genes associated with cell mobilization, cell turn-over, defense mechanisms, and cellular communication and signaling (fibronectin, adhesion/integrin) were up-regulated in *Salmonella*-infected chickens when compared with non-challenged chickens (Schokker *et al.*, 2011*a*). Further, it was found that the *Salmonella* infection abolished the well-ordered spatial and temporal development of the immune system (especially genes involved in T-cell activation) in challenged chickens (Schokker *et al.*, 2010). Based on these findings, Schokker *et al.* correlated the intestinal gene expression profiles with the severity of systemic *S.* Enteritidis infection in challenged chickens. Genes associated with metabolism, cell turnover, and tissue repair in the jejunum of challenged chickens were negatively correlated with the severity data, which was consistent with previous studies (Schokker *et al.*, 2011*b*, 2012). Further,

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Table 2. Transcriptomics studies

Pathogen	Objective	Methods	Major findings	Reference
S. Enteritidis	Identify miRNAs associated with *Salmonella* infection in laying chicken cecum	RNA-Seq (Illumina HiSeq 2500)	The micro-RNAs (miRNAs), gga-miR-125b-5p, gga-miR-34a-5p, gga-miR-1416-5p, and gga-miR-1662 contribute to *Salmonella* infection at the onset of egg laying by regulating the homeostasis between metabolism and immunity.	Wu *et al.* (2017)
S. Typhimurium	Analyze thymic responses to the challenge with *Salmonella* or *Salmonella* LPS in chickens	RNA-Seq (Illumina HiSeq 2000)	LPS of *Salmonella* induces thymocyte death and thymic atrophy in chicks through the TLR4-FOS/JUN signaling.	Huang *et al.* (2016)
S. Typhimurium	Determine the general chickens response to *Salmonella* infection independent of age at infection and, time post infection	20.6K chicken oligo array (ARK Genomics)	Phosphorylation of proteins, acetylation in the cytoplasm and lumen of other cellular components, and ATP consuming processes are the important biological mechanisms involved during chicken host response to *Salmonella* infection.	te Pas *et al.* (2012)
S. Enteritidis	Investigate the differences in the susceptibility of newly hatched chicks to oral infection with *S.* Enteritidis	*Gallus gallus* 20K v.2 single-spotted 20,460 oligonucleotides (ARK Genomics)	Intestinal barrier functions and immunological responses are the underlying factors behind the difference in the systemic spread of *Salmonella* in hatchlings of different broiler lines.	Schokker *et al.* (2012)
S. Typhimurium	Investigate the transcriptional responses of chicken intestinal tissues in response to *Salmonella* infection	4 × 44K chicken arrays (Agilent)	Genes associated with cell mobilization, defense mechanisms, cellular communication and signaling are upregulated in intestinal tissues of *Salmonella*-infected chickens.	Schokker *et al.* (2011*a*)
S. Enteritidis	Investigate the effects of *S.* Enteritidis on morphological and functional changes in chicken jejunal development	4 × 44K chicken arrays (Agilent)	Genes involved in cell turn-over are highly expressed in *Salmonella*-infected intestine in chickens. *Salmonella* infection abolished the well-ordered spatial-temporal development of the immune system in chickens.	Schokker *et al.* (2010)
S. Enteritidis	Investigate the expression of immune-related genes in heterophils of chickens, susceptible and resistant to *Salmonella* infection	44K chicken array (Agilent)	Immune-related genes, especially involved in Toll-like receptor (TLR) signaling pathway, and genes involved in T-helper cell activation, expressed differently in chicken lines of different genetic backgrounds in response to *Salmonella* infection.	Chiang *et al.* (2008)
S. Enteritidis	Investigate the correlation of intestinal gene expression patterns with severity of systemic *Salmonella* infections	E-MEXP-2042 (ArrayExpress)	Genes associated with morphological and immunological processes are up-regulated; whereas genes associated with metabolism, cell turn-over and tissue repair are down-regulated with increased severity of *Salmonella* infection in chickens.	Schokker *et al.* (2011*b*)
S. Enteritidis	Analyze the virulence mechanisms that allow *Salmonella* to colonize oviduct tissue. Unravel the mechanisms that protect *Salmonella* from environmental insults in oviduct such as antibacterial molecules of egg white	Chicken oviduct cDNA libraries	Stress-induced survival mechanisms (cell wall modifications, export antimicrobial molecules) enable the *Salmonella* to persistently colonize without inducing damage and inflammation.	Van Immerseel (2010)
S. Enteritidis	Investigate the influence of host genetic background on intestinal gene expression responses to *Salmonella* infection in chickens	cDNA and oligonucleotide microarrays (Affymetrix)	Gene expression responses associated with inflammation, wound healing, acute phase response, the fibrinogen system, and actin polymerization are the important processes involved during *Salmonella* infection in chickens and the expression responses varied according to the genetic background of chickens.	van Hemert *et al.* (2006)

(*Continued*)

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Table 2. (*Continued.*)

Pathogen	Objective	Methods	Major findings	Reference
S. Enteritidis	Identify immune-related gene markers and pathways	RNA-Seq (Illumina HiSeq 2000)	FoxO signaling pathway, especially FoxO3, was activated in *Salmonella* susceptible chickens.	Li *et al.* (2018)
S. Typhimurium	Identify a single-nucleotide change in a promoter region of African *S.* Typhimurium responsible for devastating epidemic of bloodstream infections across sub-Saharan Africa	RNA-Seq (Illumina HiSeq)	PgtE is responsible for enhanced virulence in chicken and human.	Hammarlöf *et al.* (2018)
C. jejuni	Investigate the *C. jejuni* oxidative stress defense mechanisms through transcriptional profiling	*C. jejuni* NCTC 11168 microarray	PerR, Fur, iron metabolism, and oxidative stress defense are the elements responsible for *C. jejuni* colonization of the chick cecum and/or subsequent survival.	Palyada *et al.* (2009)
C. jejuni	Investigate the genomics differences between poor- versus robust-colonizing chicken *C. jejuni* isolates, 11168(GS) and A74/C, respectively	A library of 1633 oligonucleotide probes (MWG-Biotech)	Presence of *maf*, *flaA*, *sodB*, *racR*, *pglH*, and *cadF* genes are responsible for robust colonization of *Campylobacter* in poultry.	Hiett *et al.* (2008)
E. coli O1	Investigate the changes in miRNAs associated with *E. coli* infection in chicken	RNA-Seq (GenomeAnalyzer IIx, Illumina)	miRNA, gga-miR-429, increased in *E. coli* infection in poultry.	Jia *et al.* (2016)
E. coli O1	Investigate the bone marrow transcriptomic responses to *E. coli* infection in chicken	RNA-Seq (Illumina HiSeq 2000)	Lymphocyte differentiation, proliferation, and maturation are greatly impaired in *E. coli*-infected poultry; whereas, innate and adaptive immune responses, including dendritic cells, monocytes and killer cell activity, TLR- and NOD-like receptor signaling, as well as T-helper cells and many cytokine activities are greatly enhanced.	Sun *et al.* (2015)
E. coli O1	Investigate the DNA methylome profile of poultry infected with *E. coli*	MeDIP-Seq (Illumina HiSeq 2000)	Methylation changes of genes involving cell signaling and molecular transport are required to resist *E. coli* infection in poultry.	Xu *et al.* (2014)
E. coli O1	Investigate the spleen transcriptome response in *E. coli*-infected chickens	4 × 44K chicken arrays (Agilent)	Genes associated with immune pathways (TLR, Jak-STAT, and cytokine signaling) are differentially expressed in *E. coli*-infected chickens.	Sandford *et al.* (2011)
E. coli O78	Investigate the transcriptional response of *E. coli* mutant with deleted pst gene responsible for virulence in chicken	*E. coli* Genome 2.0 Array (Affymetrix GeneChip®)	Genes associated with LPS modifications and coding for cell surface components such as type 1 and F9 fimbriae are required for *E. coli* virulence in chicken.	Crépin *et al.* (2008)

the authors showed that the differences in the intestinal barrier functions and immunological responses were the underlying factors behind the varying susceptibility of different chicken breeds/lines to *Salmonella* infections. Similar conclusions were obtained by Hemert *et al.* when they investigated the influence of host genetic background on intestinal gene expression responses to *S.* Enteritidis infection in chickens using cDNA and oligonucleotide arrays (van Hemert *et al.*, 2006). The authors found that the gene expression responses associated with inflammation (IL-8, K60, NOXO1, MHC class II), wound healing (Vanin 3, HGF1/MSP), acute phase response (avidin, antitrypsin, fibrinogen, and ovotransferrin), the fibrinogen system (fibrinogen), and actin polymerization (intestinal zipper protein) were upregulated in response to *Salmonella* infection in chickens. However, the expression of these genes varied according to the genetic background of the chickens. Overall, these transcriptomic studies provided insight into the chicken systemic and intestinal responses to *Salmonella* infection and factors behind the variable responses of chickens to infection. In the long-run, these findings can help in the development of strategies to modulate the host responses to *Salmonella* infection as well as develop *Salmonella*-resistant chicken breeds; thereby, enhancing the control of *Salmonella* in poultry.

Transcriptomics to understand host resistance as well as pathogen colonization and virulence mechanisms

The genetic background of both the host and pathogen are factors influencing the fate of an infection (Casadevall and Pirofski, 2001; Doeschl-Wilson and Kyriazakis, 2012). Analyzing the transcriptome during the host–pathogen interaction would allow the identification of factors responsible for the successful or unsuccessful infection by a pathogen, that could be used to develop novel control methods. Hiett *et al.* showed, using *C. jejuni* 11168(GS) microarray, that the *maf* (motility accessory factor),

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*fla*A (flagellin), *sod*B (superoxide dismutase), *rac*R (chemotaxis protein CheY), *pgl*H (protein glycosylation pathway), and *cad*F (*Campylobacter* adhesion to fibronectin) genes were required for a robust colonization of chicken intestine by *C. jejuni* (Hiett *et al.*, 2008). It is well known that oxidative stress defense mechanisms are essential for *Campylobacter* colonization and survival in the chicken intestine (Hermans *et al.*, 2011); however, by using the same technique as above (*C. jejuni* NCTC 11168 microarray), Palyada *et al.* discovered that PerR (peroxide-sensing regulator), Fur (ferric uptake regulator), and iron metabolism were closely associated with oxidative stress defense allowing *C. jejuni* colonization and survival in chicken intestine (Palyada *et al.*, 2009).

Li *et al.* characterized the splenic transcriptomes of *S.* Enteritidis-susceptible and -resistant chickens and found the activated FoxO signaling pathway, especially FoxO3 in susceptible chickens (Li *et al.*, 2018). This finding can be used to enhance the chicken's innate resistance against *Salmonella* infection. Immerseel *et al.* studied the virulence mechanisms that allow *S.* Enteritidis to colonize oviduct as well as the mechanisms protecting *Salmonella* from environmental insults in oviduct, such as antibacterial molecules in egg white (Van Immerseel, 2010). Based on the studies conducted using chicken reproductive tract cDNA libraries, the stress-induced survival mechanisms (cell wall modifications and antimicrobial molecules export) enabled the *Salmonella* to persistently colonize the chicken oviduct without inducing damage and inflammation. Similarly, several studies showed that the virulence and colonization ability of *Salmonella* in chickens varied according to the host and pathogen genetic background (Foley *et al.*, 2013). A recent study, using RNA-Seq, analyzed the transcriptome of *S.* Typhimurium ST313, an epidemic and highly virulent *Salmonella* strain. They found that PgtE (an outer membrane protease) was responsible for enhanced virulence of ST313 in chickens and humans compared with *Salmonella* ST19 strains (Hammarlöf *et al.*, 2018).

Besides *Salmonella* and *Campylobacter*, transcriptomic studies have also been conducted on avian pathogenic *E. coli* (APEC) since it is known to be a common bacterial pathogen of poultry and a potential zoonotic uropathogen (Guabiraba and Schouler, 2015; Liu *et al.*, 2018*a*). Further, APEC has been considered as an important source of ARGs to human pathogens (Osman *et al.*, 2018). Crepin *et al.* analyzed the transcriptome profile of virulent and avirulent (*pst* mutant) APEC O78 in chickens using an Affymetrix GeneChip® *E. coli* Genome 2.0 array (Crépin *et al.*, 2008). The study found that genes associated with lipopolysaccharide (LPS) modifications (*ept*A and *yei*U) and genes coding for cell surface components such as type 1 (*fim*A, *fim*B, *fim*C, and *fim*I) and type 9 (*ydeTSR*, *ydeQ*, *c1936*, and *c1935*) fimbriae are required for APEC virulence in chickens. These genes were also crucial in facilitating APEC colonization, biofilm formation, and resistance to serum, acid, and cationic antimicrobial peptides. Transcriptomic tools also allowed for the study of the DNA methylome (the set of nucleic acid modifications in an organism's genome or in a particular cell) using MeDIP-Seq (Xing *et al.*, 2018). For instance, Xu *et al.* observed a distinct association between the functional DNA methylation changes in APEC O1-infected chickens and the severity (from mild to severe) of the infections, suggesting that methylation changes in genes involved in cell signaling and molecular transport (*IL8*, *IL2RB*, and *IL1RAPL1*) are required for chickens to resist APEC infections (Xu *et al.*, 2014).

Overall, these studies identify factors and mechanisms of host resistance to pathogens as well as pathogen virulence, colonization, and survival mechanisms in the host. These findings facilitate chicken breeding strategies to develop resistant chicken lines as well as the development of novel antibacterial strategies.

Transcriptomics to understand the immunopathology of foodborne pathogens

The early stage of the interactions between a host and a pathogen elicit specific host immune response pathways, which once understood, might be used to help to modulate, prevent, and treat infection (Ashida *et al.*, 2011). Huang *et al.* analyzed the thymic responses to a *S.* Typhimurium challenge in chickens using RNA-Seq. The authors found that the LPS of *Salmonella* induced thymocyte death and thymic atrophy through the TLR4-FOS/JUN signaling pathway (a pathway that mediates the inflammatory processes during *Salmonella* infection), which could be a target for future therapeutic development (Huang *et al.*, 2016). In another study, Chiang *et al.* investigated the expression of immune-related genes in heterophils from chickens susceptible or resistant to *Salmonella* infection using the 44K chicken Agilent array (Chiang *et al.*, 2008). The immune-related genes, especially those involved in the TLR signaling pathway and T-helper cell activation (*B-G*, *CD4*, *IKK-ϵ*, *TRIF*, *IL-1β*, *IL-6*, *IL-8*, *CCL4*, and *K60*) were upregulated in resistant chickens but were downregulated in susceptible chickens after infection. A similar approach was used by Sun *et al.* to study the gene expression in bone marrow of healthy and APEC O1-infected chickens (both susceptible and resistant), using RNA-Seq (Sun *et al.*, 2017*a*). Innate and adaptive immune responses (dendritic cells, monocytes, and killer cell activity), TLR- and NOD-like receptor signaling, T-helper cells, and many cytokines were upregulated in APEC-resistant chickens; whereas, lymphocyte differentiation, proliferation, and maturation mechanisms were greatly impaired in APEC susceptible chickens. Similarly, genes associated with immune pathways (TLR, Jak-STAT, and cytokine signaling) were down-regulated in the spleens of severely APEC-infected chickens compared with mildly APEC-infected chickens when analyzed using the Agilent 4 × 44K chicken arrays (Sandford *et al.*, 2011). Thus, these findings unravel the profile of immune responses and associated pathways involved during infection. These findings facilitate development of immunotherapeutics which could selectively enhance protective immune responses; thereby, protecting chickens from infections (Helbig *et al.*, 2013).

Transcriptomics to develop novel vaccine and antibacterial therapies

Apart from the abovementioned studies, transcriptomics also allow investigation of the host microRNAome (set of small non-coding RNAs with a key role in physiological and pathological processes) during bacterial infection (Staedel and Darfeuille, 2013). The identified key microRNAs (miRNAs) could be candidates for the future vaccine and antibacterial development (Drury *et al.*, 2017). For instance, by investigating the cecal microRNAome response to *S.* Enteritidis infection in chickens using Solexa Sequencing, Wu *et al.* identified four miRNAs (gga-miR-125b-5p, gga-miR-34a-5p, gga-miR-1416-5p, and gga-miR-1662) up-regulated in chicken cecum (Wu *et al.*, 2017). These miRNAs are responsible for maintaining the homeostasis between metabolism and immunity; thereby, enhancing the resistance of chickens toward *Salmonella* infection. A similar study was performed in APEC O1-infected chickens

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using deep sequencing (Jia *et al.*, 2016). The authors identified gga-miR-429 miRNA, which is associated with the enhanced susceptibility of APEC in chickens, upregulated. Therefore, suppression of gga-miR-429 expression could increase the chicken's resistance to APEC infection. Overall, these findings introduce important new areas to explore the pathophysiology of bacterial infections in poultry.

In conclusion, transcriptomics offers a wealth of opportunities to solve the problems of foodborne pathogens in poultry. Looking at the trend, the transcriptomic studies are expected to increase rapidly over the coming years. Even though transcriptome studies in the past were mostly based on microarrays, RNA-Seq is more likely to be used in the future because of the high coverage and rapid development of high-throughput technologies (Lowe *et al.*, 2017). Further, the recent advancement of single-cell RNA-Seq (measures the distribution of expression levels for each gene across a population of cells) has allowed researchers to uncover new and potentially unexpected biological discoveries compared with traditional bulk RNA-Seq (measures average expression level for each gene across a large population of input cells) (Hwang *et al.*, 2018). Compared with genomics and metagenomics, which involve studying only the genome architecture, transcriptomics connect the genome to gene function and thereby provide better understanding of the complex interactions between the pathogen, host, and the environment (Manzoni *et al.*, 2018). However, like genomics, transcriptomics is costly and requires extensive bioinformatics and biostatistics tools. Bioinformatics tools that are user friendly, fast, efficient, and which do not require specialized skills are needed. Some of the recently developed bioinformatics tools such as ToPASeq (Ihnatova and Budinska, 2015), SimBA (Mariano *et al.*, 2016), TRAPLINE (Wolfien *et al.*, 2016), SPARTA (Johnson *et al.*, 2016), SAMSA2 (Westreich *et al.*, 2018), VIPER (Cornwell *et al.*, 2018), PIVOT (Zhu *et al.*, 2018), and Matataki (Okamura and Kinoshita, 2018) have made the analysis of transcriptomics data efficient and even allow data analysis to be done on a personal computer.

Proteomics and metabolomics

Proteomics is the study of the structure and function of proteins and peptides in an organism or cell (Martinović *et al.*, 2016). Proteomics programs can also be used to create a complete three-dimensional map of the cell with proteins' location (Graves and Haystead, 2002). The proteome can be studied using high-throughput techniques, including tandem-MS and differential in-gel electrophoresis (DIGE). On the other hand, metabolomics is the study of low molecular weight metabolites (organic acid, essential amino acids, sugars, and intra- and extra-cellular signaling molecules) in an organism or cell. These metabolites are required for the cellular maintenance, growth, and development (Fiehn, 2002; Deidda *et al.*, 2015). The metabolome can be studied using MS (Singhal *et al.*, 2015), vibrational spectroscopy (Ellis *et al.*, 2017), and nuclear magnetic resonance spectroscopy (Garcia-Perez *et al.*, 2017). To date, the use of proteomics and metabolomics in food safety is limited and underestimated; however, an increasing number of studies demonstrate the benefit of these techniques for the rapid detection and identification of low level of metabolites in complex samples. These techniques offer great advantages for food-safety risk monitoring (pathogen detection), characterization of the complex pathogen–host interactions, epidemiology, immunopathology, and antibiotic-resistance studies (Ramos *et al.*, 2016; Jadhav *et al.*, 2019). Details of major proteomics and metabolomics study cases presented in this review are displayed in Table 3.

Proteomics and metabolomics to detect, characterize, and differentiate foodborne pathogens

For decades, the identification of potential foodborne pathogens in a complex sample has been done using time-consuming culture-based techniques (Jasson *et al.*, 2010; Li and Zhu, 2017), and onerous immunological and molecular techniques (Anderson *et al.*, 2012). However, the development of novel metabolomics and proteomics tools have enhanced the rapidity, viability, and veracity of foodborne pathogen detection (Bergholz *et al.*, 2014; Ramos *et al.*, 2016; Jadhav *et al.*, 2018). For example, gas chromatography mass spectrometry (GC-MS) allowed the detection of changes in the metabolite profile of foodborne pathogens (*E. coli* O157:H7, *S.* Typhimurium, *S.* Muenchen, and *S.* Hartford) in chicken meat during incubation in non-selective culture media. Principal component analysis showed different metabolites and based on the overall metabolite profile of each bacteria, GC-MS was able to detect the presence of *E. coli* O157:H7 and *Salmonella* spp. at a limit of detection (LOD) of 7 ± 2 CFU per 25 g of ground chicken within 18 h post-processing (Cevallos-Cevallos *et al.*, 2011). This approach is more rapid than conventional culture-based methods (LOD ranged between 10^2 and 10^4 CFU ml^{-1}), which are also laborious, time consuming, require up to 1 week for the confirmation of pathogens' species, and have higher probability of giving false results (Lee *et al.*, 2014; Zhao *et al.*, 2014). In another study, Fourier transform infrared (FT-IR), Raman spectroscopies, and matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) were used as a metabolic and proteomic fingerprinting approach for the rapid differentiation and characterization of closely related *Campylobacter* species isolated from humans and chickens (Muhamadali *et al.*, 2016). This study clearly showed that the combined metabolic and proteomic fingerprinting approach was more rapid and sensitive than 16S rRNA sequencing. Both FT-IR and MALDI-TOF-MS techniques differentiated *C. fetus* subspecies fetus from *C. fetus* subspecies *venerealis*, while the 16S rRNA sequencing data could not. Additionally, MALDI-TOF-MS showed higher sensitivity and provided information for identification of specific proteins and peptides related to each species. Taken together, these studies suggest that metabolomics and proteomics allow early, rapid, and accurate detection, differentiation, and characterization of potential foodborne pathogens in animal and food products, which could result in reduction of (1) foodborne pathogen outbreaks, (2) testing and holding costs, and (3) product recalls; thus improving product safety and quality.

Proteomics and metabolomics to understand host interaction, colonization, survival, and adaptation of foodborne pathogens

All organisms produce thousands of proteins and metabolites with various functions at any given time; however, only few of them are essential during the interactions between the pathogen and the host (Jean Beltran *et al.*, 2017). Therefore, comparative proteomics (i.e., the comparison of the proteome of two distinct samples) can be used to identify factors essential for the pathogen or the host at a given time point and location. For example, *C. jejuni* A74C is a robust colonizer of the chicken intestine (up to 10^7 CFU ml^{-1}) while *C. jejuni* 11168-PMSRU is a poor

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Table 3. Proteomics and metabolomics studies

Pathogen	Objective	Method	Major findings	Reference
E. coli O157:H7, *S.* Typhimurium, S. Muenchen, and S. Hartford	Rapid detection of foodborne pathogens in chickens using metabolomics	Mass spectrometry (GC-MS)	Metabolomics was able to detect the these pathogenic bacteria at levels of 7 ± 2 CFU per 25 g of ground chicken.	Cevallos-Cevallos *et al.* (2011)
Campylobacter spp.	Differentiate eleven *Campylobacter* strains from six species using biochemical metabolomics fingerprints	FT-IR and Raman spectroscopies, and MALDI-TOF-MS	The cluster analysis of the data from each approach provided clear differentiation of each *Campylobacter* species, which was generally in agreement with a phylogenetic tree based on 16S rRNA gene sequences.	Muhamadali *et al.* (2016)
C. jejuni	Identify the variation between *C. jejuni* isolates in colonization aspect using proteomics	Two-dimensional gel electrophoresis	Detected the specific expression of an outer membrane fibronectin binding protein, serine protease, and a putative aminopeptidase in the robust colonizer A74C strain. While, other specific proteins such as a cysteine synthase and aconitate hydratase were detected on the poor colonizer *C. jejuni* NCTC 11168-PMSRU isolates.	Seal *et al.* (2007)
C. jejuni	Role of fatty acid metabolism in bacterial adaptation in chickens using proteomics	iTRAQ-coupled 2D-LCeMS/MS	*Ex vivo* proteomics studies combined with directed mutagenesis identified that FabG affects the fatty acid composition of *C. jejuni* 81-176, which significantly reduce *C. jejuni* persistence in the chicken gut.	Asakura *et al.* (2016)
C. jejuni	Test the effects of *pgl* gene deletion on the *C. jejuni* proteome	Label-based LC-MS/MS	Glycosylation plays a role in *C. jejuni* phenotypes such as biofilm formation, motility, cell stress, nutrient uptake and utilization, chemosensing, and electron transport.	Cain *et al.* (2019)
E. coli	Investigate the plasma proteome during *E. coli* infection in chicken	Quantitative Tandem Mass Tags-Q-Exactive-Plus MS	Plasma proteins; serum-amyloid-A, ovotransferrin and alpha-1-acid-glycoprotein are affected during *E. coli* infection in chicken.	Horvatić *et al.* (2019)
Salmonella spp.	Investigate the plasma proteomic changes in chickens infected with *Salmonella*	MALDI-TOF-MS, LC-MS/MS	Increase in fibrinogen beta-derived peptide and decrease in apolipoprotein-AII-derived peptide at 24 h post-injection.	Packialakshmi *et al.* (2016)
Salmonella spp.	Analyze the proteome of spleen CD4, CD8 and γδ T-lymphocytes during *Salmonella* infection in chickens	Quantitative LC-MS/MS	γδ T-lymphocytes up-regulate proteins related to immune response, oxidative phosphorylation and the spliceosome during *Salmonella* infection.	Sekelova *et al.* (2017)

colonizer (up to 10^4 CFU ml^{-1}). Seal *et al.* compared the proteome from both strains using 2D-gel electrophoresis and MALDI-TOF/TOF analysis to identify the differentially expressed *C. jejuni* proteins (Seal *et al.*, 2007). Three proteins (a branched outer membrane fibronectin [Fn] binding protein [*CadF*], putative serine protease [*htrA*], and a putative aminopeptidase P) were detected only in A74C. Proteins (a cysteine synthase B [*cysM*], aconitate hydratase [*acnB*], and ketol-acid reductoisomerase [*ilvC*]) specific to 11168-PMSRU methods were also identified. Previously, Fn was shown to mediate *C. jejuni* adherence to the epithelial cells via CadF (Ziprin *et al.*, 1999; Monteville *et al.*, 2003). Similarly, HtrA was shown to play a role in intracellular replication of adherent and invasive *E. coli* strain LF82 within J774-A1 macrophages (Bringer *et al.*, 2005), *AcnB* is known to control the flagella biosynthesis in *S. enterica* (Tang *et al.*, 2004), while *cysM* was reported to play a role in the survival and growth of *S. aureus* and *E. coli*, respectively (Garvis *et al.*, 1997; Lithgow *et al.*, 2004). Comparative proteomics methods were also used to identify and compare the total proteins of *S.* Enteritidis, *S.* Typhimurium, and *S.* Gallinarum isolated from humans and chickens using two-dimensional gel electrophoresis (2-DIGE) and MS (Sun and Hahn, 2012). The MALDI-TOF analysis identified proteins (β-lactamase, RfbH protein, and shikimate kinase) that are related to virulence. For example, β-lactamase expression was induced (7–8-fold) in the human *S.* Enteritidis isolate relative to the chicken isolate. Further, RfbH was highly expressed in *S.* Gallinarum and human *S.* Enteritidis, while shikimate kinase was highly expressed in *S.* Typhimurium. Comparison of the proteomics profiles of the serovars from different hosts may provide insight into the potential roles of the identified proteins in the host adaptation and pathogenicity of *Salmonella*.

Using green fluorescent protein (GFP)-expression and iTRAQ-coupled 2D-LC-MS/MS, Asakura *et al.* showed that the fatty acid metabolism is an important feature for the persistence of *C. jejuni* 81-176 in a chicken's ceca (Asakura *et al.*, 2016).

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Ten proteins (FabG, HydB, CJJ81176_0876, MscS, CetB, FlhF, PurH, PglJ, LpxC, and Icd) were significantly up-regulated (>1.4-fold) after infection. Through fatty acid methyl ester analysis and directed mutagenesis, the authors showed that the *fabG* deletion significantly reduced the unsaturated fatty acids abundance (1.3-fold) as well as the bacterial burden in chicken's ceca. This study provided evidence implicating fatty acid metabolism in *C. jejuni* 81-176 colonization of chicken.

N-glycosylation encoded by *pgl* is essential for *C. jejuni* colonization in chickens and virulence in humans, which makes it an ideal antimicrobial target (De Schutter *et al.*, 2017). However, the limited knowledge concerning the biological functions of N-glycosylation has limited drug-discovery approaches. Cain *et al.* studied the proteome response of a *C. jejuni pglB* (oligosaccharyl transferase) deletion mutant using label-based liquid chromatography/tandem mass spectrometry (LC-MS/MS) and DIASWATH-MS to understand the molecular mechanisms associated with *pglB* (Cain *et al.*, 2019). Overall, this study showed that glycosylation mediates several *C. jejuni* phenotypes including biofilm, motility, cell stress, nutrient uptake and utilization, chemosensing, and electron transport. Specifically, the deletion of *pglB* significantly increased the abundance of stress-associated proteins (*ClpB*, *GroEL*, *GroES*, *GrpE*, and *DnaK*), reduced transducer-like (*Tlp*) proteins and depleted respiration/electron transport-associated proteins (*MfrABC*, *SdhABC*, *FrdABC*, and *SucCD*).

In conclusion, proteomics facilitates the identification and characterization of proteins and molecular mechanisms essential for the survival of foodborne pathogens in specific environments, which will aid the development of novel targeted intervention strategies (attenuated vaccines and narrow spectrum antimicrobials) (Kumari *et al.*, 2008; Jagusztyn-Krynicka *et al.*, 2009; DelVecchio *et al.*, 2010).

Proteomics to identify immunological biomarkers of infection

The interactions between hosts and pathogens induce specific changes in host protein quality, expression, and/or abundance (Sethi and Brietzke, 2015). These modifications, even minimal, can be detected using proteomics-based technologies, which allow the identification of biomarkers of early infection (Kaddurah-Daouk *et al.*, 2008; Kusebauch *et al.*, 2018). Using the quantitative Tandem Mass Tags approach Q-Exactive-Plus mass-spectrometer (TMT-based LC-MS), Horvatic *et al.* quantified the changes in the proteome of chicken plasma after treating chickens with *E. coli* LPS (Horvatić *et al.*, 2019). These authors identified that pathways related to blood coagulation, exocytosis and protein excretion, vasoconstriction were activated while others related to apoptosis, extracellular matrix organization, peptidase/hydrolase regulation, drug transport were inhibited during the early period of inflammation. Previous studies reported the therapeutic potential of fibrinogen complex, which plays an important role in blood coagulation and innate and T-cell mediated immune responses, against bacterial pathogens such as *S. aureus* (Davalos and Akassoglou, 2012). Accordingly, the 87 regulatory proteins identified by Horvatić *et al.* which were significantly increased after 12 h post-LPS injection (including serum-amyloid-A, ovotransferrin, and alpha-1-acid-glycoprotein), might represent novel targets for therapeutic approaches (Horvatić *et al.*, 2019). A similar study performed using MALDI-TOF and LC-MS/MS on chickens after injection with *S.* Typhimurium LPS (Packialakshmi *et al.*, 2016) revealed a significant increase in fibrinogen beta-derived peptide and a decrease in apolipoprotein-AII-derived peptide in the blood samples at 24 h post-LPS injection. Further, LC-MS/MS data showed a significant increase of α1-acid glycoprotein, a chemokine CCLI10, and cathelicidin-2, and a significant decrease in an interferon-stimulated gene-12-2 protein in the LPS treated group.

Sekelova *et al.* studied the proteome of chicken's splenic lymphocytes (CD4, CD8, and γδ T-lymphocytes) before and after intravenous infection with *S.* Enteritidis using LC-MS/MS (Sekelova *et al.*, 2017). Overall, 171 proteins were differentially expressed in response to *S.* Enteritidis infection in at least one of the T-lymphocyte type. CD4 T-lymphocytes respond to *S.* Enteritidis infection by inducing ribosomal proteins and inhibiting the lysosomal functions, MHCII and lysosomal (cathepsin) functions. In cytotoxic CD8 T-lymphocytes, the expression of the STAT1 and MCM (minichromosome maintenance complex proteins) proteins were increased. The expression of γδ T-lymphocytes is regulated through oxidative phosphorylation and epigenetic histone modification. The T-lymphocyte specific proteins identified were identified involved in cell differentiation, cell activation, and regulation of programed cell death (Berndt and Methner, 2001; Matulova *et al.*, 2012). Identification and characterization of new proteins using proteomics is a promising approach to study proteins involved in immune response and which can be used as candidate biomarkers of inflammation and *Salmonella* infection in chickens.

In conclusion, increasing number of studies demonstrated the great potential of proteomics and metabolomics for the rapid detection and identification of foodborne pathogens in food products (Martinović *et al.*, 2016). For example, rapid identification of foodborne pathogens via the identification of species-specific proteomics profiles can lower the risks associated with the consumption of contaminated food (Seng *et al.*, 2009). In the future, the combination of high-throughput methods with MS techniques would facilitate the increased use of these technologies and make them more efficient for routine analyses of foodborne pathogens. For example, RapidFire® MS is a low cost high-throughput solid phase extraction automated system allowing analysis of up to 384 samples 10 times faster than the traditional MS/MS approach, which is ideal for drug-discovery purposes (Leveridge *et al.*, 2016; Veach *et al.*, 2019). Further, the recent development of PANDA-view, a comprehensive and flexible tool, will facilitate the analysis and data visualization of quantitative proteomics data (Chang *et al.*, 2019). In summary, all of these technological advances will facilitate the replacement of time consuming, less reliable and less sensitive, and laborious conventional food-safety detection methods by proteomics and metabolomics approaches.

Conclusion and future prospective

'Omics' metthods will likely be key to a new era in food safety. High-throughput genomic technologies, especially WGS, have revolutionized the ability to detect and attribute the source of foodborne pathogens in poultry. WGS also makes it feasible to understand the genetic diversity and evolution (Rantsiou *et al.*, 2018), surveillance of the antibiotic resistance and associated resistant genes (Oniciuc *et al.*, 2018), and elucidate the transmission dynamics of foodborne pathogens in poultry (Le and Diep, 2013). In the USA, GenomeTrkar, a genomic reference database of foodborne pathogens, based on WGS data has been successfully used for real-time surveillance of foodborne pathogens in food and environmental sources (Timme *et al.*, 2019). WGS has tremendous potential for global epidemiological surveillance of foodborne pathogens, outbreak detection, and infection control

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in the future (Rantsiou *et al.*, 2018). Transcriptomic technologies have been crucial in uncovering a myriad of information regarding persistence, virulence, host–pathogen interactions, and pathogenicity of foodborne pathogens in poultry (Salazar *et al.*, 2017). Transcriptomics have also been used to identify the colonization and survival mechanisms of foodborne pathogens in host as well as markers of host resistance to foodborne pathogens (Salazar *et al.*, 2017). Thus, transcriptomic data have great potential to be utilized for rational development of new and improved control strategies for foodborne pathogens in poultry (Bergholz *et al.*, 2014). Beyond the genomic and transcriptomic technologies, MS-based proteomic and metabolomic technologies have aided in identifying biomarkers of foodborne pathogen infections in host as well as rapid detection of foodborne pathogens in complex samples, even at low abundance levels (Jadhav *et al.*, 2018, 2019). This information could guide development of new rapid diagnostic tests as well as new therapeutic drugs and vaccines to control foodborne pathogens in poultry by identifying molecules essential for the survival of the pathogen under specific conditions.

'Omics' approaches are powerful tools requiring specific equipment and skill sets, and their use is highly associated with the cost of these approaches. For example, WGS technologies have not been widely adopted in developing countries because of the high cost related to establishing and maintaining a sequencing facility, lack of skilled personnel, and limited access to tools for genomic data manipulation and analysis (Helmy *et al.*, 2016). Further, the numerous types of equipment, analytic tools, and databases with different velocities and veracities that can be used to process the big data, can will affect the quality of the results and the biological conclusions (Brenner, 2010; Press, n.d.; Ruiz, 2017). Accordingly, standardized guidelines should be developed and freely available to normalize and facilitate future metagenomics studies. Similarly, the transcriptomics approach has not achieved its full potential because of the lack of faster, less computationally intensive, and easier to use bioinformatics tools (Salazar *et al.*, 2017). The development of user-friendly analysis tools in the future will help in the utilization of transcriptomic tools to yield solutions to foodborne pathogen infections in poultry. Proteomic and metabolomic approaches produce bounteous data and therefore require analysis of large and complex datasets using intensive computational methods (Gatto *et al.*, 2015). It is important to keep in mind that the use of 'omics' approaches in science is very recent and the field is evolving rapidly; therefore, the interpretation of the results might change with the evolution of the tools available. In summary, omics technologies have the potential to transform the fields of food safety and public health but the technology requires more "user-friendly" bioinformatics analysis tools as well as easier access of omics data at the global level. In-depth understanding of foodborne pathogens in poultry using omics technologies will enhance the food safety and thereby improve public health.

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