ISSN 1466-2523

Animal Health Research Reviews **13**(1); 89–99
doi:10.1017/S1466252312000138

The microbiome of the chicken gastrointestinal tract

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Received 12 April 2012; Accepted 26 May 2012

Abstract

The modern molecular biology movement was developed in the 1960s with the conglomeration of biology, chemistry, and physics. Today, molecular biology is an integral part of studies aimed at understanding the evolution and ecology of gastrointestinal microbial communities. Molecular techniques have led to significant gains in our understanding of the chicken gastrointestinal microbiome. New advances, primarily in DNA sequencing technologies, have equipped researchers with the ability to explore these communities at an unprecedented level. A reinvigorated movement in systems biology offers a renewed promise in obtaining a more complete understanding of chicken gastrointestinal microbiome dynamics and their contributions to increasing productivity, food value, security, and safety as well as reducing the public health impact of raising production animals. Here, we contextualize the contributions molecular biology has already made to our understanding of the chicken gastrointestinal microbiome and propose targeted research directions that could further exploit molecular technologies to improve the economy of the poultry industry.

Keywords: 16S rRNA gene, genomic analysis, nutrition, reducing pathogen load, antibiotic resistance genes, virulence genes

Introduction

The gastrointestinal tracts (GITs) of chickens harbor microbial communities, or microbiomes, that play important roles in: growth and development, including the production of energy-rich short chain fatty acids (SCFA; Dunkley *et al.*, 2007); promotion of GIT villus and crypt morphology (Shakouri *et al.*, 2009); nutrient utilization, including reduction in luminal viscosity (Shakouri *et al.*, 2009), the deconstruction of dietary polysaccharides (Beckmann *et al.*, 2006; Qu *et al.*, 2008); nutrient absorption (Cole and Boyd, 1967); and well-being of their chicken hosts, including detoxification (Hai *et al.*, 2010). The chicken GIT is inhabited by various bacteria (Qu *et al.*, 2008), methanogenic archaea (Saengkerdsub *et al.*, 2007a, b), fungi (Okulewicz and Zlotorzycka, 1985), and viruses (Qu *et al.*, 2008). Protists are more sparsely distributed (Okulewicz and Zlotorzycka, 1985) and are generally regarded as pathogens. The composition of the GIT microbiome reflects co-evolution among the inhabiting microbes, genetic, immune, and metabolic interactions with the host, and environmental influences (Yeoman *et al.*, 2011). Microbes are found across the entire length of the GIT, where they show spatial variation in community composition biogeographically (Fig. 1; Gong *et al.*, 2007) as well as between luminal and mucosa-associated populations (Gong *et al.*, 2002).

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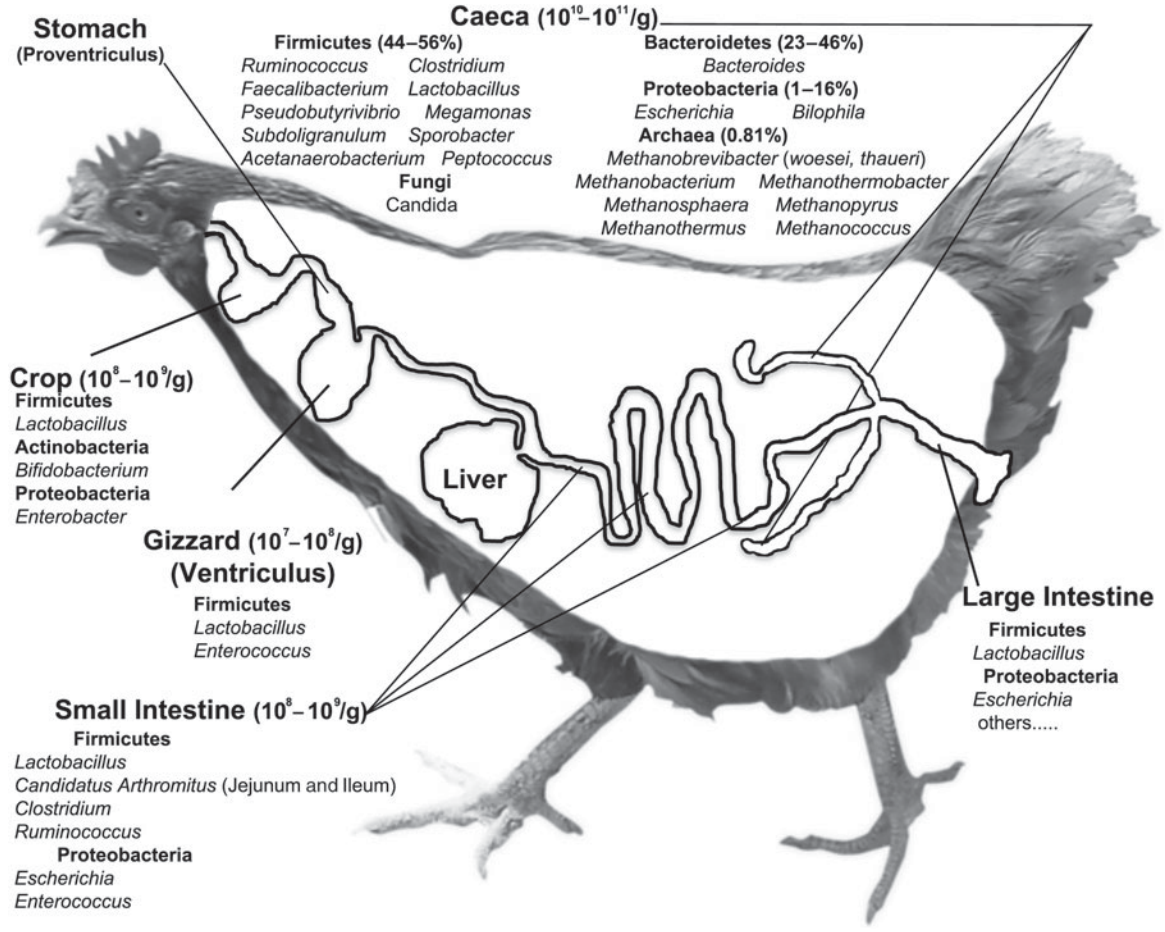

Fig. 1. Major taxa surveyed along the chicken GIT. Data on taxa and their spatial distribution are taken from Qu *et al.* (2008), Saengkerdsub *et al.* (2007a, b), and Gong *et al.* (2002). Virus and phage populations are not presented or adequately sampled and listed cecal colonists are limited to the most common and abundant taxa. Numerous other taxa have been described in the chicken ceca.

Microbial diversity and abundance are most evident in the ceca (Gong *et al.*, 2007), where more than 2200 operational taxonomic units (OTUs; 95% sequence ID; Danzeisen *et al.*, 2011) and as many as 3500 genotypes (Qu *et al.*, 2008) have been predicted. Consistently, microbial fermentation is most active in this section of the GIT.

As a result of issues that relate to zoonoses, food safety, animal nutrition, and health, the composition and function of the chicken GIT microbiome has received significant attention from researchers for almost 40 years. The original study of chicken GIT community composition by Salanitro *et al.* (1974) looked at 325 strains isolated by culture from the cecum of 5-week-old broiler hens. These strains were reported to represent up to 81% of the cultivable microbes from the chicken ceca. However, by the early 1990s it was recognized that the richness of species in all microbiomes, and indeed the Earth's biosphere, had been significantly underestimated by conventional microbial culturing methodologies (Amann *et al.*, 1995). In fact, the majority of microbial species colonizing the chicken GIT have not been cultivated. More recently, culture-independent methods have been developed to overcome cultivation biases and allow more complete and detailed information on microbial community diversity, composition, and function.

16S rRNA gene-directed microbiome composition

The use of the 16S rRNA gene as a phylogenetic marker to study bacterial and archaeal diversity and composition across various environments has resulted in tremendous quantities of information about microbial community dynamics. In particular, the increasing affordability and capability of second and subsequent-generation high-throughput sequencing platforms have made it possible to explore microbiomes at unprecedented phylogenetic depth. These surveys have uncovered the fine-grained structure of microbial communities occupying these ecosystems, exposing important features such as the existence of a rare biosphere, whose low-abundance populations dominate ecosystem diversity

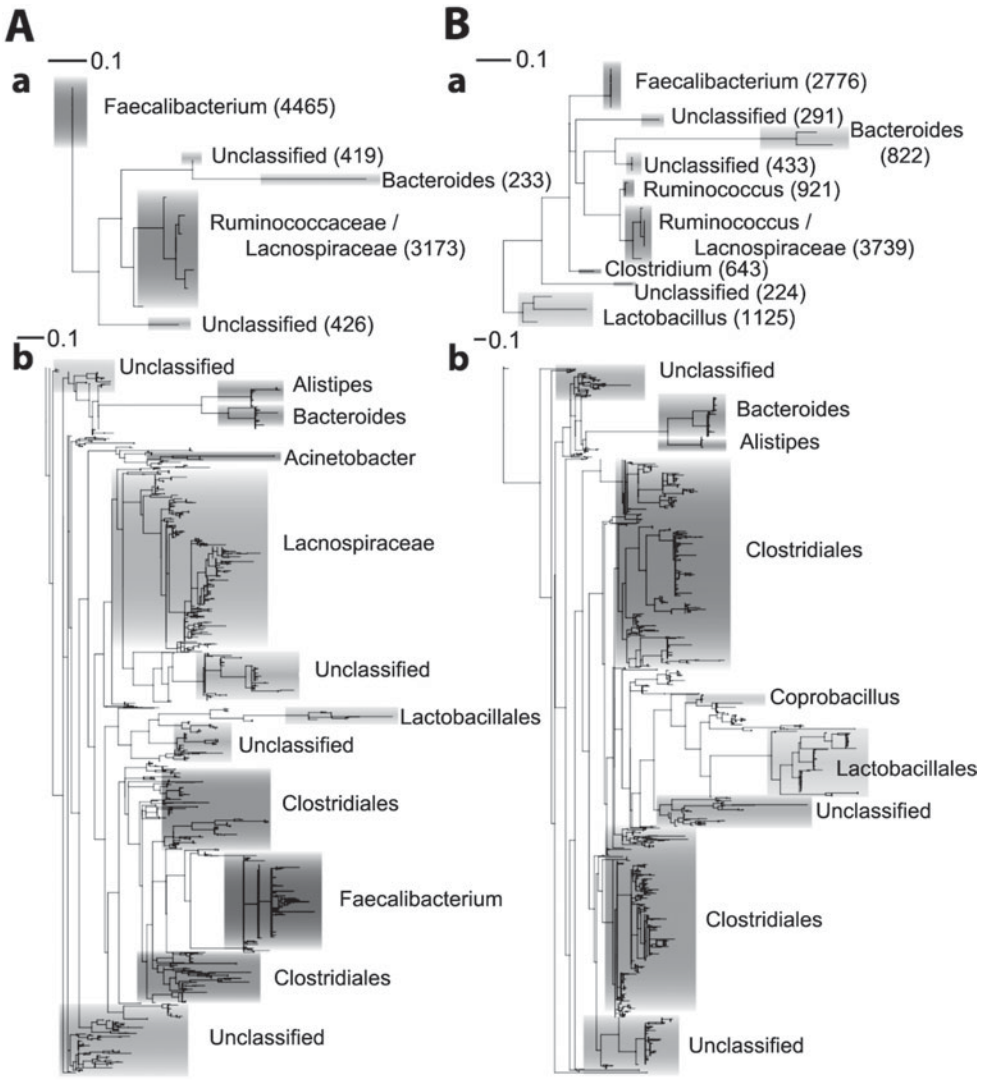

Fig. 2. Maximum likelihood trees of abundant (a) and rare (b) OTUs. Trees are built from sequence data generated by Qu *et al.* (2008) for Chick1 (Panel A) and Chick94 (Panel B) with detailed taxonomic assignments. Unclassified refers to a taxonomic classification of less than 70% confidence by RDP at Order. Taxonomic clades are shaded according to a shared taxonomic designation. The number of sequence tags in each of the OTUs in the modal biosphere is given in parentheses following their taxonomic classification.

(Fig. 2; Dethlefsen *et al.*, 2008; Huse *et al.*, 2008; Turnbaugh *et al.*, 2008). Although outnumbered by an order of magnitude by bacteriophage (Rodriguez-Valera *et al.*, 2009), bacteria are the most abundant and diverse domain of life in the chicken GIT.

Microbial density and diversity are greatest in the cecum where longer digesta transit times permit more substantial microbial fermentation (Rehman *et al.*, 2007). In the cecal pouches, bacteria are present at concentrations of 10^{10}–10^{11} cells/g cecal material, encoding more than 95% of the genetic information present (Qu *et al.*, 2008; Danzeisen *et al.*, 2011). Consistent with other host-associated microbiomes (Ley *et al.*, 2008), the bacterial phylum Firmicutes is the predominant phylum in the chicken crop, gizzard, small intestine, and cecum (Rehman *et al.*, 2007; Qu *et al.*, 2008; Danzeisen *et al.*, 2011). Firmicutes represent 50–90% of all taxa in the cecum (Qu *et al.*, 2008; Danzeisen *et al.*, 2011), while culture-dependent and -independent approaches indicate the proportion of Firmicutes (principally in the form of *Lactobacilli*) is greater than 90% in other GIT locations (Gong *et al.*, 2007; Rehman *et al.*, 2007). Archaea are less abundant, being present at concentrations of 10^{5}–10^{7} cells per gram of cecal material (Saengkerdsub *et al.*, 2007a) and encoding around 1–2% of the genetic information present in the ceca (Qu *et al.*, 2008; Danzeisen *et al.*, 2011). *Methanobrevibacter* is the predominant archaeal genus in the chicken ceca, with taxa similar to *Methanobrevibacter woesei* being the most prolific of this domain (Fig. 1; Saengkerdsub *et al.*, 2007a). Other archaeal taxa exist, and consistent with other GIT environments, all archaea appear to be involved in the methanogenic

dissipation of hydrogen produced during fermentation (Saengkerdsub *et al.*, 2007a, b).

Analyses of rarefaction curves and diversity indexes indicate that microbial richness and diversity increase with age (Danzeisen *et al.*, 2011). In the work by Danzeisen *et al.* (2011), OTUs (95% sequence ID) corresponding to *Roseburia, Coprococcus, Butyricoccus, Papillibacter* (all Firmicutes), and *Escherichia* (Proteobacteria) were found to be abundant constituents of the chicken ceca, but were not detected before 14 days of age, while other OTUs classified as *Fastidiospila, Hespellia, Lactobacillus*, and *Coprococcus* (all Firmicutes) were not detected before 35 days of age. *Methanobacteriales* were detected in the fecal samples of 25% of chickens as early as 3 days of age and found in 100% of chickens tested from 5 days of age (Saengkerdsub *et al.*, 2007b).

Although 16S rRNA gene surveys provide taxonomic information, they fail to provide information related to microbial function. While these can be uncovered for isolated microbes in culture using a variety of directed-assays, metagenomic, metatranscriptomic, and metabolomic analyses offer the ability to understand these physiological roles for individual species (including those that have not yet been cultured), *in situ,* and in the context of the entire microbiome.

Shotgun metagenomic analyses

Gene-based metagenomic surveys provide a measure of the metabolic capabilities of a microbiome. To date, two shotgun metagenomic surveys have been performed in chickens, both focusing on the ceca. The first, performed by Qu *et al.* (2008) determined the distribution of ~200,000 genes present in healthy chickens and in chickens experimentally infected with *Campylobacter jejuni*. In the second, Danzeisen *et al.* (2011) looked at differences in genes between control chickens and those fed sub-therapeutic levels of antibiotics for growth enhancement. These studies uncovered a large amount of information relating to the prevalence of mobile elements, and genes involved in nutrition, virulence, and antibiotic resistance, which will be discussed below. They also provide an unbiased look at the diversity and distribution of all types of microbes, including bacteria, archaea, viruses, and eukaryotic microbes.

To date, no studies have integrated the global gene expression patterns or metabolite profiles from the chicken GIT to a metagenomic backbone. Therefore, currently our knowledge is limited to the metabolic potential of the chicken GIT microbiome. Several studies have investigated the transcriptional dynamics of microbial isolates of the chicken GIT, including comparisons of *in vitro* and *in vivo* grown *Salmonella enterica* serovar Enteritidis PT4 and *Salmonella enteric* serovar Typhimurium (Dhawi *et al.*, 2011; Harvey *et al.*, 2011), respectively. These studies revealed significant metabolic differences between *in vitro* and *in vivo* grown cells, as well as striking differences in the expression of important virulence factors (Dhawi *et al.*, 2011; Harvey *et al.*, 2011). One of the studies (Harvey *et al.*, 2011) also revealed significant differences in the growth rate and motility of *Salmonella* Typhimurium. These studies highlight the stark contrasts between evidence obtained at the laboratory bench and the functional reality of microbes occupying GIT environments, arguing strongly for a systems biology understanding. Even these experiments are one step removed from biological reality as they were performed in gnotobiotic hatchlings; it will therefore be interesting to determine the significance of these findings *in situ* alongside fully developed microbial ecosystems.

This is not an argument for a complete switch to meta-omic techniques. These techniques provide the opportunity to survey the system-wide dynamics of a microbiome, but meta-omic techniques need to be integrated with genomic and transcriptomic information from isolated organisms so they are correctly interpreted and contextualized, leading to a more complete understanding of the ecology and evolution of the microbiome.

Genomic analyses of microbes isolated from the chicken GIT

Most genome-sequencing projects focusing on chicken isolates have been directed toward pathogenic viruses (e.g. Barbosa *et al.*, 2007; Linde *et al.*, 2010; Qiu *et al.*, 2011; Abro *et al.*, 2012; Diel *et al.*, 2012). Those projects focusing on autonomous microbial life forms have almost universally targeted zoonotic or host pathogens (Johnson *et al.*, 2007; Ahir *et al.*, 2011), including GIT isolates (Pearson *et al.*, 2007; Thomson *et al.*, 2008; Cooper *et al.*, 2011; Feng *et al.*, 2012) or opportunistic pathogens (Johnson *et al.*, 2011). A handful of bacteria have been isolated and sequenced from the chicken GIT without a clear zoonotic link, including *Bacteroides salanitronis* BL78, *Lactobacillus crispatus* ST1 and *Lactobacillus salivarius* NIAS840. The *B. salanitronis* and *Lactobaillus* genomes were all reported in the past few years and describe bacteria of potential importance to GIT health (Ojala *et al.*, 2010; Gronow *et al.*, 2011; Ham *et al.*, 2011). In addition to bacterial and viral genomes (Thomson *et al.*, 2008), genome-sequencing efforts have also successfully targeted a *Sipboviridae*-family bacteriophage, SPN3UB (Lee *et al.*, 2012) and a *Podoviridae*-family phage, *Φ*CPV1 (Volozhantsev *et al.*, 2011). Bacteriophage SPN3UB was isolated from chicken feces (Lee *et al.*, 2012), while *Φ*CPV1 was isolated from chicken intestinal contents. These phages are infectious to important zoonotic pathogens and are being explored

as alternatives to antibiotics for the control of *Salmonella* Typhimurium and *Clostridium perfringens*, respectively.

Although the molecular interrogation of the chicken GIT microbiome is only in its adolescence, its contributions to our understanding of growth, health, and development of the chicken host have been significant, and may lead to new methods for the mitigation of zoonotic diseases that use chickens as a vector.

The chicken GIT microbiome's role in host nutrition

The chicken GIT microbiome produces enzymes enabling the deconstruction of dietary polysaccharides (Beckmann *et al.*, 2006). These enzymes are critical to host nutrition because chickens, like most animals, lack the genes for glycoside hydrolase (GH), polysaccharide lyase (PL), and carbohydrate esterase (CE) enzymes that are necessary to facilitate this process (Morris, 2003). Metagenomic analyses have illustrated the significance of the cecal microbiome's contribution to carbohydrate metabolism. Genes encoding GHs, PLs, CEs, and other proteins involved in carbohydrate metabolism (transporters and those involved in central carbohydrate metabolism) have been shown to be more abundant than any other category of gene in this environment (~20% of genes; Qu *et al.*, 2008; Danzeisen *et al.*, 2011).

During the deconstruction of dietary polysaccharides, GIT bacteria produce SCFAs (Topping and Clifton, 2001; Dunkley *et al.*, 2007). The composition and proportions of these SCFAs vary depending on microbial composition, which is to some degree adaptable, and fine-tuned by the composition and structure of the fiber component of the chicken's diet (Topping and Clifton, 2001). Acetate is the primary SCFA produced in most GIT environments, including the chicken, followed by propionate and butyrate (Topping and Clifton, 2001; Dunkley *et al.*, 2007). Other SCFAs such as valerate, isobutyrate, and isovalerate are also produced in trace amounts (Dunkley *et al.*, 2007). Concentrations of butyrate are of particular physiological significance, as this SCFA is the primary energy source of colonic epithelia and has been shown to be essential to homeostasis of colonocytes and development of GIT villus morphology (Panda *et al.*, 2009; Donohoe *et al.*, 2011). The three major SCFAs (acetate, propionate, and butyrate) all appear important to colonic musculature and vasculature in the GIT (Topping and Clifton, 2001). These SCFAs are also of critical importance to host energetics and hydration. SCFAs stimulate fluid and electrolyte uptake and are absorbed transepithelially as a source of energy that contributes between 10% (humans) and up to 70% (ruminants) of the host's daily energy requirements (McNeil, 1984; Topping and Clifton, 2001; Flint and Bayer, 2008). Although their exact contribution in chickens has yet to be determined, the SCFA butyrate has been shown to improve growth performance and carcass quality characteristics in chickens (Panda *et al.*, 2009).

The GIT microbiome also contributes to nitrogen metabolism. Genes involved in the metabolism of protein (9–10% of genes), amino acids (8–9%), and nitrogen (1%) have all been shown to be abundant (Qu *et al.*, 2008; Danzeisen *et al.*, 2011). The relative proportions of genes dedicated to the metabolism of these three nitrogen sources are consistent with protein being the major source of nitrogen and depicting the major direction of nitrogen flux (protein – amino acids – nitrogenous compounds) in the GIT. The microbial metabolism of dietary protein that escaped host metabolism earlier in the GIT provides further amino acids for egg production, maintenance, and growth (Latshaw and Zhao, 2011). However, subsequent metabolic processing to ammonia or urea is of no nutritive value to the host and approximately half of the available dietary nitrogen is excreted, mostly as ammonia in chickens (Latshaw and Zhao, 2011). This hyper-production of ammonia and subsequent excretion is not only nutritionally inefficient but also underpins negative effects on performance, health, and mortality in poultry houses, and is a major environmental and public health concern (McCubbin *et al.*, 2002; Xin *et al.*, 2011).

Genes dedicated to fatty acid and lipid metabolism are also detected (1–2%; Qu *et al.*, 2008), suggesting microbial modulation of lipid profiles as has been described in other livestock (Dhiman *et al.*, 2005). Conjugated linoleic acid (CLA) is one of the best-studied microbially produced fatty acid and is produced by certain microbes as an intermediate during the biohydrogenation of the polyunsaturated linoleic acid (Palmquist *et al.*, 2005). CLA has been found to naturally occur in chicken meat (Dhiman *et al.*, 2005). Dietary supplementation of CLA has been shown to increase lean body mass in chickens and to be incorporated into tissue lipids (Simon *et al.*, 2000). It should also be noted, however, that dietary CLA also appears to affect yolk quality and embryo mortality in laying hens by altering yolk fatty acid composition and albumen and yolk pH, a feature that can be overcome by the co-supplementation of olive oil (Aydin *et al.*, 2001).

The incorporation of a molecular understanding of the microbiome with nutritional science therefore paves the way for new research that should seek to optimize the composition of the chicken GIT microbiome. Such research could provide new opportunities to enhance SCFA production, reduce nitrogen losses or optimize fatty acid profiles (which may vary between broiler and laying hens). Such benefits could lead to significant improvements in poultry production and the associated economics. SCFA and CLA production also have been linked to host health (Badinga and Greene, 2006; Wong *et al.*, 2006) and may provide additional benefit in reducing disease.

The role of the chicken GIT microbiome in reducing pathogen loads

For over 100 years chickens have been recognized as an important source of zoonotic infection (Higgins, 1898), a feature that has long plagued the poultry industry. This may be exacerbated by less active innate and humoral immune systems in chickens that are permissive to colonization by pathogenic bacteria such as species of *Salmonella* and *Campylobacter* (Toth and Siegel, 1986; Jeurissen *et al.*, 1998) and numerous host-specific or host-promiscuous viruses. Metagenomic analyses have shown us that genes associated with virulence are abundant within the chicken microbiome (~8% of all genes; Qu *et al.*, 2008; Danzeisen *et al.*, 2011). These mostly include genes for antibiotic resistance (>55% of virulence-related genes), and iron scavenging (13%), but also include genes involved in types III and IV secretion (>2%), adhesion (>1%), invasion and intracellular resistance (1%; Qu *et al.*, 2008), lipid A biosynthesis (not quantified), and type I pilus formation (not quantified; Danzeisen *et al.*, 2011). Many of these virulence genes, including those involved in type IV secretion, type I pilus formation, lipid A biosynthesis, and iron scavenging were found to represent a significantly larger portion of the total genes identified in chickens subjected to various sub-therapeutic antibiotic treatments (STAT; Danzeisen *et al.*, 2011).

The composition of the chicken GIT maintains a fine balance; disruptions to key species can enable the dramatic proliferation of pathogenic microbes (Kimura *et al.*, 1976; Morishita and Mitsuoka, 1976) and dramatic increases in the proportion of virulence genes (Danzeisen *et al.*, 2011). Conversely, a stable and healthy GIT microbiome can limit the colonization of zoonotic pathogens, such as *Salmonella* (Hudault *et al.*, 1985) and *Campylobacter* species (Soerjadi-Liem *et al.*, 1984) as well as transform clinically significant fungal mycotoxins periodically found in feed to non-toxic derivatives (Hai *et al.*, 2010). Although *Enterococcus faecium* and some *Lactobacillus* isolates have been suggested to limit the colonizing potential of some major pathogens through direct competitive interactions (Jin *et al.*, 1996; Carina Audisio *et al.* 2000), relative exclusion is strongly correlated to increasing species complexity (Hudault *et al.*, 1985; Fukata *et al.*, 1991; Schoeni and Wong, 1994). The pre-establishment of the microbiome prior to infection is an important precursor of resistance (Hudault *et al.*, 1985), perhaps relating to the order and complexity of epithelial adherence. It is therefore clear that maintaining chicken GIT health is one key to limiting pathogen loads and increasing food safety in the poultry industry. Microbial diversity in the chicken GIT is sensitive to a number of perturbing agents, including parasitic infection with *Ascaridia galli* (Okulewicz and Zlotorzycka 1985) or protozoal infection with *Eimeria tanella* (Kimura *et al.*, 1976), as well as human interventions such as the provision of antibiotics (Danzeisen *et al.*, 2011).

Antibiotics are used therapeutically to treat disease in humans and domestic animals. In the late 1940s, it was recognized that sub-therapeutic levels of antibiotics (STAT) could be used to expedite and enhance the growth of chickens (Stokstad and Jukes, 1950) and other livestock (Gustafson and Bowen, 1997). The serendipitous finding came from chickens fed fermentation waste from cyclotetracycline production as an inexpensive source of vitamin B_{12} (Stokstad and Jukes 1950). In addition, STAT was recognized as a tool to reduce pathogen loads and decrease the risks of zoonotic transmission (Gustafson and Bowen, 1997). The mechanisms that link STATs to animal productivity have not been established but because of their growth promoting activity STATs became widely used in production facilities. Today STATs are still used in the USA and several other countries around the world as they have been for more than 50 years (Collignon *et al.*, 2009; Chapman *et al.*, 2010).

Molecular methods have established that STATs act non-specifically affecting a broad range of microbial taxa. Monensin in combination with either virginiamycin or tylosin has been shown to significantly decrease bacteria of the major phylum Firmicutes, including *Roseburia, Enterococcus, Lactobacillus* and *Blautia,* and increase Proteobacteria such as *Escherichia* and Ruminococcaceae such as *Anaerotruncus, Subdoligranulum*, and *Sedimentibacter* (Danzeisen *et al.*, 2011).

In the 1960s, it was suggested that STATs could lead to proliferation of the pool of antibiotic resistance genes and allow their transfer to human pathogens (Swann, 1969). Evidence suggests a number of pathogens can colonize both the human and chicken GIT (Johnson *et al.*, 2008, 2009; Gipp *et al.*, 2011), providing opportunities for gene exchange. Gene-directed metagenomic surveys have provided clear evidence that microbes colonizing the chicken GIT are an abundant source of antibiotic resistance genes (Qu *et al.*, 2008; Zhou *et al.*, 2012). Most prolific are genes encoding resistances to fluoroquinolones, tetracyclines, cobalt, zinc, cadmium, and common antibiotics used in poultry production (Qu *et al.*, 2008; Danzeisen *et al.*, 2011). Methicillin (Qu *et al.*, 2008) and beta-lactam (Qu *et al.*, 2008; Danzeisen *et al.*, 2011) resistance genes are also common. This abundance of antibiotic resistance genes has been contrasted with the near absence of these genes in animals with no historical exposure to antibiotics and minimal interactions with humans or other animals from areas where antibiotics are frequently used (Thaller *et al.*, 2010). However, certain antibiotic resistances are ancient properties of microbes (D'Costa *et al.*, 2011). Genes encoding ampicillin and spectinomycin resistance have been detected in free-range chickens not routinely subjected to STATs (Zhou *et al.* 2012), though in the same study antibiotic-resistance genes were found to be almost four times more prevalent in conventionally raised (STAT-treated) chickens (Zhou *et al.* 2012). Danzeisen *et al.* (2011) found that antibiotic resistance genes were not enriched by

controlled short-term STAT application. This could suggest that the low dosages of STATs have limited bactericidal action and therefore elicit a limited selection pressure, but may also reflect the long-term adaptation of the chicken GIT microbiome to STAT use.

Perhaps the most disturbing aspect of this abundance of antibiotic resistance genes in the chicken ceca is that they are co-occurring in an environment that also has a high abundance of mobile DNA elements, as detected in shotgun metagenomic surveys (Qu *et al.*, 2008; Danzeisen *et al.*, 2011). The genome sequences of chicken isolates *Salmonella* Enteritidis P125109 and *Salmonella enterica* serovar Gallinarum 287/91 carry potentially mobile genomic islands (Thomson *et al.*, 2008). Plasmids carrying virulence genes have been observed in potentially zoonotic *Escherichia coli* strains of chicken origin (Johnson *et al.*, 2008). Even the avirulent *L. salivarius* NIAS840 and *B. salanitronis* Bl78 isolates carry three plasmids each (Gronow *et al.*, 2011; Ham *et al.*, 2011). A quick survey of the gene contents of these plasmids shows the largest of the *B. salanitronis* plasmids, pBACSA01, encodes apparatus necessary for conjugative and type IV DNA transfer, while all three *B. salanitronis* plasmids carry genes for mobilization, indicating that all three *B. salanitronis* plasmids could be moved between bacterial hosts. Mobile elements have different host ranges but are well described for their ability to move among very disparate microbial hosts. *Staphylococcus aureus'* recent adaptation to the chicken GIT (during the STAT era) included the acquisition of novel mobile genetic elements (Lowder *et al.*, 2009). Although antibiotic resistance genes were largely absent from plasmids that pre-date the antibiotic era (Hughes and Datta, 1983), they are commonplace among today's GIT microbes (Schultsz and Geerlings, 2012). These genes are functional across multiple host species as exemplified by Zhou *et al.* (2012), who demonstrated that antibiotic-resistance genes identified from a metagenomic clone library could be introduced into a strain of *C. jejuni* and be functionally active. Therefore mobile elements may provide a vehicle permitting the transfer of genes that facilitate antibiotic resistance and virulence to initially antibiotic-sensitive, avirulent microbes, potentially including those of clinical importance (Gyles, 2008). Consistently, surveys have demonstrated erythromycin resistance is prevalent in *C. jejuni* strains (Ladely *et al.*, 2007) and multiple antibiotic resistances and virulence traits are prevalent in strains of *E. coli* (Johnson *et al.* 2008; Glenn *et al.*, 2012) isolated from STAT chickens. A quick catalogue of the antimicrobial resistance genes present in the genomes of chicken GIT isolates *S.* Gallinarum 287/91 and *S.* Enteritidis P125109 shows these organisms each carry an impressive arsenal of genes potentially enabling increased resistance to multiple antimicrobials (Table 1). Even the avirulent chicken isolate *B. salanitronis* DSM1870 carries an assortment of antibiotic resistance genes, perhaps chronicling a strong anthropogenically imposed selection pressure of this environment.

Collectively, these studies clearly illustrate a mechanism through which the history of STAT use could have contributed to the proliferation of microbial antibiotic resistances. This is further supported by a number of indirect pieces of evidence. Yet, conclusive evidence has for a long time been lacking in this argument. Recent work in pigs has provided much clearer evidence that zoonotic transfer between humans and STAT-treated animals can lead to microbes obtaining antibiotic resistances (Price *et al.*, 2012). Although the area remains contentious, legislative steps are already in place to illuminate STAT use in USA farming practices. A mechanistic understanding of STAT-mediated growth promotion could lead to the identification of new and more broadly accepted agents to facilitate this process. Optimizing GIT microbial communities through microbiome-directed nutrition or probiotics could provide new opportunities to limit pathogen colonization. Further research into alternatives to antibiotics that are target-specific, such as lytic phages could attenuate the transmission of zoonotic pathogens without negatively impacting production.

Conclusions

Molecular interrogation of the chicken GIT microbiome has given us a new level of understanding of its composition and spatial structure. Surveys of phylogenetic markers such as the 16S rRNA gene have allowed researchers to overcome the roadblocks associated with culture-based surveys. In tandem with modern sequencing technologies, these surveys have also allowed us to describe more than the most abundant few organisms. Gene-directed metagenomic surveys have described the functional content of the microbiome illustrating its contribution to host nutrition. These surveys, along with microbial genomic analyses, have also provided clearer evidence of the abundance of antimicrobial and pathogenicity traits circulating in the chicken GIT. The prevalence of mobile elements further raises concerns about the role of poultry in exacerbating the virulence and resistance of zoonotic human pathogens. Transcriptional analyses have shown the disparity between *in vitro* and *in vivo* experimentation, and the deployment of metatranscriptomics is needed to place this information in an *in situ* and physiologically relevant context. The field of GIT microbiology is moving toward an integrated systems level understanding. Early meta-omic analyses have enabled those with an interest in the chicken GIT to also venture down this road. An improved and integrated understanding of the role of nutrition and the microbiome in mitigating disease and promoting animal growth and productivity may lead next-generation farming practices to a level that exceeds that currently achieved through STAT provision.

Table 1. Antimicrobial resistance genes in the genomes of some chicken GIT isolates

Antimicrobial resistance genes	*S. enterica* serovar Gallinarum 287/91[1]	*S. enterica* serovar Enteritidis P125109[1]	*B. salanitronis* DSM1870[1]
Anti-metabolites			
Sulfonamides[S]	SG2259		
Cell wall synthesis inhibitors			
Beta-lactams[C]	SG1598		
Ampicillin	SG2643		
Glycopeptides[C]			
Bleomycin			Bacsa_0310, Bacsa_2931
Vancomycin		SEN2177	
Fosfomycins[C]	SG1968		
Membrane function inhibitors			
Polymyxins[C]	SG2328, SG2333	SEN2286	
Polypeptides[C]			
Bacitracin	SG3101	SEN3047	Bacsa_2304
Nucleic acid synthesis inhibitors			
Quinolones[C]	SG1598		
Fluoroquinolones[C]			
Norfloxacin	SG1956		
Enoxacin	SG1956		
Novobiocin	SG2158, SG2159, SG2160		
Protein synthesis inhibitors			
Aminonucleosides[C]			
Puromycin	SG2209		
Aminoglycosides[C]	SG1860, SG4014	SEN1788	
Gentamicin	SG4014		
Streptomycin	SG3991		
Spectinomycin	SG4016		
MLSK[2,S]			
Clindamycin			Bacsa_3730, Bacsa_3731
Erythromycin	SG4004		
Phenocols[S]			
Chloramphenicol	SG1598, SG3587		
Polyketides[S]			
Tetracycline	SG1598, SG2643		Bacsa_2536, Bacsa_2537, Bacsa_2540
Other			
Fosmidomycin	SG0504	SEN0474	
Topical antiseptics			
Acriflavine	SG0485, SG0486, SG3281	SEN0456, SEN0457, SEN3224, SEN3225	Bacsa_1319, Bacsa_1327, Bacsa_1328, Bacsa_1649, Bacsa_1670, Bacsa_1893, Bacsa_1951
Other			
Bicyclomycin	SG2259	SEN2214	Bacsa_2484
Camphor	SG0634		Bacsa_0754
Cetylpyridinium	SG4181		
Heavy metal	SG0985, SG3360, SG3434, SG4166		Bacsa_1320, Bacsa_1422, Bacsa_1876
Melittin		SEN2283	
Methyl viologen	SG1553	SEN1481	
Multi-resistance	SG1598, SG2194, SG2209, SG2604, SG2722, SG3634	SEN1531, SEN1532, SEN1533, SEN1566, SEN1567, SEN2659, SEN2660, SEN3615	Bacsa_0099, Bacsa_0761, Bacsa_1555, Bacsa_1650, Bacsa_1950, Bacsa_2048, Bacsa_2660, Bacsa_2031, Bacsa_3272, Bacsa_3722
Nitroimidazole	SG0696	SEN0663	Bacsa_3112
Tellurite	SG1514, SG1515	SEN1447, SEN1448	Bacsa_0256
Tiamulin	SG3999		

[1]Locus tags of genes in this genome potentially increasing resistance to antimicrobial; [2]Macrolides, Lincosamides, Streptogramins, Ketolides; [C]Bactericidal; [S]Bacteriostatic.

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