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Mini Review

Antibiotics Related Alteration in Gut Microbiota and Compromised Immunity in Broiler Chickens

Irfan Zia Qureshi* and Ayesha Razzaq

Department of Animal Sciences, Pakistan

***Corresponding author:** Irfan Zia Qureshi, Laboratory of Animal and Human Physiology, Department of Animal Sciences, Faculty of Biological Sciences, Islamabad, 45320 Pakistan

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Abstract

Considering the widespread use of antibiotics in the poultry industry, impact of antibiotics on gut microbiota and immune system of the bird is of major concern. A better understanding of how antibiotics use change gut microbiota in relation to poultry production is thus definitively required. Technical progress in the field of next-generation sequencing, mass spectrometry and bioinformatics facilitates the study of highly complex biological samples such as taxonomic and functional characterization of microbial communities that virtually colonize all present ecological niches. Compared to the structural information obtained by metagenomic analyses, metaproteomic approaches provide, in addition, functional data about the investigated microbiota. This review focuses on the effect of antibiotic administration on the status gut microbiota of broiler chicken post hatch. The advent of-omics technologies for analyzing total DNAs, RNAs, proteins, metabolites, and bacteria will provide a better glimpse of microbiota activities at localized intestinal sites. Combining classical with modern techniques is a powerful strategy to fill gaps in our knowledge of animal intestinal microbiomes. In addition, further interdisciplinary approaches will be essential as we proceed to a new era of antibiotic use in food animals.

Keywords: Antibiotics; Microbiome; Next-generation sequencing; Broiler chicken

Abbreviations: T-RFLP: Terminal Restriction Fragment Length Polymorphism; PCR: Polymerase Chain Reaction; OTUs: Operational Taxonomic Units; DGGE: Denaturing Gradient Gel Electrophoresis; FCR: Feed Conversion Ratio; BMD: Bacitracin Methylene Disalicylate

Introduction

Animal health benefits from a stable intestinal microenvironment, for which proper development and functioning of the intestinal microbiota and immune system are essential [1]. The gastrointestinal microbiota has one of the highest cell densities of any ecosystem and in poultry, it ranges from 108 to 1010 bacteria per gram of gut content [2]. Colonization of the gut microbiota in young animals occurs simultaneously with the development of the gut tissues [3,4]. Immediately after hatch, the microbiota, mainly comprising bacteria, co-evolve with their host to form dynamic and unique microbial communities along the intestine and form a synergistic relationship with their poultry host [5,6]. Due to differences in morphology, functionality, metabolic interactions, and microenvironment, regional heterogeneity in community composition is observed along the different gastrointestinal (GI) segments [7,8]. However, the composition of the bacterial communities is believed to be influenced mainly by age, diet, and gut location. However, host genetics, rearing environment, stress, immune status, and interactions within bacterial communities are also important influencing factors [9-13].

It is believed that microorganisms can also directly interact with the lining of the gastrointestinal tract [14]. This may alter the physiology of the tract and immunological status of the bird [15]. During the first week post-hatch, the chicken small intestine grows rapidly and gets readily affected by post hatch feeding. Post hatch feeding thus alters development of gut microflora within the first two weeks post hatch [16]. It is generally known that the diversity of gut microbiota plays essential role in host metabolism, nutrient digestion, growth performance and overall health of the host [17]. Microbial flora is also believed to protect against colonization of the intestines by pathogens and to stimulate the immune response [18].

The widespread use of antibiotics during the livestock production process [19], not only changes the gut micro-ecosystem but also leads to the emergence of pathogenic bacteria resistant to antimicrobials. This issue has seriously threatened animal husbandry and human health [20]. In relation to productivity, it becomes important to have an understanding about their impact on gut microbiota. Phylogenetic analyses of antibiotic resistance genes and analyses of bacterial genome content suggest widespread and rapid distribution of antibiotic resistance genes among host-associated (especially intestinal) bacteria after ampicillin, penicillin, ciprofloxacin, and carbadox treatments [21]. Antibiotic disruption

of commensal microbiomes may remove the protective barrier, leaving the host susceptible to colonization by pathogens from food and other sources [22]. Gene expression experiments have revealed that most antibiotics, including the rifampicin, erythromycin, and tetracycline, at sub inhibitory concentrations, modulate bacterial gene expression [23].

Recent molecular approaches allow studying the biodiversity, composition and growth dynamics of gut microflora through T-RFLP (Terminal restriction fragment length polymorphism) and quantitative PCR (real-time polymerase chain reaction) [24]. In addition, advances in ribosomal DNA-based molecular techniques have made it possible to obtain new information by identifying different bacterial populations in intestinal contents and mucosal samples [25]. These techniques may prove helpful for monitoring the effect of diets and other variables on the microbial communities of the GI tract under commercial conditions [26].

Pyrosequencing approach was used by Choi et al. [27] to investigate the bacterial communities in separate GIT regions of broiler chicken. In this study, the DNA samples extracted from seven different regions along the GIT were subjected to bacterial-community analysis by pyrosequencing of the V1-V3 region of 16S rRNA gene. The results revealed that major bacterial phyla in the chicken-gut microbiota included Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Acidobacteria, but Firmicutes were mostly dominant (67.3±16.1% of the total sequence reads identified). This study indicated further that microbial communities of main segments in the chicken GIT were distinctive according to both individuals and the different segments of GIT, but their stability was maintained along the GIT [27].

Another recent study [28], elaborated and confirmed the understanding of the chicken intestinal microbial composition. Microbiota inhabiting five different intestinal locations (duodenum, jejunum, ileum, cecum, and colon) of 42-day-old broiler chickens was detected through 16S rRNA gene sequence analysis. As a result, 1,502,554 sequences were clustered into 796 operational taxonomic units (OTUs) at the 97% sequence similarity value and identified into 15 phyla and 288 genera. Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Cyanobacteria were the major microbial groups and Firmicutes was the dominant phylum in duodenum, jejunum, ileum and colon accounting for > 60% of sequences, while Bacteroidetes was the dominant phylum in cecum (>50% of sequences), but had less occurrence in the other gut sections [28].

Gut microbiota development studies in chickens by sequencing 16S rRNA gene and T-RFLP revealed that complexity of gut microbiota increased gradually with age [29], and that the most dramatic development occurred during the first week of life [30]. Likewise, it was shown that complexity of chicken microbiota gradually developed from day 1 to day 19 and among younger birds, there was greater individual variation in microbiota composition as compared to older birds from the same flock [31]. It has been also established that changes in microbial colonization in early life (the first 2wk post hatch) influence the functioning of the adult gut in broiler chickens [1].

Short-term (24h) early life exposure of day 1 old broiler chickens to orally administered amoxicillin has been shown to affect microbial colonization, mucosal gene expression and intestinal immune development over a period of 2 weeks. These data support that early life colonization of the gut by microbiota is an important driver of immune development and/or immune programming [32]. The effect of dietary supplementation with a combination of avilamycin and salinomycin on the bacterial community in the ileum of broiler chickens at different ages (7,14,21, and 35 days) was studied using PCR with denaturing gradient gel electrophoresis (DGGE) analysis and bacteriological culture. Enumeration of bacteria by culture demonstrated an effect of antibiotics on the total Lactobacillus population and *C. perfringens* population in ileal contents of broilers. Both PCR-DGGE and bacterial counts revealed that the composition of the microflora was age dependent and influenced by dietary antibiotic supplementation [33].

Caecal microbiota has been characterized by sequencing of bacterial 16S rRNA gene amplicons. In 15-day-old Cobb broiler, in which zinc bacitracin and avilamycin were administered over a 10-day period. However, avilamycin produced no effect on growth performance and exhibited little significant disturbance in the microbiota structure. On the other, zinc bacitracin reduced the feed conversion ratio (FCR) in treated birds, changed the composition and increased the diversity of caecal microbiota by reducing the dominant species. Avilamycin only produced minor reductions in the abundance of two microbial taxa, whereas zinc bacitracin produced relatively large shifts in a number of taxa, primarily Lactobacillus species [34].

Administration of antibiotics later in life (day 15 to day 20 post hatch) in broiler chicken have also studied the microbiota and immune parameters. Chickens received enrofloxacin through drinking water from 15d post hatch. Before and at 6, 16, and 27d after start of the administration of antibiotics, the composition of the microbiota in the jejunum was determined using a 16S ribosomal RNA gene-targeted DNA microarray, the CHICKChip. The results of this study indicated that the antibiotics affect the composition of microbiota and immune parameters in older birds only temporarily [35]. Neumann & Suen [36] compared the effects of two in-feed antibiotics i.e. virginiamycin and bacitracin methylene disalicylate (BMD), which are typically used by commercial poultry producers in the United States, on the chicken gastrointestinal microbiota. Quantitative PCR and 454 pyrosequencing of the V6-V8 region of the 16S rRNA gene were employed to examine the bacterial microbiota and Clostridium perfringens, respectively, in the jejunum and caecum of market-age broiler chickens. Their results suggested that virginiamycin had a more pronounced impact on broiler gastrointestinal tract bacterial communities, relative to BMD, and it manifested primarily through significant enrichments in the genus Faecalibacterium in the caecum and a distinct population of Lactobacillus [36].

The effects of avilamycin, zinc bacitracin, and flavophospholipol on broiler gut microbial community colonization and bird performance in the first 17 days post hatch were identified by T-RFLP. Significant differences were found in the gut microbiota associated with gut sections, dietary treatment, and age. Results also showed that interbird variabilities in ileal bacterial communities were reduced (3 to 7 days posthatch) in chicks fed with feed containing antimicrobial agents, while avilamycin and flavophospholipol had the most consistent effects on gut microbial communities. Identification of key bacterial species influenced by antimicrobial-supplemented feed immediately post hatch may therefore assist in the formulation of diets that facilitate beneficial gut microbial colonization and, hence, the development of alternatives to current antimicrobial agents in feed for sustainable poultry production [37].

In our laboratory, we administered enrofloxacin, a potent veterinary antibiotic, to broiler chicken (1 and 15 days) at various doses for a period of 7 days. In addition to microflora, the antibiotic radically affected the delicate balance of micro- and macro minerals in the gut tissues and as well other organs [38]. Considering our results, future studies as to how altered mineral composition as a result of antibiotics use affects the microbiota and its performance should be conducted. Dietary interventions at young age, such as the usage of (pre) starter feeds, prebiotics, probiotics and antibiotics, are considered to affect the crosstalk between microbiota and host mucosal cells in the intestinal tract, which may result in a change of immune development [39,40]. Ban on use of antibiotics as growth promotors in the European Union (EC Regulation, No. 1831/2003), and their potential restriction in other countries, has resulted in a reduction of animal performance and in an increase of enteric pathologies [41]. As a consequence, non antibiotic feed additives are a subject of increasing interest [42]. Prebiotics are defined as non-digestible dietary compounds that modulate the composition and/or activity of gut microbiota, conferring a beneficial effect on the host [43]. Successful productive results have been reported in poultry by using dry whey powder [44] and inulin [45].

In this scenario, it is clear that the antimicrobials disrupt the gut microbiota population. Thus, under these conditions, prebiotics may be selectively used as substrate for the growth of beneficial bacteria. Further work should focus not only the caecal bacterial profile in response to additive supplementation at some time points of the broiler lifespan, but should also include the characterization of different sections of the upper gastrointestinal tract. It would also be relevant to investigate, through metagenomics, the functional properties of the microbiota when broilers are fed with different non-antibiotic additives. Subsequently, Next-generation high-throughput sequencing (HT-NGS) technologies have been developed to overcome the limitations of first-generation technology that included higher speed, less labor, and low costs. Various platforms that have been developed include: sequencing-by-synthesis 454 Life Sciences, Illumina (Solexa) sequencing, SOLID sequencing (among others), and the Ion Torrent semiconductor sequencing technologies that use different detection principles. As technology advances, progress that has been made toward third generation sequencing technologies is being reported. This includes Nanopore Sequencing and real-time monitoring of PCR activity through fluorescent resonant energy transfer. The advantages of these technologies include scalability, simplicity, with increasing DNA polymerase performance and yields, being less error prone, and economically cost-effective with the eventual goal of obtaining real-time results. These technologies can be directly applied to improve poultry production and enhance food safety. For instance, sequence-based (determination of the gut microbial community, genes for metabolic pathways, or presence of plasmids) and function-based (screening for function such as antibiotic resistance, or vitamin production) metagenomic analysis can be highly informative. Gut microbialflora/communities of poultry can be readily sequenced to determine the changes that affect health and disease along with efficacy of methods to control pathogenic growth [46].

Conclusion

In conclusion, previous research has provided ample evidence that the administration of various antibiotics, at different doses regimes, has a clear impact on microbiota composition and performance measures in broiler chicken of different age groups. A detailed knowledge of broiler gut microbiota colonization and succession immediately post hatch and later in age, and how these bacteria influence bird productivities, are essential to understand impact of different generations of antibiotics for variable time periods. Moreover, studies on the effect of altered microbiota post antibiotics usage on the immune system of birds are scant. It is therefore pertinent that future studies should take this aspect into consideration. Further research is also needed to understand the effects resulting from contrasting concentrations of antibiotics, particularly by looking into possible interactions between the concentration of antibiotics, the age of the birds, the dietary treatment period and the pre-existing microbiota. Such kind of knowledge will assist in beneficial manipulation of gut microbiota via diet or additives or isolating organisms that might be used as probiotics. This would also help to develop high-productivity strategies in the poultry sector that could rely more on the use of probiotics and less on in-feed antibiotics.

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