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# An ecological perspective on U.S. industrial poultry production: the role of anthropogenic ecosystems on the emergence of drug-resistant bacteria from agricultural environments

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The industrialization of food animal production, specifically the widespread use of antimicrobials, not only increased pressure on microbial populations, but also changed the ecosystems in which antimicrobials and bacteria interact. In this review, we argue that industrial food animal production (IFAP) is appropriately defined as an anthropogenic ecosystem. This paper uses an ecosystem perspective to frame an examination of these changes in the context of U.S. broiler chicken production. This perspective emphasizes multiple modes by which IFAP has altered microbiomes and also suggests a means of generating hypotheses for understanding and predicting the ecological impacts of IFAP in terms of the resistome and the flow of resistance within and between microbiomes.

## Addresses

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## Introduction

John Muir once mused, “When we try to pick out anything by itself, we find it hitched to everything else in the Universe” [1]. These words highlight the importance of interconnectedness to ecology, which has been defined as “the science of the complex interactions that binds organisms and their environments together” [2]. As such, an ecological framework encompasses all nutrient and energy flows, biotic and abiotic factors, and life histories of all organisms. Today, the magnitude of human impacts on natural systems makes consideration of anthropogenic changes to ecosystems important. Agriculture is one such activity, because it inherently creates anthropogenic ecosystems [3], which we define as collections of organ-

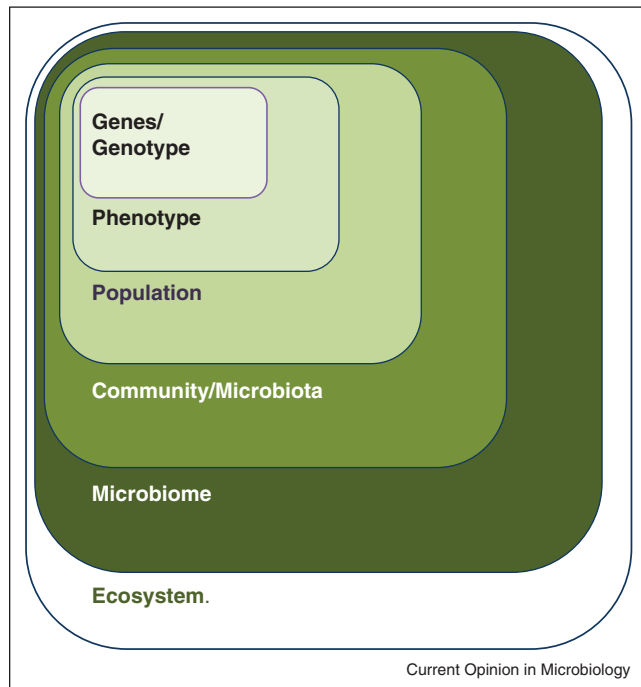
isms and physical structures under human control and manipulation. The early ecosystem changes caused by hunter-gatherers and practitioners of agronomy and animal husbandry were limited in geographic scale and impact [4]. The adoption of an industrialized model in modern food-animal production [5] has been successful in increasing global food production but also has intensified its impact through the expansion of anthropogenic ecosystems [3,4]. In contrast to earlier practices, modern industrial food-animal production (IFAP) employs methods that include constraining animal interactions, impacting natural selection through breeding and genetic modifications, and altering environments through changes to landscape structure, engineering of animal housing, and disposal of animal waste [3,5,6]. Notably, chemical interventions – primarily antimicrobials – are used regularly in the industrialized production process [7,8].

In this review, we apply an ecological framework to discuss the impact of widespread antimicrobial use in U.S. broiler production, an example that is particularly relevant because the U.S. transformation of food animal production began with the broiler chicken industry and because many of these production practices have been adopted globally [5,9]. We argue that IFAP creates anthropogenic ecosystems wherein antimicrobial use inevitably selects for antimicrobial resistance in bacterial populations within animal hosts and the environment. This alters microbial communities (**microbiomes**) and the collection of available mobile resistance genes (**resistome**) dispersed into the surrounding ecosystems [10,11,12].

## An ecological framework for microbial studies

Studying the complex interactions within microbial ecosystems using a scaling-up, systematic approach is at once powerful and elegant. This framework allows researchers to evaluate different ecosystem components at different levels of resolution and generate hypotheses that can contribute to elucidating and understanding the overall microbial ecosystem. Figure 1 illustrates conceptually how changes at the genetic level (by spontaneous mutation or horizontal gene transfer) result in bacterial phenotypes and populations that confer selective advantage in the context of antimicrobial selective pressure, and, equally important, how selection at any level of this ecological framework has the potential to impact subsequent higher levels. Changes in microbial ecology on the industrial poultry farm may

Figure 1



Conceptual representation of telescoping levels at which microbes interact with each other and with hosts within an ecosystem. At the microscopic molecular level, genetic changes are expressed as differences in phenotype for an individual. Individuals with different phenotypes within a bacterial species make up a population, and populations of different bacteria comprise a community, or microbiota. For example, an individual chicken will have a cecal community of bacteria, and on the population level for a chicken flock, this is termed the chicken cecal microbiome. Microbiomes interact with each other and also with all the biotic and abiotic factors that comprise an ecosystem.

impact other microbiomes through the porosity of the production system (see below) by which antimicrobials, antimicrobial-resistant bacteria, and resistance determinants disperse off the farm.

### Industrial poultry production systems

Food animal production has always involved anthropogenic modifications of natural ecosystems to generate protein for human consumption [3,4,6]. In early animal husbandry, because the size and impact of the anthropogenic systems was relatively small, the natural structures and dynamics of ecosystems were largely preserved [4]. A crucial component to the maintenance of natural cycling was the limit on production rates from the constraints of space, feed, and bird genetic potential for growth [4]. Nutrient availability and on-farm microbial diversity further impacted the dynamics of host and environmental microbial communities [4].

Modern IFAP is detached from the conditions of natural ecosystems in energy inputs and carrying capacity [3]. Its

practices that increase productivity also contribute to deterioration of ecological balance through nutrient and chemical pollution, and consumption of water and other local natural resources [6]. Many operations now rely on resource inputs from global sources [13]. Poultry production has become highly concentrated geographically, particularly in the southeastern U.S. [5], and each production operation now produces more animals and animal wastes than any time in the past. The number of large farms (*i.e.* with over 125,000 broilers) has increased over 10-fold between 1982 and 2002 [14]. Broiler farmers today raise a median of ~160,000 birds with a nominal spatial footprint for animal housing [14] and produce an estimated 5250 tons (~4.7 million kg) of manure per median-sized facility annually [14]. Such nutrient loads far exceed what ecological processes at the field level can absorb [15,16]. Thus, nutrient cycling has become disengaged from the local ecosystem [17]. IFAP practices such as monocropping [17] and selective breeding practices [5] have contributed to reduction in overall biodiversity, and particularly in the genetic diversity of the commercial broiler stock [18]. Today's U.S. broiler stock is dominated by a single breed, the Cornish Cross, which was bred to reach market weight in 7 weeks [18]. In other species, hosts with naturally short or artificially shortened life spans have been associated with increases in toxin gene acquisition or speed of reproduction in coevolved pathogens [19<sup>\*</sup>]. A recent study showed that modern broiler chickens, when compared to a heritage breed, had differences in both intestinal structure (length of jejunum and height of villi) and in composition of gastrointestinal bacterial communities [20]. These anatomic and microbial changes in modern broiler chickens are potentially related to genetic differences in intestinal development [20]. However, the full impact of reduced host diversity and industrialized growth conditions on host-microbe interactions and the broiler stock's susceptibility to emerging infectious diseases remains to be investigated.

### Microbial populations and communities

The anthropogenic ecosystems created by IFAP practices have extensive direct impacts on the microbial ecology of poultry hosts and the environment, and probably indirect impacts on consumers through poultry products. In nature, microbes are known to both produce and to develop resistance to antimicrobials, leading to a set of complex interactions now hypothesized to contribute to the signaling and regulation in natural microbial ecosystems [21]. However, the extent and magnitude of antimicrobial use in IFAP far exceeds, in volume and impact, that of naturally occurring antimicrobials [10<sup>\*</sup>,22]. The U.S. FDA reports that 13 million kg of antimicrobials were sold or distributed for use in food-producing animals during 2009 [23]. The particular practice of adding non-therapeutic concentrations of broad-spectrum antimicrobials to feed [24] generates an ideal setting for selecting

bacterial cells or populations that have gained resistance through mutations or horizontal gene transfer [7,25].

The process of natural selection by antimicrobial use in IFAP is reflected in observations of antimicrobial-resistant isolates from livestock, including poultry, shortly after the introduction of routine use of antimicrobials as feed additives in the 1950s and 60s [7,26,27]. At the community level, as resistant populations replace susceptible populations, genes that confer resistance to antimicrobials or other traits important to bacterial survival in one population/species are available to other populations/species through horizontal gene transfer, enabling the creation of novel multidrug-resistant bacteria and/or multidrug resistance-conferring mobile elements [12,28]. Researchers may compare the genetic relatedness of bacterial strains and resistance genes or mobile elements using PCR-based molecular techniques, as illustrated in source-tracking studies that compare enteric bacterial species, such as multidrug-resistant *Campylobacter spp.* and *Salmonella spp.*, associated with both poultry product contamination and human disease [29,30\*\*]. The impact of antimicrobial use in IFAP on the availability of this collective group of resistance genes, or the resistome, is illustrated in Figure 2 through the ‘cloud computing’ concept. It depicts that the physical events of natural selection occurring in numerous molecular settings will

increase the abundance and diversity of this conceptual, open-access repository of resistant genes [10\*]. Recent articles summarize and analyze current research on the resistome, including and beyond specific applications to agricultural ecosystems [10\*,11\*,12,21,48]. We focus our review on research along the pathways that connect the commercial poultry intestinal microbiome with microbiomes in surrounding environments.

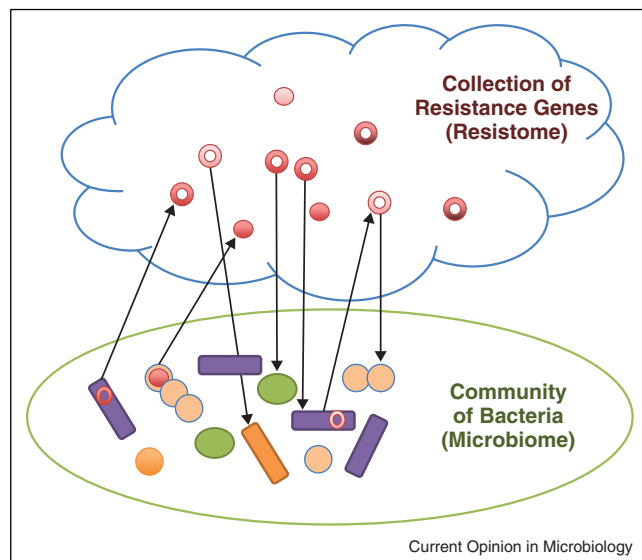
### Ecosystem connections

The impact of natural selection within the intestine of individual poultry hosts can be further scaled up to the inter-microbiome and inter-ecosystem level (Figure 3). Agricultural ecosystems interact with other ecosystems directly at local and regional levels, and more broadly through global movement of dusts and water [65] as well as economic trade in feeds, animals, and animal waste [13]. Although the industrial poultry house often is assumed to be biocontained and biosecure, multiple pathways connect it with surrounding ecosystems [8]. These include ventilation practices required to keep crowded animals alive; movement of rodents [31], wild birds [9\*], and insects [32] in and out of confinement facilities, and transfers of wastes [33]. These conditions release viable microbes and resistance determinants into surrounding environments, water systems, and wild animal reservoirs [8,34,35\*].

### The anthropogenic IFAP broiler microbiome

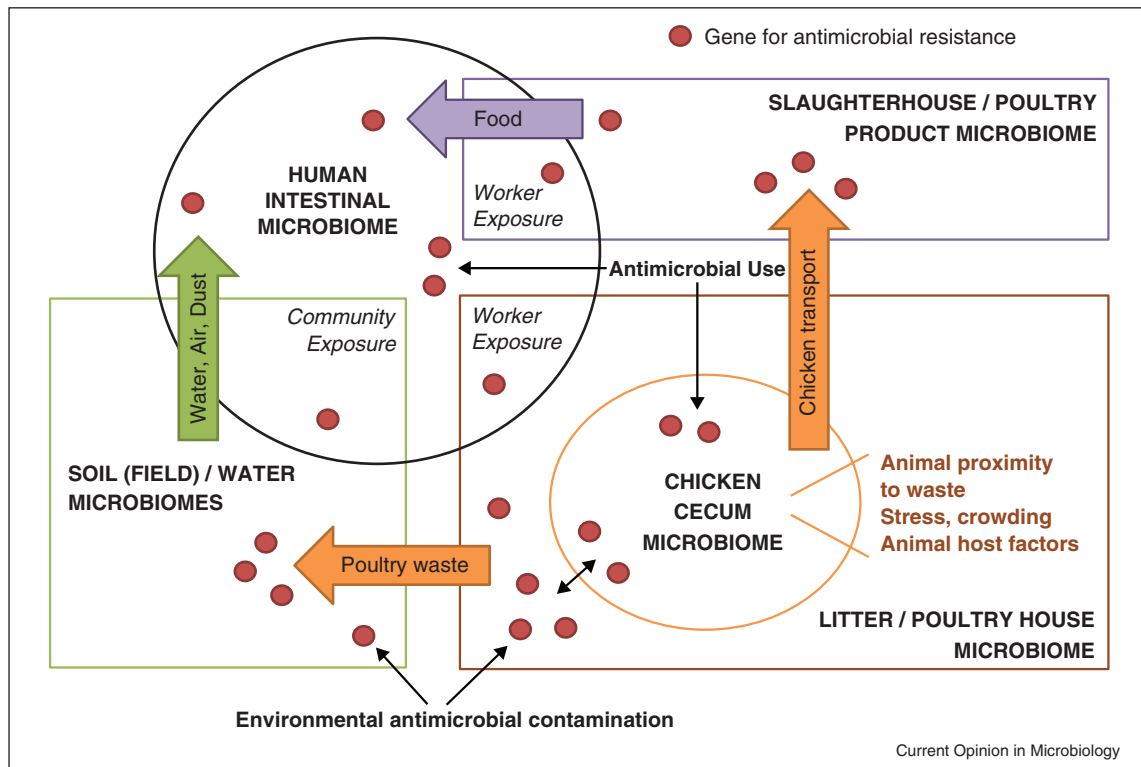
The anthropogenic broiler microbiome has relevance both in terms of host microbiome and inter-ecosystem impacts. In the broiler chicken, production conditions related to diet, environment, stress, and host factors such as breed and age contribute to the evolution of the intestinal microbiome (reviewed in [36,37]), but the impact of antimicrobial use can have immediate, profound effects. Use of antimicrobials alters the healthy enteric microbiome of the chicken [38]. Experimental depletion of normal intestinal microflora in mice with antibiotic treatment made them susceptible to *Salmonella* enteritidis; poultry treated with certain preparations of intestinal microflora were protected from *Salmonella* (reviewed in [36]). Genetic analysis of the U.S. commercial broiler cecum microbiome has demonstrated that it contained a wide array of resistance genes and genes that facilitate horizontal gene transfer [39\*\*]. Recent Canadian studies also have found widespread prevalence of virulence and resistance genes from *Enterococcus spp.*, *E. coli*, and *Clostridium perfringens* species isolated from enteric samples from conventional broilers that were fed antimicrobials [40\*,41,42]. In addition, salinomycin-treated and bacitracin-treated broilers, compared to those not fed antimicrobials, were significantly associated with increases in the presence of an aminoglycoside resistance gene, sulfonamide resistance genes, and class 1 integron genes in cecal and environmental *E. coli* isolates [42]. Of these selected genes, class 1 integron genes are known to

Figure 2



A conceptual interpretation of the resistome as parallel with (but not identical to) ‘cloud computing.’ In both cases, information is transferred to and from participants (i.e. bacteria) through networks, stimulated by selective pressures, for example, ‘need to know.’ The ‘cloud’ holds the resources of multiple users and communities. Individuals can send and receive information (in terms of genetic or other coded data) and then exchange among each other. ‘Learning’ (or selection) occurs at the level of communities, and this knowledge is transferred to the cloud on a dynamic basis.

Figure 3



Conceptual, potential role of selective pressure of antimicrobial use and other anthropogenic ecosystem alterations that impact microbiomes in the chicken cecum, poultry house environment, local soil and water environments, processing plant environment, and human intestine.

be capable of promoting movement of resistance genes among bacteria [28,42].

Two significant routes for the broiler microbiome to have inter-ecosystem impacts are via poultry waste and poultry products. Poultry house wastes or 'litter' are a complex input of cecal microbes, nutrients for microbial growth such as carbon, phosphates, and nitrogen [43], undigested feed, and feed additives including antimicrobial agents, disinfectants, and metals [13,44]. Communities of antimicrobial-resistant species have been recovered from poultry litter in the U.S. [45]. A recent Canadian study demonstrated antimicrobial chemical residues and antimicrobial-resistant *E. coli* in poultry litter related to feeding of these antimicrobials in commercial flocks and as part of a controlled trial that compared antimicrobial treated and untreated flocks [46<sup>\*</sup>]. Litter type, age, and re-use (not cleaning between flocks) may impact microbial diversity, as measured using molecular techniques, both in litter and in the intestines of young chickens raised on the litter, suggesting a bi-directional influence of microbiomes [47<sup>\*\*</sup>]. Much like the chicken cecum, poultry waste contains a significant number of resistance integrons, particularly in gram-positive bacteria [42,48]. Some patterns of resistance appear to persist in bacteria even after cessation of antimicrobial use, for

example, fluoroquinolone resistance in *Campylobacter* [49] and sulfonamide resistance in *E. coli* [46<sup>\*</sup>], and this finding may complicate interpretation of feeding trials that use a control group not fed antimicrobials.

#### The anthropogenic IFAP environmental microbiome

Much of the impact of antimicrobial use on the environmental microbiome is via poultry waste disposal. Application of litter onto open fields can impact the soil microbiome locally to regionally through run-off and air-borne drift. The U.S. has no regulatory requirements for treating animal wastes, leading to uncontrolled methods of waste storage before land disposal [33]. Simple storage methods do not affect prevalence of pathogens nor drug-resistant pathogens [50<sup>\*</sup>]. Most of the antimicrobials in feeds pass largely unchanged through the broiler gut into excreta [22]. Some drugs (including oxytetracycline and fluoroquinolone analogs) can persist in the soil environment with half-lives as long as 150–250 days with undiminished potency [22,35<sup>\*</sup>]. While the impact of agricultural practices on soil and aquatic resistomes recently has been reviewed [11<sup>\*</sup>], the ecological effects on bacterial communities from application of animal wastes to soils have not been well studied. The interaction of naturally occurring and IFAP-derived antimicrobials on the environmental microbiome is probably highly

complex and dynamic [10<sup>\*</sup>]. Singer *et al.* acknowledged this when they proposed to use landscape ecology techniques to incorporate spatial heterogeneity of risk factors in characterizing environmental processes that influence the distribution of microorganisms, antimicrobials, and resistance determinants [51].

Dispersal of antimicrobial-resistant bacteria and resistance determinants represents the inter-ecosystem effects of antimicrobial use in IFAP. Human links include vehicles, animal transport, and networks of social and commercial contact [52<sup>\*</sup>,53]. Cross-contamination of poultry during transport and at slaughter contributes to greater microbial diversity in retail chicken as compared to live birds [54,55]. *Campylobacter* isolates collected on-farm and post-slaughter [55] and at various stages of bird transport [54] were sequenced using multi-locus sequence typing (MLST), and genetic relatedness of isolates was compared using Simpson's diversity index [55] or Bayesian methods for clustering [54]. Contamination during the harvest process can impact poultry house [56] and slaughter workers [57], as well as retail chicken consumers in a global market. Indirect but compelling evidence for the impact of antimicrobial use in IFAP comes from molecular analyses of bacteria in live poultry and/or on poultry products in conjunction with analysis of human isolates [58<sup>\*</sup>,59,60]. Although this research area is too large to detail fully in this paper, numerous studies demonstrate presence of genes for antimicrobial resistance [30<sup>\*\*</sup>,40<sup>\*</sup>,45,61], strains of antimicrobial-resistant bacteria, for example, methicillin-resistant *Staphylococcus aureus* [62,63], and resistance-conferring plasmids [58<sup>\*</sup>] in humans and poultry.

## Conclusions

In 1989, Paul and Robertson suggested that ecological principles be applied to the agricultural sciences to manipulate organisms and ecosystems for commercial gain, stating “[t]he opportunity for the ecologist in modern agriculture, then, is to provide concepts and principles that can be used as tools to design resource-efficient agricultural systems.” [64]. In the same year, Jackson and Piper addressed the same issue, but “with the assumption that problems *in* agriculture are primarily derivatives of the problem of agriculture,” suggesting that ecological tools be applied to mitigate damage caused by modern agricultural practices [3]. These authors agreed that the complexity of environmental influences from agriculture deserved joint focus of the disciplines. Since that time, the integrative perspective of ecology has been less influential in guiding research and public policy related to the industrialized process of food animal production. In this brief review, we have adopted an ecological framework to examine impacts from the imbalances in poultry ecosystems—particularly from selective pressure on microbial communities from antimicrobial use. This perspective assists us in identifying important research gaps at all

levels of microbial communities and ecosystems, suggesting opportunities to expand existing knowledge through molecular tracking of resistance determinants along multiple pathways that connect animals in intensive production environments to workers, farming communities and food consumers. A key conclusion is the need to examine changes at the microbiome level. Further research is needed to evaluate microbiome impacts from reduced host diversity and industrialized production conditions; to trace antimicrobial impacts on microbial ecology throughout the broiler production cycle; and to characterize influences on an already complex soil microbial ecology from deposition of poultry litter that contains antimicrobials and antimicrobial-resistant organisms. As broiler production methods continue to evolve, an ecological perspective can inform strategies to maintain natural ecological processes. Improvements through reduced antimicrobial use and implementation of new waste management strategies are crucial to an ecologically sustainable future for the broiler industry.

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