Food-animal production and the spread of antibiotic resistance: the role of ecology

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Antibiotic-resistant pathogens increasingly threaten human health. Widespread application of antibiotics to animal populations raised for food, including chickens, cattle, and pigs, selects for resistance and contributes to the evolution of those pathogens. Despite a half century of research establishing the mechanisms and pathways by which antibiotic-resistant bacteria spread from food animals to people, scientists lack the appropriate data and models to estimate the public health burden of antibiotic-resistant human infections attributable to antibiotic use in food-animal production. Genomic technologies are enabling researchers to track the bidirectional transmissions of specific bacterial strains from livestock to people – and from people to livestock – that can amplify resistance traits. Concepts in ecology, which were developed to understand resource subsidies, metapopulations, and biological invasions, provide insight into the epidemiology of antibiotic resistance from genomic data. By applying ecological principles to highly resolved phylogenetic data, researchers can improve strategies for controlling antibiotic resistance.

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Although they play very different roles in human lives and in the landscape, hospitals and farms are ecologically connected by the dynamics of microorganisms (Figure 1). Food-animal production includes breeding, raising, and processing animals and their products – such as meat, milk, and eggs – for human consumption; for definitions of specialist terminology, see Panel 1. Globally, more than 57 million kilograms of antibiotics are used annually on food animals (Van Boeckel *et al.* 2015), creating a massive source of antibiotic-resistant bacteria that are transmitted to people via occupational exposure on farms, along the food production chain, through food itself, and through environmental pathways like contaminated soil and water. Accordingly, food-animal production is an important source of new antibiotic-resistant bacterial pathogens and a control point for decreasing resistance in the human population. Despite the public health threat of accelerated antibiotic resistance, scientists are unable to quantify how antibiotic use in food-animal production shapes the expansion of resistant microbial strains in food and medical systems. Advances in genomic technologies enable new insights into the ecological dynamics and evolutionary relationships of zoonotic pathogens. Combined with ecological theory, this knowledge has the potential to transform how scientists manage the evolution and spread of antibiotic resistance.

Through genetic sequencing, it is possible to identify and track the movements of specific strains between food animals and people in much the same way that ecologists track the movements of macroscopic organisms. Ecological principles – when applied to microbial genomics data – may help researchers understand the spread of antibiotic resistance. For example, the concept of resource subsidies describes fluxes of dead or living organic biomass between donor and recipient ecosystems, and how these fluxes are regulated by ecological variables. Metapopulation theory, describing the periodic colonization and extinction of discrete habitat patches in the environment, may help elucidate the dynamics of linked bacterial reservoirs along the food production chain. In addition, concepts used to understand biological invasions such as propagule pressure, invasibility of native ecosystems, and disturbance regimes can be successfully applied to the problem of antibiotic resistance. Integrating these ecological approaches with genomic data on antibiotic resistance could both strengthen the management of drug resistance and improve ecological theory.

In a nutshell:

- The food-animal production industry is a source of antibiotic-resistant bacteria that can infect people
- Despite the public health threat of antibiotic resistance, there is little quantitative understanding of how antibiotic use in animals affects the expansion of resistant bacteria in food and medical systems
- Genomic data are elucidating, in unprecedented detail, the pathways by which antibiotic-resistant bacteria move between food animals and humans
- Applying basic ecological principles can help organize this new knowledge to better monitor, understand, and manage the spread of antibiotic resistance

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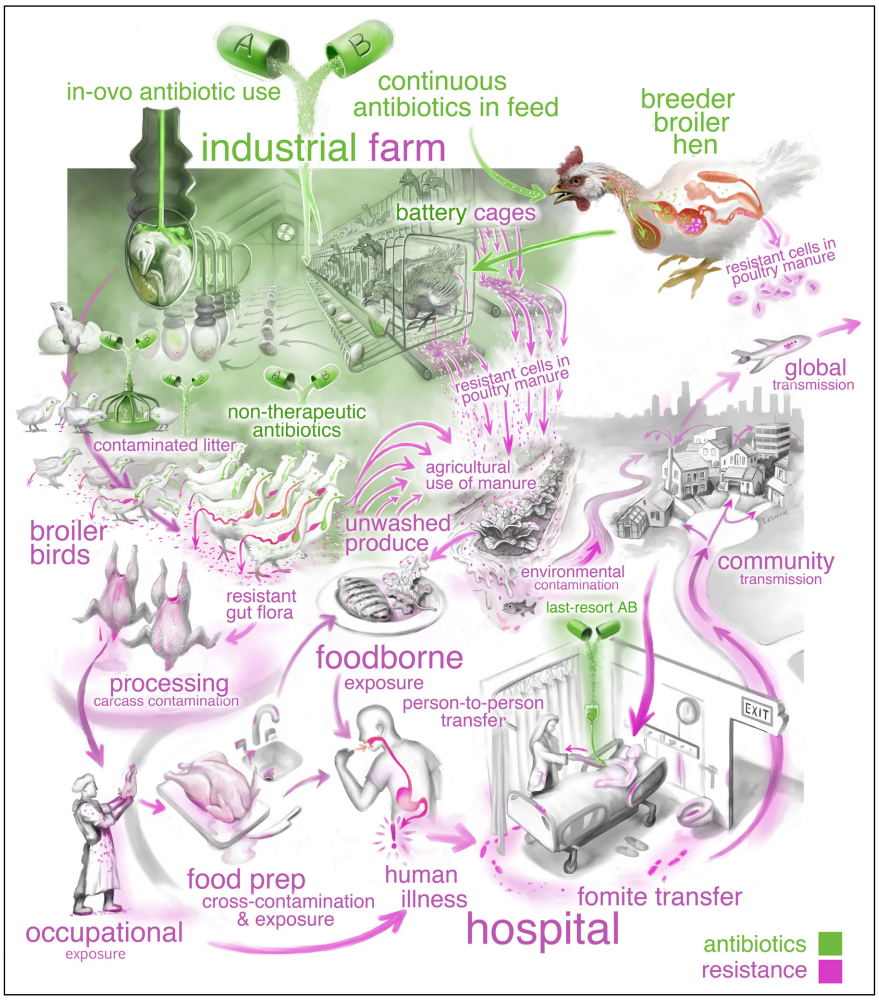

Figure 1. *Multiple pathways link antibiotic use (green) and antibiotic resistance (pink) in the food-animal and human health sectors. Most antibiotics currently sold in the US support the production of food animals. In poultry, antibiotics are administered in-ovo (in the egg) and via water and feed to promote growth and to treat, control, and prevent disease. Routine exposure to antibiotics selects for resistant bacteria that can persist on meat and in animal waste. Antibiotic-resistant bacteria can also be transmitted vertically through maternal generations of breeding stocks (not shown). Humans are exposed to and may be infected by resistant bacteria in food-animal production facilities (not shown) and in meat processing plants, as well as by preparing and consuming contaminated meat. Animal waste that contains resistant bacteria may enter nearby aquatic and terrestrial ecosystems, and may also be applied to crops, which are subsequently consumed by people. Such foodborne bacteria can cause antibiotic-resistant infections in humans. Certain types of resistant pathogens may persist in the human population and cause additional infections in the community or move back into the food-animal production domain and become re-amplified for resistance against additional drug classes (not shown).*

The problem of antibiotic use

Since the dawn of the antibiotic era, biologists have recognized both the promise and peril of antibiotic use. Although these drugs save thousands of lives each year, their use drives the evolution of resistant pathogens. Food-animal production accounts for an estimated 80% of annual antibiotic purchases in the US (FDA 2012, 2014). Antibiotics are administered to treat infections in humans but have multiple uses in food-animal production, including infection treatment, routine disease prevention, and growth promotion. Any use of antibiotics can select for resistance. But when administered to promote growth and prevent disease – in regular, low-level doses among densely stocked poultry, swine, cattle, and other food animals – antibiotics exert continuous selective pressure for resistant strains of bacteria (Silbergeld *et al.* 2008; Marshall and Levy 2011). As a result, novel strains of resistant zoonotics evolve (Silbergeld *et al.* 2008), some of which colonize and infect humans (Dutil *et al.* 2010). Antibiotic-resistance genes that evolved within the highly selective environment of food animals are also indirectly transmitted to human pathogens through horizontal gene transfer (Schwarz *et al.* 2006), and these pathways can be mediated by microbial taxa native to soils and surface waters (eg Forsberg *et al.* 2012) as well as by human commensals (Smith *et al.* 2002).

The phenomenon of animal-to-human transmission was exemplified on a large scale in 2005–2006, when the Canadian government asked Québec broiler chicken hatcheries to stop injecting chicken eggs with ceftiofur, a third-generation cephalosporin (Dutil *et al.* 2010). Upon withdrawing the drug, cephalosporin-resistant *Salmonella* and *Escherichia coli* on retail chicken products in the region plummeted and continued to decline until the drug was reintroduced in 2007. Coincident with this decline, cephalosporin-resistant *Salmonella* infections in humans also fell sharply. No other changes were implemented, showing that a single prophylactic antibiotic injection to eggs prior to hatching could exert selective pressure potent enough to carry through hatching, grow-out, slaughter, processing, and distribution, to ultimately affect the human population that consumes the final product (Dutil *et al.* 2010).

In human health, the problem of antibiotic resistance stems from excessive use of existing drugs, exacerbated by inadequate development of new drugs (Laxminarayan *et al.* 2013). Antibiotic use and bacterial transmission among patients, medical staff, and visitors drive the

Panel 1. Definitions of key terms

COP (colonizing opportunistic pathogen): A microbe that can harmlessly colonize and persist in the human body, but may cause infection when conditions are right.
CC (clonal complex): A group of phylogenetically related clones, or sequence types (STs), within a microbial species that is defined according to a specific set of housekeeping genetic loci.
ExPEC (extra-intestinal pathogenic *Escherichia coli*): A group of *E coli* strains associated with infections outside the gut, such as urinary tract infections, sepsis, pneumonia, peritonitis, osteomyelitis, and meningitis.
flux: A measure of the magnitude of biomass (or any material) moving from one ecosystem compartment to another per unit time.
horizontal gene transfer: The movement of DNA between microbes other than direct inheritance from a parent cell.
invasibility: A measure of how vulnerable an ecosystem is to colonization and disruption by non-native species.
metapopulation: A group of conspecific populations that are separated from one another in space but interact via the movement of individuals among them.
microbiome: The assemblage of microorganisms in a particular environment.
mobile genetic element: A piece of DNA that can move to a different location within or among different genomes.
MLST (multi-locus sequence typing): A technique for assigning categories of phylogenetic similarity to isolates of microbial species using variation across a set of housekeeping genetic loci.
MRSA (methicillin-resistant *Staphylococcus aureus*): A type of antibiotic-resistant bacteria that commonly occurs in people and animals.
plasmid: A type of mobile genetic element that is a common mechanism for horizontal gene transfer of antibiotic resistance genes among microbes.
propagule pressure: A measure of the number of individuals of a non-native species transported into a native ecosystem. Propagule pressure is a function of the number of transport events and the number of individuals per transport event.
resource subsidy: The movement of biomass from one ecosystem to another that may alter the ecology of organisms in the recipient ecosystem.
zoonotic: An infectious disease that can spread from animals to humans.

emergence of resistant pathogens in medical settings, eroding the effectiveness of those drugs (Levy and Marshall 2004). Antibiotic use in outpatient populations may also contribute to resistance in the community, and in many countries, antibiotics can be purchased over the counter, leading to inappropriate use and resistance, such as the failure to complete a full course of antibiotics (Laxminarayan *et al.* 2013). In the US, up to 50% of antibiotics used in the medical sector are unnecessary or are prescribed with improper dosage or duration (CDC 2013), driving additional – and avoidable – selection for resistance.

Connections between animal-borne bacteria and humans

An abundance of evidence details the spread of antibiotic resistance between food animals and people (Figure 1; Swann *et al.* 1969; Marshall and Levy 2011). Antibiotic use in food animals is correlated with antibiotic resistance among bacteria affecting human populations (Angulo *et al.* 2004; Silbergeld *et al.* 2008), which is driven by occupational exposure in the food-animal industry (eg Price *et al.* 2007) as well as by foodborne exposure among consumers, as described above (eg Dutil *et al.* 2010; Figure 2). Other routes of transmission between people and animals include food-animal waste applied as crop fertilizer (Chee-Sanford *et al.* 2009), movement of pathogens from farm workers to animals (Lowder *et al.* 2009), airborne transmission (Gibbs *et al.* 2006), and surface water and groundwater contamination (Sapkota *et al.* 2007). As the food-animal industry becomes more globalized and long-distance human travel more frequent, the pathways linking antibiotic resistance on farms to resistance in hospitals and in communities become crucial control points for minimizing the spread of resistant human pathogens.


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Figure 2. *Retail meat commonly harbors antibiotic-resistant strains of bacteria (Xia* et al. *2011). Consumers may be colonized by these strains through improper handling of meat in the kitchen.*

The recent discovery of a novel mobile colistin-resistance gene is a clear example of local-scale antibiotic use rapidly leading to global-scale patterns of resistance (Liu *et al.* 2016). Colistin is an older antibiotic that had

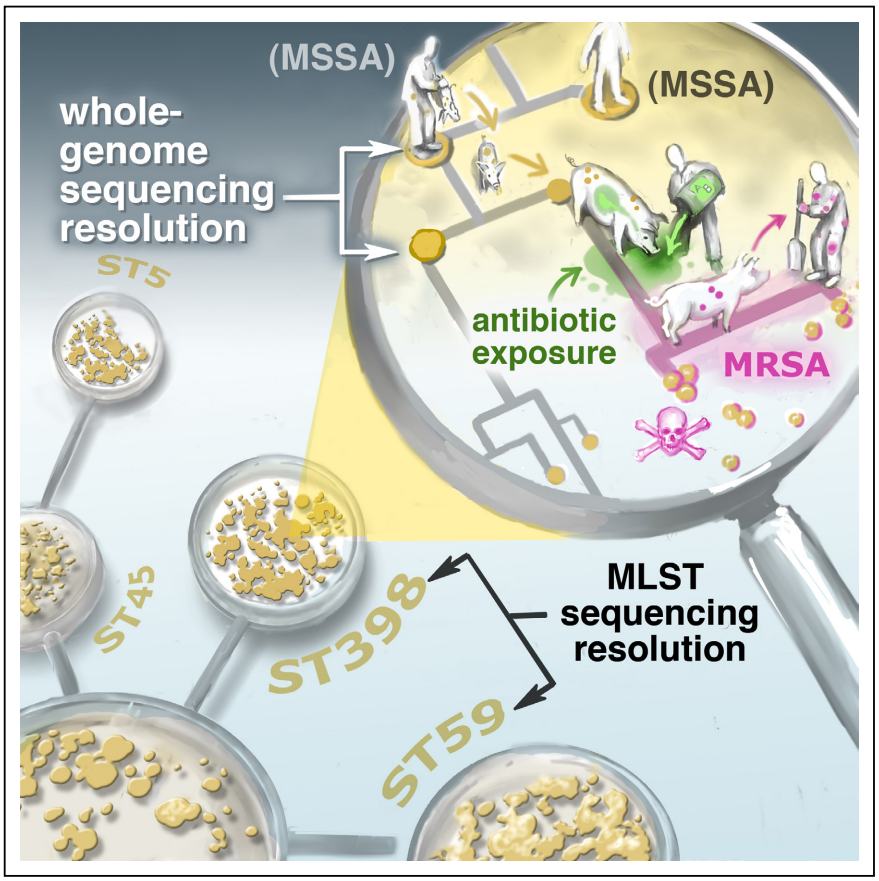

Figure 3. *Applying next-generation sequencing technologies to zoonotic pathogens can illuminate their evolutionary histories and reveal the ecological pathways leading to the emergence of antibiotic-resistant strains in humans. For example, phylogenetic relationships of lineages of methicillin-resistant* Staphylococcus aureus *(MRSA) within the multi-locus sequence typing (MLST) defined clade 398 (ST398) were only apparent after fully sequencing ST398 isolates collected from livestock and people. Whole-genome sequencing indicated that "livestock-associated" MRSA originated in humans as methicillin-susceptible* S aureus *(MSSA) before jumping to livestock populations, where it acquired both tetracycline and methicillin resistance (Price* et al. *2012). Subsequent spillover of this newly resistant lineage back into people continues to cause a growing number of MRSA infections in areas with high human–livestock contact (Graveland* et al. *2011).*

fallen out of favor for human therapy due to its toxicity (Pogue *et al.* 2011). However, in recent years colistin has become the preferred antibiotic – in many cases the only effective one – for treating most antibiotic-resistant Enterobacteriaceae infections (Perez and Van Duin 2013). Unbeknownst to many, colistin had also become a commonly used additive in Chinese food-animal production, and it was among Chinese livestock that a new mobile colistin-resistance gene was first identified (Liu *et al.* 2016). This gene, named *mcr-1*, was carried on a plasmid in *E coli* isolated from livestock, meat products, and humans. The plasmid – a common type of mobile genetic element that serves as a mechanism for horizontal gene transfer among bacteria – was readily transferred between strains of *E coli* and could be picked up by other members of the Enterobacteriaceae in the lab. In a marked display of the power of genome sequencing, public databases, and the speed of modern molecular techniques, a slew of short reports followed the initial paper documenting the occurrence of *mcr-1* in countries around the world (Web-Panel 1). The gene was found in multiple plasmids and several different strains of *E coli*, providing empirical evidence for the prevalence of *mcr-1* and the threat that it poses to people living thousands of kilometers from its original source.

Genomics can trace bacterial transmission with high precision

Microbial genes encoding antibiotic resistance have moved between the food-animal and human health sectors, resulting in illnesses that could not be treated by antibiotics (Price *et al.* 2007, 2012; Dutil *et al.* 2010). Next-generation sequencing technologies reveal the epidemiology and dynamics of antibiotic-resistant organisms with higher resolution than previous techniques (Figure 3; Köser *et al.* 2014), including differentiating, with high phylogenetic precision, which strains are the sources of resistant infections in animals and in people. For example, when "livestock-associated" methicillin-resistant *Staphylococcus aureus* (MRSA) clonal complex 398 (CC398) was discovered in Dutch swine farmers in 2004 (Armand-Lefevre *et al.* 2005), it alarmed public health officials who feared it might spread to other humans and other livestock populations. Indeed, MRSA CC398 was later found in livestock throughout Europe and the US (Smith and Pearson 2011). In recent years, human infections caused by CC398 have accounted for up to 40% of MRSA infections in parts of Europe, where they are strongly correlated with a high frequency of human–livestock contact (Graveland *et al.* 2011). Initially, researchers concluded that MRSA CC398 originated in swine populations and subsequently spilled over to infect humans, but this conclusion was based on incomplete genomic information (Van Belkum *et al.* 2008). Whole-genome sequence typing of MRSA CC398 later revealed multiple distinct lineages, including livestock-independent lineages that are transmitted among people, and a recently derived livestock-associated

lineage that primarily colonizes pigs, cattle, and poultry (Price *et al.* 2012), which also spills over to people via occupational exposure (Figure 3; Graveland *et al.* 2011; Price *et al.* 2012).

Genomic sequencing was similarly useful in identifying the source of parallel antibiotic-resistant *Salmonella* outbreaks in cattle and people in Scotland, which began in the mid-1980s. It was assumed that domestic cattle were the source of the human infections (Threlfall *et al.* 1994), but a retrospective whole-genome-based phylogenetic analysis showed that the *Salmonella* populations and resistance elements from people and domestic cattle were distinct (Mather *et al.* 2013). While occasionally misinterpreted as suggesting that food animals *in general* were not the source of the human infections, the study simply showed that *domestic* (sympatric) livestock were not the source of the human infections and that foodborne *Salmonella* strains imported to Scotland were probably the culprits.

As illustrated by these examples, the declining costs and increased speed of whole-genome sequencing enables comprehensive sampling with fine spatial and temporal resolution to track near-real-time strain dynamics over targeted animal and human populations. Ecologists commonly use physical tags, collars, or unique markings to track the movements of individual animals. Similarly, genomic "tags" can track the transmission dynamics of antibiotic-resistant pathogens (Bowers *et al.* 2016). Using whole-genome sequencing to generate such tags, Davis *et al.* (2015) showed that food-animal-associated bacterial strains entered the local human population through retail meat (Figure 2), revealing intermingled populations of antibiotic-resistant *Klebsiella pneumoniae* from concurrently sampled retail meat and clinical urine and blood samples (Davis *et al.* 2015). This spillover of genetically indistinguishable drug-resistant infectious bacteria from food into people provides convincing evidence supporting earlier correlational data linking changes in antibiotic use to congruent changes in antibiotic-resistant human infections at regional and national scales (Angulo *et al.* 2004; Dutil *et al.* 2010).

Genomic technologies can also track the movements of specific resistance genes and reconstruct bacterial transmission events among networks of individual hosts, for example, to trace outbreaks of multidrug-resistant bacteria in clinical settings. The reconstructed transmission pathways of a deadly outbreak of multidrug-resistant *K pneumoniae* implicated environmental reservoirs within the hospital and "silent" carriers (people without symptoms who transmitted the bacterium to others) in perpetuating the outbreak (Snitkin *et al.* 2012). In such outbreaks, it is now possible to detect extensive and rapid inter-bacterial transmission of mobile plasmids encoding antibiotic resistance (Conlan *et al.* 2014). Similar analyses could quantify the role of mobile genetic elements in the transmission of foodborne pathogens, and their persistence at different stages of the food-production chain – at the farm or in the slaughterhouse, for instance. In this way, plasmid sequencing has the potential to advance antibiotic resistance epidemiology by quantifying the rates at which specific antibiotic resistance-carrying genes are exchanged among different bacteria in different reservoirs such as food animals, retail meat, and humans.

Ecological models can improve understanding of resistance epidemiology

Genomic techniques can generate powerful data describing the linkages between animal-borne bacteria and humans, which, combined with sound theory, can identify management options for curbing the spread of antibiotic resistance. The acquisition and spread of antibiotic resistance traits are biotic processes with ecological and environmental dimensions. For example, antibiotic-resistant bacteria move through coupled human–natural ecosystems among a variety of reservoirs and pathways (Silbergeld *et al.* 2008; Marshall and Levy 2011), and food-animal-associated resistant bacteria persist in the environment (Gibbs *et al.* 2006), where they can affect the ecology and evolution of native microbial assemblages (Baquero *et al.* 2013), including lateral exchange of resistance genes (Forsberg *et al.* 2012). Furthermore, different strains are transmitted at different rates among livestock (Skånseng *et al.* 2007), within households (Davis *et al.* 2012), and across the globe (Banerjee and Johnson 2014). Despite this implicit focus on the ecology of the antibiotic-resistant bacteria, there have been very few attempts to explicitly apply ecological principles to understand the evolution and spread of antibiotic-resistant bacteria. Below, we explore the potential for three distinct ecological concepts or theories to yield quantitative insight into the problem of antibiotic resistance when applied to high-resolution phylogenomics data from antibiotic-resistant bacterial strains in various environmental reservoirs.

Resource subsidies

The bidirectional transmission of resistant bacteria between food animals and people can be described in the same terms that describe fluxes of resource subsidies between ecosystems (Polis *et al.* 1997). In this case, the ecosystems are the food-animal production sector and the human population. Factors such as boundary permeability, flux size, and the community composition of the recipient ecosystem govern the exchange and impact of novel bacteria (Cadenasso *et al.* 2004; Price *et al.* 2007). Unlike passive subsidies of dead organic matter (eg leaves falling into a stream), subsidies of living organisms can have direct functional consequences for recipient ecosystems (Flecker *et al.* 2010); therefore, cross-ecosystem fluxes of resistant bacteria have the added potential to alter recipient bacterial communities. Furthermore, food-animal and human ecosystems impose

Panel 2. The asymmetric exchange of antibiotic-resistant bacteria between food animals and humans

The flow of antibiotic-resistant bacteria from food animals to humans far exceeds the flow of bacteria in the opposite direction. On the basis of retail poultry sales, we estimate that there were at least 15 billion potential foodborne exposures to food-animal bacteria in 2012 (assuming an average exposure portion of 2 pounds [0.9 kg] for the 34.9 billion pounds [15.8 billion kilograms] of poultry sold in the US in 2012; USDA 2014a). At least 89% of retail poultry harbors bacterial strains resistant to at least one type of antibiotic (We estimated the proportion of retail poultry with antibiotic-resistant bacteria by multiplying the reported prevalence of bacteria in meat by the proportion of bacteria isolated from meat that was resistant to at least one antibiotic. We performed the above calculations separately for five common foodborne bacterial taxa [*Campylobacter*, *Enterococcus*, *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*] and we made the conservative assumption that bacterial taxa were maximally correlated in their occurrence patterns on meat. We combined data from chicken and turkey meat to represent poultry. Data were taken from Waters *et al.* [2011] and NARMS [2011]). If only 1% of the potential exposure events involving antibiotic-resistant bacteria resulted in successful transmission to people, then there are roughly 130 million instances of resistant strains moving from poultry to people via food annually in the US. This estimate includes transmissions due to consuming undercooked meat and improper handling of raw meat prior to cooking, but does not consider occupational exposures (Figure 4), food-animal waste on harvested food crops, and contaminated water, all of which could increase further the flux of resistant microorganisms from food animals to people.

The flux of bacteria from humans to food animals is much smaller. Although the food-animal industry is large, it is highly concentrated and has implemented procedures to minimize contact between workers and food animals (Graham *et al.* 2008). An estimated 94,000 US workers come into contact with birds in poultry houses annually (assuming an average of two full-time workers at each poultry operation; USDA 2014b). If each of those workers transmits novel human bacteria to their flocks 10 times each year, then the flux of bacteria from humans to food animals is approximately 1% of the flux of resistant strains in the opposite direction.

Figure 4. *Inside a broiler production house, a veterinarian wears protective clothing. Most poultry workers interact with their flocks several times a day, often without protective equipment (Graham* et al. *2008).*

different selective pressures on incoming and resident bacteria, owing to different patterns of antibiotic use on the farm and in human medicine. This selection gradient could accelerate evolutionary change within bacterial strains that move repeatedly between animals and people, leading to novel combinations of antibiotic resistance and virulence.

The resource subsidy perspective may provide insight into the ecological controls on the spread of resistance. How permeable is the boundary between food animals and people, and how does this vary among bacterial taxa? What are the within-ecosystem pool sizes and cross-ecosystem fluxes for specific bacterial strains that move between the food-animal and human health ecosystems? To what extent does the microbiome of the recipient ecosystem determine the successful introduction of resistant strains or genes? Answering these questions through metagenomic monitoring of the food-animal production chain and prospective clinical and non-clinical studies would diversify and strengthen existing efforts to control the spread of antibiotic resistance. For instance, target bacterial strains and gut microbiomes could be sampled and sequenced over time in a prospective cohort composed of multiple networks of host individuals, each centered on a single, clinical index case. Combining such data with concurrent genomic sampling of retail meat (eg Davis *et al.* 2015) could quantify strain-level transmission rates between human and food-animal sectors. Furthermore, potential factors regulating transmission, such as microbiome composition, could be explored quantitatively.

Published data permit coarse estimates of transmissions between food animals and people, and reveal those transmissions to be bidirectional yet extremely lopsided (Panel 2), with implications for the spread of antibiotic-resistant human pathogens. Even if human-adapted pathogens only rarely cross species boundaries into food animals, the routine use of antibiotics in food-animal production increases the likelihood that those pathogens will acquire resistance. The magnitude of the flux of resistant bacteria from food animals back to people (Panel 2) virtually ensures that one of those pathogens – with newly acquired antibiotic resistance – will re-enter the human population. Strong genomic evidence indicates that this mechanism is responsible for the rise in infections of livestock-associated MRSA CC398 in Europe. Whole-genome sequencing of MRSA CC398 isolates suggests that this lineage originated in humans before jumping to livestock, where it gained resistance to multiple antibiotics (Price *et al.* 2012). The massive flux of bacteria from food animals to people enables MRSA to

re-enter the human population, and is likely driving the observed increase in MRSA infections (Figure 3).

Recent genomic data have further elucidated the pathways of bidirectional MRSA CC398 transmissions. From 1999 to 2011, MRSA CC398 infections in Denmark increased rapidly among people who had no known livestock exposure, either directly or through household contact (Larsen *et al.* 2015). Health officials feared that MRSA CC398 might be establishing in the human population. Researchers found a parallel increasing trend among the livestock-exposed population and extensive spatial clustering among livestock-exposed and unexposed cases, with very few infections in urban-dwelling Danes (Larsen *et al.* 2015). It looked as though the unexposed cases were indirect spillover from the livestock reservoir, and that the strain was not becoming independently established in the community. These data also suggested that foodborne transmission was minimal, despite substantial MRSA CC398 contamination among Danish pork products (Larsen *et al.* 2015).

However, a more comprehensive analysis later showed that a small number of urban-dwelling Danes with no livestock exposure had been infected by a novel MRSA CC398 strain (Larsen *et al.* 2016). This strain was special in that it carried mobile phage-encoded genes from the innate immune evasion complex (IEC), which protects *S aureus* from the human immune system (Cuny *et al.* 2015). The presence of these genes indicated that the strain was uniquely adapted to infect humans. The strain was also unusual in that it had never been isolated from Danish livestock but had been isolated from poultry flocks in other EU countries and from poultry products imported into Denmark (Larsen *et al.* 2016). All together, these data implicate poultry meat as the source of these urban infections and underscore the need to understand how multiple concurrent cross-ecosystem fluxes of bacteria interact to produce observed patterns of antibiotic resistance in human populations.

Quantifying cross-ecosystem "bacterial subsidies" can improve current understanding of how antibiotic resistance spreads. Combining this ecological concept with sequencing techniques to track individual bacterial strains can yield strain-specific data on the pools and fluxes of resistant and susceptible microorganisms and the factors that control those fluxes (as defined by Nakano and Murakami 2001). Such data can be valuable in parameterizing and building quantitative models of the consequences of antibiotic-resistant bacteria moving between food animals and people (eg Huxel and McCann 1998).

Metapopulations

The ability to genomically identify individual bacterial clones allows for the application of metapopulation theory to better understand the persistence of antibiotic-resistant strains in coupled human and food-animal ecosystems. Modeling bacterial exchange in this way could elucidate the cryptic ecology of a widespread class of infectious bacteria, the colonizing opportunistic pathogens (COPs; Price *et al.* in press). COPs are unique because they can be transmitted harmlessly to a healthy individual and can persist asymptomatically for an indefinite period prior to causing infection. *S aureus* and extra-intestinal pathogenic *Escherichia coli* (ExPEC) are two common bacterial COPs (Russo and Johnson 2003; Nadimpalli *et al.* 2015) associated with antibiotic resistance and food-animal production. Between six and eight million Americans suffer from *E coli* urinary tract infections each year (Russo and Johnson 2003), and contaminated meat and poultry likely serve as a source of ExPEC strains that cause some of those infections (Nordstrom *et al.* 2013). Antibiotic-resistant urinary tract infections are increasing (Russo and Johnson 2003; Price *et al.* 2013), and antibiotic-resistant ExPEC strains are commonly found in retail poultry and pork (Xia *et al.* 2011). Genomic sequence data from ExPEC strains

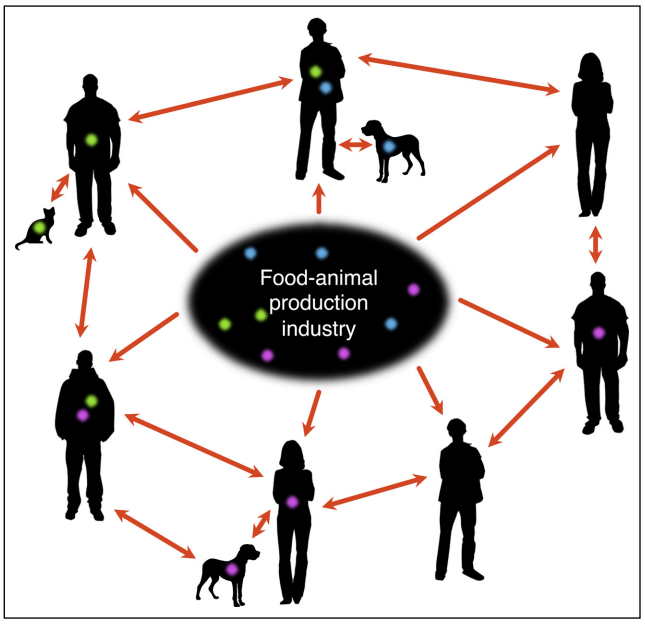

Figure 5. *Many antibiotic-resistant zoonotic bacteria such as extra-intestinal pathogenic* Escherichia coli *(ExPEC) and methicillin-resistant* Staphylococcus aureus *(MRSA) are colonizing opportunistic pathogens (COPs), which are capable of asymptomatic transmission and persistence within hosts for indefinite time periods. COPs may later cause infections or they may be shed from a host completely. Such host-level colonization and extinction dynamics can be modeled as a metapopulation. High-resolution genomic tools enable testing whether different strains (colored circles) vary in their colonization and extinction rates. The food-animal production sector may act as a source – or mainland – reservoir of resistant strains, thereby promoting persistence of the metapopulation in the community. Arrows indicate potential transmission pathways. Individuals without colored circles are uncolonized.*

isolated from concurrently sampled human urinary tract infections and retail meat (eg Davis *et al.* 2015) could test the hypothesis that food contributes to rising antibiotic-resistant ExPEC infections in people.

COPs like ExPEC and MRSA can colonize multiple reservoirs (eg people, pets, food animals, food), persisting in each for a variable period of time (Nordstrom *et al.* 2013; Nadimpalli *et al.* 2015), and so may be characterized as metapopulations (Figure 5). In metapopulations, if the rate of colonization drops below the rate of local extinction, the metapopulation goes extinct (Hanski 1999). For multidrug-resistant COPs, metapopulation models may suggest novel ways of managing their spread. Hygiene practices or probiotic treatments that lower the colonization rate below the threshold required for persistence could halt the spread of antibiotic-resistant strains. Similarly, treatments that promote colonized hosts shedding the target strain (ie where it no longer persists in the host microbiome) could help limit the spread of resistance. On the other hand, if an antibiotic-resistant COP strain is continually colonizing the host population via the large reservoir of the food supply, then source–sink dynamics may dominate, and the strain will persist until the source reservoir is controlled. Genomic techniques provide a means to quantify the colonization and extinction dynamics of individual antibiotic-resistant bacterial strains (WebTable 1), enabling tests of metapopulation theory and management strategies for disrupting strain persistence.

Species invasions

Invasion biology provides another set of ecological principles to understand the spread of antibiotic-resistant pathogens among food animals and people. Colonization is a crucial step in the persistence and spread of antibiotic resistance and is akin to an invasive species establishing and thriving in a previously unoccupied ecosystem. Invasion biologists have traditionally considered three factors when predicting species invasions: (1) propagule pressure and biotic characteristics of the invading species (Daehler 2003; Von Holle and Simberloff 2005); (2) biotic features of the native community, like biodiversity (Naeem *et al.* 2000); and (3) abiotic characteristics of the native ecosystem, such as resource availability and disturbance (Hobbs and Huenneke 1992; Davis and Pelsor 2001). For antibiotic-resistant bacteria originating on the farm and transmitted to people via food, propagule pressure can be extremely high (Panel 2). Furthermore, standard courses of antibiotics disturb the human microbiome (Costello *et al.* 2012), creating niche opportunities (Shea and Chesson 2002) well matched to the drug-resistant traits of the invading bacteria. Compared to animal and plant invaders of macroecosystems that rely on unique phenotypes (Kolar and Lodge 2002; Strauss *et al.* 2006) and plasticity (Daehler 2003), antibiotic-resistant bacteria also adapt rapidly to local conditions via horizontal gene exchange (Schwarz *et al.* 2006).

Invasions are difficult to predict, and their outcomes vary among different environments (Lodge 1993; Mack *et al.* 2000). Using metagenomics to characterize a host's native microbiome provides opportunities to test and extend models of ecological invasions. For example, carefully designed observational studies of people whose microbiomes are exposed to novel antibiotic-resistant bacteria through occupational exposure within the food-animal industry or through travel (eg Arcilla *et al.* 2014) could provide powerful new insights into the relative roles of propagule pressure, native taxonomic composition, and disturbance in conferring resistance to invasion.

Genomic data from antibiotic-resistant bacteria could also shed light on the role of evolution in driving population dynamics of bacterial strains in ecological time (Prosser *et al.* 2007). Horizontal gene transfers of mobile genetic elements encoding for drug resistance can initiate the rapid spread of antibiotic resistance (Bowers *et al.* 2015) and may ultimately drive niche displacement of individual strains within host microbiomes. How important is this mechanism to the process of community assembly, which is typically explained by competitive interactions (Tilman 1982) or stochastic processes (Hubbell 2001) rather than evolutionary forces?

Conclusions

The ecological concepts of resource subsidies, metapopulations, and invasive species offer models for understanding the epidemiology of antibiotic resistance, and advances in genomic techniques can provide phylogenetic data for testing these models (WebTable 1). Uniting ecological theory with observations of microbial ecosystems in the genomic age is only beginning (Prosser *et al.* 2007). For example, primary succession, recovery from disturbance, and invasive species are useful ecological paradigms for understanding community assembly of the human microbiome in newborns, after antibiotic treatment, and during pathogen invasion (Costello *et al.* 2012). Similarly, testing ecological theory at the intersection of human health, food-animal production, and antibiotic resistance is likely to advance both ecology and epidemiology.

Managing the spread of antibiotic resistance requires understanding the ecological interactions of resistant bacteria throughout the microbiosphere. The widespread use of antibiotics in food-animal production accelerates the evolution of resistant strains, an important source of new antibiotic-resistant bacterial pathogens in people. Genomic technologies are illuminating the evolutionary histories of some emergent resistant pathogens and their patterns of occurrence and transmission between food animals and humans, together revealing a dynamic and integrated ecosystem of antibi-

otic resistance (Figure 1). Applying existing ecological principles to these data can not only yield new insights into the epidemiology of antibiotic resistance and the human burden it causes but also improve how scientists monitor and manage its spread.

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