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ANTIBIOTICS IN AGROECOSYSTEMS: STATE OF THE SCIENCE

Not All Antibiotic Use Practices in Food-Animal Agriculture Afford the Same Risk

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Abstract

The World Health Organization has identified quinolones, third- and fourth-generation cephalosporins, and macrolides as the most important antibiotics in human medicine. In the context of agricultural use of antibiotics, the principle zoonotic agents of concern are *Salmonella enterica*, *Campylobacter* spp., *Escherichia coli*, and *Enterococcus* spp. Antibiotic exposure provides a selective advantage to resistant strains of these bacteria relative to their susceptible conspecifics. This is a dose-dependent process, and consequently antibiotic use practices that involve higher doses will exert greater and longer-lasting selective pressure in favor of resistant bacterial populations and will therefore increase the probability of transmission to people and other animals. Oral administration has a greater impact on enteric flora with the exception of fluoroquinolone treatments, which appear to affect the enteric flora equally if administered orally or parenterally. The use of quinolones in agriculture deserves heightened scrutiny because of the ease with which these broad-spectrum antibiotics favor spontaneously resistant bacteria in exposed populations. When present at sufficient concentrations, excreted antibiotics have the potential to selectively favor resistant bacteria in the environment and increase the probability of transmission to people and animals. The bioavailability of antibiotics varies greatly: some antibiotics remain active in soils (florfenicol, β-lactams), whereas others may be rapidly sorbed and thus not bioavailable (tetracycline, macrolides, quinolones). When considering the risks of different antibiotic use practices in agriculture, it would be prudent to focus attention on practices that involve high doses, oral delivery, and residues of antibiotics that remain active in soils.

Core Ideas

- The use of antibiotics in agriculture is thought to contribute to antibiotic resistance worldwide.
- Risk assessment should focus on the largest potential contributors to antibiotic resistance.
- Antibiotic dose and administration practices are key variables.
- Excreted antibiotics may play an important role, but not all antibiotics remain active in soil.
- The use of quinolones in agriculture deserves special scrutiny.

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The rapid emergence and expansion of antimicrobial resistance is considered by many people and organizations to be a major global health challenge of the 21st Century. The challenge of antibiotic resistance can be summarized as overlapping processes of emergence, amplification, persistence, and dissemination. Many antibiotic-producing organisms exist in nature, and thus it should be no surprise that resistance traits also exist without anthropogenic influence (Davies and Davies, 2010; Bhullar et al., 2012; Nesme et al., 2014; Lok, 2015). Resistance traits are typically recognized only after they have become sufficiently prevalent to be clinically important (i.e., emergence). Once resistant organisms have emerged they are far more likely to be transmitted to new hosts when their numbers are selectively favored (i.e., amplification). The degree that resistance traits persist in a population in the absence of antibiotic selection pressure is primarily a function of the fitness cost that is imposed on the host bacterium for carriage of the resistance trait. Traits that exert little to no fitness cost are likely to persist for extended periods of time, particularly when they are linked to other selectively advantageous traits (Khachatryan et al., 2006; Eberhart et al., 2014).

With respect to the use of antibiotics in agriculture, the World Health Organization (WHO) has called for "internationally recognized principles for risk assessment....related to antimicrobial resistance owing to non-human use of antimicrobials" (WHO, 2011). This WHO guidance document presents a very useful exercise for identifying the most critical antibiotics, of which quinolones, third- and fourth-generation cephalosporins, and macrolides met the criteria that were reviewed or developed in this report. Quinolones, third-generation cephalosporins (ceftiofur), and macrolides are used in food-animal agriculture (Table 1). Although fourth-generation cephalosporins are not used in this manner, the use of earlier-generation cephalosporins may selectively favor fourth-generation cephalosporin resistance. Thus, although emergence to fourth-generation cephalosporins will not occur due to direct use of these antibiotics in food animals, it is possible that use of other cephalosporins will select for this trait once it enters food-animal populations. In the United States, sales of medically important antibiotics for use in food

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Abbreviations: OR, odds ratio.

Table 1. Antibiotics approved for use in veterinary medicine (FDA, 2015). Dosages and applications are generalized descriptions intended to inform the reader of the range of uses. This information should not be used as a basis for application decisions. In the near future it is likely that antibiotics will no longer be labeled for growth promotion in the United States (FDA, 2013).

Antibiotic	Dose	Purpose	Withdrawal time	Sources for guidelines
Aminocoumarins				
Novobiocin	150–400 mg per quarter, cattle	mastitis	none	Federal Register (2014)
Aminoglycosides				
Gentamicin	2–5 mg kg^{-1}, cattle; 2.5–4 mg kg^{-1}, pig; 100 mg per quarter, cattle	therapeutic	2–3 d infusion (milk); 20–30 d oral (beef), 3 d (pork); 100–200 d parenteral (pork)	Boothe (2015a); MacNeil (2015)
Neomicin	10–22 mg kg^{-1}, cattle and pig; 10–30 mg kg^{-1}, chicken; 150–300 mg per quarter, cattle	therapeutic	2–3 d infusion (milk); 20–30 d oral (beef), 3 d (pork); 100–200 d parenteral (beef), 40 d (pork)	Livingston (2015); Boothe (2015a)
Spectinomycin	15–30 mg kg^{-1}, cattle; 11 mg kg^{-1}, pig; 100 mg kg^{-1}, chicken	enteric and respiratory diseases	2–3 d infusion (milk); 20–30 d oral (beef), 3 d (pork); 100–200 d parenteral (beef), 40 d (pork)	Committee for Veterinary Medical Products (2000); Boothe (2015a)
Amphenicols				
Florfenicol	20 mg kg^{-1}, cattle; 15 mg kg^{-1}, pig	therapeutic	28 d (milk and meat); 18 d (pork)	Schering Plough Animal Health (1996); MSD Animal Health (2015)
Cephalosporins				
Ceftiofur	1.1–2.2 mg kg^{-1}, cattle, sheep & goat; 3–5 mg kg^{-1}, pig; 0.08–0.5 mg kg^{-1} poultry, day-old	therapeutic	4 d (meat); none (sheep and goat)	Jaglan et al. (1989)
Cephapirin	200–300 mg $quarter^{-1}$, cattle	therapeutic	4 d (meat); 3 d (milk)	Thomson Micromedex (2003)
Diaminopyrimidines				
Ormetoprim	15% of premix feed, poultry	coccidiostat	5 d (meat)	Zoetis (2015a)
Fluoroquinolones				
Danofloxacin	1.25 mg kg^{-1}, cattle; 5–50 mg kg^{-1}, chicken	respiratory diseases (nondairy, nonlaying)	not available	Heitzman (2004)
Enrofoxacin	2.5–5 mg kg^{-1}, cattle and pig; 10 mg kg^{-1}, chicken	respiratory diseases	28 d (beef); 7 d (chicken)	Bayer Healthcare (2014); NOAH (2013)
Glycopeptides				
Bambermycin	0.1–2 kg t^{-1}, chicken; 10–20 mg $animal^{-1}$ d^{-1}, cattle	growth promotion	none	Hoechst-Roussel Agri-Vet Co. (1993); Huvepharma (2015a)
Ionophores				
Laidlomycin	30–150 mg $animal^{-1}$ d^{-1}, cattle	growth promotion	none	Syntex Animal Health (1994)
Lasalocid	10–30 mg $head^{-1}$ d^{-1}, cattle	growth promotion	none	Regassa et al. (2015) ; Zoetis (2015a)
	68–90.7 g t^{-1}, chicken	coccidiostat	none	
Monensin	1–2 mg kg^{-1}, cattle	increased milk production	none	Bizec and Sanders (2012)
Narasin	19.8 mg kg^{-1}, cattle	growth promotion	none	Martin and Friedlander (2015)
	60–80 mg kg^{-1}, chicken feed	growth promotion	none	
Salinomycin	10–30 ppm, lambs and calves; 60 ppm, chicken (nonlaying); 25–50 ppm, pigs	coccidiostat	5 d (chicken meat); none (other meat)	Huvepharma (2015b)
Lincosamides				
Lincomycin	10 mg kg^{-1}, cattle	therapeutic	48 h (meat)	Boothe (2015b)
	20 g t^{-1}, pig; 40–200 g t^{-1}, pig	growth promotion; prophylaxis	none	
	2–4 g t^{-1}, chicken	growth promotion	none	Zoetis (2015b)
Pirlimycin	50 mg per quarter, cattle	therapeutic	none	The Upjohn Company (1993)
Macrolides				
Erythromycin	8–15 mg kg^{-1}, cattle	therapeutic	14 d (meat)	Boothe (2015c)
Gamithromycin	6 mg kg^{-1}, cattle	therapeutic	63 d (meat)	Boothe (2015c)
Tildipirosin	4 mg kg^{-1}, cattle	respiratory diseases	NA	Merck Animal Health (2015)

Table 1. Continued.

Antibiotic	Dose	Purpose	Withdrawal time	Sources for guidelines
Tilmicosin	10 mg kg^{-1}, cattle	respiratory diseases	7 d (meat)	Boothe (2015c)
	181–360 g t^{-1}, pig	growth promotion	7 d (meat)	Regassa et al. (2015)
Tulathromycin	2.5 mg kg^{-1}, cattle	respiratory diseases	18 d (meat)	Zoetis (2015c)
Tylosin	10–20 mg kg^{-1}, cattle	respiratory diseases	21 d (meat), 28 d (milk)	Regassa et al. (2015)
	4–50 g t^{-1}, chicken	growth promotion	none	
	10–110 g t^{-1}, pig	growth promotion & prophylaxis	none	
	8–10 mg $head^{-1}$ d^{-1}, cattle	prophylaxis	none	
Tylvalosin	50–100 ppm, poultry and pig	respiratory disease	2 and 3 d (meat)	ECO Animal Health (2015)
Penicillins				
Amoxicillin	4–10 mg kg^{-1}, cattle	therapeutic	30 d (meat)	Boothe (2015d)
Ampicillin	5–10 mg kg^{-1}, cattle	therapeutic	6 d (meat)	Boothe (2015d)
Cloxacillin	500 mg per quarter, cattle	therapeutic	30 d (meat)	DailyMed (2015)
Penicillin	10,000–30,000 IU kg^{-1}, cattle	therapeutic	10 d (meat), 3 d (milk)	Boothe (2015d), Zoetis (2015d)
	10–50 g t^{-1}, pig	growth promotion	none	
	2.4–50 g t^{-1}, chicken	growth promotion	none	
Pleuromutilins				
Tiamulin	1.8–15 mg kg^{-1}, pig	therapeutic	3–7 d	Novartis Animal Health (2015)
Polypeptides				
Bacitracin	35–70 mg $head^{-1}$ d^{-1}, chicken	growth promotion	none	Butaye et al. (2003)
	10–30 g t^{-1}, pig	growth promotion	none	
	4–50 g t^{-1}, chicken	growth promotion	none	
	250 g t^{-1}, pig	prophylaxis	none	
Quinoxalines				
Carbadox	55 ppm, pig	growth promotion & prophlyaxis	28 d (meat)	Food and Agriculture Organization (2003)
Tetracyclines				
Chlortetracycline	350 mg $head^{-1}$ d^{-1}, cattle	prophylaxis	2 d (beef)	Regassa et al. (2015)
	10–50 g t^{-1}, pig; >50 g t^{-1}, pig	growth promotion; prophylaxis	none	
	10–100 g t^{-1}, chicken	growth promotion & prophylaxis	none	
Oxytetracycline	75 mg $head^{-1}$ d^{-1}, cattle	growth promotion & prophylaxis	none	Regassa et al. (2015)
	10–50 g t^{-1}, pig	growth promotion & prophylaxis	5 d (pork)	
	5–50 g t^{-1}, chicken	growth promotion & prophylaxis	0–3 d (meat)	
Sulfonamides				
Sulfadimethoxine	27.5–55 mg kg^{-1}, cattle	therapeutic	5–7 d (meat), 60 h (milk)	The United States Pharmacopeia Convention (2007)
	0.05%, chicken	therapeutic	5 d (meat)	
Sulfamethazine	220 mg kg^{-1}, cattle	therapeutic	10 d (meat), 96 h (milk)	The United States Pharmacopeia Convention (2007)
Streptogramins				
Virginiamycin	6–10 g t^{-1}, pig	growth promotion	none	Regassa et al. (2015)
	>25 g t^{-1}, pig	prophylaxis	none	
	5–20 g t^{-1}, chicken	growth promotion	none	
	10–25 mg $head^{-1}$ d^{-1}, cattle	growth promotion & prophylaxis	none	

animals is dominated by tetracyclines (63%), followed by penicillin and cephalosporin β-lactams (8%), macrolides (5%), sulfonamides (4%), aminoglycosides (3%), lincosamides (2%), and fluoroquinolones (<1%). Others that are medically important but not independently reported include florfenicol, ormetoprim, and virginiamycin (15%) (FDA, 2015) (see Table 1 for examples of antibiotic applications). These sales figures are likely to change as growth promotion practices are phased out of food production (FDA, 2013).

When attempting to identify the most significant potential risks from antimicrobial use in agriculture, it is important to recognize several caveats. First, as noted by the WHO report (WHO, 2011), the primary zoonotic agents of food-animal origin include *Salmonella enterica*, *Campylobacter* spp., *Escherichia coli*, and *Enterococcus* spp. All of these bacteria normally reside in the gastrointestinal tract, and this fact provides an important point of focus when we consider how antibiotics are administered to and eliminated from animals. These organisms can also survive ex vivo, meaning that it is also possible for antibiotic selection pressure to be exerted outside of the host when bioavailable antibiotics are excreted from treated animals. If this ex vivo selection process is a significant risk factor, this also provides a potentially important but largely unappreciated point where more control efforts could be directed (Call et al., 2014).

When we consider risk in agricultural applications, it is also important to understand that transmission of resistance traits is not a "free for all." That is, there are genuine biological and phylogenetic constraints to gene transmission, and thus the dissemination of resistance traits between species is not a random process. For example, although it is possible for antibiotic resistance traits to move between Gram-positive and Gram-negative organisms, this is clearly uncommon and thus probably not a significant risk factor relative to other contributors to the antibiotic resistance challenge. Furthermore, with the exception of *Campylobacter*, these organisms are not naturally competent; thus, although environmental DNA (i.e., naked DNA) can be acquired by bacteria in the environment (Goetsch et al., 2012), this does not pose a significant risk factor for transmission of resistance traits to these pathogens. Even in the case of *Campylobacter*, the pool of horizontally transmissible resistance genes is quite distinct from those of *E. coli* and *Salmonella*. For example, resistance to tetracycline is typically conferred by *tet*(O) in *Campylobacter* (Bae et al., 2007; Abril et al., 2010) but not in *E. coli*. In contrast, there are 12 different tetracycline resistance genes that confer resistance to *E. coli*, and many of these are also found in *Salmonella* (Chopra and Roberts, 2001). Thus, risk should also be considered in the context of the specific pathogens of interest.

The mechanisms, pathways, and network interactions that potentially contribute to emergence, amplification, persistence, and dissemination of antibiotic-resistant bacteria are complex and overlapping (Davies and Davies, 2010). The WHO report (WHO, 2011) provides a model to consider this conceptual challenge; that is, when we consider the nearly infinite combination of interactions and selective pressures that contribute to the antibiotic resistance challenge, we need to focus on those that are the principle contributors from agricultural practices. As an example, therapeutic treatment of rainbow trout with oxytetracycline probably contributes much less to this crisis compared with therapeutic administration of fluoroquinolones in poultry. Consequently, focusing on poultry applications is probably a better investment of limited time and resources.

In this review we explore how different antibiotic administration practices affect enteric microflora of livestock, the short-term fate of antibiotics in livestock environments, and the types of resistance traits that might provide useful information when assessing the risk of different antibiotic use practices. The overriding goal is to highlight ideas that better inform risk assessment strategies so that policymakers can have the greatest positive impact.

Dose Matters

A large body of literature addresses the many potential and hypothetical mechanisms by which low concentrations of antibiotics, however defined (subtherapeutic, subinhibitory, sub-subinhibitory; see Table 1 for examples of different doses for different antibiotic applications), might contribute to the evolution of antibiotic resistance (e.g., Gullberg et al., 2011; Sandegren, 2014). Biologically, however, natural selection from antibiotic exposure is a dose-dependent process whereby higher doses result in higher concentrations within treated animals, and this imposes a commensurately greater selective advantage for antibiotic-resistant bacteria. For example, Cazer et al. (2014) developed a pharmacokinetic model that predicted the concentration of chlortetracycline in the large intestine of a 300-kg steer. An orally administered growth promotion application produces an estimated maximum drug concentration of 0.3 μg mL^{-1}, whereas correspondingly higher concentrations were predicted for doses used for disease prevention (1.7 μg mL^{-1}) and treatment (31.5 μg mL^{-1}). Other models from human medicine have drawn similar conclusions (Austin et al., 1999; Opatowski et al., 2010).

These predictions are borne out by empirical data where, in most cases, lower doses (e.g., growth promoting) produced no measurable effect relative to prophylactic or therapeutic doses (Table 2). If proliferation of antibiotic-resistant bacteria is considered a significant risk factor from antibiotic use, then disproportionate attention should be focused on high-dose, therapeutic applications of antibiotics. Ironically, if growth promotion and prophylactic concentrations (lower doses) prevent disease, these practices will also limit the demand for therapeutic doses, whereas eliminating these practices may increase the demand for therapeutic applications (Berge et al., 2005), with the unintended consequence of causing a net increase in the prevalence of resistant bacteria (see also Phillips [2007]). Clearly, a preferred strategy would be to emphasize targeted therapy (Berge et al., 2009) and alternative strategies to improve animal health and reduce the overall demand for therapeutic applications in food animal production (Palmer and Call, 2013).

Route of Administration

The route of administration (i.e., via ingestion of food or water, intramuscular or subcutaneous injection, or intramammary route) affects how bacteria are affected because the concentration in different tissues varies according to pharmacokinetic properties of the antibiotics being used. One might expect that oral administration will have a greater impact on the

gastrointestinal microflora simply owing to the direct route of exposure. Zhang et al. (2013) tested this idea by orally inoculating mice with a mixture of *tet*(M)-carrying *Enterococcus* spp. or bla_{CMY-2}–carrying *E. coli* and then treating those mice either orally or intravenously with identical doses of ampicillin or tetracycline. As expected, the copy number of targeted resistance genes increased significantly more in mice that were treated orally. From a risk perspective, greater attention to oral administration practices may be more useful, although this should be confirmed empirically for different antibiotics and animals. For example, Devreese et al. (2014) found similar concentrations of enrofloxacin in the cecum and colon of broiler chickens that were treated orally or by intramuscular injection. Unexpectedly higher concentrations were present in the cloaca after systemic injection compared with oral administration. Wiuff et al. (2003) also reported that emergence of resistance to fluoroquinolones in pigs was independent of route of administration (oral or intramuscular), dose, or time of treatment. Furthermore, it might be possible to adjust dosage guidelines to minimize the impacts on nontarget organisms. For example, Vasseur et al. (2014) cured rats of *Klebsiella pneumoniae* lung infections by treating them with either 5 or 50 mg kg^{-1} cefquinome (subcutaneous). The number of cefotaxime-resistant enteric bacteria increased over 4 log with the 50 mg kg^{-1} treatment, whereas the 5 mg kg^{-1} treatment had no measurable effect on the enteric bacteria.

Dry-cow therapy and mastitis treatment often involve intramammary infusions that do not affect the gastrointestinal tract. Nevertheless, *E. coli*, *Klebsiella*, and other enteric bacteria can cause mastitis, and it is worth considering these treatments because of their potential to amplify resistant populations that could be transmitted to people and animals. For example, Saini et al. (2013) examined the herd-level association between antimicrobial use and antimicrobial resistance for environmental mastitis in cattle (n = 394 farms). Intramammary administration of cloxacillin, penicillin-novobiocin, and cephapirin was highly correlated with ampicillin-intermediate or -resistant *E. coli* (odds ratios [ORs], 26, 32, and 189, respectively, for dry cows). For lactating cows, a similarly high correlation was evident for intramammary administration of ceftiofur (OR, 162), whereas systemically administered penicillin was less correlated (OR, 2.7). In this latter case, less penicillin probably reached the mammary gland after systemic administration. These results are consistent with a higher dose of antibiotic having a greater selective effect on resistant bacteria compared with low doses. Further work is needed to determine if selection of resistant bacterial populations from these practices warrants increased attention in risk analysis.

Quinolones: A Special Case

Resistance to most antibiotics is conferred by genetically encoded traits such as efflux pumps, enzymes, and proteins that exclude access to the antibiotic target (e.g., ribosomal protection proteins). Quinolones are a special case because resistance can be conferred by single-nucleotide mutations in the chromosomally

Table 2. Effects of different doses of antibiotics on resistant bacteria recovered from animal feces.

Animal	Antibiotic	Dose†	Effect	Reference
Feeder pigs	chlortetracycline	2× [350 mg kg^{-1} (7 d) + 50 mg kg^{-1} (14 d)] vs. 50 mg kg^{-1} (35 d), in feed	High dose selected for multidrug resistant plasmid containing *E. coli*; low dose had no impact.	Johnson et al. 2015
Calves	penicillin G	five doses, 0–50 μL kg^{-1}, in milk	Dose-dependent increase in resistant bacteria	Langford et al. 2003
Piglets	ciprofloxacin	1.5 mg kg^{-1} d^{-1} vs. 15 mg kg^{-1} d^{-1}, oral	Dose-dependent increase in resistant bacteria	Nguyen et al. 2012
Steers	chlortetracycline & sulfamethazine	44 ppm each, in feed		
	chlortetracycline	11 ppm, in feed		
	monesin	25 ppm, in feed		
	tylosin	11 ppm, in feed		
	virginiamycin	31 ppm in feed	Over a 314-d period, the combination of chlortetracycline and sulfamethazine increased the prevalence of tetracycline and ampicillin-resistant *E. coli* in feces; diet also influenced shedding.	Alexander et al. 2008
Steers	tilmicosin	10 mg kg^{-1}, single dose subcutaneously	All three treatments caused an increased proportion of erythromycin-resistant fecal enterococci.	
	tulathromycin	2.5 mg kg^{-1}, single dose subcutaneously		
	tylosin	11 ppm, in feed, 28 d		Zaheer et al., 2013
Mice	cefquinome	50 mg kg^{-1}, twice daily, subcutaneously, 4 d; 5 mg kg^{-1}, daily, subcutaneously, 4 d	High dose led to a 4-log increase in the number of resistant bacteria; low dose had no effect.	Vasseur et al. 2014
Steers	chlortetracycline	22 mg kg^{-1}, in feed, mixed exposure periods	Transient but significant increase of prevalence of tetracycline-resistant *E. coli*	Platt et al. 2008
Dairy calves	oxytetracycline	26 μL kg^{-1}, in milk, 4–6 wk	Three tetracycline-resistant multidrug resistant subpopulations increased (<5%); five other subpopulations were unchanged.	Khachatryan et al. 2004
Poultry	enrofloxacin or danofloxacin	10 mg kg^{-1}, in water, 5 d	Nearly 100% resistant *Campylobacter*	Humphrey et al. 2005

† The unit mg kg^{-1} refers to mass of antibiotic per kg body weight; ppm refers to parts per million relative to feed; μg mL^{-1} is in reference to the identified fluid.

encoded gyrase and topoisomerase IV genes (Aldred et al., 2014). There is no reason to invoke hypermutator models or adaptive mutation mechanisms to explain this process. Spontaneous mutations occur with every generation of binary fission, and a very small proportion of the bacterial progeny ($\sim 10^{-8}$) will have the necessary point mutations to confer resistance to quinolones. This "background" is only evident under selective pressure from antibiotics that allow these few strains to multiply in the presence of quinolones, whereas sensitive strains cannot. This is graphically illustrated by the near immediate and complete conversion of sensitive *Campylobacter* populations in chickens into ciprofloxacin-resistant populations after metaphylactic treatment with fluoroquinolones (Humphrey et al., 2005). Findings such as this led to the FDA ban on use of fluoroquinolones for treatment of poultry flocks (Nelson et al., 2007). It also appears that fluoroquinolone treatment in livestock affects the gastrointestinal flora equally for oral or systemic treatment (Wiuff et al., 2003). From a risk perspective, the use of quinolones and fluoroquinolones in food animal production probably needs very close scrutiny.

Ionophores and Nonmedically Important Antibiotics

Nonmedically important antimicrobials only present a risk for human medicine if resistance to these compounds is genetically encoded and when these traits are genetically linked with traits that encode resistance to medically important antibiotics. Under this scenario, the use of nonmedically important antimicrobials could coselect for important resistance traits. Of the nonmedically important antimicrobials, ionophores represent ~30% of all domestic sales of antimicrobials for use in food animals (FDA, 2015). No genetically encoded resistance trait has been described for ionophores (Calloway et al., 2003), and these compounds probably represent a best practice with respect to improving animal health and performance without causing risk to public health (Butaye et al., 2003). Carbadox, a quinoxaline compound, has been shown to induce prophage activity in *Salmonella* that could lead to enhanced horizontal transmission of antibiotic resistance traits (Bearson et al., 2014). Tiamulin-resistant *E. coli* have been described, although the trait involves target modification by chromosomal mutation that is not horizontally transmissible (Bosling et al., 2003). Resistance to novobiocin has been "engineered" by chromosomal mutation and thus is demonstrably possible (Hardy and Cozzarelli, 2003). Bacitracin resistance in *Clostridium perfringens* is horizontally transmissible (Charlebois et al., 2012) and has also been described for *Enterococcus fecalis* (Matos et al., 2009). In the long term, these genetically encoded resistance traits could play a role in perpetuating resistance to medically important antibiotics through coselection, but this has not been documented to date.

Biodegradation of Excreted Antibiotics

There is a significant body of literature concerning the fate and transport of antibiotic residues in the environment (Thiele-Bruhn, 2003; Sarmah et al., 2006; Wang and Wang, 2015). Conclusions about the risks from these residues are mixed in part because the biological effects of residues are dose-dependent, and dilution below certain thresholds renders these compounds unimportant (Schwab et al., 2005). Another reason that the conclusions are mixed is that not all antibiotics remain bioavailable in the environment. Some compounds are degraded or are rapidly sorbed to soil particles (Subbiah et al., 2011a; Subbiah et al., 2012).

When antibiotics are degraded, the degree that the degraded products remain bioactive is dependent on the presence or absence of functional groups, and this varies depending on the mechanism of degradation. For example, ceftiofur is degraded in vivo by hydrolysis, which results in the formation of desfuroylceftiofur. Desfuroylceftiofur retains bioactivity similar to its parent compound. In contrast, biodegradation of ceftiofur results in the formation of cef-aldehyde products that contain a cleaved β-lactam ring and thus retain no significant bioactivity (Li et al., 2011). In general, degradation products exhibit equal or less bioactivity relative to their parent compound. For example, tylosin-B is a metabolite of tylosin and retains 83% bioactivity (Wegst-Uhrich et al., 2014). Tylosin-B can also be formed as a degradation product after hydrolysis in acidic water (pH 4) (Mitchell et al., 2015). Mohring et al. (2009) found that hydroxylated sulfadiazine retained less than 10% bioactivity compared with sulfadiazine. In one very interesting case, an important degradation product from tetracyclines (anhydrotetracycline) selectively favors susceptible *E. coli* over tetracycline-resistant *E. coli* that express a *tet*(A) efflux pump (Palmer et al., 2010).

Manure can enhance antibiotic degradation rates in soil. This observation highlights the importance of considering how degradation rates are estimated. For instance, Chen et al. (2014) reported that the degradation half-life of oxytetracycline in soil was reduced significantly in the presence of manure. Consequently, degradation calculations for antibiotics in soil are likely to be overestimated for cases where manure inputs are likely. Bacteria from both soil and gastrointestinal flora can contribute to antibiotic biodegradation. For example, enteric bacteria, including *Bacillus cereus*, *Bacillus mycoides*, *Bacteroides* spp., *Eubacterium biforme*, *Bifidobacterium breve*, and several *Clostridium* spp. found in the gastrointestinal tract of untreated cattle produce enzymes that degrade ceftiofur (Wagner et al., 2011); Dantas et al. (2008) reported that some bacteria (including Enterobacteriales and Pseudominadles) found in the natural soil can degrade almost all important antibiotics that are frequently used in animals and people (including penicillins and ciprofloxacin). Degradation of ceftiofur was faster in fresh mixtures of soil and manure compared with the same mixtures that were autoclaved, and this process was temperature dependent (Subbiah et al., 2012). Li et al. (2011) also found an increased rate of biodegradation of ceftiofur when mixed with animal waste.

Sorption of Excreted Antibiotics

Besides dilution and degradation, sorption to soil can determine the impact of antibiotics in the environment. For example, fluoroquinolones have a high affinity for soils, and little remains unbound to interact with bacteria (Leal et al., 2013). As a consequence, typical concentrations of these residues in soils are mostly unavailable, a prediction that is borne out empirically (Rosendahl et al., 2012; Youngquist et al., 2014). In contrast, florfenicol has almost no affinity for soil particles (Subbiah

et al., 2011a), and thus residues of florfenicol could affect soil microbiota if sufficient concentrations are present. The degree of sorption for a specific compound is also dependent on the physicochemical properties of the soil. For terrestrial food animals it is particularly important to consider how excreted residues sorb to animal bedding and soils. These interactions are measurable and predictable, and this helps to identify which antibiotic residues deserve more attention from a risk perspective.

Wang and Wang (2015) provide a detailed review of the factors responsible for the differential adsorption of antibiotics in soil. Depending on the antibiotic, sorption increases with increasing clay content or with increasing organic matter concentration. For example, fluoroquinolones and sulfonamides adsorbed better to manure with a high organic matter content, whereas oxytetracycline and tylosin were less responsive to the organic matter content (Marengo et al., 1997; Loke et al., 2002). Other antibiotics are affected by cation exchange capacity (ciprofloxacin adsorbs well to soil with high effective cation exchange capacity [Carrasquillo et al., 2008]), pH, or other soil properties (Sassman and Lee, 2005; Strock et al., 2005; Sassman et al., 2007; Kim et al., 2012; Leal et al., 2013; Williams et al., 2013). Lincomycin sorption is dependent on soil pH (Williams et al., 2013), and oxytetracycline adsorption to clay minerals and humic substances is dependent on the pH (Figueroa et al., 2004). Sittig et al. (2012) found that the easily accessible fraction of sulfadiazine in soil was low, "indicating a low bioavailability."

In general, antibiotics can sorb very tightly to soil particles and are not available for microorganism uptake. Under permissible conditions, however, antibiotics can desorb (reversible sorption) into an aqueous solution (Ortega-Calvo et al., 2015), but it is not clear if this occurs commonly under field conditions. For instance, Subbiah et al. (2011a) attempted to desorb tetracycline at room temperature by mixing tetracycline-exposed and washed soil with a low volume of water. This slurry was inoculated with a resistant *E. coli* strain and a sensitive isogenic strain, but there was no evidence of a selective advantage for the resistant strain. In contrast, when Chander et al. (2005) compared recovery of bacteria in the presence of tetracycline- or tylosin-adsorbed soils at 37°C, they found a dose-dependent reduction for both antibiotic-sensitive and antibiotic-resistant bacteria. Bansal (2012) reported that tetracycline adsorbs less to soil particles at higher temperatures, which could explain some of the differences between Subbiah et al. (2011a) and Chander et al. (2005). If temperature is an important factor in natural systems, then lower subsurface soil temperatures will be conducive to adsorption of antibiotics like tetracycline, thereby limiting desorption into groundwater (Harmsen, 2007). In addition, higher pH can affect sorption because ionizable antibiotics are greatly affected by pH (Wang and Wang, 2015). For example, in the presence of 1 mol L^{-1} $MgCl_2$ (pH 8.5), adsorbed oxytetracycline is released into the aqueous phase of soil-water solutions (Kong et al., 2012). The quantity of oxytetracycline that desorbed was highly dependent on soil properties where the presence of clay and organic matter decreased desorption of oxytetracycline, whereas Zhang et al. (2014) reported that the presence of organic acids enhances the bioavailability of tetracycline in water.

Antibiotic degradation products or metabolites may have similar or decreased sorption affinities compared with their parent compounds (Wegst-Uhrich et al., 2014). Consequently, as contaminants degrade they may become more water soluble. This could be problematic when the degradation products or metabolites retain a degree of antimicrobial activity and the concentration is sufficiently high to affect bacterial populations. Overall, these studies indicate that sorption can effectively "neutralize" some antibiotics, but under favorable conditions the sorbed antibiotics may be released and become bioavailable. Nevertheless, it remains to be determined if desorption occurs with sufficient frequency under field conditions to consistently affect populations of antibiotic-resistant bacteria.

Several studies have estimated soil distribution coefficients (K_d), where antibiotics with a relatively high K_d have a higher affinity for soil sorption. Thus, higher K_d values denote antibiotics that may have limited bioavailability in soils once equilibrium is reached. This may be affected by temperature, pH, clay, organic matter, metals, etc. Beta-lactams, tetracyclines, and other antibiotics have acid/base properties, and therefore soil constituents other than organic matter can affect availability. Gong et al. (2012) developed a model to estimate sorption for oxytetracycline, norfloxacin, and sulfamethazine. Model parameters included pH, clay, free Fe oxides, free Al oxides, Al, Ca, and organic matter content, and the hypothesized mechanism of adsorption was surface complexation (Gong et al., 2012). Wegst-Uhrich et al. (2014) stated that sulfonamides and macrolides primarily sorb to soils via electrostatic forces, but, consistent with Gong et al. (2012), they concluded that tetracyclines and fluoroquinolones sorb to soils through cation exchange, surface complexation, and cation bridging.

Antimicrobial sorption to soil can be summarized for medically important compounds (Fig. 1 and 2) and nonmedically important compounds (Fig. 2) on the basis of empirically determined sorption coefficients. Most antibiotic sorption can be modeled by a linear equation (yielding a K_d coefficient) or a nonlinear Freundlich equation (yielding a K_f coefficient). Larger soil sorption coefficients denote compounds that sorb more to soil particles. For example, danofloxacin, enrofloxacin, chlortetracycline, oxytetracycline, tetracycline, and tylosin have, on average, relatively large K_d values (>1000 L kg^{-1}) (Fig. 1). Tylosin transformation and degradation products tylosin A-aldol and tylosin D have similar sorption coefficients even though the chemical structures have been marginally modified. Antibiotics and degradation products that completely sorb to soils are less bioavailable to soil microorganisms in general. In contrast, ormetoprim, sulfadimethoxine, sulfamethazine, and florfenciol have smaller K_d values (<50 L kg^{-1} on average), consistent with incomplete sorption to soil particles (Fig. 1). Antibiotics that do not completely sorb to soils can be bioavailable to soil microorganisms until the antibiotics are effectively degraded.

Studies have also reported K_f values for antibiotics when nonlinear sorption is observed (i.e., when antibiotic sorption is a function of antibiotic concentration). A lower sorption coefficient results from nonlinear Freundlich sorption compared with linear sorption. Comparing K_f values for tetracyclines, lincomycin, and amoxicillin shows that lincomycin and amoxicillin have smaller sorption coefficients on average (<50 $mmol^{1-N}$ L^N kg^{-1}) (Fig. 3). This means that lincomycin and amoxicillin do not sorb to soil particles completely.

The addition of manure to soil can also affect antibiotic sorption and abiotic degradation because manure adds organic

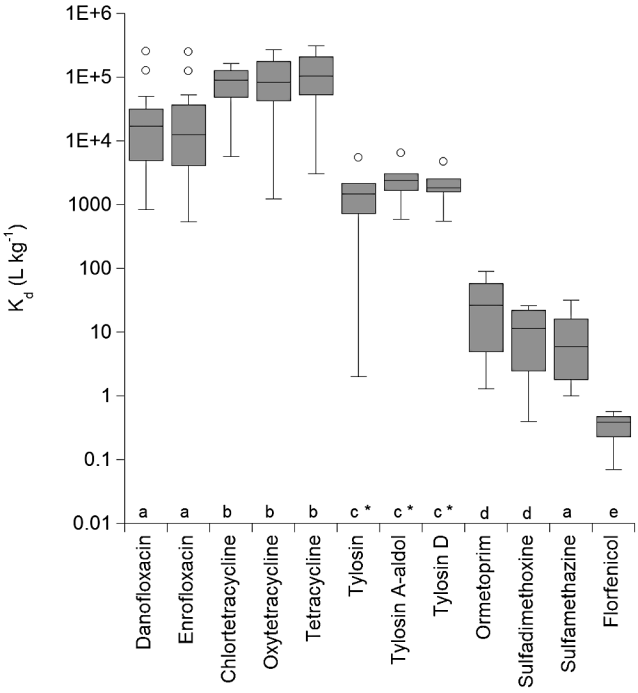

Fig. 1. Soil distribution coefficients (K_d) for medically important antimicrobials used in animal husbandry in the United States. Data were collected from the following sources: (a) Leal et al. (2013), (b) Sassman and Lee (2005), (c) Sassman et al. (2007), (d) Sanders et al. (2008), and (e) Yates et al. (1996). * Sorption coefficient values calculated based on Freundlich sorption coefficients (K_f) and a specified antibiotic concentration.

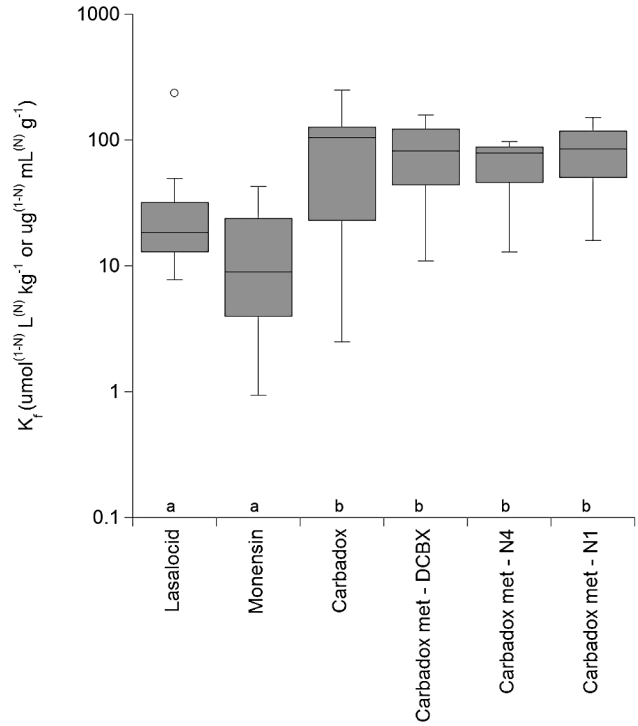

Fig. 2. Freundlich sorption coefficients (K_f) for nonmedically important antimicrobials used in animal husbandry in the United States. Data were collected from the following sources: (a) Sassman and Lee (2007) and (b) Strock et al. (2005). Lasalocid and monensin data are reported in μmol$^{(1–N)}$ L$^{(N)}$ kg^{-1}. Carbodox and metabolite data are reported in μg$^{(1–N)}$ mL$^{(N)}$ g^{-1}. Met, metabolite. DCBX, desoxycarbadox.

matter to the soil and alters moisture level and pH (Naramabuye and Haynes, 2006) that, in turn, affect both adsorption and hydrolytic degradation of antibiotics (Chee-Sanford et al., 2009; Wang and Wang, 2015). Organic matter in soil increases the availability of functional groups (-COO-) that contribute to adsorption of antibiotics (Sibley and Pedersen, 2008). Dissolved organic matter can have the opposite effect and can contribute to desorption of antibiotics in soil (Loke et al., 2002). Furthermore, Thiele-Bruhn and Aust (2004) reported that the addition of manure can decrease adsorption of sulfonamides in soils because constituents in the soil competitively occupied sorption sites, thereby decreasing the capacity of the soil to sorb the antibiotic.

It is possible that antibiotic sorption to soil may be a predictor of sorption to some animal bedding materials, such as compost. Wood shavings or sand bedding materials may have limited sorption potential, so antibiotic residues in these materials are likely to remain bioavailable. Sand does not sorb antimicrobials efficiently, but the population of bacteria is lower compared with organic bedding material (Hogan et al., 1989). Low sorption by bedding materials such as wood shavings means that confined pens with treated animals might represent "hot spots" for reservoirs of antibiotic resistance on farms. In general, for soils and bedding materials, antibiotics and degradation products that have lower K_d and K_f values warrant greater attention in risk analysis compared with those with greater values.

The Distribution of Resistance Genes

Agricultural environments are frequently affected by animal wastes, and therefore antibiotic resistance traits will also be present. Some investigators consider this "pollution" to be a

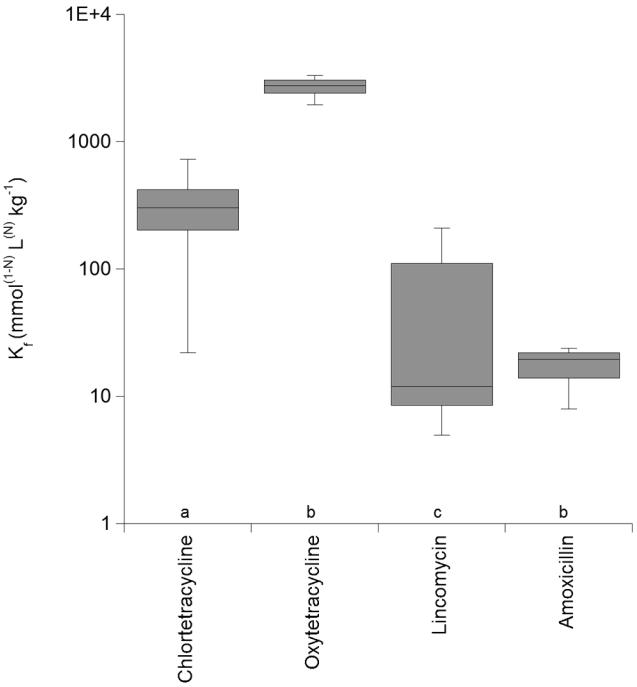

Fig. 3. Freundlich sorption coefficients (K_f) for medically important antimicrobials used in animal husbandry in the United States. Data were collected from the following sources: (a) Sassman and Lee (2005), (b) Kim et al. (2012), and (c) Williams et al. (2013).

significant risk factor to the environment and for dissemination to human pathogens (Martinez, 2009). The magnitude of such risk has never been quantified, but in most environmental situations the abundance of our target enteric organisms (*E.*

coli, *Salmonella*, *Campylobacter*, and *Enterococcus*) is likely to be orders of magnitude less than is found in the gastrointestinal tract of people and animals. This does not mean that horizontal gene transfer does not occur in lower-density populations, but such dilution necessarily reduces the likelihood of frequent gene transfer in the environment when compared with the more densely populated gastrointestinal lumen. An exception might be environments that are heavily affected both by high concentrations of antibiotic residues and large numbers of bacteria being added through fecal shedding (e.g., in a calf pen after the animal is treated with an antibiotic). In these select situations, a high local density of resistant bacteria will, by definition, increase the likelihood that the bacteria will share resistance traits and increase the probability that resistant microbes will be transmitted to other host animals through direct contact.

In theory, environmental DNA in soil and manure could contribute to horizontal transmission of resistance traits, but natural competency has either never been demonstrated or is very rare for *E. coli* (Sinha and Redfield, 2012), *Salmonella* (MacLachlan and Sanderson, 1985), and *Enterococcus* (Bourgogne et al., 2008). *Campylobacter* is naturally competent (Lorenz and Wackernagel, 1994), but this has mostly been seen in the context of DNA sharing between *Campylobacter* strains and species (Vegge et al., 2012). Consequently, for these organisms "naked DNA" per se is unlikely to represent a risk factor in agricultural environments. Indeed, environmental contamination with resistance genes probably falls more often into the category of "less important" compared with other risk factors that result in log-fold increases in the abundance of antibiotic-resistant bacteria.

Beyond learning about broad-scale dissemination of resistance traits (e.g., Koike et al., 2007), it is useful to consider what information can be gained by quantifying the distribution of resistance genes in environmental contexts. That is, the distribution of genes becomes a "read out" for effects of different practices rather than being considered a risk factor itself. In this respect, resistance phenotypes and genotypes could be used to track the dissemination of bacteria (e.g., to identify dissemination pathways) or to quantify the extent that genes can be detected from a point source (e.g., a farm) (Koike et al., 2007). This assumes that the presence of these traits represents a biological impact of importance to public health outside of hypothetical scenarios. Unfortunately, unlike chemical signatures that can be considered in the context of mass-balance equations, the abundance of bacteria is subject to a variety of stochastic factors that will limit the ability to infer impact over space and time based on the abundance of resistance traits. For example, two populations might experience a similar migration rate of resistant bacteria, but chance events could allow disproportionate amplification of these resistant bacteria in one population and not the other. Consequently, abundance would no longer reflect true migration rates.

Judicious selection of resistance traits might provide insight into the magnitude of recent selection pressure. For example, some antibiotic resistance traits or transmissible plasmids have a measureable fitness cost (Subbiah et al., 2011b) that would lead to their eventual extinction from a given population of bacteria. In theory, fitness coefficients for these genes and plasmids might be combined with information about the presence and abundance of these genes or plasmids to infer how recently a population experienced antibiotic selection pressure or the magnitude of selection pressure after a defined treatment. Unfortunately, fitness costs can vary at a strain level (Johnson et al., 2015), so such inferences might be limited. Stochastic events and coselection would also decouple the relationship between abundance of these traits and a targeted event such as introduction of a new antibiotic on a farm.

Another complicating issue is that antibiotic resistance can persist in production environments (and clinical environments; Enne et al. [2001]) in the absence of corresponding antibiotic use (Walk et al., 2007). Under these circumstances, the abundance of resistance genes will not reflect antibiotic use practices. This can happen when resistance traits confer a growth advantage regardless of antibiotic selection pressure (Luo et al., 2005) or through coselection when resistance traits are linked to other genes that encode resistance to antibiotics, heavy metals, and other toxins (de Lorenzo et al., 1984; Delgado-Iribarren et al., 1987; Martinez and Perez-Diaz, 1990; Hernandez et al., 1998; Stepanauskas et al., 2006; Nilsson et al., 2009). In one case, chromosomally encoded resistance for streptomycin, sulfonamide, and tetracycline antibiotics (Khachatryan et al., 2004; Khachatryan et al., 2008) was harbored in *E. coli* strains that also produced a microcin (Eberhart et al., 2012). Production of the microcin, particularly in the dairy calves that received a nonmedicated milk supplement, provided a selective advantage against conspecific, antibiotic-sensitive *E. coli* (Khachatryan et al., 2006; Eberhart et al., 2014). Other nonresistance traits or toxins are encoded on plasmids that could provide a coselective advantage for maintenance of antibiotic resistance traits in the absence of antibiotic use (Sanchez et al., 2002; Linares et al., 2005). Collectively, these limitations make it unlikely that risk analysts would be able to make a priori decisions about the most informative genotypes for assessing risks in the context of antibiotic use. This is not unlike bacterial source tracking applications, where analyzing the distribution of bacterial genetic markers can easily produce misleading inferences (Leach et al., 2008).

Conclusions

Antibiotic-resistant zoonotic bacteria from food animals principally concern enteric bacteria (*E. coli*, *Salmonella*, *Campylobacter*, and *Enterococcus*), and therefore oral and therapeutic doses of antibiotics are likely to have greater impact on antibiotic resistance compared with parenteral and low-dose applications. Quinolones deserve greater scrutiny given the speed with which bacterial resistance can sweep through populations and the fact that parenteral administration can affect enteric flora as much as oral administration. Medically important antibiotics that remain bioavailable in soils deserve greater attention where high concentration exposures are evident. In this regard, the antibiotics of greatest concern include florfenicol, penicillins, cephalosporins, sulfonamides, ormetoprim, and lincosamides. Questions remain regarding the magnitude of impact that is generated from excreted antibiotics and the best mitigation strategies. Notably, empirical data for K_d and K_f are lacking for many antibiotics and associated metabolites. There is also uncertainty regarding the contribution from intramammary infusions that are used to treat mastitis.

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