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Altered Egos: Antibiotic Effects on Food Animal Microbiomes*,†

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†We dedicate this review to Dr. Abigail Salyers (1942–2013), whose research and teaching inspired a generation of intestinal microbial ecologists.

Keywords

gut microbiology, growth-promoting antibiotics, livestock, poultry, antibiotic resistance, subinhibitory antibiotics

Abstract

The human food chain begins with upwards of 1,000 species of bacteria that inhabit the intestinal tracts of poultry and livestock. These intestinal denizens are responsible for the health and safety of a major protein source for humans. The use of antibiotics to treat animal diseases was followed by the surprising discovery that antibiotics enhanced food animal growth, and both led to six decades of antibiotic use that has shaped food animal management practices. Perhaps the greatest impact of antibiotic feeding in food animals has been as a selective force in the evolution of their intestinal bacteria, particularly by increasing the prevalence and diversity of antibiotic resistance genes. Future antibiotic use will likely be limited to prudent applications in both human and veterinary medicine. Improved knowledge of antibiotic effects, particularly of growth-promoting antibiotics, will help overcome the challenges of managing animal health and food safety.

Contents

THE ANIMAL INTESTINAL MICROBIOME

Animals are vehicles for the single-celled organisms that inhabit their bodies. Animals, including humans, have over 10^{14} cells, of which only about 10% are animal cells. The vast majority are microbial cells residing primarily within the gastrointestinal (GI) tract (132). These microbial cells encompass all domains of life: Bacteria, Archaea, and Eukaryota (anaerobic fungi, yeast, and protozoa). A healthy intestinal microbial community is in a dynamic equilibrium with itself, with the host, and with abiotic components of the environment. The abiotic components are the physical and chemical ingredients of their microhabitats—dietary substances, bacterial viruses (phages), host and microbial cell products, osmolality differences, variable viscosity, low oxygen concentrations and redox potentials, and pH (5.5 to 6.9). The bacteria are the dominant microbial population in the gut and will be the focus of this review.

Gut commensal bacteria are the coevolved partners of their animal hosts and harbor competitive fitness (niche) adaptation traits to benefit their own survival. They share general characteristics that allow them to succeed in the complex gut environment. These traits are considered useful for identifying and studying microbes most likely to be key contributors to GI microecology. Indigenous microbes in the GI tract (as summarized by Savage, 132)

1. can grow anaerobically,
2. are always found in normal adults,
3. colonize particular areas of the tract,
4. colonize their microhabitats during succession in infant animals,
5. maintain stable population levels in climax communities in normal adults, and
6. may associate intimately with the mucosal epithelium in the area colonized.

Bacteria indigenous to avian and mammalian GI tracts contribute to the health and well-being of the host animal (60, 64, 85, 98, 154). The relationship is, on balance, a mutualism (win-win, for both partners), and host health is affected when that microbiota is either perturbed or eliminated. Investigations comparing germfree or ex-germfree with conventional animals, antibiotic-treated with untreated animals, and developing neonates with mature adults have revealed the importance of the intestinal microbiota to the host's physiology, metabolism, nutrition, immunology, and ability to resist pathogens (34, 61, 106, 133, 147) (see sidebar, Contributions of the Gastrointestinal Microbiota to Host Health).

CONTRIBUTIONS OF THE GASTROINTESTINAL MICROBIOTA TO HOST HEALTH

1. Directly affect intestinal health, functions, and products: microbiota-associated characteristics,[1] cross feed butyrate (72, 82, 90, 145), degrade mucin (33, 89), affect intestinal gene expression (26), influence intestinal morphology (134)
2. Facilitate maturation and functioning of innate and adaptive immune system (2, 49, 110, 148, 150)
3. Affect host physiology and nutrition: generate short-chain fatty acids used by host (63), metabolize bile acids (steroids) (14), supply microbial proteins for ruminants (29)
4. Biotransform diet components: metabolize plant polysaccharides (45); remove toxic dietary compounds, such as oxalate (37) and mimosine (57)
5. Provide first line of defense against microbial pathogens: colonization resistance (112, 121, 143, 150)
6. Affect distal host tissues: pulmonary, central nervous system (perhaps behavior) through chemical and immunological signaling (30, 54, 150)

Intestinal microbial habitats are diversified vertically (longitudinal axis, mouth to anus) and horizontally (radial axis, lumen to mucosal epithelium) (52, 53, 87, 125, 132, 162, 164). The differences in host physiology of these compartments yield distinct bacterial communities. *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* constitute much of the bacterial community of animal ceca, large intestines, and feces (5, 31, 36, 39, 65, 73, 80, 84, 88, 162), whereas the *Firmicutes* dominate the small intestines (ilea) of swine and chickens (36, 87, 125, 162, 164). The host animal initiates mechanical and chemical digestion in the proximal GI tract, absorbing nutrients from food (and microbial products in ruminants) in the small intestine. The microbiota of the large intestine breaks down complex molecules such as plant cell walls to release and ferment small molecules. Hindgut-fermenting animals, including pigs, derive as much as 10–30% of their maintenance energy requirement from microbial production of short-chain fermentation acids in the cecum and proximal colon (63). Based on the human large intestine microbiota, members of the phylum *Bacteroidetes* predominantly encode the machinery to break down the complex, fibrous molecules (94). Although fecal samples are practical for describing intestinal bacterial activities, it should be kept in mind that feces are a derived composite of upstream intestinal compartments, with a bias toward the heavily populated lower GI compartments. Additionally important, yet more challenging, to study are the microhabitats on or near mucosal epithelial surfaces that foster the most intimate interactions between microbes and host cells.

ANTIMICROBIALS IN UNITED STATES ANIMAL AGRICULTURE

An important tool for maintaining health and improving productivity of farm animals has been in-feed antibiotics. In the United States, the Food and Drug Administration establishes guidelines for

[1]Microbiota-associated characteristics (MACs) are differences in intestinal biochemical properties between conventional animals and animals with modified or not-yet-developed microbiotas (27, 28, 61, 100). They include short-chain fatty acid production, mucin breakdown, cholesterol-to-coprostanol conversion, dehydroxylation of bile acids, and degradation of glycosphingolipids. MACs can be useful barometers of intestinal microbiota activities.

REGULATING AGRICULTURAL ANTIBIOTICS IN THE UNITED STATES

Legislated efforts and voluntary recommendations to restrict or ban antimicrobial growth promoters from agriculture have centered on concerns that the widespread use of growth promoting antimicrobials created animal reservoirs of antibiotic resistance that could spread to humans. Indeed, the World Health Organization has defined antibiotics of high medical importance, and protecting the efficacy of these is of particular interest (160). The US FDA Center for Veterinary Medicine recently reviewed key reports and scientific literature describing the impacts of antibiotic use on antibiotic-resistant intestinal bacteria and on the exchange of antibiotic resistant bacteria among humans and farm animals. The analysis led to recommendations for the judicious use of medically important antimicrobial drugs in food-producing animals (44). Judicious or prudent use will require veterinarian oversight for prophylactic and therapeutic treatments. Administering medically important antimicrobials for growth promotion constitutes an injudicious use and is not recommended. Non-medically important antimicrobials outside of the FDA's guidance, such as quinoxaline antibiotics (carbadox) and perhaps certain ionophores (salinomycin) would presumably remain categorized as judicious for growth-enhancing uses. The FDA document currently contains recommendations and it is unclear whether or when these recommendations will become legal requirements.

the judicious use of antimicrobials in animal management (44; see sidebar Regulating Agricultural Antibiotics in the United States). Various antimicrobials have been approved as dietary additives for acute therapy, prophylactic therapy, and performance enhancement (nontherapeutic) purposes for chickens, turkeys, swine, and beef cattle (**Table 1**). Acute therapy is treating sick animals with diagnosed disease for a limited time. For prophylactic therapy, antibiotics are administered to healthy animals at management stress points to prevent disease development and transmission. For example, for treatment of shipping fever respiratory disease following transportation stress, cattle with clinical signs receive injections of antibiotics (acute therapy) whereas neighboring animals without signs receive diets containing broad-spectrum antibiotics (prophylactic therapy, 350 mg chlortetracycline per animal per day) (11, 55).

The third use of antibiotics in agriculture is for enhancing performance, which is also known as improving feed efficiency (weight gain/weight of food consumed/specific time period). Animals are given diets containing antimicrobials at concentrations lower than those used for therapy, resulting in subtherapeutic doses. Chlortetracycline, for example, is approved at 10–50 g per ton of feed for growing pigs (44 to 110 lbs), 8- to 40-fold less than doses approved to treat enteric diseases (11). Unlike therapeutic antibiotic uses, there is generally no time limit for growth-promotion applications. In practice, however, growth-enhancing benefits of antimicrobials decline in adult animals, and so they are not often fed antibiotic-containing diets for performance. Importantly, all antibiotics have withdrawal times before the animals go to market, to eliminate drug residues in meat products.

HISTORY OF GROWTH-PROMOTING ANTIMICROBIAL USE IN LIVESTOCK AND POULTRY

Because of the development of large-scale production of antibiotics for controlling human infections during World War II, antibiotics became sufficiently economical for use in farm animals. In the mid-to-late 1940s, different research labs examined the effects of antibiotics administered to food animals (25, 70, 103). One of the first applications of antibiotics in animals was the treatment of bovine mastitis with penicillin in the mid-1940s (55).

Table 1 Antimicrobials historically approved by the U.S. Food and Drug Administration as dietary additives for chickens, turkeys, cattle, and swine in the United States[a,b]

Antimicrobial	Uses/therapies[c]
Clopidol, narasin, nicarbazin, robenidine, salinomycin, semduramicin	Prevent coccidiosis (C)
Decoquinate	Prevent coccidiosis (C, BC)
Diclazuril, halifusinone, zoalene	Prevent coccidiosis (C, T)
Amprolium	Prevent coccidiosis (C, T, BC, DC)
Lasalocid	Prevent coccidiosis (C, T, BC), increase rate of weight gain/feed efficiency (BC)
Clopidol	Prevent leucocytozoonosis (*Leucocytozoon smithii*) (T)
Bacitracin (BMD) Bacitracin (Zn)	Increase rate of weight gain/feed efficiency (C, T, S) Increase egg production (C) Aid to prevent/control enteritis (C,T) Treat chronic respiratory diseases (air sacculitis) and blue comb (C) Control swine dysentery, clostridial enteritis (S) Reduction in feedlot liver abscesses (BC) Increase rate of weight gain/feed efficiency (C, T, S, BC)
Bambermycin	Increase rate of weight gain/feed efficiency (C, T, S, BC)
Carbadox	Increase rate of weight gain/feed efficiency (S) Control swine dysentery (*Brachyspira hyodysenteriae*), enteritis (salmonellosis) (S)
Chlortetracycline[d]	Increase rate of weight gain/feed efficiency (C, T, S, BC) Control infectious synovitis (mycoplasma) (C, T) Control respiratory diseases: air sacculitis (C), shipping fever (BC), *Pasteurella* pneumonia (S) Reduce mortality of *Escherichia coli* infections (C) Control hexamitiasis and blue comb (T) Control of anaplasmosis: *Anaplasma marginale* infections (BC) Reduce mortality of *Salmonella enterica* Typhimurium infections (T) Decrease incidence of jowl abscesses (Group E *Streptococcus*), leptospirosis (S) Treatment and control of bacterial enteropathies: *Lawsonia intracellularis* (S), *E. coli* (BC, S)
Florfenicol	Control respiratory diseases (S)
Laidlomycin	Increase rate of weight gain/feed efficiency (BC)
Lincomycin	Increase rate of weight gain/feed efficiency (C, S) Treat and control swine dysentery (*B. hyodysenteriae*) (S), *Lawsonia* proliferative ileitis Reduce severity of mycoplasma pneumonia (S)
Monensin	Prevent coccidiosis (C, T, BC) Increase rate of weight gain/feed efficiency (BC) Increase milk production efficiency (DC)
Neomycin/oxytetracycline[e]	Increase rate of weight gain/feed efficiency (C, T, S) Control infectious synovitis, fowl cholera, and chronic respiratory diseases, air sacculitis (*Mycoplasma* and *E. coli*) (C) Control hexamitiasis (*Hexamita meleagridis*) and infectious synovitis (*Mycoplasma synoviae*) (T) Treat bacterial enteritis, bacterial pneumonia Control colibacillosis (*E. coli*) (S, BC) Control and treat leptospirosis (S) Increase rate of weight gain/feed efficiency (BC) Reduce liver abscesses (BC)

(*Continued*)

Table 1 (*Continued*)

Antimicrobial	Uses/therapies[c]
Penicillin	Increase rate of weight gain/feed efficiency (C, T, S)
Roxarsone[f]	Increase rate of weight gain/feed efficiency (C, T) Treat swine dysentery (*B. hyodysenteriae*) (S)
Sulfadimethoxine/ormetoprim	Prevent coccidiosis (C, T) Aid to prevent infectious coryza (*Haemophilus gallinarum*), colibacillosis (*E. coli*), fowl cholera (*Pasteurella multocida*) (C, T)
Tiamulin	Control of swine dysentery (*B. hyodysenteriae*), proliferative ileitis (*L. intracellularis*) (S)
Tilmicosin	Control of respiratory diseases (S, BC, BD)
Tylosin	Increase rate of weight gain/feed efficiency (C, S) Aid in control of chronic respiratory diseases (C) Control of swine dysentery (*B. hyodysenteriae*) and proliferative ileitis (*L. intracellularis*) (S) Reduce liver abscesses (BC)
Tylosin/sulfamethazine	Lower incidence and severity of atrophic rhinitis (*Bordetella bronchiseptica*) (S) Prevent swine dysentery (*B. hyodysenteriae*) Control bacterial pneumonias (*P. multocida*, *Arcanobacterium pyogenes*) Reduce incidence of jowl abscesses (Group E *Streptococcus*)
Virginiamycin	Increase rate of weight gain/feed efficiency (not used in egg layers) (C, T, S, BC) Prevent necrotic enteritis (*Clostridium perfringens*) (C) Control and treatment of swine dysentery (*B. hyodysenteriae*) (S) Reduce liver abscesses (BC)

[a]Abbreviations: BC, beef cattle; BMD, bacitracin methylene disalicylate; C, chickens; DC, dairy cattle; S, swine; T, turkeys.
[b]Adapted from Feed Additive Compendium 2012 (11). The list is limited to compounds whose spectrum of activity targets microbes; i.e., they have antibacterial or antiprotozoal (e.g., coccidian) properties.
[c]Approved use (amounts and duration) of any drug depends on animal species, body weight (growth stage), age, combination with other drugs, application, and restrictions (withdrawal times before shipping to market).
[d]Not approved for use in poultry egg production; oxytetracycline is approved for similar but fewer applications than chlortetracycline.
[e]Most of the antimicrobials in the table are approved for use in combinations of two or three antimicrobials with different activity spectra and for different applications. For example, tylosin plus sulfamethazine is an approved combination to treat various swine diseases.
[f]Roxarsone is an organo-arsenic compound with currently suspended use due to detection by the Food and Drug Administration of high levels of inorganic arsenic in broiler chicken feed.

Antibiotic enhancement of the nutritional value of animal feeds emerged from research to supplement plant-based diets with microbial products (55, 70). Plant products in feed (soy and corn) were an important accommodation for the war effort to avoid expensive animal protein additives (e.g., fish meal). Plant-based diets, however, lacked essential B vitamins and methionine. Jukes and colleagues (70) at Lederle Laboratories discovered that culture biomass and end products recovered from large-scale production of chlortetracycline (Aureomycin) by *Streptomyces aureofaciens* were as effective as animal liver extracts for enhancing the growth of chicks deficient in vitamin B_{12}. Following a report that streptomycin or sulfathiazole (Sulfasuxidine) enhanced the growth rate of chick poults (103), purified aureomycin and penicillin were found to have growth-enhancing effects on chicks and pigs (70, 91).

The commercial benefits of enhancing animal feed efficiency led to a flood of patent applications for antibiotics for that purpose. Noteworthy applications include chlortetracycline mash by American Cyanamid (69); penicillin by Merck (108); oxytetracycline by Pfizer (116); kanamycin by Bristol (19); spiramycin by Rhone-Poulenc (126); tetracycline, sulfonamide, and penicillin

combination by American Cyanamid (58); and quinoxaline dioxides (carbadox, Mecadox) by Pfizer (117). Ionophore antibiotics were found to increase the feed efficiency of foregut animals (sheep, goats, beef cattle) and led to a patent for monensin, dianemycin, and nigericin by Eli Lilly and Company (123). A valuable resource for locating agricultural antibiotic patent information is the Espacenet website (**http://worldwide.espacenet.com/advancedSearch?locale=en_EP**).

THE ANTIBIOTIC GROWTH EFFECT

Daily feeding of performance-enhancing antibiotics to farm animals was rapidly and broadly implemented, creating steady economic returns for pharmaceutical companies and savings in feed costs for animal producers. Despite the benefits, the precise mechanism of growth promotion has remained elusive. Proposed mechanisms for the antibiotic growth effect (AGE) as mediated through the intestinal microbiomes of the animals include (*a*) reduction of growth-depressing microbial metabolites, (*b*) reduction of microbes competing for host nutrients, (*c*) inhibition of subclinical infections, and (*d*) enhanced uptake of nutrients through thinning of the intestinal walls. Numerous studies of performance-enhancing antibiotic effects on animal intestine functions and intestinal microbiomes have been performed (35, 48, 135). The first three of the proposed AGE mechanisms have support from microbiological studies.

Some early studies of the AGE effect included animals afflicted with respiratory and digestive diseases of unknown etiology (13, 25). Consequently, it is possible that performance antibiotics suppressed subclinical infections in those studies and may continue to do so today under suboptimal management conditions. Subclinical infections are immunologically and metabolically costly to hatchling or postweaning food animals. Some of the growth-enhancing effects of the ionophore salinomycin in poultry might be due to suppression of subclinical infections of *Clostridium perfringens* in the intestinal tract (41, 67). *C. perfringens* strains can cause necrotic enteritis in poultry.

Support for the AGE mechanisms of reduction of growth-depressing microbial metabolites and reduction of microbes competing for host nutrients comes from in vitro and in vivo studies of the ionophore class of antibiotics that is used to treat poultry coccidiosis and enhance performance in ruminants. Beef cattle in feedlots are given dietary ionophore antibiotics, such as monensin, lasalocid, and laidlomycin, to increase feed efficiency by as much as 10%. Ionophores are inhibitory (but not exclusively) for certain gram-positive species (*Firmicutes*). They accumulate in the cytoplasmic membranes of sensitive bacteria, dissipating ion gradients and uncoupling ATP hydrolysis from functions essential for cell growth and survival. On a macro level, monensin and other ionophores affect key bacterial populations involved in rumen metabolism, specifically increasing energy available to the animal by shifting reducing equivalents from methane and acetate production toward propionate, a gluconeogenic volatile fatty acid. They affect producers of lactic acid and thus can subdue damaging effects of acidosis. They inhibit amino acid–fermenting bacteria, which deprive the host animal of an important dietary nitrogen source. The combined actions of the manipulation of fermentation stoichiometry, lactate suppression, and protein flow in ruminants through effects on the microbiota provide explanations for the AGE of ionophores (24, 129, 155).

Another AGE possibility is that antibiotics inhibit microbes that metabolize bile acids. Bile acids (steroids) are essential for host lipid metabolism (fat absorption) and are chemically modified by numerous hindgut bacteria (14, 42). Bile acid deconjugation in chicken ileal homogenates was reduced by performance-enhancing antibiotics (42). More recently bile acids have been found to be involved in endocrine and metabolic signaling (22). The possible influence on these activities from bile acid modification by intestinal microbes is yet to be determined.

Antibiotic growth effects have not been detected in germfree poultry and swine and are detectably greater for animals under poor management conditions (28, 153). AGE is associated with multiple antibiotic classes (**Table 1**). Although these observations point more toward direct antibiotic effects on microbial populations, effects on host tissues are worth considering. Niewold (105) has proposed a nonantibiotic, anti-inflammatory mechanism for AGE, namely that performance-enhancing antibiotics accumulate in (intestinal) inflammatory cells and directly inhibit host-damaging inflammatory responses. Collateral effects on host tissues and organs have been noted for sulfonamides and erythromycins, and immunomodulatory effects have been reported for macrolides, lincosamides, β-lactams, and tetracyclines (7, 111).

ANTIBIOTIC RESISTANCE IN FOOD-PRODUCING ANIMALS

Arguably, the greatest impact of antibiotic use on the intestinal microbiotas of food animals has been as a selective force driving the evolution of both antibiotic-resistant bacteria and bacterial subspecies. Antibiotic resistance, however, did not originate as a product of agricultural antibiotic use. Antibiotic resistance is an ancient bacterial trait, existing in soil bacteria (the soil resistome) and carried on plasmids (e.g., serine β-lactamases) millions of years before the dawn of agriculture (3, 6, 95). Phylogenetic analyses led Aminov & Mackie (8) to conclude there are multiple resistance lineages for the naturally occurring antibiotics erythromycin, vancomycin, and certain β-lactams and tetracyclines. Environmental bacteria are the closest progenitor sources of antibiotic resistance genes now found in veterinary and human clinics and prevalent in food animals (8).

Similar to antibiotic-resistant clinical isolates that rapidly appeared in humans (8), antibiotic-resistant bacteria quickly appeared in farm animals receiving antibiotics (35, 136). Streptomycin-resistant coliform bacteria in turkeys fed that antibiotic were reported in 1951 (35). Chickens were found to carry chlortetracycline-resistant *Enterococcus faecalis* strains soon after they were fed that antibiotic (40).

The taxonomic diversity and prevalence of antibiotic-resistant bacteria in and around farm animals fed antibiotics also increased. H.W. Smith estimated that a majority of *Escherichia coli* in British swine herds had become tetracycline-resistant after 18 years of antibiotic feeding (136). Tetracycline-resistant lactobacilli and enterococci were found in pigs on farms feeding tetracycline (47). In a retrospective analysis of 1,729 *E. coli* isolates collected from humans, cattle, chickens, and pigs between 1962 and 2002, Tadesse and colleagues (146) detected significant increases in resistance to 11 of 15 tested antibiotics, including resistances to ampicillin, tetracycline, kanamycin, and sulfonamides. Increases in gentamicin, kanamycin, and trimethoprim/sulfamethoxazole resistances were more common in *E. coli* from animals than in *E. coli* from humans. A recent temporal analysis of agricultural soils in the Netherlands revealed that levels of resistance genes rose over time from the preantibiotic era (1940s) to 2010 (74). Human *Enterobacteriaceae* from culture collections predating the antibiotic era contain conjugative plasmids lacking resistance genes, an indication that now-ubiquitous resistance gene transfer cassettes had not yet evolved (62).

ANTIBIOTICS AND THE EVOLUTION OF ANIMAL INTESTINAL MICROBIOMES

The effect of an antibiotic on a bacterial population or community is dependent on the concentration of the antibiotic. Therapeutic doses of antibiotics are defined for the animals being treated and are designed to achieve concentrations that are inhibitory to bacterial targets. However,

subinhibitory antibiotic concentrations are often experienced by bacteria, either intentionally in subtherapeutic (growth-promoting) uses of antibiotics or unintentionally based on the antibiotic's inability to penetrate biofilms or infiltrate microhabitats. It is unclear which and how many commensal bacteria experience subinhibitory antibiotic concentrations because of the technical challenges of sampling remote gut microhabitats and of detecting low amounts of compound. However, it is likely that antibiotics administered at any level result in subinhibitory concentrations somewhere in the body (12). Indeed, an important early observation was made by famed microbiologist H.W. Smith in comparing antibiotic use to treat disease with antibiotic use for growth performance: "There is no essential difference between the emergence of resistant strains of bacteria as the result of the use of antibiotics in the treatment of clinical disease and as a result of their use as feed [performance] additives. . . When antibiotics are used in the treatment of clinical disease the pressure is high but of short duration and when they are used as feed additives the pressure is lower but of longer duration" (136). The antibiotic revolution unleashed an antibiotic resistance evolution.

Both phylogenetic analyses of antibiotic resistance genes and analyses of bacterial genome contents point to horizontal gene transfer events (**Figure 1**) as the basis for the widespread and rapid distribution of antibiotic resistance genes among host-associated (especially intestinal) bacteria (8, 115, 157). The conduit mechanisms for resistance gene transfer among studied intestinal bacteria are largely plasmids and integrative and conjugative elements (115, 130, 161). Subinhibitory concentrations of antibiotics have been shown to induce the transfer of antibiotic resistance genes carried on these elements, such as transfers of erythromycin in *Lactobacillus plantarum* (43) and tetracycline in *Bacteroides thetaiotaomicron* (137).

Horizontal gene transfer is not limited to exchanges among strains of bacterial species. Intergeneric transfers of antibiotic resistance elements have been experimentally demonstrated in turkeys (118) and in swine (18). Species from several genera of gram-positive bacteria in chicken litter were found to carry class I integrons, genetic elements traditionally associated with antibiotic resistance in gram-negative *Enterobacteriaceae* spp. (104). Postulated taxonomic barriers to horizontal gene transfer among intestinal bacteria seem to have been overcome or reduced or never to have existed. This could be due to high population densities of diverse bacteria in close proximity, including exchanges between cross-feeding metabolic synergists.

Other mediators of horizontal gene transfer are bacteriophages (phages) and gene transfer agents (GTAs). The results of metagenomic studies in swine and mice suggest that certain oral antibiotics increase phage activities in the gut (5, 102). Ampicillin, penicillin, ciprofloxacin, and carbadox are among those antibiotics shown to modulate phage activities, including the transfer of antibiotic resistance genes. In *Streptococcus* spp., β-lactam antibiotics were shown to weaken the cell wall and increase susceptibility to lysis by exogenous phages (152). Ciprofloxacin induces prophages in *Clostridium difficile* (99), and carbadox induces prophages in *E. coli* (77), *Salmonella enterica* (15), and a prophage-like GTA in *Brachyspira hyodysenteriae* (141). A notorious consequence of prophage and GTA induction is gene transfer, which promotes both the transfer of antibiotic resistance genes and pathogen evolution (20, 141).

In addition to the instant effects on gene transfer, subinhibitory antibiotic concentrations have evolutionary effects on bacterial populations. This is due to increased mutation rates and nonlethal selective pressure for beneficial mutations (10). The consequences of this can be diversification of bacterial populations (50) and selection for multidrug resistance (46, 68, 76), both of which have been established in laboratory experiments with *Pseudomonas aeruginosa*.

Additionally, a largely unstudied bacterial diversity at the subspecies level seems to exist within intestinal microbiomes, undetectable by high-throughput DNA sequencing techniques.

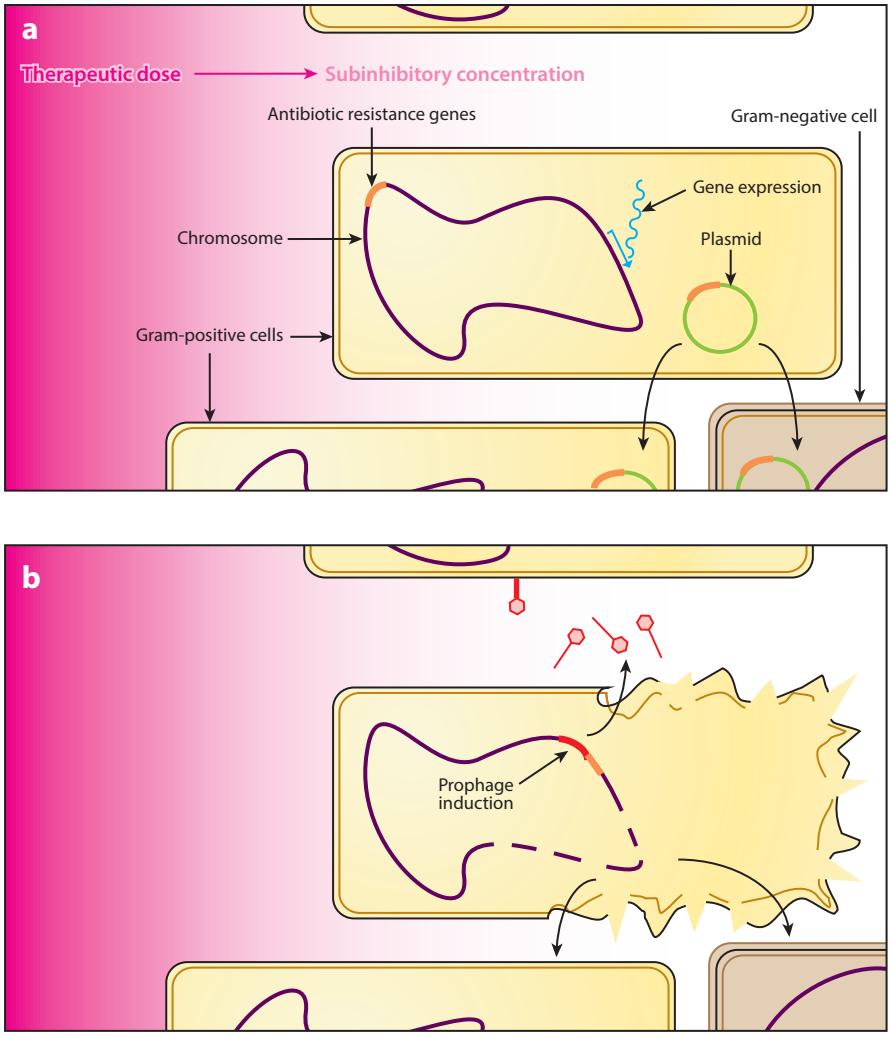

Figure 1

Effects of subinhibitory antibiotic concentrations on intestinal bacteria. Bacteria (*yellow* rectangles represent gram-positive cells with *purple* chromosome; *tan* rectangle represents a gram-negative cell) living in various microhabitats of intestinal ecosystems are likely exposed to subinhibitory antibiotic concentrations (*light pink*) even when antibiotics are administered at therapeutic doses (*dark pink*). (*a*) Subinhibitory concentrations of antibiotics select for antibiotic resistance genes (*orange*), stimulate horizontal gene transfer (e.g., *green* plasmid), and induce gene expression (*blue*). (*b*) Prophage induction (*red*) is another effect of subinhibitory antibiotic concentrations. Phages mediate bacterial evolution by transferring fitness genes and promote nutrient turnover by lysing host bacteria in the intestinal ecosystem.

Subspecies diversity is enhanced through horizontal gene transfer, enabling bacteria to adapt to their existing environment and to invade other environments (157). Intestinal bacteria ranging from commensals (*Megasphaera elsdenii*, 139) to pathogens (*S. enterica*, 114; and *C. perfringens*, 75) have shown subspecies differences extending to antibiotic resistance profiles. Evolution through recombination of antibiotic resistance genes (mosaicism) also contributes to intestinal subspecies diversity (17, 140, 151).

PERSISTENCE OF ANTIBIOTIC RESISTANCE

Antibiotic-resistant bacteria exist and stably persist in the environment, including in animal intestinal ecosystems. Even animals on farms where antibiotic use has been curtailed continue to harbor common antibiotic resistance genes (1, 88, 142). Numerous explanations have been provided for this persistence of resistance (9, 131, 138). Among these are bacterial subspecies diversity, coselection of clusters of fitness genes, and stimulation of horizontal gene transfer by subinhibitory antibiotic concentrations. The persistence of resistance in commensal bacteria creates a reservoir for both bacterial pathogens of animals and human foodborne pathogens originating from animals to acquire resistance genes under selective pressure (46, 157). Furthermore, antibiotic-resistant bacteria from farm animals are shed into the environment (66). The environment provides an opportunity for bacteria from disparate sources to comingle, mixing agricultural and anthropogenic sources of resistance genes in watersheds, for example (119). The persistence and dissemination of resistance genes in the environment complicates efforts to determine the direction of antibiotic resistance dissemination (animals to humans or vice versa).

COLLATERAL EFFECTS OF ANTIBIOTICS ON GUT BACTERIA

A healthy microbiota is critical to host health, and microbiota contributions can be influenced by antibiotic exposure (56, 101, 106, 113). Antibiotic disruption of commensal microbiomes can remove a protective barrier, leaving the host susceptible to colonization by pathogens and especially ingested pathogens (112, 143, 144, 150, 158, 159). Well-known examples of antibiotic-impaired colonization resistance are the cephalosporin-induced *C. difficile* infections and the streptomycin-treated mouse model for *Salmonella enterica* Chronic *C. difficile* infections respond to colonic microbiome repopulation with fecal bacteria taken from healthy donors (109, 127, 128). The human commensal species susceptible to antibiotics and antagonistic to *C. difficile* have not as yet been identified, although members of the *Lachnospiraceae* family could play a role (124). Unlike *C. difficile* in humans, the mouse intestinal microbiota is intentionally disrupted by streptomycin to create an *S. enterica* serovar Typhimurium infection model (121). In this model, volatile fatty acids (acetic, propionic, butyric, valeric acids) were identified as inhibitory to *Salmonella* spp. at cecal pH values (120). Neither the species that protect nor the mechanisms that cause colonization resistance are well studied. In general terms, either direct effects (intermicrobial competition for habitats or niches or suppression by chemical/molecular weapons), indirect effects (host immune suppression or activation of immune responses), or a combination of both could be involved (81).

In addition to imbalances in the gut microbial community, subinhibitory antibiotic concentrations evoke a wide variety of unintended effects on bacteria themselves that expand well beyond those of antibiotic resistance gene transfer and evolution. These effects have long been appreciated at least morphologically, and over the years they have been further defined. Gene expression experiments revealed that most antibiotics, including rifampicin, erythromycin, and tetracycline, at subinhibitory concentrations, modulate bacterial gene expression (32, 51, 86). Additionally, virulence genes are among those that are commonly found to be upregulated by subinhibitory antibiotic concentrations in pathogenic bacteria. This has been shown with tetracycline and quinolones in *S. enterica* serovar Typhimurium (16, 163) and tobramycin and tetracycline in *P. aeruginosa* (86).

A NEW ERA OF ANTIBIOTIC USE

Antimicrobial use in agricultural animals has been driven by farm management practices. Concerns about the collateral effects of antibiotics, particularly surrounding antibiotic resistance gene

evolution, spread, and persistence, have been mounting for decades. Restrictions on antibiotics used for performance enhancement in food animals have been implemented in some countries and have been recommended in the United States (44). Judicious antibiotic use encompasses both an awareness of collateral effects and a cost-benefit analysis of antibiotic uses by veterinary and medical practitioners (83). Antibiotic alternatives in farm management deserve greater scientific scrutiny so as to preserve our ability to treat infectious bacterial diseases (4, 23), and national strategies to discover, develop, and adopt effective antibiotic alternatives for agriculture and human use should be encouraged (83, 138).

Little progress has been made in understanding AGE mechanisms at the microbiota level, particularly in hindgut-fermenting animals. This is likely due to the complexity of the intestinal microbiome as well as the research challenge of linking (statistically significant) animal performance measurements with retrospective and dynamic intestinal microbiota effects. The advent of -omics technologies for analyzing total DNAs, RNAs, proteins, metabolites, and bacteria will provide a better glimpse of microbiota activities at localized intestinal sites. For example, a high-throughput analysis of the effect of multiple antibiotics on the human fecal microbiota showed that the number of damaged cells increased whereas the number of active cells stayed the same (96), suggesting that there could be a relative increase in the turnover of microbial-derived small molecules and cellular subunits in the large intestine. *Firmicutes* were more severely affected by antibiotics than were other bacterial phyla, suggesting that the primary fermenters of polysaccharides (*Bacteroidetes*) remain functional in the bacterial food chain of the large intestine. In addition to the potential direct effect of antibiotics on interbacterial nutrient exchange, the antibiotic induction of prophages indirectly contributes to microbial nutrient cycling and merits further investigation. Improved understanding of gut microbial ecology, and in turn of AGE mechanisms, will lead to the design of efficacious alternatives. Discussions found elsewhere in this issue provide data from other fields that could further inform these ideas (38, 71 97, 156).

Perhaps the greatest limitation to understanding and controlling the ecology of the intestinal microbiome to improve food animal health is our currently limited knowledge of the important players and their contributions. But how much has been learned in the last several decades! Traditional culture-based isolations and characterizations of individual gut bacteria (21, 59, 64, 78) provided the foundation for modern, high-throughput census taking (e.g., 16S rRNA gene surveys) and functional analyses (e.g., metagenomics). Molecular technologies revealed gaps and limitations of culture-based approaches by detecting diverse, yet-to-be cultured microbial taxa in animal and human intestinal tracts (107, 122, 149). A recent strategy combining the benefits of both culture and molecular techniques revealed 174 new bacterial species in the human gut (79). Bacterial culturing continues to be required to assign functions to unknown bacteria and genes (82, 89). Combining classical with modern techniques is a powerful strategy to fill gaps in our knowledge of animal intestinal microbiomes, and further interdisciplinary approaches will be essential as we proceed into a new era of antibiotic use in food animals.

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