Alternatives to antibiotics are urgently needed in animal agriculture. The form these alternatives should take presents a complex problem due to the various uses of antibiotics in animal agriculture, including disease treatment, disease prevention, and growth promotion, and to the relative contribution of these uses to the antibiotic resistance problem. Numerous antibiotic alternatives, such as pre- and probiotics, have been proposed but show variable success. This is because a fundamental understanding of how antibiotics improve feed efficiency is lacking, and because an individual alternative is unlikely to embody all of the performance-enhancing functions of antibiotics. High-throughput technologies need to be applied to better understand the problem, and informed combinations of alternatives, including vaccines, need to be considered.

Introduction: the need for antibiotic alternatives

Antibiotics have long been used for treating disease, preventing disease, and improving feed efficiency in conventional livestock and poultry production. Their use was implemented in the 1950s as a way to meet the increasing demand for food. Antibiotics given to pigs were estimated to save as much as 20% of feed per pound of weight gain [1]. Whether the same performance enhancement continues in the present remains unclear [2]. Concurrent with antibiotic use, antibiotic-resistant bacteria were isolated from animals receiving antibiotics from the earliest days. Concerns quickly arose about the development of resistant pathogens associated with animal and human diseases, as well as increases in the antibiotic resistance gene pool in commensal bacteria, but the risk was outweighed by the benefits of reduced cost to the industry [3]. In addition to improving feed efficiency, antibiotics in agricultural animals are used to improve animal welfare, and so there must be a balance between antibiotic use and preserving antibiotic efficacy for both human and animal health. Sixty years later, the debate continues in the USA and abroad. Concerns over the spread of antibiotic-resistance genes to human and animal pathogens continue to drive the debate [4].

European nations have implemented bans on the use of growth-promoting antibiotics, and the practice in the USA is under increasing regulatory and political scrutiny. The Center for Veterinary Medicine of the US Food and Drug Administration (FDA) recently issued a ‘Guidance for Industry’ that describes requirements for label claims and recommended restrictions on uses of antibiotics in food-producing animals [5]. This document outlines voluntary limitations on the use of antibiotics based on the risk assessment of resistance development and on the importance of a given antibiotic to human therapy. The two guiding themes for the risk assessment were that antibiotics should only be used for prevention, control, and treatment of specific animal diseases and a requirement for veterinarian involvement in the decision to use antibiotics. Although the new FDA guidance allows antibiotic use in food-producing animals to control specific diseases, the use of antibiotics for growth promotion, increased performance, and improved feed efficiency will no longer be permitted. Additionally, certain antibiotics of critical importance, such as third-generation cephalosporins, are likely to be restricted to human use in the near future even if they are important for animal disease treatment [6]. This is in part because of the demonstrated potential for veterinary antibiotics (e.g., tylosin) to coselect for resistance to antibiotics of human importance (e.g., vancomycin) [7]. It is important to recognize that the FDA guidelines may lead to more sickness and to an increased demand for therapeutic antibiotic treatment in livestock (as was seen in Denmark [8]). Alternatives to growth-promoting antibiotics are therefore only a fraction of the problem; we also need alternatives for disease prevention and control, and treatment of animals (Box 1).

Challenges of antibiotic alternatives

Alternatives to antibiotics in food-producing animals are urgently needed but present a difficult problem in part because of the complexity of the gastrointestinal (GI) ecosystem. The GI tract is an intricate organization of epithelial cells (the mucosal barrier), the mucosal immune system, and microbiota. The epithelium with its mucus layer separates the microbiota, pathogens, and unfavorable environmental conditions from the host, and is also the main site of nutrient absorption. The GI microbiota competes with intestinal pathogens for nutrients and binding sites, produces
chemical modulators of intestinal health such as butyrate, and influences immune maturation. A healthy microbiota filled with beneficial microbes is certainly important to animal health, but both a healthy microbiota and its converse, dysbiosis, are poorly defined. Metagenomics, metatranscriptomics, and other ‘omics’ technologies provide an opportunity for defining the microbes and microbial activities that compose and maintain a healthy microbiota [9,10]. Of particular importance is the homeostasis between a healthy microbiota and the immune system because the microbiota modulates innate immune responses to prevent barrier dysfunction and regulates the function of adaptive immune mediators [11–13]. In turn, the host exerts immune tolerance, moderates inflammation, and competes with the microbiota for nutrients, all of which incur an energy cost.

Knowledge about the mechanism of how antibiotics enhance animal growth is important to the development of viable alternatives. How antibiotics increase performance is not clear, but possible mechanisms may include a reduction in total bacterial load, suppression of pathogens, thinning of the mucosal layer, and direct modulation of the immune system [14,15] (Figure 1a). Some gut bacteria may decrease the energy cost to the immune system, yielding surplus calories for weight gain. Additional growth-promoting effects of antibiotics could include increased nutrient absorption by the host or bacterial community remodeling in favor of non-antagonistic or beneficial bacteria and functions [16,17]. Defining the effect of antibiotics and alternatives on the host and its microbiota will facilitate the development of efficacious solutions.

The different potential mechanisms of antibiotic growth promotion beget different alternatives (Figure 1b). Using targeted approaches to reduce the carriage of specific pathogens or to alter the host immune response will be important to prevent or reduce disease burden and positively influence growth performance without the collateral effects of antibiotic treatment [18]. If the mechanism is dependent on the microbiota or its interaction with the immune system, then feed additives such as pre- or probiotics are appropriate. If the mechanism of growth promotion is via disease prevention or reduction, then the most appropriate alternatives would be vaccines or health-promoting pre- or probiotics. Below we will discuss some of the advantages and disadvantages of various alternatives to antibiotics in agricultural animals.

Feed additives

The nutritional components of animal feed are continually adjusted to optimize the effects on animal health and growth while being largely dependent on feed input costs. Dietary supplementation may also include prebiotics, probiotics, and organic acids. Prebiotics are selectively fermented components of feed (either inherent or added) that modulate the gut microbiota to benefit host health, such as the competitive exclusion of pathogens or the stimulation of health-promoting metabolites [19]. Primary examples of prebiotics include dietary fibers and oligosaccharides. Like prebiotics, in-feed organic acids can be inherent or added, and they function by decreasing the pH of an environment, limiting feed spoilage, and resulting in lower pathogen survival in the gut [20]. Organic acid delivery ranges from the addition of a single component such as lactic acid to complex blends created by fermentation. Probiotics confer benefits analogous to prebiotics but are living cells such as Lactobacillus, Streptococcus, Bifidobacterium, Bacillus, and yeasts [21,22]. Traits important to a probiotic strain include being nonpathogenic, resistance to stomach acids and bile, having the potential to colonize the host, production of nutrients, being free of antibiotic resistance genes or having reduced gene transfer functions, and antagonism of pathogens.

The potential for the above additives to replace antibiotics is well established, and numerous pre- and probiotic products are commercially available and in active use [23–25]. However, the true efficacy of pre- and probiotics in agricultural animals remains unclear because of inconsistent experimental results [26,27]. Explanations for the disparities between studies include differences in experimental conditions, animal age, genetics, and health status. Additionally, the inconsistent results could be attributed to a lack of understanding of the mechanism of action for either pre- or probiotics, as well as unknown interactions among these products, the host, and the GI microbiota. For example, there have been studies that quantify some aspects of the GI microbiota in response to probiotics [28]. However, the methods applied to the microbial community in those studies have not been able to fully characterize the community,
leaving the true effect on the microbiota by the probiotic (and vice versa) largely unknown. Thorough study of the changes in the microbiota and host responses to feed additives using next-generation sequencing technologies combined with systems biology approaches will greatly advance this field.

**Phage therapy**

An additional antibiotic alternative that has enjoyed renewed traction is bacteriophage (phage) therapy. Phage therapy involves the use of bacterial viruses (phages) to attack a specific bacterium or narrow group of bacteria with the advantage over antibiotics being that autochthonous bacteria are unharmed and no dysbiosis occurs [29]. The success of phage therapy is dependent on numerous factors. Phages have a narrow bacterial host range and do not target multiple bacterial pathogens, so the efficient use of phage therapy requires the identification of the pathogen or at least a high suspicion of their presence. It is most efficacious when the bacteria being treated are readily accessible, such as the historical treatment of dysentery [29] or the modern treatment of burn wounds [30]. In addition to being accessible, the numbers of target bacteria need to be high. Experiments using lytic phages to counter the foodborne pathogen *Salmonella enterica* serovar *Typhimurium* in chickens [31] and pigs [32] have reduced but not eliminated the *Salmonella* load. One confounding factor was that the inoculated phage only persisted in the gut as long as *Salmonella* remained abundant [31]. Also, therapy is most effective when phages are administered soon after bacterial infection. The seminal work of H.W. Smith and colleagues showed that K1 phages injected intramuscularly are 100% effective at curing mice of *Escherichia coli* O18ac:K1:H7 ColV+ infections when injected immediately following bacterial inoculation [33]. The efficacy of the phage treatment was lost, however, when phages were administered 16 h after infection, thus limiting phage therapy to prophylactic or immediate-treatment situations. Another reason why the efficacy of phage therapy needs constant monitoring is that the host immune response may neutralize phages (although this probably only occurs after repeated treatment) [34]. Finally, concern over the target bacteria becoming resistant to the phage often necessitates the generation and administration of phage cocktails [29]. The somewhat boutique nature of phage therapy – requiring specific, accessible, and abundant target bacteria and administration soon after infection – continues to challenge its adoption as a viable antibiotic alternative in Western countries [35].

In addition to the technical challenges, the biological and evolutionary consequences of phage therapy need to be considered. For example, it is important to avoid temperate phages for therapeutic application because of the potential for transfer of virulence or antibiotic resistance genes from the phage to the host bacterium, although even obligate

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**Figure 1.** Antibiotics and their alternatives have many effects on the gut microbiome. Shown is a schematic representation of a longitudinal section of the gut, with the lumen in the center (brown shading), surrounded by the mucosa (epithelium is dark pink, lamina propria is light pink) containing immune cells (red and green nucleated ovals). (a) Antibiotics exert positive (arrows) and negative (bars) effects on a variety of factors in the gut: they can inhibit the mucosal immune system (green nucleated cells), inhibit pathogens (yellow rods), or modulate the microbiota by stimulating some members (red cocci) while inhibiting others (dark blue cocci), or all of the above. (b) A potentiated prebiotic is presented as an example of mixed additives, an approach that might be the most comprehensive alternative to antibiotics because each separate component (i.e., anti-inflammatory, prebiotic, and vaccine) replicates a different effect conferred by the antibiotic.
lytic phages harbor genes of unknown function that could also result in undesired gene transfer [36]. One way to avoid this is the use of purified phage gene products such as lysins to selectively kill target bacteria. Phage lysins could be applied to a bacterial infection, particularly on an accessible mucosal surface, and attenuate the infection by lysing the bacteria from without [37]. The discovery and development of novel phage-derived therapeutics could benefit by the application of functional metagenomic analyses, which are a high-throughput way of bioprospecting for functions of interest such as phage lysins [38].

**Vaccines**

Vaccines are an underappreciated antibiotic alternative despite the availability of many effective vaccines and a general understanding of vaccine immunology. This is compared with other proposed alternatives such as prebiotics or probiotics where limited mechanistic information is wrought with highly variable efficacy. Based on the more comprehensive understanding of immune responses and protection, vaccines should be a promising antibiotic alternative for reducing the burden of animal diseases and human pathogens in food-producing animals. Additionally, it is important to note that vaccination could also reduce the use of therapeutic antibiotics because of the reduction in clinical infections. As an example, vaccination against the swine pathogen *Lawsonia intracellularis* reduced the need for therapeutic oxytetracycline administration in Danish pigs [39]. Similar decreased need for therapeutic antibiotics might also be anticipated following widespread adoption of vaccines for other pathogens.

Broad discussion of all possible vaccines targeting animal or foodborne pathogens is beyond the scope of this manuscript due to the specificity of host–antigen interactions. We will therefore consider potential vaccines for one example—the immediate post-weaning period in swine—because it is likely to be a time during which growth-promoting antibiotics are most effective at bacterial disease reduction [40]. Major enteric pathogens that cause disease problems and production losses during the post-weaning and later periods in swine include enterotoxigenic and shigatoxigenic *E. coli* (the causative agent of post-weaning diarrhea and edema disease), *Brachyspira hyodysenteriae* (the causative agent of swine dysentery), *L. intracellularis* (the causative agent of porcine proliferative enteropathy), and *Salmonella enterica* serovar Choleraesuis (the causative agent of systemic salmonellosis in swine). Oral vaccination of weaned pigs with live attenuated bacterial vaccines is thought to be the most effective approach for reducing enteric diseases in swine. Oral live vaccination is the strategy for the commercial vaccines available for the reduction of *L. intracellularis* [41] and *S. Choleraesuis* [42]–associated diseases, but the promise of this approach for swine pathogenic *E. coli* has not progressed to commercial products (e.g., [43]). Efficacious parenterally administered toxoid or adhesin subunit vaccines against experimental *E. coli* infections have been reported but have not been commercialized (e.g., [44]). Experimental vaccines against *B. hyodysenteriae* have been reported as subunit vaccines as well as whole cell bacterins, but evaluation and efficacy data are limited (e.g., [45]). Development of effective vaccines to prevent disease and associated production losses during the post-weaning period should be a priority in the search for replacements for growth-promoting antibiotics. Acceptance and widespread use of vaccinations as alternatives to antibiotics will depend on cost and ease of use. Cost comparisons may be difficult, but administration of live oral vaccines in feed or water could be comparable to administration of antibiotics by these routes.

**Mixing additives: potentiated probiotics and synbiotics**

Combinations of antibiotic alternatives hold the promise of potentiating each other’s efficacy and duplicating the effect of in-feed antibiotics (Figure 1b). The term potentiated probiotics refers to such combinations of probiotics with other additives (e.g., vaccines or organic acids) with the goal of synergistically increasing the effect of the probiotic [23,46]. For instance, it is possible that a probiotic that only confers gastrointestinal health benefits could support the growth of, and be simultaneously delivered with, a probiotic that competitively excludes a potential pathogen. The most common pairing that has been tested is probiotics with probiotics, and this combination is termed synbiotic. Like studies utilizing probiotics or prebiotics individually, synbiotic studies have found inconsistent results, with some studies reporting gains in animal performance or decreases in food borne pathogens (reviewed in [26]), but others have not (e.g., [28]). Other combinations such as probiotics and vaccines for food safety have rarely been tested, but a combination of competitive exclusion cultures and a *Salmonella* vaccine resulted in a greater protective effect than either treatment alone [47]. Another attempted approach was a probiotic *E. coli* that produced a microcin that can inhibit growth of *Salmonella*, but *in vivo* experiments were unsuccessful at reducing *Salmonella* shedding [48]. A better understanding of the effects and mechanisms of action of the various components, as enabled by high-throughput sequencing, will allow for more rational potentiated probiotic designs, guiding the selection of antibiotic alternatives that best complement each other and best replicate the effect of growth-promoting antibiotics.

**Concluding remarks**

No ‘magic bullet’ alternative exists to cover the spectra of antibiotic classes and antibiotic uses in agricultural animals. Alternatives such as vaccines or bacteriophages, although limited to the control of specific bacterial species or strains, benefit from not having antibiotic side effects of perturbing entire microbial populations. Vaccine combinations or phage gene products would yield a broader bacterial target range. Interdisciplinary translational research emphasizing all three components of host health – gut microbiota, intestinal physiology, and immunity – holds promise for discovering antibiotic alternatives (Box 2). This approach is now feasible through new technologies allowing integrated research to simultaneously examine genomes, metagenomes, transcriptomes, and proteomes. As with any animal management approach, a significant challenge for antibiotic alternatives will be low cost per animal, and this challenge should diminish as demand increases. Despite the obstacles, many alternatives have been proposed and productive collaborations among biochemists, microbiologists,
Box 2. Considering alternatives in the context of host-microbe evolution

The effect of antibiotics and their alternatives on an animal and its gut microbiota is usually examined before, during, and after antibiotic administration. However, evolutionary factors are worthy of consideration, such as the vertical transmission of the hologenome (the combined genetic information of the host and its microbiota). It is important to assess the impact of any antibiotic treatment or alternative in terms of future outcomes (e.g., subsequent generations) in addition to immediate outcomes (e.g., disease prevention, increased weight gain, etc.).

The homeostatic symbiotic relationship between hosts and their microbiota is an ancient product of a long co-evolutionary process, and it appears to be vertically transmitted (49–51). This vertical transmission is tied to evolution because although selection acts on individual genes (both host and microbial), gene selection is influenced by ecological forces such as interactions among microbes and host factors (52). Host genetics, by shaping the microbial community (51), and ecological forces such as antibiotics and their alternatives combine to influence host-microbial interactions. One theory of evolution, the hologenome theory, is notable in its inclusion of both the host and its microbial community. The hologenome theory considers the holobiont (the host and its microbiota), acting in concert with its total combined genetic information (the hologenome), as a unit of selection in evolution (49).

In the context of the hologenome theory of evolution, it is possible that some of the desired effects of antibiotics are perhaps being vertically transmitted in the microbiota or the host or both, and therefore maintained by the holobiont without continued antibiotic application. The influence of modern production practices, such as directed breeding, on this vertical transmission is unclear. It is additionally unknown whether or not the relatively short history of antibiotic use is sufficient time for an evolutionary change to be detected, but it is tempting to speculate that at some point the holobiont could inherit the benefits of antibiotic treatment and that these benefits would continue in the absence of antibiotics. If that is the case, then the search for alternatives in agriculture animals should focus on maintaining the evolutionary changes brought about by antibiotics in addition to replicating other effects of antibiotics.

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References

3 Agriculture Board, National Research Council (1966) Proceedings, First International Conference on the Use of Antibiotics in Agriculture, National Academy of Sciences
22 Guo, X. et al. (2008) Screening of Bacillus strains as potential probiotics and subsequent confirmation of the in vivo effectiveness of Bacillus subtilis MA139 in pigs. Antonie Van Leeuwenhoek 90, 139–146