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Alternatives to antibiotics: a symposium on the challenges and solutions for animal production

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Abstract

Antibiotics are one of the most important medical discoveries of the 20th century and will remain an essential tool for treating animal and human diseases in the 21st century. However, antibiotic resistance among bacterial pathogens and concerns over their extensive use in food animals has garnered global interest in limiting antibiotic use in animal agriculture. Yet, limiting the availability of medical interventions to prevent and control animal diseases on the farm will directly impact global food security and safety as well as animal and human health. Insufficient attention has been given to the scientific breakthroughs and novel technologies that provide alternatives to antibiotics. The objectives of the symposium 'Alternatives to Antibiotics' were to highlight promising research results and novel technologies that could potentially lead to alternatives to conventional antibiotics, and assess challenges associated with their commercialization, and provide actionable strategies to support development of alternative antimicrobials. The symposium focused on the latest scientific breakthroughs and technologies that could provide new options and alternative strategies for preventing and treating diseases of animals. Some of these new technologies have direct applications as medical interventions for human health, but the focus of the symposium was animal production, animal health and food safety during food-animal production. Five subject areas were explored in detail through scientific presentations and expert panel discussions, including: (1) alternatives to antibiotics, lessons from nature; (2) immune modulation approaches to enhance disease resistance and to treat animal diseases; (3) gut microbiome and immune development, health and diseases; (4) alternatives to antibiotics for animal production; and (5) regulatory pathways to enable the licensure of alternatives to antibiotics.

Keywords: antimicrobials, immune modulation, gut microbiome, antibiotic growth promoter, pre/probiotics, antibiotic resistance, feed additives, One Health, lytic enzymes, bacteriophage, antimicrobial peptides, phytochemicals

Introduction

There is worldwide concern over the present state of antimicrobial resistance (AMR) among zoonotic bacteria that potentially circulate among food-producing animals including poultry, beef and dairy cattle, goats, sheep and aquaculture (Gyles, 2008; Prescott, 2008). This has resulted in the general public's perception that antibiotic use by human beings and in food animals selects for the development of AMR among food-borne bacteria that could complicate public health therapies (DuPont, 2007). A major issue is that AMR may not only occur among disease-causing organisms but has also become an issue for other resident organisms in the host (Yan and Gilbert, 2004). Although antibiotic growth promoters (AGPs) have

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been successfully utilized during food-animal production since their efficacy was first described during the 1940s, the exact modes of action are not fully understood and are probably multi-factorial (Gaskins *et al.*, 2002; Dibner and Richards, 2005; Niewold, 2007). Sub-therapeutic use of antibiotics as growth promoters in animal feeds was discontinued in the European Union (Regulation EC No. 1831/2003 of the European parliament and the council of 22 September 2003 on additives for use in animal nutrition; Castanon, 2007). The concern over AMR and use of AGPs may be justified with increasing incidences of antibiotic resistance among bacterial pathogens (NAS, 2006; Gyles, 2008; Prescott, 2008) including bacteria from healthy animals (Persoons *et al.*, 2010). Consequently, there is a need for developing novel intervention methods including narrow-spectrum antimicrobials and probiotics that selectively target pathogenic organisms while avoiding killing of beneficial organisms (NAS, 2006).

However, AGP bans have had a negative impact on animal health and productivity in some countries (Casewell *et al.*, 2003). Therefore, reducing AGPs creates challenges for the animal feed and feed additive industries. Effective alternatives to AGPs are urgently needed to help maintain current animal production levels without threatening public health and this should stimulate new research (Millet and Maertens, 2011).

Because of the need for alternative or novel approaches to conventional antibiotics (NAS, 2006; Lloyd, 2012), a symposium entitled 'Alternatives to Antibiotics: Challenges and Solutions in Animal Production' was hosted by The World Organisation for Animal Health (formerly the Office International des Epizooties, or OIE) in Paris, France on 28–29 September 2012. This meeting focused on novel antimicrobials for animal production, animal health and food safety (http://www.ars.usda.gov/alternativestoantibiotics/). There were five principal subjects that included: (1) alternatives to antibiotics, lessons from nature; (2) immune modulation approaches to enhance disease resistance and to treat animal diseases; (3) gut microbiome and immune development, health and diseases; (4) alternatives to antibiotics for animal production; and (5) regulatory pathways to enable the licensure of alternatives to antibiotics. Although the symposium focused primarily on technologies that could potentially lead to new options and alternative strategies for preventing and treating diseases of animals, some of the new technologies could also provide the means for a 'One Health' approach (http://www.onehealthinitiative.com/) and could have direct applications as medical interventions for human health and food safety.

Session 1: alternatives to antibiotics, lessons from nature

The observation of the antagonistic effects that one microbe can exert on another led to the discovery of antibiotics, such as penicillin produced by *Penicllium notatum*, followed by isolation of actinomycin and streptomycin that resulted in tremendous successes for treating human and animal diseases caused by bacteria (Demain, 2006, 2009). Consequently, the discovery of additional antimicrobials from nature could potentially lead to even more wide-ranging novel medical interventions alternatives to conventional antibiotics (Joerger, 2003). Gene-encoded natural antibiotics that have gained recent attention include host-derived antimicrobial peptides (AMPs) such as defensins and cathelicidins that provide a protective response against bacterial infection and are a principal component of innate immunity in vertebrates (Sang and Blecha, 2008). For instance, antimicrobial activities of porcine host defense peptides (HDPs) are a large group of innate immune AMPs that possess antibacterial activity (Sang and Blecha, 2009). Cathelicidins are HDPs that were first described in mammals and are also found in birds that exhibit both antimicrobial and immunomodulatory activities (van Dijk *et al.*, 2009, 2011) that could potentially be used to control pathogens such as *Campylobacter jejuni* (van Dijk *et al.*, 2012). Other peptides such as lactoferricin B (LfcinB), a 25-residue peptide derived from the N-terminal domain of bovine lactoferrin (bLF), and synthetic derivatives of this peptide cause depolarization of the cytoplasmic membrane in susceptible bacteria and have antimicrobial activity (Liu *et al.*, 2011). AMPs, such as cecropins (Boman *et al.*, 1991) and magainins (Zasloff, 1987) are produced by insects and amphibians, respectively, while bacteriocins produced by lactic acid bacteria (LAB) generally function to suppress competitor species that are primarily active against other Gram-positive bacteria (Cotter *et al.*, 2005) that could be used to control deleterious bacteria.

Prebiotic and probiotic approaches entail the use of microbial food supplements that beneficially affect the host by improving its intestinal microbial balance (Gibson and Roberfroid, 1995). Dietary administration of spore-forming bacteria can be applied so that the natural balance of an animal gut microflora can be restored and returned to its normal nutrition, growth and health status (Fuller, 1989). Investigators have used the term 'synbiotic' to describe the use of probiotic and prebiotic mixtures that may have beneficial effects on animal or human gastrointestinal systems (Kolida and Gibson, 2011). These approaches have been utilized during food-animal production to improve health but there remains a need to assess their effectiveness and mechanisms of action (Huyghebaert *et al.*, 2011; Kenny *et al.*, 2011). Dietary administration of mannanoligosaccharides (MOSs) induced changes of gut morphology and lowered the pH of excreta reflecting a reduced bacterial challenge in the intestine of pigeons, and therefore, MOS has potential as a prebiotic strategy in birds (Abd El-Khalek *et al.*, 2012). Probiotic bacteria have a positive effect on gastrointestinal function, such as newly described bacterium isolated

from the cecum of broiler chickens, *Butyricicoccus pullicaecorum*, which was reported by Dr Richard Ducatelle (Eeckhaut *et al.*, 2008). Patients with inflammatory bowel disease have lower numbers of *Butyricicoccus* bacteria in their stools and oral administration of this bacterium improved gastrointestinal epithelial barrier function, indicating the bacterium may be a useful probiotic (Eeckhaut *et al.*, 2012). Yeast species have also been used as probiotics (Hatoum *et al.*, 2012) and for delivery of enzymes in animal feeds (Beg *et al.*, 2001; Haefner *et al.*, 2005). Consequently, development of genetically engineered yeast and bacterial cells expressing antibacterials may have potential as probiotics (Biliouris *et al.*, 2012).

Bacteriophages have been utilized as treatments for bacterial diseases in Eastern Europe (Sulakvelidze, 2005), and there are reports of successful use of bacteriophages in poultry (Atterbury *et al.*, 2007) and of early work in cattle (Smith *et al.*, 1987), but much remains to be done to convince the pharmaceutical industry in Europe or North America that the approach is effective (Pirnay *et al.*, 2011; Brüssow, 2012). A bacteriophage cocktail that targets *Listeria monocytogenes* contaminants on ready-to-eat (RTE) foods containing meat and poultry products was granted approval during 2006, which was the first time that the US Food and Drug Administration (FDA) accepted the use of a bacteriophage preparation as a food additive (Bren, 2007). Preparations of bacteriophages are commercially available in many parts of the world and Dr Kim Jae-Won from South Korea presented the use of bacteriophage applications to reduce mortality in poultry due to *Salmonella* Gallinarum and Pullorum. Another report at the conference included the use of a lytic phage to treat a fatal neonatal meningitis *Escherichia coli* infection of rats (Pouillot *et al.*, 2012). An important extension to bacteriophage therapy is the use of phage lytic enzymes (PLEs) that digest the bacterial peptidoglycan, especially of Gram-positive bacteria, as a novel class of alternative antimicrobials (Fischetti, 2008; Schmelcher *et al.*, 2012a). Dr David Donovan reported that PLEs can be applied externally and have a variety of biochemical activities that can be fused into recombinant chimeric molecules that synergistically retain their parental activities to digest bacterial cell walls thereby avoiding resistance development (Schmelcher *et al.*, 2012b). Many of these enzymes are highly species-specific (Simmons *et al.*, 2010) and their cell wall binding (CWB) domains can also be used for bacterial detection systems (Schmelcher *et al.*, 2010).

Although there initially may be concerns over using recombinant DNA produced enzymes as feed additives for food production animals, recombinant synthesized enzymes such as phytases and carbohydrases are commercially produced and sold for feed additive purposes during monogastric food-animal production (Adeola and Cowieson, 2011). Proteases added to broiler feed were reported to have a beneficial effect by increasing the feed conversion ratio and lowering levels of *Clostridium perfringens* in the ileum (Buttin *et al.*, unpublished data). There are a wide variety of enzymes marketed commercially for poultry feed additives, many of which are produced as recombinant proteins in yeast commercially and sold as a lysate, which argues for the economic feasibility of further developing enzyme additives (see http://www.dsm.com/en_US/html/dnpna/anh_enzymes_home.htm; http://www.ublcorp.com/; http://biosciences.dupont.com/industries/animal-nutrition/enzymes/; http://www.novozymes.com/en/solutions/agriculture/animal-nutrition/). Production of enzymes by *Pichia pastoris* can serve as a potential source for biochemical or animal feed studies (Johnson *et al.*, 2010) and dietary use of encapsulated lysozyme (Zhong and Jin, 2009), as a feed additive in the diet of chickens significantly reduced the concentration of *C. perfringens* and gastrointestinal lesions due to the organism in the ilium (Liu *et al.*, 2012a). Interestingly, xylanase added to a wheat-based diet alleviated the pathological effects of *C. perfringens* in broiler chickens (Liu *et al.*, 2012b).

Phytogenic feed additives comprise a wide variety of herbs, spices and products derived from these materials that include essential oils have proven to benefit food-animal production (Windisch *et al.*, 2008; Wallace *et al.*, 2010). Following immunization and infection with *Eimeria tenella*, chickens fed phytonutrient-supplemented diets showed increased body weights, higher antibody levels and greater lymphocyte proliferation compared with non-supplemented controls (Lee *et al.*, 2011). At the conference it was reported that pyrosequencing was utilized as an improvement over manual counting of fecal oocysts to demonstrate reduced *Eimeria maxima* in the gastrointestinal system of broiler chickens following feeding of phytonutrients (Lillehoj *et al.*, unpublished data). More specifically, allyl methyl sulfide (AMS) as a lead compound of volatile garlic metabolites was reported to exhibit an antibacterial effect against the swine pathogen *Actinobacillus pleuropneumoniae* that also resulted in reduced pathology from disease (Becker *et al.*, 2012). Organic acid feed supplements such as caprylic acid reduced *Salmonella enterica* serovar Enteritidis colonization in broiler chickens and potentially reduced the pathogen's ability to invade intestinal epithelial cells by down-regulating key bacterial invasion genes (Kollanoor-Johny *et al.*, 2012). Also of note is that copper (Cu) and zinc (Zn) at greater than physiological levels have been proposed to be used as alternatives to antibiotics during food-animal production. However, resistance to copper can be conferred by a plasmid-borne transferable copper resistance gene (*tcrB*) reported in *Enterococcus faecium* and *Enterococcus faecalis*, and a higher prevalence of *tcrB*-positive enterococci in piglets fed elevated copper compared to that in piglets fed physiologic copper levels suggests that supplementation of copper in swine diets selected for resistance (Amachawadi *et al.*, 2011).

Session 2: immune modulation approaches to enhance disease resistance and to treat animal diseases

Session 2 of the 'Alternatives to Antibiotics' conference focused on immune modulation of the host and immune-derived therapeutics as approaches to enhance disease resistance and treat infections during food-animal production. As noted, HDPs were first investigated because of their AMP activity, but have since been studied because of their immunomodulatory activities. For example, cathelicidins can activate antigen presenting cells (APCs) stimulating immune responses (Wuerth and Hancock, 2011). In mice, a cathelin-related AMP regulates both B- and T-cell functions during adaptive immune responses (Kin *et al.*, 2011). Interestingly a truncated version of fowlicidin-1, a chicken cathelicidin AMP, was not toxic to eukaryotic cells and protected mice from lethal infections induced by methicillin-resistant *Staphylococcus aureus* (Bommineni *et al.*, 2010). Furthermore, cathelicidins in chickens are expressed in a broad range of tissues, indicating their important role in avian immune defense (Achanta *et al.*, 2012). A novel lymphocyte-derived pore-forming protein, chicken NK-lysin, has cytotoxic activity against invasive sporozoites of *Eimeria acervulina* and *E. maxima*, but exhibited no bactericidal activity (Hong *et al.*, 2008). Identification of NK-lysin from a chicken intestinal cDNA library led to synthetic peptides that had direct killing activity on apicomplexan parasites and could be utilized for protection against coccidiosis during poultry production (Lillehoj, unpublished data). Interleukins (ILs) and interferons (IFNs) are cytokines produced by a variety of cell types that stimulate development and differentiation of cells of the immune system or induce protective responses to pathogens such as bacteria and viruses (Steinbach *et al.*, 2010). An update was presented on using a IL-2-based low-dose treatment that was effective in preventing mastitis in dairy cows (Zecconi *et al.*, 2009), and a replication-defective adenovirus vector expressing IFN-α or porcine GMSF (granulocyte colony-stimulating factor) was capable of reducing symptoms caused by certain viruses (Brockmeier *et al.*, 2009, 2012).

LAB have been utilized as probiotics during food-animal production (Huyghebaert *et al.*, 2011; Kenny *et al.*, 2011) and dietary supplementation with direct fed microbials (DFMs) may result in energy re-partitioning to the immune system with an increase in antibody production (Qiu *et al.*, 2012). Dietary feeding of probiotic-supplemented feed reduced intestinal inflammatory cytokine expression and enhanced growth performance in poultry (Higgins *et al.*, 2011). Furthermore, *Bacillus subtilis* strains may have anti-inflammatory effects in mice reducing symptoms of inflammatory bowel disease that are dependent on immunomodulatory responses (Foligné *et al.*, 2012). Approaches utilizing pathogen-specific antibodies in animal feeds are based on the fact that transfer of avian maternal antibodies from serum to egg yolk can confer passive immunity to embryos and neonates as was observed more than 100 years ago (Klemperer, 1893; Tini *et al.*, 2002). Consequently, passive immunization by oral administration of specific antibodies is a possible approach as an alternative to antibiotic treatment to reduce gastrointestinal pathogens in human beings and animals. Specifically, based on treatment with specific antibodies targeting *E. coli* adherence-associated proteins (Cook *et al.*, 2007), orally administered pathogen-specific antibodies may alleviate enteric diseases. This approach has been taken by using chicken egg-yolk antibodies (IgY) to lower gastrointestinal pathogens in broiler chickens and swine (Maiti and Hare, 2010).

Session 3: gut microbiome and immune development, health and diseases

The gut microbiome and immune development, health and disease during food-animal production were the subjects of the third session. Resident microbes of the gastrointestinal system have become the subject of extensive investigations and it is becoming increasingly recognized that gastrointestinal organisms play important roles in health and disease (Clemente et al, 2012; Honda and Littman, 2012; Hooper *et al.*, 2012; Isaacson and Kim, 2012; Kohl, 2012; Lozupone *et al.*, 2012). The first plenary presentation by Dr Bret Finlay was a report on how intestinal microbiota, particularly during early human infancy, play critical roles in regulating immune responses associated with the development of allergic hypersensitivity and how associations between particular gut microbes and different disease phenotypes, as well as identified immune cells along with their mediator molecules are involved in allergy development. Interestingly, he reported a direct association between the use of antibiotics early in life and the development of increased severity of asthma with age (Russell and Finlay, 2012). Probably more related to food-animal production was the rumen microbiome research presented by Professor R. John Wallace and how the organisms involved with biomass conversion in the rumen may lead to discovery of new enzymes for production of biofuels (Hess *et al.*, 2011) as well as the importance of the rumen microbiome to health and disease of the host (Khafipour *et al.*, 2011; Mao *et al.*, 2012; Newbold *et al.*, 2012). Viruses, in particular bacteriophages, have a major impact on microbial communities (Mokili *et al.*, 2012; Reyes *et al.*, 2012) and within a microbial community the presence of clustered regularly interspersed short palindromic repeats (CRISPR) is an indicator of bacteriophage–host interaction (Bhaya *et al.*, 2011). The rumen microbiome reportedly contains up to 28,000 different viral genotypes with prophage sequences outnumbering potential lytic phages by 2 : 1 with the most abundant types associated with the

dominant rumen bacterial phyla *Firmicutes* and *Proteobacteria* (Berg Miller *et al.*, 2012).

Poultry have become one of the most, if not the most, prominent sources of animal protein worldwide, so it is no surprise that the chicken gastrointestinal microbiome is of major interest to investigators attempting to improve growth, health and food safety of poultry (Wise and Siragusa, 2007; Gyles, 2008; Kohl, 2012; Yeoman *et al.*, 2012; Oakley *et al.*, 2013). There appears to be a decrease in microbial diversity of the chicken gut at 14–16 days post-hatch that is associated with an alteration from skeletal to muscle growth (Lumpkins *et al.*, 2010). Also, growth performance may differ between chicken breeds that could be associated with the gastrointestinal microbiome. However, there may always be variation among individuals probably due to initial bacterial colonization at post-hatch. It was reported that jejuna microbiota was dominated by lactobacilli (over 99% of jejuna sequences) and showed no difference between birds with high and low feed conversion ratios, while the cecal microbial community displayed higher diversity with 24 unclassified bacterial species significantly differentially more abundant between high versus low performing birds (Stanley *et al.*, 2012).

Session 4: alternatives to antibiotics for animal production

Antibiotics in feed, as stated previously, have been successfully utilized since the 1950s to promote growth during food-animal production (Gaskins *et al.*, 2002; Dibner and Richards, 2005; Niewold, 2007). Consequently, there is a need to develop alternatives to AGPs that not only have antibacterial activities, but may also have a positive impact on feed conversion and/or growth of food-animals. Phytonutrients added to feed during food-animal production was previously discussed relative to antibacterial action, but these additives may also have beneficial effects such as improvement of host immunity or animal growth and production (Lee *et al.*, 2011; Liu *et al.*, 2012c). Professor Sergio Calsamiglia Blancafort whose principle interest is rumen physiology reported on a variety of approaches to regulate rumen function, including immunization with antigens against specific bacteria (Calsamiglia *et al.*, 2010). Vaccine formulations or treatment with passive antibodies against *Streptococcus bovis*, *Lactobacillus* spp., and *Fusobacterium nechrophorum* reportedly reduced bacterial counts, improved rumen pH and increased average daily weight gain accompanied by greater feed efficiency (Calsamiglia, unpublished data). One of the more interesting approaches reported was that the growth-promoting effect of AGPs is highly correlated with the decreased activity of intestinal bile salt hydrolase (BSH), an enzyme that is produced by various gut microflora and that is involved in host lipid metabolism (Begley *et al.*, 2006). BSH catalyzes conversion of conjugated bile salts to un-conjugated bile salts, and conjugated bile salts are needed to maintain efficient lipid digestion for absorption of fatty acids. Therefore, the decreased intestinal BSH activity in AGP-treated animals would increase a relative abundance of conjugated bile salts. Consequently, a BSH with broad substrate specificity from a chicken *Lactobacillus salivarius* strain was utilized to discover novel BSH inhibitors as feed additives to replace AGPs for enhancing the productivity and sustainability of food animals (Wang *et al.*, 2012).

Session 5: regulatory pathways to enable the licensure of alternatives to antibiotics

The worldwide animal health market is estimated to be worth $20.1 billion (USD), with the majority of this occurring in the USA and the EU (Hunter *et al.*, 2011). The animal health industries are generally represented by the International Federation for Animal Health (IFAH; http://www.ifahsec.org/), which is comprised of member companies and other associations (http://www.ifahsec.org/who-we-are/members-associations/) with interests in veterinary medicines, vaccines or other animal health products. Although there are a wide variety of alternatives for antibiotics being investigated, the actual number of new commercial antimicrobials with antibiotic-like outcomes marketed has been minimal in number due to a variety of reasons. This has principally been due to the discontinuation of antibiotic research and development by pharmaceutical companies for more profitable drugs that require long-term treatment of human diseases or conditions (Shryock, 2004; Fox, 2006; Hunter *et al.*, 2011). Certainly, intellectual property issues will be of concern because there is apparently no 'safe harbor research exemption' for a veterinary biological product manufactured using recombinant DNA or other site-specific genetic techniques in the USA (Lu *et al.*, 2011).

Development of new antimicrobials must adhere to commercial development and registration processes that follow initial discovery and should include an assessment of animal health needs that will result in return on commercial investment along with consideration of the intellectual property (Hunter *et al.*, 2011). Consequently, commercialization of a drug will involve a private sector sponsor that has contact with the FDA Center of Veterinary Medicine (CVM) or, for a biologic, the USDA Center for Veterinary Biologics in the USA. Dr Steven Vaughn of the US FDA directs the Office of New Animal Drug Evaluation and presented an overview on the initiatives for improving products (http://www.fda.gov/AboutFDA/ReportsManualsForms/Reports/ucm274333.htm). He also reviewed a history of the legislative statutes that provide the basis for regulatory oversight in the USA, which include the Federal Food, Drug and Cosmetic Act, the Federal Meat, Poultry Products and Egg Products

Inspection Acts, Virus-Serum-Toxin Act and the Food Safety Modernization Act (Berry and Martin, 2008). Professor David K. J. Mackay, Head of Veterinary Medicines and Product Data Management for the European Medicines Agency, discussed the EU Action Plan against AMR that identifies seven areas of action that are most necessary, including development of new effective antimicrobials or alternatives for treatment (http://ec.europa.eu/health/antimicrobial_resistance/policy/index_en.htm). Dr Huiyi Cai, Deputy President of the National Feed Industry Association, Peoples Republic of China, and General Director of the Feed Research Institute of the Chinese Academy of Agricultural Sciences, presented information on feed additives used during food-animal production that is regulated by the Ministry of Agriculture in China.

Industry representative Marike Dussault, Director, Regulatory Affairs & Pharmacovigilance at Pfizer, Inc., Canada, stressed that initial proof of efficacy of an antimicrobial compound is rarely the rate-limiting step, but that rather animal safety, delivery method and economics are usually the more stringent factors for advancing candidate drugs for commercial development. This also includes large-scale production accompanied by good manufacturing practices (GMPs) requirements that can inhibit transition to full-scale antimicrobial production. Octavia Panyella of Lohmann Animal Health reported that, unlike in the USA, no bacteriophage products have been registered in the EU and the regulation standards would follow those for a feed additive or for veterinary medicine. In the final analysis, it will be necessary for the private sector to partner with government or university investigators to bring any new or novel antimicrobials to commercial development. This will have to mean more involvement from the initial stages of development on the part of companies that have the ability to complete large-scale production of a product followed by clinical or efficacy trials.

Needs and recommendations from the panel discussions

Panel discussions at the end of each session were organized to capture problems, solutions and recommendations for advancing the research and development of alternatives to antibiotics. Three overarching themes that resonated across all panel discussions included concerns over the shortages of antimicrobials, further restrictions on their use and reservoirs of resistance genes accompanied by their transfer to pathogenic bacterial strains. The shortage of antimicrobials, either commercially available or under development for treating microbial infections of animals and, in particular, products that are effective against pathogens with antibiotic-resistant genes is a critical issue for animal agriculture. There are concerns that the effectiveness of many or all antibiotics produced will eventually be confounded by resistance development in the target pathogens. There is a critical need to develop innovative antimicrobials that provide alternatives to conventional antibiotics and that are refractory to resistance development. Second, eliminating the use of antibiotics for animal production may have adverse consequences on the production, health and welfare of animals. Although the mechanisms by which antibiotics enhance animal production and health have not been fully elucidated, scientific advances resulting from new research tools such as metagenomics and other genome-enabled technologies are providing insights into the ecology of the gut microbiome, host–pathogen interactions, immune development, nutrition and health. These advanced research tools provide new opportunities for developing alternative strategies to enhance the production and health of livestock, poultry and fish. Lastly, commensal bacteria can serve as a reservoir of antibiotic resistance genes for eventual transfer to pathogenic strains. One potential strategy to avoid selecting for resistance genes in commensal bacteria is to develop alternative antimicrobials that are limited in their target pathogen range. One potential solution is to consider the selection of multiple products that can work synergistically, such as the production of phage cocktails that would target numerous pathogens simultaneously. Also, probiotics and enzymes could be utilized that target specific pathogens but potentially competitively favor establishment of beneficial microbes early in life. Several needs were identified, key among them were: (1) a need to conduct scientific studies to determine the efficacy and safety of alternative products; (2) a need to conduct studies under field conditions in target animal species; (3) a need to integrate nutrition, health and disease research; (4) the need for alternatives to antibiotics to be regulated as a drug, a biologic, a feed additive or possibly all; (5) alternatives to antibiotics should be developed according to national and international standards to meet the requirements for efficacy, safety and quality; (6) a need to engage regulatory agencies early in the process; (7) the need to link academia, government researchers, feed industry, pharmaceutical industry, regulatory agencies and livestock industries; and (8) stakeholders and the scientific community must accurately define the scope of future research, development and applications for alternatives to antibiotics.

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