

Antimicrobial resistance in livestock: antimicrobial peptides provide a new solution for a growing challenge

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Implications

- The rise in antimicrobial resistance is widely acknowledged as a result of misuse and overuse of livestock antibiotics. Once transferred to human beings, these strains can cause diseases that are not treatable by antibiotics.
- Antibiotics can largely accumulate in the environment through livestock excretion and eventually threaten public health.
- Current government regulations on antibiotics cannot effectively and immediately stop the increase in antimicrobial resistance. There is an urgent need to find alternatives that do not lead to resistance in the future.
- Antimicrobial peptides hold promise as effective alternative as they have efficacious antimicrobial effects and weak resistance induction ability.

Key words: antibiotic alternatives, antimicrobial peptides, antimicrobial resistance, livestock

Introduction

Animal husbandry, the agricultural practice of breeding and raising livestock, is a major food-producing industry worldwide. Compromised gut health due to improper nutrition and management is a significant challenge for this industry as it can result in poor development and growth, disease, morbidity, and mortality in livestock. Poor gut health is often associated with leaky gut, intestinal atrophy, infection, and inflammation, particularly when animals are young. Antibiotics are widely used in modern livestock production as growth promoters primarily due to their preventative effects on livestock diseases. Global consumption of antibiotics from food animal production was estimated to be 63,151 tons in 2010 and is predicted to rise dramatically by 67% by 2030 (Van Boeckel et al., 2015). This practice has been

linked to the spread of antimicrobial-resistant pathogens in both livestock and humans, posing a significant public health threat. Antimicrobial resistance is becoming a worldwide concern, and without effective countermeasures, it is predicted to kill more than 10,000,000 human beings and cause \$100 trillion economic loss annually by 2050 (O'Neil, 2014). Due to the widespread use of antibiotics for treatment, prophylaxis, and growth promotion, livestock have become a reservoir of antimicrobial-resistant bacterial strains and genes. According to prevalence studies and homology sequence analyses, resistant strains have been extensively identified in animals, and they can be transferred to farmers, slaughterhouse workers, and veterinarians through direct contact, and even consumers through the food chain (Marshall and Levy, 2011; Vishnuraj et al., 2016). Concerns about this issue have risen rapidly, with a surge of published articles in the past 10 yrs (from 4 to 73, based on a search of "antimicrobial resistance" and livestock in PubMed database). Notably, resistance to the last-resort antibiotic, colistin, has been found in livestock, alarming us to the impending threat of antimicrobial resistance (Irrgang et al., 2016; Liu et al., 2016). Moreover, antibiotic residues in excrement may cause a high level of antibiotic accumulation in the environment, increasing the exposure and occurrence of antibiotic resistance, and leading to more profound and complex impacts.

In the face of the antimicrobial resistance threat, authorities of major economies, including the European Medicines Agency and the Food and Drug Administration (FDA) in the United States, have imposed regulations on antibiotic growth promoters (AGP) and have tried to put treatment antibiotics under official surveillance to decrease antimicrobial resistance through reduced antibiotic use. However, withdrawal of antibiotics from livestock productions can result in a number of challenges, including compromised gut health and a rise in gut diseases. Concurrently a number of effective alternatives have also been reported; for example, antimicrobial peptides, which play multiple roles in bacterial elimination, immune response, epithelial reinforcement, and combating diseases like cancer (Chu et al., 2015; de la Fuente-Núñez et al., 2017). Antimicrobial peptides further show biofilm destruction on multi-resistant strains while rarely resulting in antimicrobial resistance (Ageitos et al., 2017). Antimicrobial peptides can exhibit favorable therapeutic effects on piglet diarrhea depending on direct antimicrobial effects, intestinal barrier enhancement,

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and inhibition of inflammation (Yi et al., 2016, 2017). In this review, we summarize the current status of antibiotic use in livestock production and potential challenges associated with the use of antibiotics and then elaborate on the antimicrobial effects of antimicrobial peptides, their underlying mechanisms, and the potential of antimicrobial peptides in livestock production.

Threats from Antibiotics Use in Livestock Production

Selection of antimicrobial-resistant bacteria

The livestock industry is observing rising levels of antimicrobial resistance, similar to what has been happening to antimicrobial resistance findings in human clinical isolates (O'Neill, 2015a). Based on EU official statistics, we made a summary about antimicrobial resistance dynamics of the most prevailing food-borne pathogens: *Escherichia coli* and *Salmonella* spp. isolated from pigs, cattle, and broilers (Tables 1 and 2). Antibiotics ranked as critically important or important to human medicine by WHO are ones that become less effective through inappropriate use and in turn lead to failures of bacterial disease treatment. Through both rigid restrictions and public awareness, the goal is to see a decrease, or at least delay, in antimicrobial resistance development. However, the current moment is critical, as evidenced by *E. coli* data that shows an obvious upward trend in antimicrobial resistance based on a high level in pigs and broilers, while resistant ratio in *Salmonella* is much smaller and the rising trend in resistance to some antibiotics is partially reverted. Remarkably, resistance to our last-resort antibiotic, colistin, is commonly detected in both bacteria from the tested species. Considering strict regulations on the use of antibiotics in livestock production in the EU, the survey indicates that current policies may not be effective enough to tackle increased antimicrobial resistance.

Antibiotic residue in the environment

Antibiotics are not easily degraded in the animal body and thus enter into the environment through excreted urine and feces (Lim et al., 2013). Due to the farming scale, livestock farms have been reported to discharge a large amount of antibiotics into nearby surface water, soil, and sludge (Watanabe et al., 2010,

Zhu et al., 2013). In China, 84.0% of total antibiotic excretion comes from farming animals (pig: 44.4%, chicken: 18.8%, other animals: 20.9%; Zhang et al., 2015). Residual antibiotics usually have negative impacts on organisms, food security, and water security. These can result in a selection of antimicrobial strains and accumulation in the human body through the food chain and drinking water (Sarmah et al., 2006; Näslund et al., 2008; Underwood et al., 2011; Qian et al., 2012; Boonsaner and Hawker, 2013; Jia et al., 2017). According to a comprehensive survey of total antibiotic emissions in river basins of China, an overlap with animal industry area and the basin province is clearly evident: the areas with a high level of antibiotic residue produce nearly 1/2 of all slaughter hogs and 1/3 of overall meat (Zhang et al., 2015). Antibiotic residues from livestock also occur in some developed countries. Oxytetracycline and sulfadiazine are antibiotics commonly used in livestock and can be detected to quantify the animal contribution. Kolpin et al. (2002) carried out a survey of 139 streams in the United States, and the maximum concentration of oxytetracycline was 340 ng/L. In the United Kingdom, the maximum concentrations of the two antibiotics were 4490 and 4130 ng/L, respectively (Environmental Agency, 2005). Although there is a lack of recent survey data about antibiotic residue in water, we speculate that there should be a rising trend in the natural environment in recent years as a result of the worldwide boost in animal product consumption, the corresponding growth in farming size, and our current sewage treatment methods.

Solutions to the Threats Caused by Antibiotic Use in Livestock Production

Official regulation and surveillance

Facing the serious threat of livestock-use antimicrobial drugs, the governments of major economies have already imposed a series of policies, acts, and guidelines to ensure the responsible use of antibiotic drugs. In the United States, the FDA issued a series of documents concerning antimicrobial use in food-producing animals, claiming that until 2016 no antibiotics deemed medically important can be used as AGP. However, annual sales of antibiotics from 2009 to 2015 grew dramatically, with little changes in market ratios of medically important and not currently medically important antibiotics (Figure 1).

Table 1. *Escherichia coli* antimicrobial resistance (%) isolated from food-producing animals in European Union

Category	Year	AMP	CTX	CHL	CIP	CST	GEN	NAL	SXT	TET
Pig	2010	21.0	1.0	7.0	2.0	-	2.0	2.0	37.0	48.0
	2015	39.3	1.4	18.3	10.5	0.4	3.3	6.0	44.2	54.7
Cattle	2010	28.0	3.0	17.0	15.0	-	9.0	13.0	34.0	38.0
	2015	31.0	1.7	15.4	11.4	0.9	3.8	8.7	36.6	45.4
Broiler	2010	35.0	5.0	8.0	29.0	-	4.0	26.0	34.0	31.0
	2014	58.6	5.1	21.6	65.7	0.9	11.6	62.6	53.1	50.1

Data from The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals, and food in 2010, 2014, and 2015.

AMP, ampicillin; CTX, cefotaxime; CHL, chloramphenicol; CIP, ciprofloxacin; CST, colistin; GEN, gentamicin; NAL, nalidixic acid; SXT, sulfamethoxazole; TET, tetracycline.

Table 2. *Salmonella* antimicrobial resistance (%) isolated from food-producing animals in European Union

Category	Year	AMP	CTX	CHL	CIP	CST	GEN	NAL	SXT	TET
Pig	2010	21.0	1.0	7.0	2.0	-	2.0	2.0	37.0	48.0
	2015	39.3	1.4	18.3	10.5	0.4	3.3	6.0	44.2	54.7
Cattle	2010	28.0	3.0	17.0	15.0	-	9.0	13.0	34.0	38.0
	2015	31.0	1.7	15.4	11.4	0.9	3.8	8.7	36.6	45.4
Broiler	2010	35.0	5.0	8.0	29.0	-	4.0	26.0	34.0	31.0
	2014	58.6	5.1	21.6	65.7	0.9	11.6	62.6	53.1	50.1

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As one of the largest pork exporters, Denmark has taken serious steps in tackling antimicrobial resistance by becoming one of the first countries to ban all AGP. AGP were gradually banned from 1995 to 2000 (Jensen and Hayes, 2014) after the Denmark government introduced a Yellow Card scheme to punish farmers using excessive prescriptions. The antibiotic restrictions proved to be successful with regard to antimicrobial consumption and economic income (O'Neill, 2015b). As for the primary purpose of imposing antimicrobial resistance control, however, it is a little disappointing to stop the uprising trend of antimicrobial resistance through regulations on consumption and using methods (Jensen and Hayes, 2014). Together with increased morbidity in piglets, the ban on AGP and antibiotic limitations requires better management and feeding practices, even in Denmark, a developed country with rich experience and technical talent (Jensen and Hayes, 2014).

In spite of the governmental progress aimed at controlling livestock-use antibiotics, there is a lack of a unified classification for antibiotics, which continues to exacerbate issues of overuse and misuse. The U.S. FDA has ranked the importance of antibiotics into three categories: critically important, highly important, and important. The WHO, however, only has two classification levels: critically important and highly important. There are also some differences in the detailed items. In addition, no unified evaluation system is widely executed for antimicrobial resistance regulation. These multiple and somewhat incongruous criteria also complicate antimicrobial resistance surveillance and antibiotic drug regulation.

Alternatives to antibiotics

The current solutions to antimicrobial resistance for livestock-use antibiotics, such as a ban on AGP and strict regulations on veterinary use, proved to be unsatisfactory when evaluated based on antimicrobial resistance change. To avoid the negative effects of antibiotics, novel alternative approaches to antibiotics should not easily induce resistance in bacteria. Additionally, the absolute replacement of antibiotics is not realistic in the near future. Preventive therapies or adjunctive treatments with antibiotics that enhance the efficacy and consequently reduce normal doses should be regarded as an ideal approach. According to this standard, there are nine categories (antibodies, probiotics, lysins, wild-type or engineered bacteriophages, immune enhancers, vaccines, antimicrobial peptides,

host defense peptides, and antibiofilm peptides) that have great promise for commercialization in the next decade (Czaplewski et al., 2016). Except for lysins, bacteriophages, host defense peptides, and antibiofilm peptides, each alternative has two development directions. Besides, some alternative categories are partially overlapped, for example, immune stimulation, host defense peptides, innate defense peptides, and antibiofilm peptides usually function through antimicrobial peptides. Due to public awareness of antibiotic-related issues and according to business requirements, this list is rapidly expanding through the continuous efforts of academic institutions and pharmaceutical corporations. Antibiotic alternative research is a hot topic and requires further development in the pharmaceutical pipeline. Recently one product, Bezlotoxumab, was approved by the U.S. FDA in 2016 as an anti-infection drug.

Antimicrobial Effects of Antimicrobial Peptides and Their Modes of Action

Antimicrobial effects of antimicrobial peptides

Antimicrobial peptides, also known as antibacterial peptides or host defense peptides, are a 5,000-member family of short cationic peptides (less than 100 amino acids) which constitute part of the innate immune defense existing in nearly all classes of organisms (Ganz, 2002; Waghue et al., 2014; Ageitos et al., 2017). All of them commonly share a small size, linear or cyclic structure, and enjoy an 80-yr application history (Dubos and Cattaneo, 1939; Boman, 1995).

Of all the bioactivities discovered, antimicrobial effect is the first discovered and consequently drew the most attention with regard to antibiotic alternatives (STEINER et al., 1981). In terms of minimal inhibitory concentration, antimicrobial peptides exhibit significantly high effective inhibition on bacteria (Table 3). Although smaller minimal inhibitory concentrations do not necessarily mean reduced antimicrobial resistance occurrence, it can at least decrease the likelihood of unnecessary contact between targeted bacteria and antimicrobials. Secondly, antimicrobial peptides usually have a broad-spectrum activity against either Gram + or Gram - bacteria. Among 136 natural antimicrobial peptides from microorganisms, aquatic organisms, and terrestrial organisms, 90 antimicrobial peptides (66.2%) have inhibitory effects on both Gram + and Gram - ,

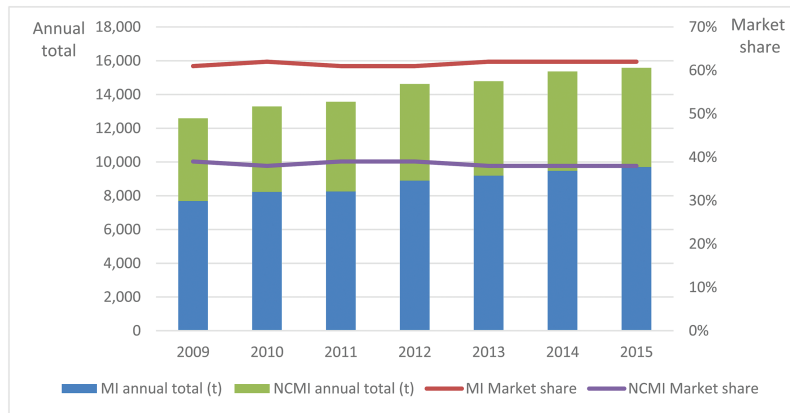


Figure 1. Antimicrobials sold in the United States classified by medical importance. MI, medically important; NCMI, not currently medically important. Data from antimicrobials sold or distributed for use in food-producing animals from (issued by FDA of the United States) 2010 to 2015.

and 23 (16.9%) and 23 (16.9%) antimicrobial peptides show single effects on Gram+ and Gram- bacteria, respectively (Ageitos et al., 2017).

Furthermore, antimicrobial peptides can enhance the efficacy of antibiotics while reducing their concentration, particularly on antimicrobial-resistant strains. Soren et al. investigated the combinative effects of novicidin with rifampin, ceftriaxone, or ceftazidime, on corresponding antibiotic-resistant bacterial strains. All formulas show synergistic effects on resistant bacteria with 70%, 89.7%, and 94.1% isolates inhibited, respectively, with reduced MIC and less hemolytic activity, whose synergetic effect is related to cytoplasmic membrane damage induced by novicidin (Soren et al., 2015). Similar results were found in combinations of azithromycin and LL-37 or colistin on multi-drug-resistant isolates (Lin et al., 2015).

Mechanisms for the antimicrobial properties of antimicrobial peptides

Mechanisms for the antimicrobial properties of antimicrobial peptides are like a pipeline composed of initial attraction and interaction, concentration-dependent threshold,

self-association and multimerization, antimicrobial actions, followed by induced cellular activities (Lee et al., 2016). Inasmuch, antimicrobial models are the most extensively investigated, with 21 models used to elucidate antimicrobial peptides' damage on bacteria. These models can be divided into a non-permeabilizing category (2 models) and a permeabilizing category (19 models), the latter of which can be further classified by pore formation or not (Wang et al., 2015). For example, two main models, barrel-stave and toroidal wormhole, are attributed to pore formation. In the barrel-stave model, ampicillin (AMP) will transform and acquire amphipathy after binding to a membrane. During accumulation to threshold concentration, antimicrobial peptides will gradually form into bigger molecules by which peptides can inset more deeply and form a "barrel," a ring-like pore, and a "stave," which is made of individual spokes around the ring. In this model, the hydrophobic domains locate outward and the hydrophilic regions locate inward, avoiding exposure to residues with the opposite hydrophilic preference (Breukink and de Kruijff, 1999). The toroidal wormhole model is similar to the barrel-stave model and is mainly reported in helical antimicrobial peptides (Matsuzaki, 1998; Hara et al., 2001; Hara et al., 2001). However, in this

Table 3. Minimal inhibitory concentration of selected antimicrobial peptides toward food-borne pathogens

Antimicrobial peptides	Minimal inhibitory concentration (μM)				References
	<i>Escherichia coli</i>	<i>Salmonella</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	
Gageotetrins	-	-	0.02 to 0.06	-	Tareq et al. (2014)
Discodermin A	1	-	1	2	Matsunaga et al. (1984)
Thanatin	<1.2	<1.2	-	<5	Fehlbaum et al. (1996)
Maximin 3	0.3	-	1.1	-	Lai et al. (2002)
CPF-St5	1	-	1	-	Roelants et al. (2013)
buCATHL4C	12.5	-	0.2 to 0.4	-	Brahma et al. (2015)
Indolicidin	0.8	-	3	-	Falla et al. (1996)
TAP	3 to 6	-	6 to 12	-	Diamond et al. (1991)
GNCP-1	5	-	3	-	Yamashita and Saito (1989)
HBD-3	1	-	0.6	-	Joly et al. (2004)
HNP-1	0.5	-	0.6	1.9	De Smet and Contreras (2005)
LL-37	0.1	0.4	-	-	Turner et al. (1998); De Smet and Contreras (2005)

model, AMP hydrophobic residues are not exposed to the lipid head groups in the cell membrane (Zhu et al., 2015). Carpet mechanism does not rely on pore formation and will non-specifically cover the surface like a carpet (Taubes, 2008), whereas it is sometimes regarded as a final step in the toroidal wormhole model (Fernandez et al., 2012). Along with membrane damage, many important pathways are unable to function (Melo et al., 2009). All of these steps take place in a few minutes, while it takes days for antibiotics to exert their antimicrobial effects (Tossi et al., 1997).

Prospective of Antimicrobial Peptides as Antimicrobial Drugs

Improvement and production

Further therapeutic development still requires all-round improvements in antimicrobial efficiency, cytotoxicity, and stability in physiological conditions. Due to the biofilm destruction, which is a lack of distinguishable markers, the antimicrobial effect of natural antimicrobial peptides usually combines with an attack on host cells (Lee et al., 2016). In addition, the greatly decreased bioactivity in plasma is another issue that obstructs the medical practice. LL-37, for example, exerts less than 1/64 bioactivity toward *Staphylococcus aureus* in the plasma-simulated solution (plasma:PBS = 1:1; de Breij et al., 2018). Improvements on the mentioned sides are usually via: 1) hybrids of active fragments from different antimicrobial peptides, 2) antimicrobial peptides modifications, 3) innovations not based on natural antimicrobial peptides, and 4) computer-aided technology based on an antimicrobial peptides database.

The hybrid method is mostly applied in antimicrobial peptide synthesis aimed at increasing antimicrobial efficacy, changing targeted bacteria, and compromising cytotoxicity (Wade et al., 1990). CAMELO is a hybrid of cecropin A and melittin A with lower minimal inhibitory concentration level (*E. coli*, 1 μM) compared with original melittin (Gram + /Gram -, 1 to 8 μM ; Oh et al., 2000; Park et al., 2011). RW-BP100, a modified protein based on cecropin A-melittin hybrid protein, has enhanced effects on both Gram + (minimal inhibitory concentration 0.3 to 1 μM) and Gram - bacteria (minimal inhibitory concentration 0.1 to 2.5 μM), but with greater cytotoxicity (Badosa et al., 2007; Torcato et al., 2013). RN7-IN6 is an improved hybrid of indolicidin and ranalexin with more potent efficacy than indolicidin and ranalexin.

Antimicrobial peptide modification happens on residues of only one kind of AMP. Pexiganan is an analog of magainin 2, showing a wide antimicrobial spectrum. A total number of 3,108 bacteria strains can be inhibited, among which 87% minimal inhibitory concentration ranges from 0.8 to 6.5 μM . More importantly, the strains are mostly resistant to antibiotics of medical importance to human beings (Gottler and Ramamoorthy, 2009). Other modified antimicrobial peptides, like Dhvars (originated from histatin; Welling et al., 2007), FL9 (originated from Fallaxin; Gottschalk et al., 2015), and

WMR-NH2 (originated from Myximidin; Cantisani et al., 2014), show outstanding inhibitory effects, particularly on drug-resistant strains.

As for the innovated antimicrobial peptides, they have more precise targets and surprising inhibitory effects. V-peptide, targeted on lipopolysaccharide and the constituent lipid A, is able to inhibit Gram - strains at minimal inhibitory concentration between 0.004 and 0.692 μM (Freceer et al., 2004). WLBU2 can inhibit bacteria at a low minimal inhibitory concentration regardless of the salt concentration. Applications of computer-aided designs represent the next generation highlighted by high potency and precise prediction based on enriching AMP databases like APD3, CAMPR3, and LAMP (Zhao et al., 2013; Waghui et al., 2016; Wang et al., 2016).

Currently, antimicrobial peptides are generally produced by chemical methods, not by bioreactors, due to the cytotoxicity produced in the latter pipeline. Even so, some antimicrobial peptides, including LL-37 and β -defensins, have been successfully expressed in *E. coli*. As a promising antibiotic alternative, in both humans and animals, it requires further breakthroughs in production method, microbial efficacy, security, and acceptable cost.

Delivery methods

As an important step in pharmaceutical research, it is necessary to investigate the delivery systems that carry antimicrobial peptides to targeted sites. However, very few articles focus on this topic, which is unfortunate given its role in enhancing antibacterial effects, reducing degradation, controlling release rate, and decreasing cytotoxicity.

Up to now, inorganic and organic materials are the two main kinds of delivery method. Inorganic delivery materials include mesoporous silica, titanium, metal nanoparticles, quantum dots, carbon-based nanoparticles, and related materials. Mesoporous silica shows good incorporation with antimicrobial peptides like LL-37, which enhances stability against degradation, specific adsorption, extended release period, and synergetic effects with antimicrobial peptides (Braun et al., 2016). The synergetic effects are probably related to surface enrichment in the membrane resulting in membrane disruption, and therefore, minimal inhibitory concentration will be lower than normal (Li and Wang, 2013). Apart from most of the mentioned characteristics, titanium materials can markedly reduce the cytotoxicity of some antimicrobial peptides (Kazemzadeh-Narbat et al., 2013). Metal nanoparticles researched are mainly Au, Pt, Ag, and Cu and have highlighted enhancement of antimicrobial effects. For example, when delivered by gold nanodots, surfactin minimal inhibitory concentration toward methicillin-resistant *S. aureus* can be lowered by more than 80-fold (Nordström and Malmsten, 2017). This may be a result of released ions and induction of reactive oxygen species (Hajipour et al., 2012). Quantum dot is reported to be system-specific, varying in cytotoxicity and antimicrobial effects (Park et al., 2011; Chen et al., 2015; Galdiero et al., 2016). Carbon-based nanomaterials, including graphene and

carbon tubes, are drawing more attention because of their imposed membrane damage and induction of oxidative stress (Hajipour et al., 2012), which can also enhance antimicrobial activity (Nellore et al., 2015).

Organic delivery materials, or polymeric materials, include particles and fibers, gels, multilayers, and conjugates. Beyond their own antimicrobial and synergetic effects, organic materials also possess outstanding plasticity and can be designed according to specific requirements. For example, polymer multilayers can be used to build more complex particles and surface coatings. Since materials in solution are gradually removed in the deposition process, it can sustain ambient conditions and the various parameters can be varied between each layer (Nordström and Malmsten, 2017).

Endogenous AMP regulation

Due to its excellent antimicrobial effects and other activities in immune enhancement, endogenous AMP regulation through nutrients or other chemicals is developed as a prevention method. Considering its oral administration method and high security (nutrients in feed), it would be convenient to use in a whole herd as prophylaxis. Butyrate and its derivatives, including 4-phenylbutyrate and sodium butyrate, show improvements in endogenous AMP LL-37 expression in both epithelial cells and macrophages (Schauber et al., 2003; Raqib et al., 2006; Steinmann et al., 2009a). Vitamin D can trigger expression of LL-37 in epithelial tissue as well as leukocytes, which was applied in treatment to *Mycobacterium tuberculosis* infections (Zasloff, 2006). A derivative of Vitamin D, 1, 25-dihydroxyvitamin D₃, has similar enhanced effects on LL-37 and exhibits synergetic effects with 4-phenylenediamine (Gombart et al., 2005; Steinmann et al., 2009b). Stimulator aroylated phenylenediamine is the latest discovery and has shown significant stimulation in LL-37 synthesis by 20- to 30-fold (Ottoosson et al., 2016). Despite its comparatively short history, it has already been listed as one of the most promising antibiotic alternatives (Czaplewski et al., 2016).

Conclusion

Antimicrobial resistance poses a worldwide threat to public health, which may be partially associated with using AGP in livestock production. The European Union banned the use of AGP in animal food production in 2006. The U.S. FDA placed restrictions on antibiotic use in animal production in December 2016; more countries are expected to follow. However, a withdrawal of antibiotics from livestock production can result in a number of challenges, including a rise in gut diseases. Therefore, the development of antibiotic alternatives for sustainable livestock production is urgently needed as the livestock industry complies with these new regulations. Antimicrobial peptides represent one of the most promising alternatives to antibiotics, with several beneficial properties including a lower risk of inducing antimicrobial resistance, excellent inhibitory effects, easiness of degradation, and host immunity enhancement. However, their application in

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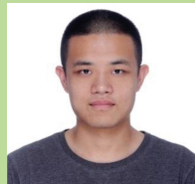


Dr Yizhen Wang has been awarded with Qiushi Professor at Zhejiang University. During the 30-year career, he devoted in biological feed stuffs, animal nutrition, immune regulation, and meat quality improvement. He has published more than 110 SCI articles including *Nature Communications*, *Biomaterials*, *Journal of Immunology* and *Journal of Animal Science*.

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livestock production has been limited, largely due to some evidenced disadvantages, including stability, susceptibility to proteolysis, low activity under physiological conditions, and a high cost of production. Their inconsistent efficacy and the only partial understanding of their modes of action have also prevented antimicrobial peptides from reaching the market place. A better understanding of the effects of antimicrobial peptides on the three components of the gastrointestinal ecosystem—gut microbiota, gut physiology, and immunology—and the mechanisms behind them will possibly allow us to make the better use of antimicrobial peptides for economically effective and sustainable livestock production. Proper delivery methods, including microencapsulation and nanotechnology, provide promising tools to deliver antimicrobial peptides to the animal gut and improve the efficacy of antimicrobial peptides in livestock production. Finally, the potential risks in using antimicrobial peptides for livestock production need to be evaluated systemically.

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