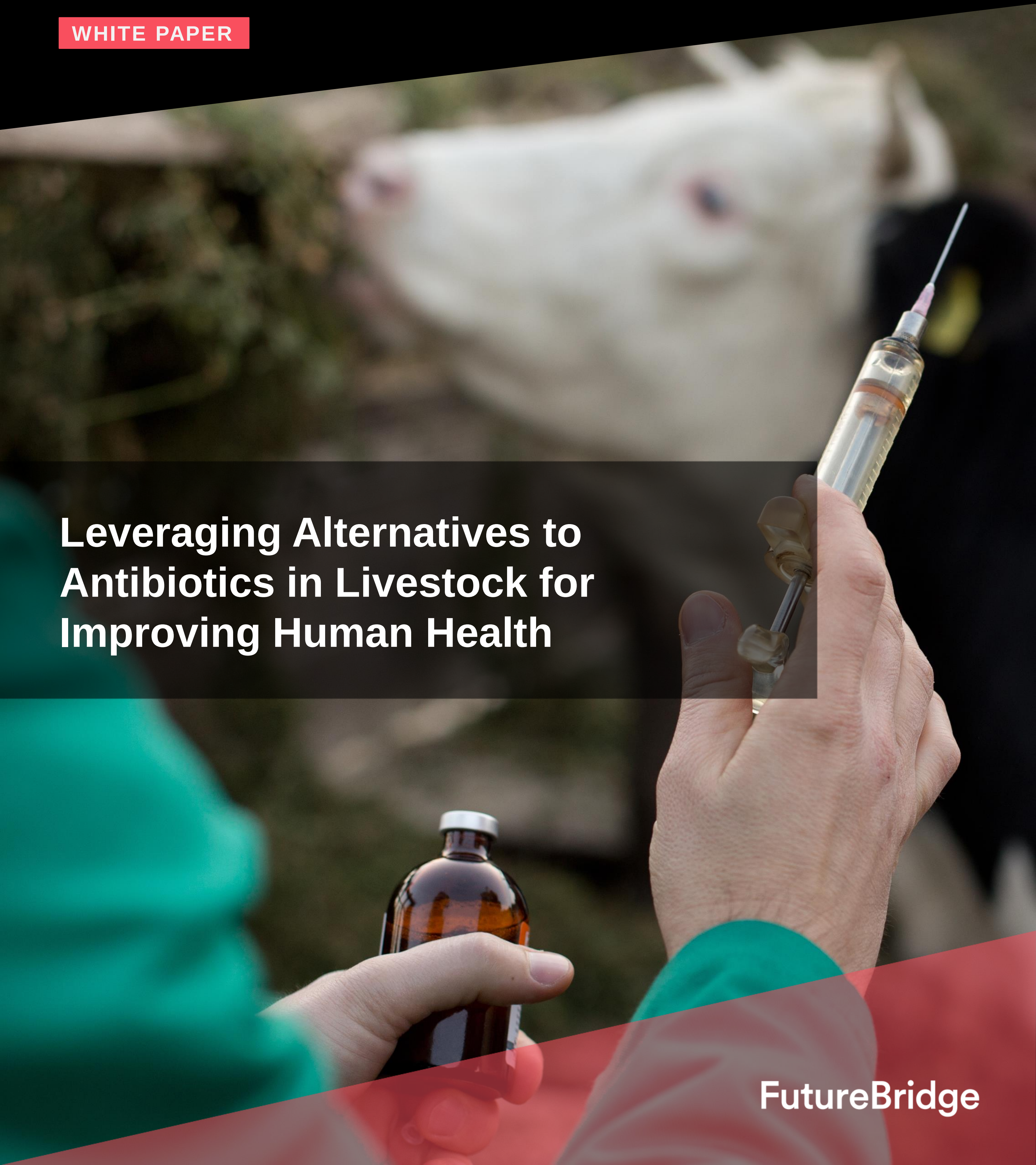
WHITE PAPER

Leveraging Alternatives to Antibiotics in Livestock for Improving Human Health



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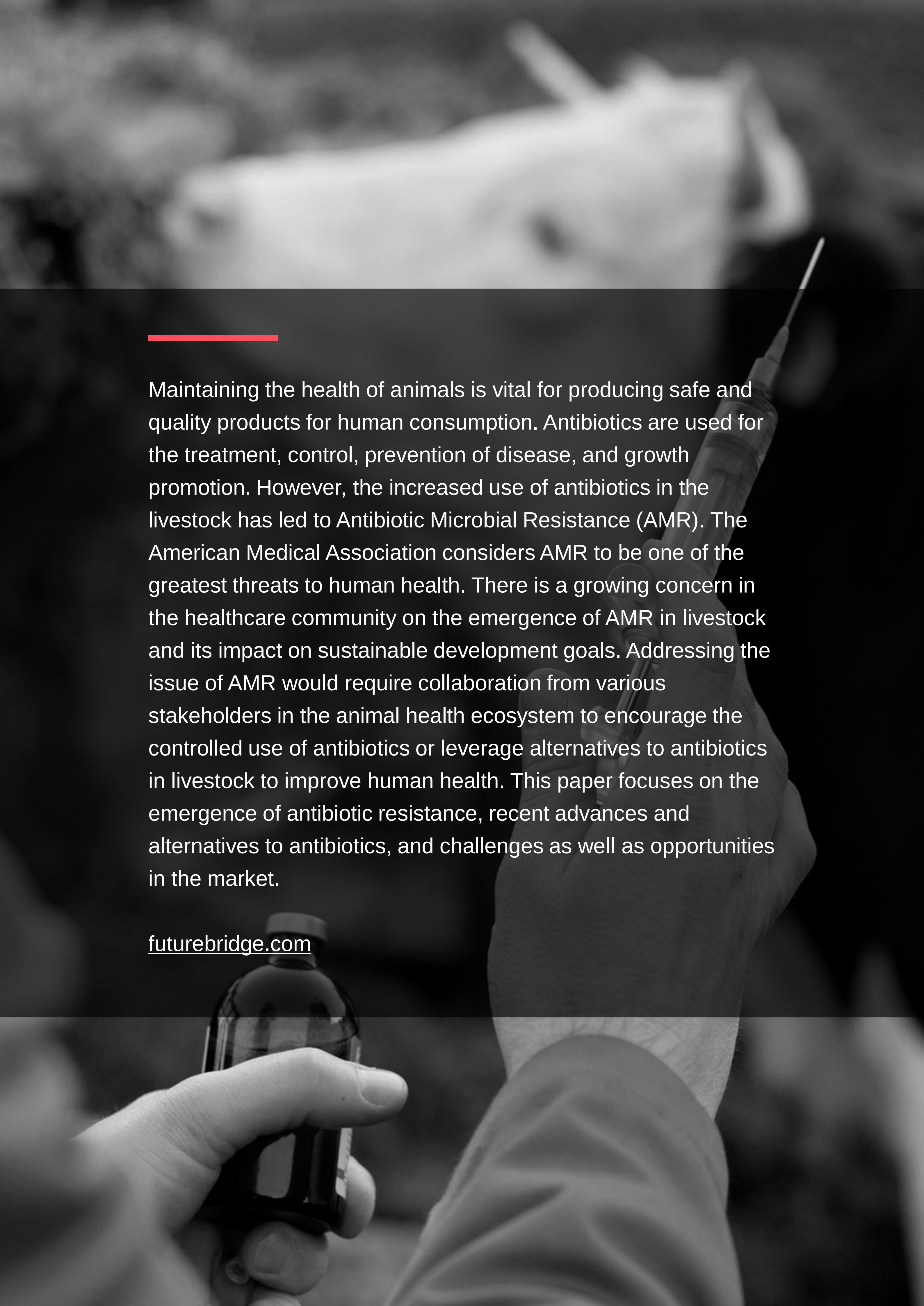

Maintaining the health of animals is vital for producing safe and quality products for human consumption. Antibiotics are used for the treatment, control, prevention of disease, and growth promotion. However, the increased use of antibiotics in the livestock has led to Antibiotic Microbial Resistance (AMR). The American Medical Association considers AMR to be one of the greatest threats to human health. There is a growing concern in the healthcare community on the emergence of AMR in livestock and its impact on sustainable development goals. Addressing the issue of AMR would require collaboration from various stakeholders in the animal health ecosystem to encourage the controlled use of antibiotics or leverage alternatives to antibiotics in livestock to improve human health. This paper focuses on the emergence of antibiotic resistance, recent advances and alternatives to antibiotics, and challenges as well as opportunities in the market.

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Summary

Maintaining the health of animals is vital for producing safe and quality products for human consumption. According to an article published on the World Organization for Animal Health, it is estimated that 20% of livestock are lost annually due to diseases, which is indeed a significant loss of food and resources. The mastitis disease alone affects one in four dairy cattle. Therefore, antimicrobial use is inevitable for the health maintenance of livestock. Antimicrobials are used for the treatment, control, prevention of disease, and growth promotion.

The increased use of antibiotics in the livestock has led to Antibiotic Microbial Resistance (AMR). Resistant bacteria are transmitted from the livestock to humans through the consumption of meat, direct contact with colonized animals, or manure spread in the environment. Annually more than 2 million people in the US are infected with antibiotic-resistant bacteria, leading to 23,000 deaths. It is predicted that globally, deaths attributable to antimicrobial resistance will reach 10 million by 2050.

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The American Medical Association considers AMR to be one of the greatest threats to human health. There is a growing concern in the healthcare community on the emergence of AMR in livestock and its impact on sustainable development goals, i.e., ensuring healthy lives and promoting overall wellbeing. In countries wherein policies are introduced to reduce antibiotic use in agriculture, a strong correlation is observed between interspecies pathogen transmissions. In the late 1990s, the European Union banned glycopeptide avoparcin, which led to a decrease in the prevalence of vancomycin-resistant enterococci in biological samples.

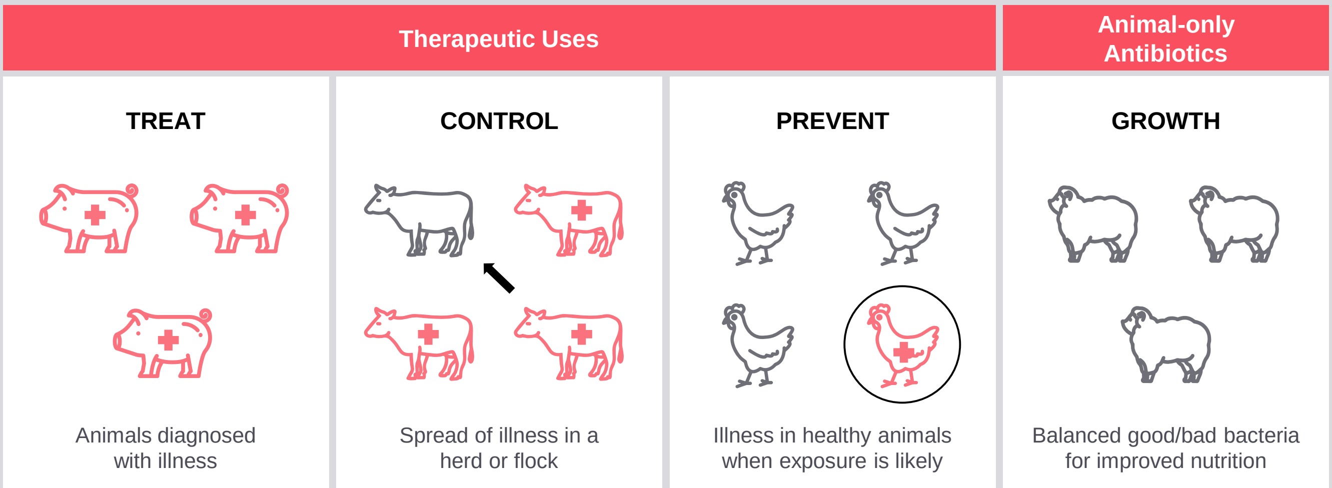
According to the United Nations report, the global population is expected to reach 9.8 billion by 2050. The increasing population has propelled the demand for affordable proteins, including meat, milk, and eggs. By 2050, it is estimated that meat production will reach 470 million tons. Farmers may prefer to employ antibiotics to prophylactically prevent diseases in their livestock, thereby addressing the growing demand for meat production. However, this may lead to alarming health concern in the human community due to the use of AMR. Addressing the issue of AMR would require collaboration from various stakeholders in the animal health ecosystem to encourage the controlled use of antibiotics or leverage alternatives to antibiotics in livestock to improve human health.

Antibiotic Usage in Livestock

Antibiotics are being used for various purposes in animal health (refer to *Exhibit 1*). Based on the antibiotics usage in livestock, they can be broadly classified into four categories:

- **Treatment:** Antibiotics are used for the treatment of animals diagnosed with diseases.
- **Control:** In the event of an outbreak, antibiotics help control the spread of an illness in a farm or ranch.
- **Prevention:** Animals live in close contact, as they share water and feed; this makes them vulnerable to contracting infectious diseases. At times, veterinarians recommend antibiotic use to prevent diseases, mainly when the livestock is at risk (for instance, during weaning from the mother).
- **Growth promotion:** Antibiotics are included in livestock feeds, as they can destroy certain bacteria in the gut and helps convert feed to muscle easily, thereby promoting rapid growth. Starting in January 2017, the FDA no longer permits the use of this class of antibiotics.

EXHIBIT 1: Antibiotic usage in Livestock



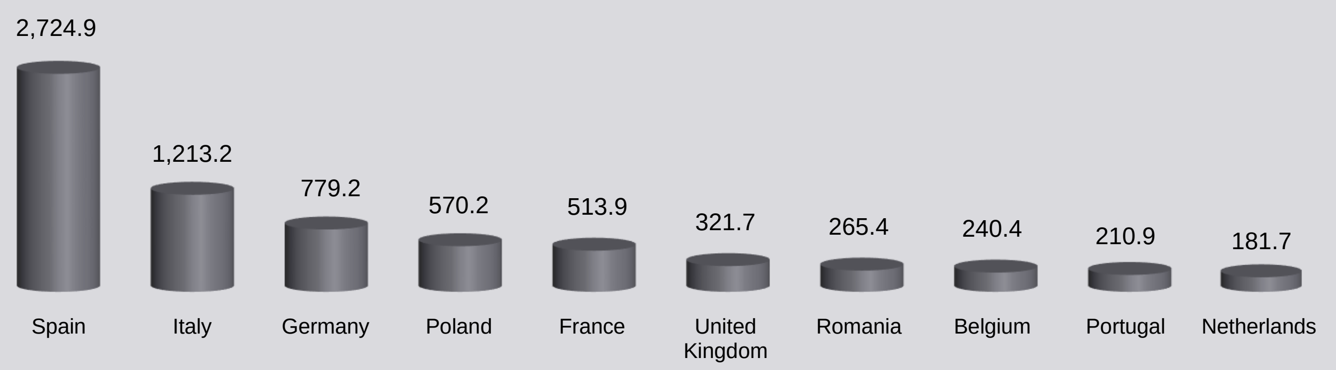
Source: Elanco (Antibiotic Stewardship Progress Report)

The sale of various veterinary antimicrobial agents in European countries provides an overview of the usage of antimicrobials in food-producing animals (refer to *Exhibit 2*). Statistics state that antibiotic usage is vital to identify and quantify risk factors for resistance development. The highest antibiotic usage (in volume) among European countries is noticed in Spain, Italy, Germany, Poland, and France.

Excessive and improper usage of antimicrobials has led to the development of resistance, such Methicillin-resistant *Staphylococcus Aureus* (MRSA) and Extended-spectrum β-lactamase (ESBL) producing bacteria in animals.

EXHIBIT 2: Sales of Veterinary Antimicrobial Agents for Food-producing Animals

(in Tons)

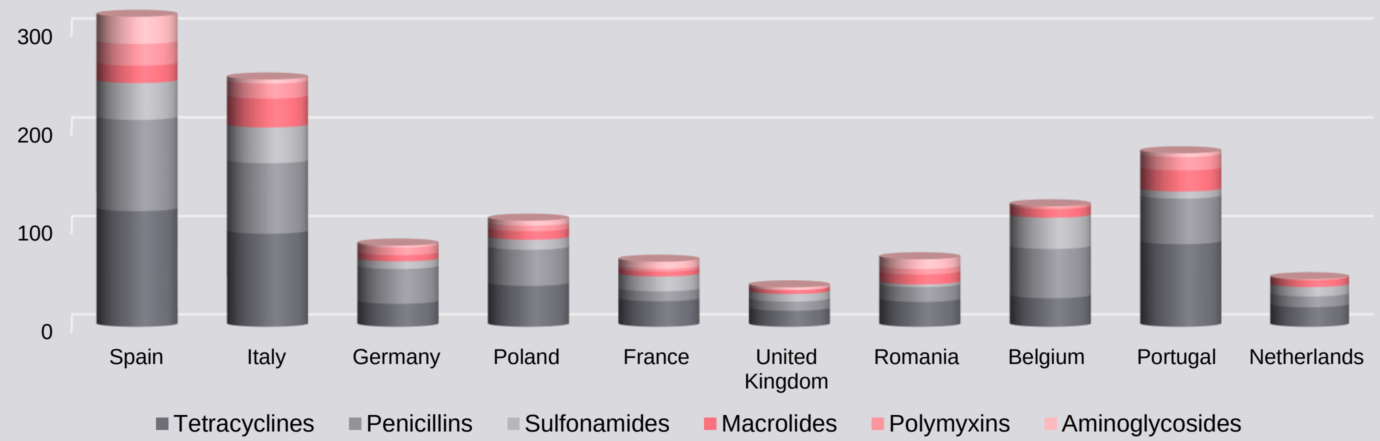


Note: Veterinary antimicrobial agents (i.e., active ingredients)
Source: Eighth ESVAC Report

Antimicrobial sale has spread to various classes; the most widely used antimicrobial classes for food-producing animals are Tetracyclines, Penicillins, Sulfonamides, Macrolides, Polymyxins, and Aminoglycosides (refer to *Exhibit 3*).

EXHIBIT 3: Antimicrobial Sales by Classes for top 10 European Countries in 2016

(in mg/PCU)



Note: mg per population correction unit (mg/PCU)
Source: Eighth ESVAC report

According to a research paper, between 2010 and 2030, the global consumption of antimicrobials in livestock is expected to increase by 67%, wherein up to one-third of the increase will be attributed to shifting production practices in the middle-income countries. The shift will be primarily from extensive farming systems to large-scale intensive farming operations, which routinely use antimicrobials in sub-therapeutic doses. The increase in total livestock antimicrobial consumption will be 99%, up to seven times the projected population growth in the countries - Brazil, Russia, India, China, and South Africa.

The global consumption of antimicrobials in animals is double than that of humans. Antimicrobials are used as growth promoters and in therapeutics; they are also used to sustain the growing demand for milk and meat, which is contributing to higher selection pressure for resistant strains of bacteria to evolve.

Emergence of Antibiotic Resistance

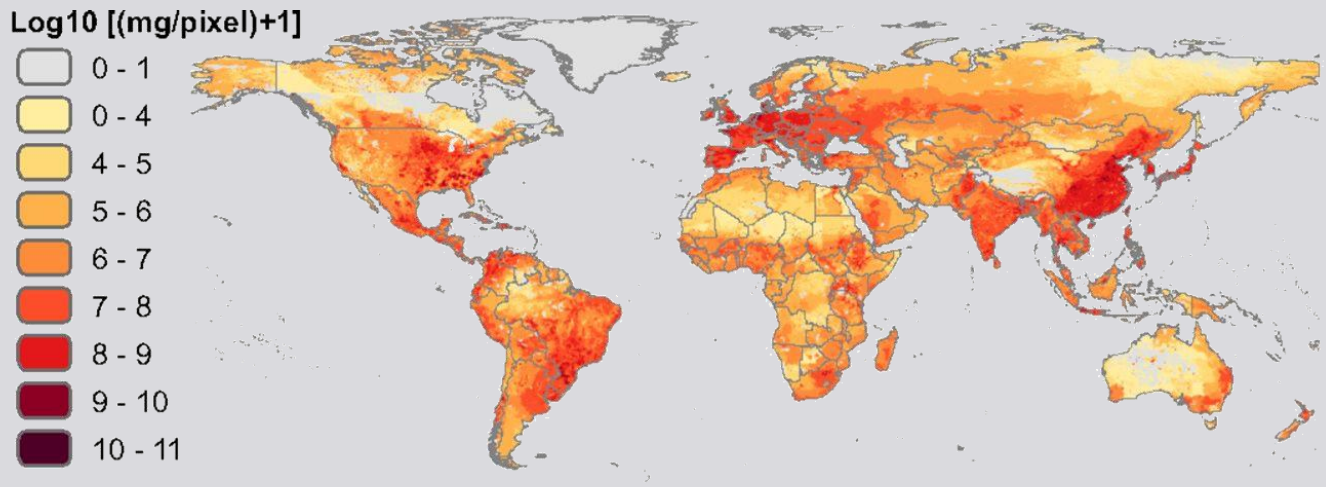
Across the globe, among veterinary animals, a high amount of antibiotics is used in swine and poultry. In the United States, particularly in Argentina and Brazil, antibiotics are used in beef cattle. Similar antibiotics are used for the human population and animal production. About 27 different antimicrobial classes are used for animals; of these only 9 are exclusively indicated for animal use. The highest sale is contributed from the classes, macrolides, penicillins, and tetracyclines; the World Health Organization (WHO) categorizes them as critically important for human.

Antibiotic Resistance Rates in Food Animals

Genetic resistance to certain diseases is developed based on the interactions between genotype and the environment of animals. It is observed that certain breeds of animals develop susceptibilities for diseases, such as Foot and Mouth disease, Sarcocystis infection, and African swine fever in pigs. Several other diseases that are common in pigs include Atrophic Rhinitis, Aujeszyky's, Porcine Circovirus Associated Disease, Salmonella, and Enterotoxigenic E. coli (F4 and F18). Enterotoxigenic E. coli strains expressing F4 fimbriae are the major cause of diarrhea among pre-weaned pigs. This kind of infection in pre-weaned pigs causes significant economic losses to the industry. The genetic tolerance of all animals evolves at the population level due to environmental stress, based on the ability of the breed.

The global antimicrobial consumption displays geographic heterogeneity across continents (refer to *Exhibit 4*). Lack of global surveillance systems for monitoring the antibiotic resistance fails to provide an exact global scenario. National level surveillance systems collect data on regular basis. Data from the recent publications in the United States (NARMS 2011) and Europe (EFSA and ECDC 2015) are summarized below.

EXHIBIT 4: Global Antibiotic Consumption in Livestock (milligrams per 10 km2 pixels), 2010



Source: Van Boeckel et al., 2015

> Lack of global surveillance systems for monitoring the antibiotic resistance fails to provide an exact global scenario.

The European Center for Disease Prevention and Control (ECDC), European Commission, and the European Food Safety Authority collect surveillance reports on antibiotic resistance in food animals from all member countries at regular intervals. These reports include resistance rates for bacterial infection from swine, cattle, and poultry; a few of the bacterial infections include E. coli, Campylobacter, Salmonella, and Methicillin-resistant Staphylococcus aureus (MRSA).

The U.S. National Antimicrobial Resistance Monitoring System (NARMS), in collaboration with the Centers for Disease Control and Prevention (CDC), the U.S. Department of Agriculture (USDA), and FDA collects antibiotic resistance reports among bacterial isolates from cattle, chickens, turkeys, and swine during their slaughter. Some of the bacterial isolates found in these animals include E. coli, Campylobacter, non-Typhi Salmonella, and Enterococcus. The reported resistance levels vary based on the animal and the antibiotic used (refer to *Exhibit 5*).

EXHIBIT 5: Antibiotic-resistant Isolates in Food-producing Animals in Europe and the US

Antibiotics Tested	Animal Type (Antibiotic-resistant Isolates in %)					
	Poultry	Swine	Cattle	Poultry	Swine	Cattle
	Salmonella spp.			*Escherichia coli*		
Ampicillin	5-98	77-87	NA	55	30	14
	7-27	11	17	16	NA	NA
Cefotaxime or Ceftriaxone	0-10	0-1	NA	6	1	1
	6-12	2	14	10	NA	NA
Chloramphenicol	0-12	12-28	18	15	15	8
	1	7	18	2	NA	NA
Ciprofloxacin	10-98	6-7	2	56	6	5
	NA	NA	2	NA	NA	NA
Gentamicin	1-85	4-8	NA	6	2	2
	4-17	NA	2	49	NA	NA
Nalidixic Acid	10-97	3	NA	52	4	5
	NA	NA	2	2	NA	NA
Sulfonamides or Sulfisoxazole	5-85	76-91	NA	45	42	20
	8-22	18	20	55	NA	NA
Tetracycline	85	72-91	NA	43	53	23
	41-46	41	31	48	NA	NA
Streptomycin	NA	NA	NA	46	48	18
	22-36	19	19	51		

Europe US

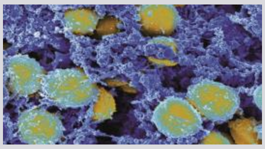
Source: Center for Disease Dynamics, Eco

Recent Advances and Alternatives to the Use of Antibiotics

Reducing the use of antibiotics in livestock is vital for the control of infectious diseases in humans and animals. Listed below are alternative strategies to prevent antibiotic resistance:

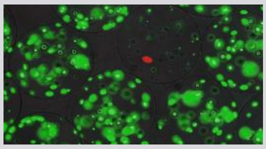
CRISPR/Cas Gene Editing System

Antibiotic Conjugates



Bacteriophages as Antimicrobial Agents

Engineered Antimicrobial Peptides



Vaccines

Chicken Egg-Yolk Antibodies

Zinc Supplement

Eubiotics

Clustered Regularly Interspaced Palindromic Repeats (CRISPR)/Cas Gene Editing System

Currently, the treatment of infections involves the use of a broad spectrum of antibiotics that leads to indiscriminate killing of beneficial commensal bacteria, thereby leading to the development of drug resistance. CRISPR-Cas technology effectively removes bacterial strains that carry genes, including those determining drug resistance. The technology helps selectively remove the AMR encoding plasmids. CRISPR-Cas systems are able to target virulence factors and antibiotic resistance genes in bacteria, providing an opportunity for the development of programmable and sequence-specific antimicrobials. It helps design antimicrobials with specified spectrum of activity to efficiently kill a target bacterial population. Unlike the existing technologies, CRISPR is effective in cases where it is desirable to eliminate only a select group of bacteria.

The greatest potential of CRISPR lies in addressing two of the most critical challenges associated with antibiotics. These challenges are listed below:

- Prevent indiscriminate eradication of beneficial intestinal bacteria
- Decrease the resistance by allowing the non-target population to thrive and occupy the ecological niche

Antibiotic Conjugates

The conjugation of antibiotics offers the targeted delivery of antibiotics to the specific site of action for the treatment or prevention of several bacterial diseases. Antibiotics in the natural form are associated with low bioavailability, biodistribution, toxicity, target specificity, and efficacy. Amidst these drawbacks, antibiotics have fast and short-acting effects, which require the administration of high doses to maintain therapeutic concentrations. The lack of specificity leads to the damage of commensal microbiota. The combination of antibiotics with conjugates avoids antibiotic-resistant pathways to revitalize antibiotics towards resistant bacteria.

THIOMAB is being developed based on the novel concept of Antibody Antibiotic Conjugate (AAC). It is currently under research and aims to treat S.aureus infections. The AAC consists of three units, namely, antibiotic, antibody, and linker, to attach the payload to antibody. The antibiotic helps target the delivery of antibiotics to the bacteria.

Bacteriophages as Antimicrobial Agents

Bacteriophages are viruses that can affect and eliminate bacteria. They are highly specific in lytic phages; bacteriophages disrupt host cells leading to bacterial cell death. DNA of the bacteriophage enters into the cytoplasm of the host through tail penetration in the cell wall. Inside the host cell, the phage genome encodes synthesis of specific enzymes to divert the host cell's protein and DNA synthesis to generate new phase particles. During the end of the phage cycle, rapid cell destruction takes place due to the formation of pores in cell membranes by phage-encoded proteins. The lytic phages are capable of replicating exponentially to eliminate even antibiotic resistance profiles.

Engineered Antimicrobial Peptides

Antimicrobial peptides are one of the most promising next-generation antibiotics that fight antimicrobial resistance. These are small amphipathic peptides (29 to 42 AA) that are positively charged and exhibit a wide spectrum of antimicrobial activity against bacteria, virus, and fungi. Compared to conventional antibiotics, antimicrobial peptides rapidly reduces the number of microbes and displays lower propensity to develop resistance. Since these class of compounds are under research at various stages, they might offer effective solution to the rising problem of gram-negative resistant bacteria.

Vaccines

The field of vaccines offers the most efficient way to prevent animal infections, thus avoiding the need for antibiotics. Vaccines are available for a multitude of diseases, and new vaccines under development are critical to tackle the antibiotic resistance as well as reduce the percentage of preventable diseases.

> An ounce of prevention is worth a pound of cure.
>
> **– Benjamin Franklin**

The whole pathogen immunization has been exploring new alternative techniques, such as subunit, DNA, and RNA vaccines.

Chicken Egg-Yolk Antibodies

Antibodies administered orally are effective in the control of enteric diseases due to their high specificity, effectiveness, and rapid onset of action. Oral antibodies are obtained from mammalian serum, colostrum, and monoclonal antibodies. Though these therapies are effective, affordability becomes a key concern. The chicken egg-yolk immunoglobulin (immunoglobulin Y) is gaining importance due to its cost-effectiveness, availability, and high yield of antibody. Immunoglobulin Y is used to prevent and treat a large number of microbial diseases in mammalian, avian, and aquatic species. In a study, Systematic Review and Meta-Analysis published by Diraviyam et al. (2014), it has been found that immunoglobulin Y is effective in controlling diarrhea in poultry, mice, pigs, and calves.

Zinc Supplementation

The supplementation of zinc oxide (1,500 to 3,000 ppm) is preferred in piglet diets to suppress bacterial adhesion and invasion, thereby preventing weight loss and diarrhea. Considering the effect of dietary zinc oxide on environmental pollution, Europe allows a maximum of 150 ppm of zinc concentration in pig feeds. Oral administration of zinc supplements in low concentrations prevents intestinal diseases caused by pathogenic organisms. Zinc in the lipid encapsulated form is preferred for oral feeding.

Eubiotics

Eubiotics are widely accepted as an alternative to antibiotic growth promoters in the animal feed industry. These eubiotics (e.g., CRINA and CYLACTIN) help maintain the intestinal flora in farm animals to enhance their health status and performance. Further, exploring new genetic approaches has made it possible to create novel probiotics or strengthen the existing probiotics that offer oral immuno-therapeutic applications.

Challenges and Opportunities

Antibiotics are considered as one of the greatest inventions in the 20th century, widely being used to treat various infections. Due to the growing concern on antimicrobial resistance, globally, the challenge is to replace them gradually and identify effective alternate treatments. The antimicrobial resistance has a greater impact not just on animal health but also has repercussions for human health and the environment. Considering the gravity of the situation, several global and national organizations are set out to identify the urgency and devise necessary strategies to address the issue of antibiotic resistance. These organizations are continuously accessing the situation, creating awareness at the ground level, and providing appropriate guidelines to farmers, veterinary practitioners, and other stakeholders.

Primary objectives of animal health organizations are to reduce the usage for preserving the efficacy of existing antibiotics, refinement to enhance their activity by using novel technologies, and find alternatives that can replace the use of antibiotics. These efforts will maintain the efficacy of the existing antibiotics and allow their use when most needed. The other growing concern is that the antibiotic pipeline is running dry, with only 15 new antibiotics approved from the year 2000. Of these new antibiotics, a very few class of antibiotics work through novel mechanisms.

Since these are at various stages of development, further research is inevitable to demonstrate their safety and efficacy to overcome antimicrobial resistance.

Addressing the AMR requires collaboration from various stakeholders in the ecosystem of animal health. It also requires cross-sector collaboration among farmers, veterinarians, pharmaceutical organizations, NGOs, and public sector entities (refer to *Exhibit 6*).

EXHIBIT 6: Stakeholders in Animal Health Ecosystem

Source: Elanco (Antibiotic Stewardship Progress Report)

Recent studies have identified that the poor quality of antibiotics is silently contributing to antimicrobial resistance in animals. Sub-standard medicines will not effectively eliminate the complete microbial population, as they offer sub-therapeutic doses of active medicament, whether due to ineffective release, impurities, or degradation of components when exposed to microbes.

The growing resistance to the existing antibiotics provides opportunities for innovation to develop new antibiotics, alternate therapies, and novel delivery systems. Unhygienic conditions in animal husbandry are the biggest contributor to the spread of infection among animals, and from animals to humans. New technologies, as well as innovative approaches in the area of waste management and feed management, are required to maintain hygiene in animal husbandry.

It is important to identify infected animals to avoid further spread of diseases and provide appropriate treatment at the earliest. Early diagnosis plays an essential role in the detection of diseases. With advancement in the Point-of-Care (POC) devices, several diagnostics tests can be performed in remote locations with immediate availability of test results. Rapid Diagnostic Tests (RDTs) reduce the time interval for test results and helps in quick decision-making on whether or not to initiate antibiotic treatment. These tests also provide opportunities to ensure the judicious use of antibiotics and improve economic outcomes.

Technological advancements provide enormous opportunities for research & development of novel approaches to prevent AMR and provide alternatives to antibiotic therapy. The recent trend indicates in-depth research in areas of CRISPR/Cas gene-editing system, antibiotic conjugates, bacteriophages as antimicrobial agents, antimicrobial peptides, vaccines, eubiotics, and zinc supplementation. Presently, with the advent of novel technologies, it has become possible to provide therapeutic solutions to microbial infections.

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