Selection for antibiotic resistant bacteria due to veterinary drug use

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Highlights:
1) The overuse of antibiotics in veterinary medicine is selecting for resistant strains of bacteria that can prove difficult to treat.
2) Antibiotic resistance can spread from veterinary bacteria to human bacteria, with implications for human health.
3) The development of resistance can be avoided through management of existing populations of resistant bacteria use of alternative treatments and investment in reversing existing resistant populations.
Antibiotics are as commonly used in domestic animals as they are in human medicine. The inability for patients to communicate their ailments and the importance of maintaining healthy food animals often leads to the over-administration of antibiotics. However, the overuse of the drugs has led to the evolution of some antibiotic resistant strains of bacteria, such as *Pasteurella* spp. and *Campylobacter* spp. This resistance has the possibility of spreading into humans, causing illness, which in turn can impact human medicine. Steps must be taken in order to ensure that the further development of antibiotic resistant bacterial strains in veterinary medicine is avoided. Additionally, further action must be taken in managing existing resistant bacteria populations, specifically stopping, or limiting, selection for the resistant trait.
Antibiotics: The double-edged panacea

The creation of antibiotics was hailed as a medical miracle, with treatments and cures for many diseases now just a simple pill or injection away. These new techniques were quickly extended into veterinary medicine, with much of the same success [1]. In addition to treating diseases, food animal producers now had a new means of promoting growth of their livestock [2]. Antibiotic usage in veterinary medicine and food animal production has reached high levels, with an estimated annual usage of 16 million kg in the United States, and 5396 tons within the European Union [2, 3]. The success of antibiotics in the treatment and prevention of disease within companion pets and food animals has helped to control the spread of infectious diseases and improve the quality of life for many animals [4]. However, the creation, and subsequent overuse, of a wide variety of antibiotics since the past century has led to the development of antibiotic-resistant strains of bacteria [5].

The evolution of this resistance began with natural variation within the bacterial populations, as a result of mutations. In stable conditions, this resistance isn’t necessarily selected for. But when antibiotics are introduced into the environment, the beneficial genes, such as those providing resistance, are selected for via natural selection [4]. If the environmental disturbance is favored for long periods of time, the mutations providing the antibiotic resistance can proliferate throughout a population [1]. The lack of attention to this proliferation of the resistant strains has led the point where many species of bacteria are resistant to select antibiotics, and a few species have become completely resistant to all existing antibiotics [6]. This resistance has the ability to transfer into human medicine and antibiotic applications through multiple mechanisms [7]. Now is the
time to prevent further antibiotic-resistant strains of bacteria from developing. This paper will discuss methods by which this can be achieved. Drugs with improved antimicrobial activity and better control programs, particularly in agricultural settings, need to be developed in order to combat this issue [8]. But in order to successfully implement any changes, the reasons why antibiotics are so widely used in veterinary medicine, as well as the mechanisms by which antibiotic resistance develops, first need to be understood.

**Why are antibiotics so widely used in veterinary medicine?**

The treatment of patients who are unable to verbally communicate their illnesses requires well-developed powers of deduction. Oftentimes, in order to eliminate, or at least narrow down, their options, veterinarians prescribe a course of antibiotics in an attempt to clear up any possible bacterial infections that may be contributing to a patient’s symptoms [9]. The patient’s response to the drugs will help indicate if a sickness is bacterial in origin.

Veterinarians rely heavily on antibiotics as part of their standard post-surgical protocol [10]. Animals, particularly canines and outdoor felines, are more likely to come into contact with bacteria in their daily routines, especially if they go outside to relieve themselves. [11] Additionally, certain types of surgery are more prone to resulting in infections. Such surgeries include pyometra removal and orthopedic repair. In order to limit the possibility of a post-surgical infection, many veterinarians prescribe a course of antibiotics as a precautionary measure [12].

Food animal production is an industry that relies heavily on the use of antibiotics. The animals that are raised for slaughter are often kept in cramped, sometimes unsanitary, conditions, where bacteria can proliferate and infections within the animals
can quickly occur [13]. The animals often experience stress as well, adding pressure to
the immune system to fight off any potential bacterial invaders [14]. If an infection were
to occur, it could spread quickly throughout the group, infecting multiple animals, and
potentially causing the loss of thousands of dollars in meat and other products [15]. In an
effort to protect their profits and limit the spread of any disease, farmers and industry
workers often administer prophylactic (see Glossary) rounds of antibiotics in their herds
and flocks, in an attempt to stave off any potential infection [16]. For example, shipping
fever is a bovine respiratory infection caused by *Pasteurella haemolytica* or *Pasteurella
multocida* [17]. It is brought on by stressful situations, most commonly the movement of
cattle from one feedlot to another [18]. In order to prevent this infection and limit the
financial impact the disease could potentially have, feedlot managers often administer
antibiotics to all of their cattle prior to shipping [1].

Should a few members of the herd or flock fall ill, it is common for large-scale
farmers and feedlot managers to administer metaphylactic (see Glossary) rounds of
antibiotics in all of the animals [13]. For example, keratoconjunctivitis, or pink eye, is a
common disease in cattle that is highly communicable, especially in close quarters [19].
Keratoconjunctivitis is easily treated by the antibiotic penicillin, and as a result, many
farmers give rounds of penicillin to all of their calves in an attempt to prevent the spread
of infection [20]. In poultry, the protozoan infection coccidiosis is very contagious, and
all birds within a large production facility are treated with anticoccidials should any of
them show symptoms of infection [1].

Farmers and their hired help often administer antibiotics themselves, so as to
avoid incurring veterinary costs. In many cases, these administrations are extra label, or
at non-recommended concentrations [21]. Nonetheless, if the farmers or managers are able to save money by treating an animal themselves, some will attempt to do so before paying for professional help [21]. Veterinarians themselves also sometimes implement extra-label use of antibiotics. This extra-label usage is done because the veterinarian feels that the benefits of a drug are worth the administration of that drug, even if those benefits aren’t acknowledged as legitimate uses by the Food and Drug Administration [22]. An example of an extra label use is the administration of the antibiotic ceftiofur in cattle as a treatment for mastitis [23]. Ceftiofur is labeled for use against foot rot and respiratory disease in most farm animals, including cattle, but it has shown effectiveness against mastitis, or inflammation of the udder, that is sometimes hard to treat [24]. For that reason, veterinarians may choose to include ceftiofur in their course of treatment of the mastitis, even though that specific use isn’t recognized by the FDA.

The ability to grow an animal to finishing weight (see Glossary) in as fast of a time period as possible is essential in order for the food animal production industry to make a profit. In order to achieve this, the administration of antibiotics as a growth enhancer is a common practice [21]. The antibiotics are mixed in the feed of the animals, and serve to bolster the immune system to quickly eliminate any infection or disease that the growing animals may encounter. This supplementation, in turn, allows the food animals to remain healthy and have their energy allocated to growing and gaining weight [22]. The porcine industry, particularly, relies heavily on antibiotics as growth enhancers [23]. Without the antibiotics, the amount of feed required for piglets to reach weight milestones will increase by 2-3 kg [24]. Additionally, the mortality rate will likely see a
10-15% increase, as the control over the subclinical diseases via antibiotic supplementation will be eliminated [24].

What antibiotics are losing their effectiveness in veterinary medicine?

Many of the same antibiotics that are utilized in human medicine are also used within veterinary medicine [8]. Drugs such as tetracyclines, penicillins, sulfonamides, fluoroquinolones and β-lactam antibiotics have been widely used in animals, and thus bacteria have evolved resistance to these drugs [25]. This resistance develops as a result of selective pressures within the bacterial environment, namely the presence of antibiotics [26]. The resistance is a beneficial trait to the bacteria living within that environmental context, and as such it is proliferated throughout the population [27].

Modes of resistance vary, depending on the species of bacteria and the drug that is used against them. The most common mode of bacterial resistance to tetracycline is through the presence of a special protein that coats the bacterial ribosomes. The ribosomes are the target site of the drug [28]. The protein prevents the tetracycline from binding to the ribosome, and thus it prevents the interruption of the bacteria’s ribosomal processes. This lack of antibiotic binding makes the bacteria resistant [29]. This protein is, in part, encoded for by the tet gene, as determined by PCR analyses of bacterial isolates [30]. Tetracycline can also acquire resistance through increased efflux of tetracycline out of the cell [31]. There are two types of efflux mechanisms, those being drug-specific or multidrug mechanisms [32]. Drug specific mechanisms tend to be the result of plasmids, whereas the multidrug mechanisms are largely due to encoded mutations of the bacterium’s regulatory genes. [33]. In either case, if the bacterium is
able to remove the antibiotic from within itself before the drug has a chance to harm the
cell, the bacteria essentially become resistant.

Sulfonamides developed resistance through their acquisition of genes that encode
for a drug-insensitive enzyme [34, 35]. Those genes are known as the \textit{sul1, sul2, and sul3}
gen [35]. The normal mode of action for sulfonamides is to target the enzyme
dihydropteroate synthase (DHPS) within the bacteria’s folic acid pathway [36]. This
enzyme is responsible for folate synthesis, which the bacterium requires for growth [37].
Resistant mutants who possess the \textit{sul} class genes were able to encode insensitive
variants of the dihydropteroate synthase enzyme, allowing them to become impervious to
the antibiotic, and continue their growth and eventual reproduction [38, 39].

Fluoroquinolones typically target bacterial DNA gyrase and topoisomerase IV to
prevent the supercoiling of the chromosomal material [40]. The drug creates
conformational changes in those enzymes that results in the blocking of the progression
of the replication fork during DNA synthesis [41]. This leads to the cell’s inability to
grow, rapidly resulting in death [42]. In some resistant strains, there have been
chromosomal mutations in the bacterial efflux system, similar to those seen in
tetracycline resistance. These changes eliminate the effect of the drug on the bacteria,
effectively making it resistant [42].

Examples of bacterial resistance

\textit{Camplyobacter} spp. are a pathogenic bacteria found within the intestinal tract of
animals [43]. In veterinary medicine, these bacteria are commonly associated with
gastrointestinal infections such as gastroenteritis and diarrhea, especially within canines
and pigs [44, 45]. Specifically, the species \textit{C. jejuni} and \textit{C. coli} are responsible for the
majority of gastrointestinal infections [46]. Infections due to *Campylobacter* spp. can be
costly to treat, so many farmers and food animal production managers treat their livestock
with fluoroquinolones upon recognition of symptoms, especially chronic diarrhea, within
the group [47]. This metaphylactic use of fluoroquinolones has led to the development of
resistance within the *Campylobacter* spp to fluoroquinolones, specifically with use in
porcine and poultry industries [48].

*Pasteurella* spp. are a naturally occurring bacteria found on the mucosal surfaces
of vertebrates [49]. These bacteria are commonly implicated in widespread respiratory
infections of animals, especially ruminants (see Glossary) and domestic rabbits [50].
Symptoms of *Pasteurella* spp. infection typically include fever, nasal discharge, poor
appetite and lethargy, although the symptoms may also resemble pneumonia [51]. The
high occurrence of infection has led to the use of multiple antibiotics as forms of
treatments. This, in turn, has led to the development of resistance in *Pasteurella* spp. to
many antibiotics, including streptomycin, β-lactam antibiotics, penicillin, sulfonamides
and tetracycline [50, 52]. Furthermore, there have been discoveries of strains of
*Pasteurella* spp. that have developed resistance to combinations of drugs, such as
streptomycin, penicillin and tetracycline [52]. This multi-drug resistance serves to show
how overused the antibiotics were in the treatment and prevention of *Pasteurella* spp.
infections.

*Staphylococcus aureus* naturally occurs on the skin and mucosal surfaces of
animals, especially canines and felines [53]. Infection occurs through the introduction of
the bacteria into a wound or abrasion [54]. Classic symptoms of infection are cutaneous
inflammation, abscesses and respiratory disease. It can also be a cause of bovine and
caprine mastitis [55]. The large degree of antibiotics use to treat the infections has led to the evolution of antibiotic resistance. In particular, *S. aureus* is known to be resistant to the antibiotic methicillin, as seen with resistant strain referred to as methicillin-resistant *S. aureus* (MRSA) [56]. *S. aureus* has also shown resistance to multiple other antibiotics, including tetracycline, chloramphenicol, aminoglycosides, cephalosporins and β-lactams [57, 58].

**What is the potential impact on human medicine?**

**Transfer via physical contact**

The resistance that evolves within veterinary bacteria has the potential to impact human medicine. Zoonosis (see Glossary) of the resistant strains is able to occur, posing a risk to human health [59]. People who are employed at farms and/or food animal production facilities are at a higher risk of infection with a resistant strain of bacteria [2]. Zoonotic acquisition of resistant *Campylobacter* spp. has occurred in Spain, where farm workers became infected after coming into contact with feces from poultry and porcine that contained the resistant bacteria [60]. The bacteria they encountered were resistant to treatment with ciprofloxacin and fluoroquinolone [60]. A similar event happened in Germany, where pig farmers were found to have streptothricin-resistant strains of *E. coli* in their gut flora [61]. This occupational pathway is a mechanism by which the resistant bacteria can be released into society [62]. The same strain of bacteria found within the German pig farmers caused urinary tract infections in the people who had no contact with the farm, but lived within the same area. This transfer was probably mediated by the town’s water supply, which would have spread the bacteria the required distance and speed to match the time of infection [3, 63]. These cases illustrate how the constant
exposure to antibiotics impacted the bacteria of humans, and the ease at which resistant
strains can enter society and quickly impact human medicine.

Physical contact with animals that possess resistant bacteria is one of the easiest
pathways for the bacteria to cross over to humans. *Pasteurella* spp. bacteria are common
in the mouths of household pets, and as such can be transmitted from canines and felines
to humans through bite wounds [64]. Should the animal possess resistant strains, those
bacteria can then enter the wound on the human. From there, complications can ensue,
including septicemia and abscesses that may prove difficult to treat with antibiotics [65].

There have also been cases where humans have acquired MRSA infections from their
canine companions, and vice versa [66]. It has been suggested that MRSA infections can
cycle between pets and their owners unless both receive appropriate treatment [67].

Perhaps one of the most severe examples of zoonotic antibiotic resistance can be
seen with the infections, and resulting deaths, of two patients infected with resistant
strains of *Salmonella typhimurium* [60]. Both elderly patients showed symptoms
indicating gastroenteritis, and were treated with multiple antibiotics before their
subsequent deaths [68]. The first patient died from perforation of the intestines and colon
and the resulting peritonitis. The second patient died from perforation of the intestine and
bladder and the resulting peritonitis. The resistant *S. typhimurium* was cultured from the
peritoneal fluids of the second patient [68]. The source of infection was from resistant
*Salmonella typhimurium* transferred to the patients from pork [60]. While the *S.
typhimurium* infection wasn’t implicated as the sole cause of death, it was a major
factor, and had they not been infected, both patients may have lived longer. While this
case may be an extreme example, it serves to show the potential outcomes of some
infections with antibiotic resistant bacteria, and that certain groups of people, in this case the elderly, are at higher risk should they become infected.

**Transfer without physical contact**

The development of antibiotic resistance in human bacteria doesn’t require humans to have direct exchange with the resistant animal bacteria. Resistance can spread from animal-associated bacteria to human-associated bacteria via gene transfer, such as through conjugation or transformation. In conjugation, a recipient cell receives DNA from a donor cell via transfer with a sex pilus, which extracts the DNA from the donor bacteria [69]. In transformation, a bacterial cell picks up DNA from the environment that has been released by a lysed bacterium [70]. Were human bacteria to receive resistance via DNA uptake, that resistance could proliferate within the species, which in turn could potentially lead to an increase in treatment failures in human illnesses [59]. Conjugation poses a larger risk than transformation, as conjugation can occur between different species and genuses, whereas transformation seems to be limited at the species level [70]. Documented examples of such cases include the transfer of ampicillin and streptomycin resistance from *E.coli* bacteria to *Havia alvei*, and the transfer of vancomycin resistance from *Enterococcus faecalis* to *Staphylococcus aureus* [58, 60]. Additionally, this horizontal gene transfer may be able to occur between organisms that are distantly related, such between gram-positive and gram-negative bacteria, furthering the possibility of resistance development [71].

Antibiotic residue in food products derived from animals can select for resistance in human gut bacteria. Residues can be sequestered within the meat of the animal, or in other products, such as the milk of cows and goats and the eggs of poultry [72]. Animals
administered antibiotics must the specified withholding period (see Glossary) before their
meat can be consumed or their products, such as milk, can be sold [73]. However, trace
amounts of the drugs are almost always left behind [74]. This near-constant exposure to
low levels of antibiotics allows the gut bacteria of the consumer to develop resistance
relatively easily [72]. Were those intestinal bacteria ever to cause illness or disease, such
as gastroenteritis or diarrhea, then treating the illness and managing the bacteria
population would be difficult, lengthy, and more than likely expensive, as different drugs
and/or different drug combinations may be required. Furthermore, resistant bacteria can
be transferred to meat and food products during the slaughter process [60]. If the meat
isn’t cooked properly, the bacteria may survive and infect whoever ingests it. Similarly, if
the milk isn’t pasteurized properly, the resistant bacteria may persist within the product
[70]. Either case would expose the consumer to resistant bacteria, increasing their risk for
infection.

The presence of antibiotic residue within food products also can pose a risk to
some individuals with autoimmune disorders. Guillain-Barré syndrome is an autoimmune
disorder that is characterized by acute neuromuscular paralysis and limb weakness [75].
Episodes of this syndrome are often triggered by infections of Campylobacter spp.
infections, and are relieved when the infection is treated or weakens in its intensity [76].
However, in individuals who are infected with a resistant strain of Campylobacter spp.,
treatment of the infection takes longer, resulting in longer periods of neuromuscular
symptoms [77]. This resistance can arise from ingestion of residue-tainted meat, which
selects for antibiotic resistance within the person’s gut flora, increasing their risk for
prolonged infections and autoimmune attacks [72].
How can the development of resistant bacteria be avoided?

If a future that includes the use of antibiotics is to be expected, then steps must be taken now in order to ensure that the development of resistance within bacteria is avoided. One of the first steps that should be taken is the enactment of legislation that regulates the use of antibiotics. Indeed, some countries already have enacted such legislation, including Sweden and Denmark [78]. Sweden was the first to pass laws controlling veterinary antibiotic use. In 1986, they banned the use of antibiotics as growth promoters in all food animals [79]. Following suit, in 1997 the European Union banned the use of the antibiotic avoparcin as a growth promoter, and the use of spiramycin, virginiamycin, bacitracin and tylosin as growth promoters in 1999 [80]. Finally, in 2006, the European Union banned the use of all antibiotics as growth promoters for food animals [81]. Denmark has taken their action a step further, by erecting a monitoring system in order to track the development of resistant bacterial strains. This system is known as the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) [82]. Cofounded in 1995 by the Danish Ministries for Health and for Food, Agriculture and Fisheries, DANMAP monitors the yearly consumption of antimicrobial agents within Denmark, and tracks the occurrence of resistance within bacterial populations [83]. As a result of the Swedish ban, the use of antibiotics within animals decreased by around 55%, and the observed resistance levels within bacteria hasn’t grown [84]. The same is true when Europe is examined as a whole; while antibiotic use for treatment of infections has increased, as the animals no longer have a constant amount within their systems, the overall usage of antibiotics has decreased significantly [81].
But not all countries have such rigorous monitoring and tracking systems. The United States has no public records regarding the amount of antibiotics used within veterinary medicine, and no monitoring system to track the usage of antibiotics [85]. All figures regarding the amount of antimicrobial drugs used within the United States are estimates based on the reported sales figures [2]. The FDA is currently working on implementing new restrictions on the use of antibiotics as growth promoters within the food animal industry, but that only goes as far as asking drug manufacturers to voluntarily eliminate the growth promotion claims from their antibiotic labels [86]. The successes seen within the European countries should serve as a model for the United States in order to limit the overuse of antibiotics, and thus reduce the selection for the resistant strains of bacteria.

Another step that would aid in the avoidance of bacteria developing resistance is increasing the education of veterinarians, pet owners, farmer and food animal production workers. Veterinarians need to be reminded of their role within public health [87]. The potential zoonosis of resistant bacteria means veterinarians need to be more cognizant of outbreaks and the potential transmission to owners and handlers of the animals, and the reporting processes for those outbreak events [88]. Additionally, the importance of conservative use of antibiotics should be stressed, to ensure that they remain an effective form of treatment [89]. This includes reducing the use of antibiotics as a form of diagnosis by ruling out bacterial infections based on response to treatment with the drugs. Pet owners should be educated on the potential zoonosis of any infection, in an effort to limit the potential spillover of resistance into human-associated bacteria [90]. Farmers and food animal workers should be made aware of the potential danger in using
antibiotics as growth promoters [87]. They should also be educated on limiting the
administration of antibiotics without the approval from a veterinarian who has conducted
a physical exam of the sick patient [91]. Increasing the education of all people involved
with the administration of antibiotics to animals will help reduce the overuse of
antibiotics within veterinary medicine, thus limiting the selection for resistance in the
bacteria the drugs are used against.

The development of alternative solutions instead of resorting to the use of
antimicrobial drugs should also help minimize the development of more bacteria that are
resistant to antibiotics. Estimates have been made that state that upwards of 80% of
antibiotics could be removed from veterinary use without severe consequences [87]. To
solve the problem of needed growth promoters within livestock, different feeds should be
evaluated for nutritional content. Feeds with differing levels of starch and nonstarch
polysaccharides, based on the animal species they are being fed to, have been shown to
aid in the growth of the livestock, when compared to standard grain feed [92]. Probiotics,
prebiotics and enzyme supplements have also been shown to aid in the growth of poultry
[93].

If the use of drugs cannot be avoided or replaced with an alternative, then
consideration and funding should be given to the development of new drugs that are able
to hinder the development of resistance. An example of a drug that would be capable of
doing this would be a compound that could avoid efflux pumps [94]. As aforementioned,
efflux of the antibiotic out of the cell before the drug is able to reach its target is one
bacterial mode of resistance. If, somehow, an antibiotic compound could be created that
is able to avoid this mechanism, then that mode of resistance would be useless towards
that drug. However, much care must be put into the development of such a drug to ensure it won’t be harmful to non-target cells.

Resistance can further be avoided by keeping the living conditions of animals clean and sanitary, and limiting their risk of infection. In domestic pets, that entails clean living conditions and prompt treatment of any open wounds or potential sources of infections [95]. It also means limiting the animal’s contact with any humans who may be infected with a potentially zoonotic disease, such as MRSA [67]. For food animals, improved living conditions also include the daily removal of manure, room for the animals to move and lay down, a dry environment and shelter from extreme temperatures [96]. Having a clean environment limits the possibility of infection, and decreases the stress the animals may feel, allowing the immune system to deal effectively with any potential bacterial pathogens.

Consideration should be given to the withholding times for animal meat that had exposure to antibiotics. It is largely the responsibility of the farmers and managers to adhere to the withholding guidelines, as it is not feasible for slaughterhouses to test every piece of meat to ensure antibiotic levels inside the products are within limits [73]. In order to aid the farmers in achieving the proper withholding time, simple field tests should be developed, and existing tests refined, so that the farmers can quickly and cheaply test their products, especially milk, for the presence of high antibiotic levels [97]. The recommended withholding times should also be reevaluated based on modern day doses and concentrations of the antibiotics, in order to ensure all recommendations are correct [98]. Limiting the drug residue within edible animal products will help avoid the development of resistant in the gut flora of the consumers.
What are the next steps in managing the resistant bacteria?

Unfortunately, selection for resistance within bacterial populations has occurred. Changes need to be implemented in order to prevent the proliferation of this trait throughout larger populations of bacteria. Any infection within an animal that does not respond to treatment with antibiotics should be considered a matter of biosecurity (see Glossary), in order to best prevent the spread of the resistant bacteria [99]. Animal patients who are found to have infections stemming from antibiotic-resistant bacteria should be isolated [100]. Additionally, any people or other animals that have contact with the infected animals or their excretions, including milk, eggs and manure, should observe proper sanitation protocols [101]. Such protocols include removing any clothing that may have come into contact with the animal or its wastes, disinfecting any tools/instruments used on the animal and proper use of biomedical waste disposal systems [102]. For animals that are located on a farm, protocols can be extended to include the removal and subsequent bleaching of the shoes/boots worn within the pen or stall of the sick animal [103]. The excretions from the animal should be disposed of in a proper manner, so as to avoid the introduction of any antibiotics or antibiotic-resistant bacteria into the environment (Box 1). Following proper sanitation protocol should help eliminate the movement of the resistant bacteria into new environments and/or hosts.

When treating animals that appear to be infected with resistant bacteria, any treatment utilizing antibiotics that the animal may be undergoing should be halted at once. The continual administration of the antibiotics into the animal will only continue to select for the resistance within the bacteria [104]. Instead, alternative treatments should be considered, including euthanasia of the animal should either the infection prove too
difficult to treat or the risk of spreading the bacteria is too high [105]. The use of drug combinations should also be eliminated, or reduced as much as possible. The administration of multiple different drugs to treat one infection, in the hopes of one of them being able to work, is just a band aid solution that will only temporarily solve the problem; while it may clear up that specific infection, it also may allow for the development of resistance to multiple drugs, in either the bacteria being targeted for treatment or in the gut flora of the animal [106].

The overall widespread use of antibiotics must be controlled in order to manage existing populations of bacteria who have developed resistance. This is due to the resistance being a beneficial genetic trait, as determined by natural selection [107]. If the antibiotics are removed from the bacterial environment, which in this case would be the infected animal, then the ecological disturbance that is the antibiotics will end, and so too will end the selection for the resistant genes [105]. Indeed, the resistance may have high fitness costs in other environments, allowing for it to be outcompeted by susceptible bacteria in a drug-free environment [108]. Without the drugs, there would be no evolutionary benefit to having the resistant genes, allowing the non-resistant strains of bacteria to increase in population size, thus lowering the overall concentration of resistant cells [104]. Stopping the widespread use of antibiotics means removing them from animal feed as a growth promoter. As discussed before, their continual presence within the animals allows for continual selection for resistant bacteria. Should the amount of antibiotics in the meat product derived from that animal be high, they can go on to impact the consumer, by selecting for resistant gut bacteria within them. Managing existing
resistant populations means an end needs to be brought to the selecting factor [105]. That can only be accomplished with an end to the overuse of antibiotics.

Perhaps the best way to manage existing populations of antibiotic resistant bacteria would be to somehow reverse the resistance. Non-therapeutic chemicals that have demonstrated the capability of doing this are labeled non-antibiotics [109]. Examples of such compounds include methylene blue, amitryptiline and phenothiazines [110]. These compounds are particularly useful when used in combination with antibiotics. This combination of antibiotics with non-antibiotics is known as synergy [111]. In synergy, the non-antibiotics are able to remove or overcome the bacterial mechanisms of resistance, allowing the antibiotic to work as it should [110].

One of the ways in which the non-antibiotics are able to reduce resistance is by eliminating the resistant cell’s capability to efflux the antibiotics out before they have a change to reach their target [110]. The non-antibiotics that are able to reduce the efflux of the drugs are termed efflux pump inhibitors [112]. These efflux pump inhibitors work by obstructing the cell membrane polypeptides and efflux proteins, preventing them from recognizing and binding to antimicrobial compounds [113]. With the efflux pumps disabled, the antibiotic is then able to reach its target site within the bacterial cell and work as it normally would. While there are no data on or examples of the successful implementation of non-antibiotics within veterinary medicine, successes have been shown within human medicine. *Mycobacterium tuberculosis* is the bacterium responsible for the disease tuberculosis within humans [114]. After the development of antibiotics, it was treated aggressively with multiple different drugs. As a result, *M. tuberculosis* has developed resistance to a large number of antibiotics [115]. The mechanism of resistance
for *M. tuberculosis* is through its membrane efflux pumps. These pumps are able to recognize the structurally diverse antibiotics, and eliminate them from the bacteria cell before they have a chance to work [116, 117]. This resistance to a multitude of antibiotics leaves modern day infections difficult to treat, and dangerous to the community [115]. However, recently there has been evidence that supports the use of non-antibiotics as a mechanism to overcome resistance within resistant strains of *M. tuberculosis*. The non-antibiotic thioridazine, a type of phenothiazine, shows the most promise in the treatment of tuberculosis infections [118]. Thioridazine eliminated the effects of efflux pump, making the *M. tuberculosis* population susceptible to eradication with antibiotics that typically could not be used as treatment options. This successful use of synergy to overcome bacterial resistance shows the promise that these non-antibiotics can hold for veterinary medicine. However, clinical trials for these drugs have yet to begin, so widespread use of the compounds isn’t expected for years [112]. This means managing existing populations through other means, like those aforementioned, is still important to reduce the spread of resistance.

**An uneasy future**

The creation of antibiotics helped to usher in a new medical era, where previously untreatable diseases and infections could be cured quickly. But the overuse of antibiotics in veterinary medicine allowed for the selection of resistance within the bacterial populations the drugs were used against, creating problems for animals and humans alike. This connection has been known for years, but some countries still don’t have the measures and legislation in place to help monitor and control the spread of antibiotic resistance within bacterial populations. Veterinarians, pet owners, farmers and food
animal production workers need to do their part in helping to curb the spread of the
resistance. Antibiotic usage needs to be limited to use in treatment of bacterial infections,
not as metaphylactic or prophylactic supplements or in livestock food as growth
promoters. This restricted use will help to ensure that they remain viable treatment
options for future illnesses. Research needs to be continued on the best ways to avoid the
development of resistance, and how to best deal with existing resistant populations. Only
then can there be a future that includes antibiotics.
The antibiotics and antibiotic resistant bacteria that are present within the manure of infected animals poses a threat to the environment. Drugs such as tetracycline, aminoglycosides and sulfonamides have all been detected within soils surrounding large-scale farms or food animal buildings [119]. In a process very similar to that seen within the human gut, those antibiotics that are leeched into the environment can select for antibiotic resistant soil microbes [120]. These resistant microbes can serve as reservoirs for resistant genes, and increase the genetic diversity of the bacterial populations in which resistance is present [121]. This increase can serve to spread resistance to bacteria populations that are still susceptible to antibiotics, through routes such as conjugation or transformation. Additionally, antibiotics that are present within animal manure can pollute nearby water supplies [122]. Again, the introduction of antibiotics into the environment can select for resistant bacteria, potentially altering the ecosystem and creating large gene pools of bacterial resistance [120].

The extent to which the resistance remains within the soil bacteria depends on multiple factors, including the concentration of antibiotics, climate conditions, if the antibiotic runoff is constant or a rare occurrence and other environmental conditions [2]. For example, soil samples from four different Danish farmlands were tested for soil microbe resistance levels to three common antibiotics over an eight month period [123]. Resistance levels increased when fertilizer slurry made from the manure of pig treated with antibiotics was applied to the area [123]. Once the manure began to break down, resistance levels then declined slowly, indicating that the addition of antibiotics from within the manure was the source of the observed increase. A similar study conducted in
Japan illustrated how the long-term use of pig manure on farm soil can elevate the levels of antibiotic resistant soil microbes [124]. The same study also showed that antibiotics that were added to the livestock feed led to the development of multidrug resistance within the soil microbes, instead of just resistance to one antibiotic [124]. These studies illustrate how the overuse of antibiotics, particularly within the food animal production industry, can impact the environment, leading to the development of antibiotic resistance within natural soil bacteria. This introduction of antibiotics should be as limited as possible, as the environmental impacts may lead to larger ecological problems in the future.
GLOSSARY

Biosecurity: ensuring the protection of animals and humans from any potential agent of infection

Finishing weight: When an animal raised for food production reaches the required weight for slaughtering

Metaphylactic: The administration of antibiotics to a whole group of animals when a small number in the group become sick

Prophylactic: The administration of antibiotics to an animal or group of animals without any signs of illness in an attempt to prevent the development of any illnesses

Ruminants: Ungulate mammals who have a four-chambered stomach and digest plants via fermentation

Withholding period: The minimum amount of time between administration of a drug into a food animal and the use of that animal’s meat or products, including eggs and milk

Zoonosis: An infection or disease that humans can acquire from animals
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