Complexities in understanding antimicrobial resistance across domesticated animal, human, and environmental systems

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Antimicrobial resistance (AMR) is a significant threat to both human and animal health. The spread of AMR bacteria and genes across systems can occur through a myriad of pathways, both related and unrelated to agriculture, including via wastewater, soils, manure applications, direct exchange between humans and animals, and food exposure. Tracing origins and drivers of AMR bacteria and genes is challenging due to the array of contexts and the complexity of interactions overlapping health practice, microbiology, genetics, applied science and engineering, as well as social and human factors. Critically assessing the diverse and sometimes contradictory AMR literature is a valuable step in identifying tractable mitigation options to stem AMR spread. In this article we review research on the nonfoodborne spread of AMR, with a focus on domesticated animals and the environment and possible exposures to humans. Attention is especially placed on delineating possible sources and causes of AMR bacterial phenotypes, including underpinning the genetics important to human and animal health.

Keywords: antimicrobial resistance; antibiotic use; animal agriculture; fecal matter; soil and wastewater

Introduction

Antimicrobial resistance (AMR) is a global problem that impacts both human and animal health. Although AMR primarily derives from antibiotic and antimicrobial use, strong evidence suggests that wider spread of AMR is fueled by inadequate local sanitation, pollution, and other nonuse factors, with the natural environmental being an important conduit.[1–3] Explaining AMR and then developing informed approaches to mitigate it are difficult because many technical, scientific, and behavioral factors come into play. Factors range from understanding the evolution of resistance at the molecular level within a given organism, to transmission mechanisms and pathways across organisms, to wider dissemination between human and animal hosts and across the wider environment, including soil and water. Further, due to the urgency in addressing the problem, researchers from many disciplines are studying AMR from a variety of angles and progressively learning more about it at different levels. However, given the diversity of research perspectives and complexity of the problem, sometimes contradictory answers are being provided to key questions, such as the primary drivers of AMR, the relative role of the natural environment in AMR spread, and actual health risks associated

with different types of environmental and other exposures.

Such general queries are also true of AMR within the food animal production industry. The environment is clearly a possible source of enteric and other bacteria, including AMR strains, that can affect food animals. In fact, it is suspected that the majority of microorganisms enter animals through ingestion of water and/or soil, or through contact with other animals and/or humans in their proximity, which include antimicrobial-resistant bacteria (ARB) and antimicrobial resistance genes (ARGs). AMR sources are now common in nature, although some ARGs are also ancient; but with increased antimicrobial drug use after World War II,[5] ARB with recently acquired AMR has dramatically increased.[6] In terms of domesticated animals, this phenomenon appears to have been exacerbated by some management practices, particularly in food animals.[7] Therefore, it has become critical to understand wider factors that impact AMR in animal systems, including possible exposures to contaminated water and soils by wastewater, manure, and other sources, which are considered probable drivers of AMR transmission and dissemination pathways to and from food animals.[8]

The intrinsic complexity of AMR makes these relatively simple questions difficult to answer. Specifically, there is debate about how ARB enter wastewater and soils; how long they survive, retain viability, and infectivity; how best to quantify AMR, either ARB or ARGs, or both; and how to define and contrast risks when environmental sources are often overlapping or nebulous. Addressing these questions is further challenged by difficulties in detecting and identifying ARB and associated ARGs in environmental samples.[9] Abundances of ARGs are often less than 0.1% of microbial DNA sources,[10] and linking an ARG or suites of ARGs to conferred phenotypic resistance is difficult, especially in mixed microbial communities common in nature. For example, an ARB phenotype may result from one specific ARG to an antibiotic in an expressible location, whereas the same phenotypic response can also result from genes for less specific purposes, such as efflux pumps, which are not directly related to a specific antimicrobial agent.

Alternatively, if antibiotics used in food animals are the same or structurally similar to agents used for humans, animal use may amplify ARB of public health concern.[11,12] Such "indirect" ARB selection also occurs when ARGs conferring different types of resistance are closely located on a chromosome or plasmid, which means the selection for one type of resistance might confer other types of resistance. In turn, enteric ARB shed in feces can spread to other livestock and farm workers, reentering the environment and exposing other animals through drainage water and manure. This is analogous to AMR spread via human open defecation, which places raw fecal matter in close proximity to other individuals, increasing the risk of enteric ARB and ARG spread across human populations associated with poor sanitation.[2]

Finally, commensal bacteria such as *Staphylococcus aureus* can be transmitted to and from food animals and humans who work on farms or live in close proximity to livestock.[13] In fact, recent evidence from the Netherlands suggests that immediate proximity to livestock, such as being a farm worker versus a member of their family, significantly increases their carriage of methicillin-resistant *S. aureus* (MRSA); 38% versus 16%, respectively, in nasal and throat swabs (much higher than wider Dutch populations).[14] This study also found that only ~7% of the workers were persistent MSRA carriers, and that such exposure effects did not appear to translate to carriage in wider communities.[15] Similar conclusions were reported from a study comparing livestock-associated MRSA among farm and slaughterhouse workers in Italy.[16]

When considering the above factors and more, one can see why it is difficult to delineate clear routes of AMR dissemination among food and other domesticated animals, human populations, and the environment. Within this context, the intent of our review here is not to exhaustively discuss all links, methods, and pathways because these have been summarized in previous reviews.[11,17,18] Instead, our goal is to flag the complexity of AMR as a general problem with the hope of disentangling sometimes contradictory information into useful guidance for the food animal production industry. The article is based on presentations and discussions of an integrated discussion group associated with the conference "Minimizing the Risk of Antimicrobial Resistance from Food Animal Production," hosted by the New York Academy of Sciences on May 8 and 9, 2018.
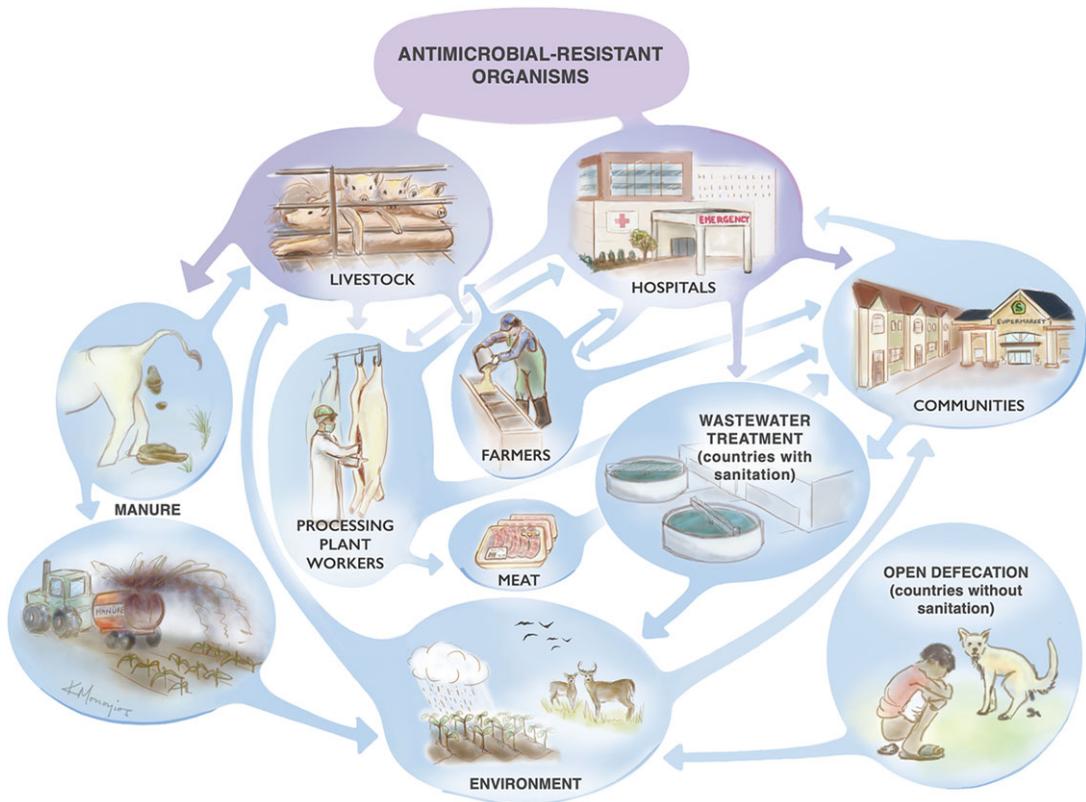
Figure 1. Potential sources and sinks for antimicrobial resistance across domesticated animals, humans, and environmental systems (adapted from Ref. 63).

Complexity of antimicrobial resistance across domesticated animals, humans, and the environment

The potential for evolution, exposure, and/or spread of AMR spans almost all elements of rural and urban life. Antimicrobial use and other factors that drive AMR are diverse and often interlinked, which are displayed within the context of the food animal production industry in Figure 1. Although Figure 1 shows an array of interactions, the reality is more complex than the figure suggests because underlying the links are even more diverse microbial and genetic phenomena, which are not well understood. Therefore, it is not surprising that AMR is such a difficult problem to address, because everything affects everything else. Therefore, we address only a few considerations to exemplify points of particular importance that overlap the food industry. These include the impact of AMR in fecal matter entering the environment; the fate of AMR in soils and water;

dissemination of ARB and ARGs among people and domesticated animals; and the need for more targeted surveillance.

Fecal matter and the environment

The massive amounts of fecal matter generated by humans and animals around the world can hugely impact local and global biomes. Humans and their domesticated animals constitute approximately 96–98% of the global terrestrial mammalian biomass.[19] The fecal matter associated with such huge populations has been estimated to be increasing by over 52 billion kilograms per year since 2003; and the total fecal matter produced is expected to reach at least 4.6 trillion kilograms per year by 2030.[20] Food animals, particularly cattle, chickens, and sheep, produce about four times more fecal matter than humans,[20] which implies that appropriate fecal matter management in food animal systems must be globally influential. Further, food animal and truly domesticated animals (e.g., companion animals) often reside close

to human habitations, especially in less developed countries, and can contribute to fecal matter exposures.

Although most of the developed world has effective wastewater treatment for human wastes, 73% of the world has no major waste treatment.[3] The extent of suitable waste management options is even less for domesticated animals, which implies that massive quantities of untreated animal fecal matter are entering the environment, potentially contaminating food and water, and also threatening public health. A strong push to address the problem is currently occurring at the World Health Organization (WHO), the World Bank, and other international agencies,[21,22] but more effort is needed, especially incrementally improving sanitation and waste treatment as strategies to reduce AMR,[2] both for human and animal fecal sources.

Wastewater.   Wastewater represents an important potential route for spreading antibiotic-resistant organisms to and from animal agriculture.[23] For example, wastewater around the world can contain carbapenem-resistant Enterobacteriaceae (CRE)[24] and other medically important ARB, including particularly resistant strains in human hospital effluents.[3,25] Although secondary and tertiary wastewater treatment plants substantially reduce bacterial loads, typically reducing ARB by 10 to over 1000 times,[17] such treatment processes do not remove all resistant bacteria. The fate of ARB and potential risks associated with their release to the environment heavily depends on the type and level of wastewater treatment. However, regardless of treatment, ARB in effluents often enter surface waters that can potentially impact downstream water users. This is particularly true where wastewater or treated effluents are used in irrigation systems (common around the world), such as for feed grains, where bacteria, including ARB, can associate with plant surfaces, especially leafy vegetables consumed by livestock or humans. Further, wastewater irrigation is also a means for surface water contamination, which can impact water quality and livestock.[11]

Although wastewater-mediated ARB and ARG spread is almost certain, its relative importance to promoting AMR is less known, partially because it is very difficult to determine sources of specific bacteria in wastewater. Previous work has suggested that hospitals and agriculture are important sources

to consider,[3] and there may be overlapping concerns. As an example, one study detected bla_{KPC-2} in 72 beef cattle lots in the United States, both conventional and raised without antibiotic operations, and many samples contained functional bla_{KPC-2} genes (i.e., genes recovered from samples that can be transformed and expressed in a competent cell).[26] bla_{KPC-2} is also a very common carbapenemase gene found in hospitalized human patients,[27] which implies that overlap between ARB in animal agriculture and hospital sources is probable. However, ARB detected in wastewater are not exclusive to hospital sources. In fact, some studies have shown that hospitals are not major sources of ARB, on a total mass basis, especially compared with parallel community sources.[28] Further, international migration of resistance is believed to be very common,[29,30] although exact pathways and the frequency of occurrence are not yet well defined.[31,32]

Despite the emerging evidence, many questions remain about the spread of ARB among human populations, the environment (soils, wastewater, and surface waters), and food animal production. One of the challenges in addressing these questions is determining how to quantify specific ARB of concern within a complex chemical matrix and mixed microbial community, especially identifying the presence of specific ARB from a distinguishable source. Recent ARG and microbiome source-tracking methods have been used to stochastically distinguish between community- and hospital-sourced ARB,[33] but this was a case in which key sources were clear. The method overlays genetic high-throughput quantitative polymerase chain reaction (HT-qPCR) and 16S sequencing data to develop resistomes and microbiomes for each source and sink,[33] and then uses similarity analysis to infer proportional relatedness among sources and sinks.[34] Given the complexity of many links shown in Figure 1, generating this type of source tracking analysis is difficult in many cases. However, it might be a useful approach for teasing out possible sources in some cases, an example of which might be allocating the relative importance of humans versus proximal domesticated animals to local environmental exposures.

Livestock fecal matter.   Healthy soil is a vital living system essential for crop agriculture. Important components for healthy soil include organic matter and microbes, including bacteria and fungi. Organic

matter provides food for microbes, stabilizes soil structures, and increases soil fertility. In agricultural systems, manure is often applied to fields as an additional source of organic matter. However, manure almost certainly contains microbes that include ARB.[35] The gastrointestinal tract of domesticated livestock contains many zoonotic pathogens and commensals that are shed in large numbers in feces. Unprocessed or uncomposted manure used in crop production may therefore contain AMR pathogens that can contaminate food and water, potentially contributing to foodborne and waterborne exposures.[36]

Therefore, livestock and other animal fecal matter can potentially contribute to ARB and ARGs in their local environments, though pathways to exposure and external release are varied. For example, humans and animals physically close to points of defecation are at higher risk of acquiring or exchanging bacteria or genes. This might occur at intensive animal production facilities (see below), but might also occur on more local scales between humans and their pets, and via soil or water impacted by the fecal matter.

Manure generated in food animal operations can be transferred through various pathways, but broad conclusions on the relative importance of each pathway are hard to determine due to contradictory results. As an example, a 2006 study of cattle feedlots in the Midwestern United States found that tetracycline ARG abundances statistically differred between waste lagoons at feedlots where antibiotics were used only therapeutically and feedlots where antibiotics were used both therapeutically and nontherapeutically at higher levels.[37] Both groupings that used antibiotics had 100 to 1000 times higher levels of ARGs than control feedlots that did not use antibiotics. The authors concluded that similarities between therapeutic-only and therapeutic/nontherapeutic lagoons were probably due to cattle in therapeutic-only herds being returned after therapy and exposing other cattle to their antibiotic-impacted feces. This is analogous to what has been seen in human populations in densely populated areas without adequate immediate sanitation.[38] Conversely, other researchers have found limited relationships between the use of different levels of antimicrobial drugs and AMR trends in fecal *Escherichia coli*.[39]

These opposite observations again demonstrate the complexity of AMR. In both cases, carefully designed studies were performed, and it is likely the studies are correct in themselves. This suggests that tertiary factors, such as how systems were sampled, the specific AMR detection methods, and local specifics to each animal operation were sufficiently different to provide opposite answers to the same question. This argues for greater unification among methods and study designs to provide less ambiguous results. A recent review concluded that there was a broad lack of quantitative causal research that associates AMR sources and increases in environmental ARB, suggesting that improved study design, greater consideration of *a priori* bias, and standardization of analytical tools are important to understanding better how ARBs in the environment affect human and animal health.[40]

Wider consideration of direct fecal releases. According to the WHO and UNICEF, in 2015 2.3 billion people lacked basic sanitation and many millions practice open defecation, which is the direct release of human fecal matter to the environment. Although this does not directly relate to AMR in food animal production, analogous fecal releases are common with food animals, and translatable knowledge can be gained from a brief review of the consequences of open defecation. As background, most people who practice open defecation live in Central and South Asia and Sub-Saharan Africa.[41] Poor sanitation and open defecation increase the environmental microbial burden and public health risk of disease. Making the situation worse, many countries with poor or nonexistent sanitation allow the sale of high value over-the-counter antibiotics, increasing the use of antibiotics and the risk of antibiotic resistance.[42]

Thus, fecal matter is potentially an important source of antibiotic resistance spread among people or animals in areas with inadequate consideration of sanitation.[42,43] For example, researchers detected higher levels of emerging resistance genes, such as bla_{NDM-1} (a carbapenem ARG), in water and sediment samples collected in the Upper Ganges River in northern India in June—at the beginning of the monsoons and coinciding with times of mass pilgrimages from urban areas—compared with February (one of the driest months).[38] Locations along the river with some fecal containment, such

as latrines, had 4-5 orders of magnitude lower CRE concentrations than stretches of the Ganges where there was open defecation.[2]

Although this is predominantly a human example, similar releases and AMR exposures almost certainly occur in food animal operations where population densities are high and local sanitation is inadequate, major concerns in any operation that includes animal rearing. Experts recently have predicted that ARB in livestock will arise in the Far East because of heavy use of antibiotics in Asian farms, which implies that significant shedding of ARB must occur in Asian animal feces. In contrast to those results, however, research conducted through the Food and Agriculture Organization of the United Nations (FAO) in 2014 found that levels of resistance to many antibiotics were actually higher in isolates from food animals in the United States and Europe than in Asia.[44]

Such differences exemplify the difficulty of AMR because comparable and plausible studies do not always provide black and white answers and can generate opposite conclusions. In the above cases, we suspect that the FAO data may not represent all food animal–related activity, particularly aquaculture in South East Asia where AMR is reported to be rampant.[45] Again, this shows how conclusions from one study can provide different answers than those from parallel studies. A useful way of discerning valuable information from the literature is through more careful examination of details within each study, and then noting those differences when drawing more general conclusions.

Agricultural soil and water

Antibiotics and ARGs can be enriched in ground and surface waters surrounding animal agriculture operations, especially where animals receive antibiotics and are close to soil and water resources.[46–50] Consequently, soils and water have the potential to be important players in the spread and persistence of ARB. Substances that are applied to the soil can quickly enter bodies of water, particularly in areas where artificial subsurface drainage systems are installed. ARB also naturally exist in soil and water environments, and a key challenge for understanding the impact of agricultural management practices on the spread and persistence ARGs and ARB is differentiating between ARGs that spread from anthropogenic sources versus ARGs already present in the environment.

Antibiotics in irrigation water can be taken up into crop tissues (mostly roots), but levels of uptake are typically low unless antibiotic concentrations are very high.[51,52] However, whether uptake occurs depends on the specific crop–antibiotic combination. No general rules have yet been established for which antibiotic–crop combinations lead to greater uptake, but, overall, uptake tends to be limited, quite selective, and plant and antibiotic specific.[52] While there is a substantial amount of literature on the plant uptake of antibiotics, there is less information on the uptake of ARGs and ARB into crop tissues, especially where exposure concentrations are within normal ranges and the experiments have been well designed with controls.[52–54] Nonetheless, evidence exists that ARGs and ARB can accumulate on plant surfaces associated with manure applications, and it has been suggested that associated crops and soils should be fallowed prior to harvest.[53] Additionally, recent work has shown that swine manure application can be associated with increases in observed levels of phenotypic ARB, as well as ARGs in soils and drainage waters. However, the abundance of different ARGs is highly variable and can range up to a year after the manure application.[50] In addition, specific crops and crop rotation can influence the types of resistance genes found in soils; for example, the ARGs in soils from corn and soybean crop production systems were found to be quite different.[35,47]

It is still unclear the extent to which resistance and related exposure in soils receiving manure and/or antibiotics translates to a human health risk. A recent study on archived soil samples in Denmark from fields receiving only either manure or inorganic fertilizer for over 100 years showed that only manured fields had significantly higher levels of extended spectrum β-lactamase ARGs (a critical form of AMR in hospitals). Further, the relative appearance of specific ARGs in the manure field was almost simultaneous (within 6 months) with appearance in hospitals.[55] It is not possible to determine whether detected ARGs first appeared in the manure or hospitals, but the results suggest that cross-location spread occurred between the original cattle (possibly via soil) and the human populations. In general, there is an urgent need to better understand the linkage between antibiotic usage and resistance across environmental compartments.

Currently, this is limited by the availability of data on the usage of antibiotics coupled directly with environmental surveillance of resistance. Further, many studies often focus on different types of ARGs or antibiotics, making it difficult to compare results between efforts. Moving forward, integrated and systematic efforts will be necessary to develop models that estimate the spread and persistence of ARB in the environment and how an indicated source of bacteria contributes to human health. This is among the most critical knowledge gaps in understanding the interconnections and consequences of the complex issue of AMR.

Transmission of antimicrobial resistance among domesticated animals and humans through direct contact

Direct contact with food animals

As mentioned earlier, bidirectional exchange of ARB and ARGs can occur between food animals and people who are in direct contact with the animals or are proximally close to animal products, such as farmers and processing plant workers.[56] Researchers have largely focused on understanding the spread of bacteria from pigs to farmers, but there is evidence that people can also spread staphylococcus and other bacteria to pigs.[56] In the case of the skin-associated bacterium *S. aureus*, which has been studied the most, there is still much uncertainty concerning the frequency with which livestock workers become colonized from food animal farms, as well as the frequency with which acquisition of this skin bacterium leads to symptomatic infections.

Determining how occupational exposure becomes an occupational hazard has been difficult to assess for several reasons, some related to how studies have been performed, but also because of genetic variation in *S. aureus* phenotypes and general difficulties in detecting effects. Researchers have conducted cross-sectional studies to assess this. For example, a group at the University of Iowa performed a cross-sectional study to identify staphylococcal infections in pig farmers by asking farm workers whether they had been diagnosed with certain bacterial infections.[57] This study relied on farmers to accurately report their medical information, and it was difficult to trace an infection back to exposure because it can take weeks to months after contact with *Staphylococcus* to develop symptoms.[58] To

aid such research, ideally researchers should collaborate with physician groups who treat patients with potential *Staphylococcus* infections, although such longitudinal studies are costly, they require staff to follow up with patients, and they must be institutional review board certified. Further, large groups of patients are needed because the subset of farmers and workers infected with *Staphylococcus* is small compared to the overall patient population.[59] A few studies exploring this issue were confined to limited geographical areas,[16,59–62] hence, additional studies need to be conducted across areas and in more locations.

To further complicate matters, there is no predominant strain of *S. aureus* linked to the majority of pig farms that can be probed specifically through genomic analyses.[63] For example, while ST398 is the predominant strain of livestock-associated *S. aureus* in Europe and has been recognized as an occupational hazard for people working in the pig industry,[64,65] in the United States several types of *S. aureus*, including ST398, ST9, and ST5, have been found in pig farms, as well as on people who work with pigs.[63,66] The proportion of these different strains varies geographically between Iowa, North Carolina, and elsewhere around the Midwest, though the breakdown of strains has not been reported for most of the United States.[60,63]

The type of *S. aureus* is also important because it can influence colonization rates. For example, strains such as ST5 seem to colonize people efficiently, whereas others such as ST398 appear to be poorly adapted to humans.[14] Studies found that ST398 colonization of veterinarians is lost fairly quickly after they spend time away from the farm.[67] Host-specific factors also appear to influence colonization rates; for example, a longitudinal study of swine veterinarians found that the majority of them was only transiently colonized, whereas a sizable minority was permanently colonized, although follow-up work suggests that colonization may be lost after a couple of years.[68] Nevertheless, short-term colonization may still result in transfer of resistance genes, such as the *mec* resistance genes in MRSA,[69] from farm-acquired *S. aureus* to strains that colonize humans.

Beyond the farm, there are data that hint that MRSA may conditionally spread from pig farms to humans who do not work in the pig industry.[15] Studies in the Netherlands have found

cases of livestock-associated MRSA[70] that may be attributable to living in areas with high animal density, although routes of dissemination are unknown. One possibility is exposure to livestock workers, resulting in secondary person-to-person transmission away from the farm source.[71] Studies in the United States have shown that proximity to concentrated animal feeding operations or areas where pig manure is applied to fields can increase the risk of MRSA carriage[72] or infection.[73]

Finally, the spread of specific ARGs from humans to livestock may also occur. One school of thought is that humans carrying MRSA have cross-infected pigs: while transient in the pig, the *mec* resistance genes from human-adapted MRSA transferred to pig-adapted MRSA, consequently giving rise to MRSA in pigs.[56] This hypothesis is supported by the fact that livestock-associated MRSA (i.e., in pigs) associates with the same *mec* genes found in human community MRSA, although this is an inferred possibility.

Although the above examples focus on pig–human interactions, they are good examples of another type of complexity in explaining AMR observations. Such issues are exacerbated by the challenge to obtaining farm-level data on antibiotic use. Although the FDA in 2016 began requiring sponsors of antimicrobial drugs to provide estimates of sales for each major food-producing species, the agency does not collect information about the geographical areas to which drugs are sold.[74] These types of data would complement U.S. maps of distribution of *Staphylococcus* and other bacterial species among farms, farmers, and the environment, and would also allow more fine mapping of resistance distribution and correlation of resistance to on-farm use of antibiotics.

What can be learned from companion animals?

Companion animals (e.g., pets) represent an often ignored, but potentially important source of ARB and ARGs in human populations because of the intimacy between humans and their pets.[18] Pets have little to do with the food animal industry, but considering them provides analogous evidence of how close personal contact between domesticated animals and humans can influence AMR spread. In Europe, veterinary pharmaceutical companies established the European Animal Health Study

Centre, a Brussels-based international, nonprofit association that conducts scientific and economic studies including, from 2008 to 2010, antimicrobial susceptibility surveillance in sick companion animals in 10 EU member states.[75] However, surveillance has been limited to skin, ear, and soft tissue infections, urinary tract infections (including prostatitis in dogs), upper respiratory tract infections, and periodontal infections. Further, surveillance of AMR markers in healthy companion animals has not been done, although what has been learned may translate to issues in the food animal industry.

Some useful examples exist, such as the possible affect pets may have had on vancomycin-resistant *Enterococcus faecium* (VRE) infections in seriously ill patients in European hospitals in the 1980s.[76] As background, VRE cases were initially attributed to food animal agriculture after VRE was isolated from livestock in Europe over time.[77] The theory was that the use of avoparcin (a vancomycin analog of the glycopeptide class of antibiotics) for animal growth promotion created selection pressure that led to VRE.[77] This resulted in the European Union banning the use of avoparcin in animal agriculture in 1997, and then the use of any antibiotic for growth promotion in 2006.[78] Although VRE on farms dropped precipitously following the 1997 ban, the VRE problem in European hospitals inexplicably persisted.[79] It was not until whole-genome sequencing was performed on clinical VRE isolates that it became evident that genotypes were not from farm isolates but probably from ampicillin-resistant *E. faecium* common in dogs in Denmark.[80–82]

There are other examples of resistance being transmitted from pets to humans that provide useful templates for food animal AMR studies. For example, exposure to puppies can introduce resistant *Campylobacter* spp. infections to humans.[83] Additionally, humans with MRSA infections should consider having their pets examined as potential MRSA reservoirs.[84] Even though MRSA is not adapted to dogs, cats, and other animals, these animals can become colonized with MRSA and then serve as vectors for spread to humans in the same household.[85]

More can be learned by the food animal industry by understanding the role of companion animals in the transmission of antibiotic resistance to humans, the environment, and to food animals. However, this research falls outside the purview of funding agencies such as the National Institutes of

Health and the U.S. Department of Agriculture.[86] The use of antibiotics in companion animals may be dismissed as less important because, according to sales data, the volume is low compared to the use of antibiotics in humans and livestock. However, the drugs that pets receive are generally more potent and injectable, and map more closely to the pattern of critically important classes prescribed to humans than to food animals, whereas food animals generally receive less important antibiotics often in their feed.[87] Understanding such relationships between specific antibiotics used for food animals and those used for pets can enrich our understanding of animal–human contact as pathway of AMR spread. Pets might spread critical antibiotic resistance to people who in turn become vectors for antibiotic-resistance dissemination to livestock and the environment.[88]

Wildlife: another complication in animal systems

Although not central to this review, brief mention of wildlife-mediated AMR is needed because it highlights another difficult-to-detect but potentially important factor related to AMR dissemination in animal–human–environmental systems. Direct contact between humans and wildlife is typically not as intimate as with livestock or domestic animals; but wildlife, especially birds, has been shown to act as a vector for AMR transmission across the environment.[89] For example, growing data suggest that ARB and ARGs may be dispersed by wild birds after exposure to sewage near rivers,[32] spreading of sewage sludge onto farmland,[90] and via direct exposure to the feces from livestock and companion animals.[91] However, such exposures and associated dissemination pathways are a complexity that is rarely considered in AMR studies, although this pathway might be the best explanation for rapid rates of AMR spread on global scales.

A recent study examining the apparent accumulation of anthropogenic ARGs in the High Arctic found that bla_{NDM-1} and other clinically important ARGs were elevated in Arctic soils near bird, reindeer, and arctic fox-watering areas, suggesting possible wildlife mediation in ARG movement.[92] In this case, ARG movement may also be mediated by human wastes; but in general, wildlife activity and associated fecal matter might explain the appear-

ance of AMR almost anywhere, including places with functionally no antimicrobial use or evident influence. Therefore, similar to companion animals, AMR carried by wildlife, possibly acquired after exposure to human, livestock, and other fecal matter, should also be considered within AMR assessments, adding further complexity to explaining cause and effects.

Considerations for AMR surveillance

Among the greatest complexities and questions that have concerned AMR scientists for many years is how best to measure AMR when studying water, soil, or other environmental systems, including for diagnostics and/or practical surveillance.[93] Metagenomics analyses offer a broad picture of the presence and relative and absolute abundances of putative ARGs across microbiomes, and can also help expand ARG databases to improve and guide future studies.[94] However, such methods do not reveal whether detected ARGs are actually functional or whether they can or are conferring phenotypic resistance in bacteria. Further, metagenomics methods cannot be easily used to link the presence of an ARG to a specific ARB, which can only be done stochastically in most scenarios. Finally, whether an ARG detected by metagenomics is present in viable bacteria, is sited alone in a genome or is part of a multidrug-resistance cassette (e.g., on a plasmid) are facts not easily determined from metagenomics data alone.[4,95] Such problems can be partially resolved by parallel culturing of organisms (from the same source as the metagenomics data), selection of specific phenotypes, and determining the genetic context of specific genes in such isolates. However, the majority of organisms found in environmental systems cannot be cultured under standard laboratory conditions, which poses a problem for linking ARB and ARG metagenomics data within environmental compartments.[10]

In contrast to metagenomics, more targeted methods for quantifying specific ARGs, such as qPCR, have been used successfully for a number of years. qPCR is still a very useful method because it is quantitative, although it does not allow the identification of genetic precursors of ARB or of previously unknown putative resistance genes. This is demonstrated by the mistaken attribution of VRE emergence in European hospitals to livestock.[96] Conversely, qPCR can be performed very quickly

and is useful for tracking specific ARGs in systems where the ARGs have already been established as important; qPCR can use known ARGs that are biomarkers for exposure and other related work. Further, a new HT-qPCR method is now available that allows hundreds of ARGs to be quantified simultaneously—a method used successfully to study ARGs in Chinese swine operations[97] and other applications.[92]

However, whole-genome sequencing has advantages, and has reduced in cost over the last 10 years. It is an approach that can inform how bacterial strains might associate with each other within a wider microbial community and how they can also be stochastically linked with antibiotic resistance.[98] However, whole metagenome sequencing and data interpretation requires considerable bioinformatics skill to decipher the resulting large and complex data sets, which currently makes surveillance requiring metagenome sequencing less practical for routine AMR screening. Fortunately, new methods are being developed, including more exact ways of determining specific bacterial hosts,[99] and simplified bioinformatics methods for generating more immediately useful data.[100] This may ultimately make such methods useful for routine work, as well as for deeper genetic investigations. As part of the One Health Initiative, which fosters collaborations between stakeholders in human and veterinary medicine, there is a push to conduct whole-genome sequencing–based surveillance of human and animal hospitals and farms, i.e., to make such methods more routine among a wider set of users.[101]

Despite such advances, current surveillance should still include traditional culturing methods, qPCR or similar targeted quantitative genetic methods, and, ideally, genome and metagenome sequencing. In fact, any study that does not include elements of all three approaches will likely not provide useful information. Although the current trend is toward the use of genetic methods for studying environmental AMR, resistance genes themselves do not automatically reflect phenotypic-resistant pathogens, which is the real veterinary and medical concern. Therefore, until sequencing-based and other genetic methods can more easily and accurately predict AMR phenotypes of health concern, traditional culturing must be a key part of surveillance. Hopefully, as databases develop this will change, especially for early screening and diagnostics, because of the intrinsic limitations of culturing to detect AMR and its capacity to spread in most of the microbial world.

Conclusions

AMR arises in bacteria through a wide variety of spontaneous mutations and/or gene transfer events, which are embedded with the global environment. Despite many knowledge gaps, it is clear that ARB and ARGs can spread among animal agriculture, the environment, farms, and human populations and households, including companion animals. The dissemination of resistance occurs on a global scale— for example, through the trade of animal food products, international travel, and wildlife migration.

However, some settings have a particularly high potential for generating, amplifying, and spreading ARG and ARGs, especially the nexus that crosses domesticated animals, humans, and the wider environment. This nexus should be a focus of mitigating AMR in broader terms because of typically higher use of antibiotics, greater animal and human population densities, and more intimate contact between humans and animals—all common in food animal operations. To reduce the development and spread of AMR, it is critical to better elucidate the myriad variables that can influence the levels of resistant bacteria and resistance genes.

As this review hopefully shows, the web of factors that can impact AMR and the potential for AMR spread is diverse and complex. It includes factors and pathways that are very difficult to define and test, and only incidental data exist to suggest their possible significance. This complexity extends to how AMR is defined and measured; and differences in methods and a broad lack of standardization in defining and detecting AMR may partially explain contradictions in existing data. Regardless, it is hoped that this brief summary helps to inform the discussion surrounding AMR, especially for the food animal industry, and will fuel a more homogenized approach to addressing questions that must be answered to mitigate globally increasing AMR.

Competing interests

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