Antibiotic Resistance Genes in the Human Impacted Environment: a One Health Perspective

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ABSTRACT

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Antibiotic resistance and its environmental component is gaining more attention as part of combating the growing healthcare crisis. The One-Health framework, promulgated by many global health agencies, recognizes that antimicrobial resistance is a truly inter-domain problem in which human health, animal agriculture and the environment are the core and interrelated components. This prospectus presents the status and issues relevant to the environmental component of antibiotic resistance, namely the needs for advancing surveillance methodology: the environmental reservoirs and sources of resistance, namely urban wastewater treatment plants, aquaculture production systems, soil receiving manures and biosolids and the atmosphere which includes longer range dispersal. Much recent work has been done describing antibiotic resistance genes in various environments; now quantitative, mechanistic and hypothesis driven studies are needed to identify practices that reduce real risk and maintain effectiveness of our current antibiotics as long as possible. Advanced deployable detection methods for antibiotic resistances in diverse environmental samples are needed to provide the surveillance information to identify risk and define barriers that can reduce risk. Also needed are practices that reduce antibiotic use and thereby reduce selection for resistances, as well as practices that limit dispersal or destroy antibiotic resistant bacteria or their resistance genes that are feasible for these varied environmental domains.
Key words: Antibiotic Resistance, Transmission, Agriculture, One-Health

INTRODUCTION

The overuse and misuse of antibiotics for human therapy and livestock production around the world over the past decades have increased antimicrobial resistance in diverse environments (Berendonk *et al.*, 2015; Garbisu *et al.*, 2018). The prevalence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) can reduce antibiotic effectiveness which is a serious threat to human health. In December 2017, United Nations Environment Programme announced environmental antibiotic resistance as the top of six emerging issues of concern. Together with climate change, water

stress and environmental damage, widespread antibiotic resistance should be regarded as one of the global challenges humans face in this century.

ARGs have been widely identified in various environments, including wastewater and sludge (Manaia *et al.*, 2018), livestock farms and soil (Zhu *et al.*, 2013), river water and sediment (Muziasari *et al.*, 2017), drinking water (Ma *et al.*, 2017a), glacier environments (Segawa *et al.*, 2013) and even the Antarctic (Wang *et al.*, 2016). The use of antibiotics in therapy and livestock production has greatly promoted the development and spread of antibiotic resistance making impacted environments into large reservoirs of ARGs (Larsson *et al.*, 2018; Manaia *et al.*, 2018). Furthermore, the prevalence of mobile genetic elements, e.g. transposons, integrons and plasmids, could promote the horizontal gene transfer of ARGs to other bacteria including human pathogens, exacerbating the antimicrobial resistance issue (Gillings *et al.*, 2015; Ma *et al.*, 2017b).

Previously studies on antibiotic resistance mainly focused on clinical microorganisms to address the direct threat of emerging antibiotic resistant bacteria on public health. With the emergence of superbugs (multidrug resistant human pathogens) in medical treatment, both medical and public concern has increased. This has led to recognition that this is a problem that requires an interdisciplinary vision since it clearly involves not only human medicine but also agriculture and veterinary medicine as well as a range of environmental domains. The result has been formulation of the "One-Health" concept to engage scientists and practitioners across these disciplines to study the problem and its remedies in a cross-disciplinary manner. Antibiotic resistance has been described as the "quintessential" One–Health issue (Robinson *et al.*, 2006). It involves antimicrobial resistance (AMR) in three main domains (Figure 1): Human health, animal agriculture and the environment. The United Nations through its agencies: the World Health Organization (WHO), the Food and Agriculture Organization (FAO) and the World Organisation for Animal Health (OIE) work jointly and take collective action to minimize the emergence and spread of AMR. As illustrated in Figure 1, soil is a central component in One-Health since it harbors a large natural resistome but also receives ARB and

ARGs from both human and animal wastes which can be returned to humans in vegetable and animal products, in surface, ground and reclaimed water, and via aerosols. This is particularly important for soil science research because of its central role as a reservoir hence potential source for resistance traits, the many routes of dissemination including to humans and the large natural background of resistance making it more difficult to assess human-enhanced resistance and high risk AMR.

DETECTION OF ANTIBIOTIC RESISTANCE GENES IN THE ENVIRONMENT

One of the major needs to address the antibiotic resistance problem is improved AMR surveillance incorporating clinical, agricultural and environmental data at local, national and global levels (Hughes *et al.*, 2016). This has been a challenge for the environmental domain since a legacy of methods tuned to the environment did not exist. In the last decade, molecular methods to quantify ARGs in the environment and their distribution and propagation in wastewater and sludge, aquaculture, soil and atmosphere have been developed or improved. These are primarily of three types: PCR based amplification of ARGs, hybridization of DNA to ARG gene fragments and searching metagenomic sequence data for ARGs. The former includes the polymerase chain reaction (PCR), quantitative real-time PCR (qPCR), DNA microarray and DNA hybridization (Zhang *et al.*, 2009). The traditional target sequence amplification-based methods, PCR and qPCR, have been widely applied to detect and quantify ARGs in diverse environmental samples because of their high sensitivity for ARGs detection under low abundance in natural environments. However, due to the limitations of primers available for ARGs amplification, PCR and qPCR approaches could only effectively detect well-studied ARGs. Besides, ARGs primers may cause biases and false negative/positive results, and only several ARGs could be detected in one set using traditional methods (Laht *et al.*, 2014; Titilawo *et al.*, 2015; Xiong *et al.*, 2015). In recent years, improvements have provided high-throughput (highly parallel) qPCR for detection of hundreds of ARGs in one run (Looft *et al.*, 2012). To further improve its efficiency and coverage, 294 primer sets for ARGs and some mobile genetic elements (MGEs) were developed (Zhu

et al., 2013) and validated by sequencing its amplicons (Johnson *et al.*, 2016). This was subsequently expanded to 384 primers (Wang *et al.*, 2016). Recently, the results from over 500 diverse samples were evaluated, and together with updated reference data and a new primers design tool, EcoFunPrimer, Primer set 2.0 for highly parallel qPCR was tested and reported (Stedtfeld *et al.*, 2018).

The rapid development of high-throughput sequencing (HTS) technology in the past decade has enabled sequence-based metagenomic analysis to be widely applied in diverse ecological, medical, engineering and physical studies (Kircher and Kelso, 2010), including in assaying ARB and ARGs in environmental samples (Zeng *et al.*, 2015; Ma *et al.*, 2016). HTS technique can rapidly generate large amounts of sequence data at a relatively low cost (Kircher and Kelso, 2010). Metagenomic analysis is a culture-independent molecular approach, which uses the generated metagenomic sequences to search, annotate and predict targeted genes. For annotation and detection of ARGs in environmental samples, the generated metagenomic sequences are filtered for quality and then compared against a reference ARG database that contains sequences of the known ARGs. With the increasing concerns of ARB and ARGs in both medical treatment and environmental monitoring, several ARG databases have been constructed to facilitate the ARGs annotation, including ARDB (Liu and Pop, 2009), CARD (Jia *et al.*, 2017), ARG-ANNOT (Gupta *et al.*, 2014) and ResFinder (Zankari *et al.*, 2012). One structured database derived from combining ARBD and CARD was designed to search metagenomic data and classify the results into 24 ARG types and 1208 subtypes (Yin *et al.*, 2018).

A variation of the above approach of searching for ARGs in metagenomic data is to first assemble the short-read ARG fragments to gain more reliability in gene identification. This can be done by gene-targeted assembly tools such as Xander (Wang *et al.*, 2015) or Mega GTA (Li *et al.*, 2017). One study reports this result in soils for 35 ARGs (Dunivin and Shade, 2018).

Challenges remain on improving the detection limit and identifying untargeted ARGs, which can be potentially emerging problematic ARGs. All of the above molecular methods are based on sequence information from known ARGs. None of these methods can detect the yet unknown ARGs. Further,

many ARG sequences may be part of the natural resistome, code for functions that by themselves do not confer resistance, or may not be expressed. Tong Zhang in Hong Kong University is working on ARGs identified with high risk, thereafter paving a way for establishing the priority in mitigating ARG transmission and designing effective control strategies.

The comparison of antimicrobial resistance among different environmental niches with culture-based clinical (pathogen) resistance is necessary for improving our knowledge on the evolution, spread and risk of ARGs in the environment. However, standard methods and criteria specifically designed for meeting this need in environmental samples is lacking. One challenge for use of culture-based methods for environmental samples is that > 99% of environmental microorganisms are not cultivable, and another is that pathogen recovery can be inefficient due to their injury or stress from being in the environment. Therefore, improvement of methods through combining molecular methods, perhaps informed by markers from clinical or culture-based studies, with high-throughput sequencing will provide a comprehensive overview of antimicrobial resistance as well as specific areas of higher risk in diverse environmental samples. Future attention should also be paid to tracking antimicrobial resistance from pollution sources versus natural environments to human-related environments, as important guidance and reference for management and control of antimicrobial resistance.

ANTIBIOTIC RESISTANCE GENES IN WASTEWATER AND SLUDGE

The major environmental sources of AMR, and especially those that are clinically relevant, that have the potential to reach humans are from i) urban wastewater treatment plants, ii) use in aquaculture, iii) use in animal agricultural for growth promotion and therapeutic use. Other sources, not discussed here, but should not be neglected, are from antibiotic production and formulation factories and use on certain food crops (this use is extremely minor for antibacterials but not so for antifungal compounes, e.g triazole fungicides). Use of antibiotics in pets, and transmission of ARB by wildlife, including birds, and in reclaimed water, e.g. for irrigation of vegetable crops are sources to also be recognized

(Fig.1).

Domestic wastewater treatment plants, also known as urban wastewater treatment plants (UWTPs), also receive ARGS and ARBs from domestic and clinical sources but cannot remove them nor most antibiotics by the most commonly used technologies applied in the treatment of domestic effluents. Therefore, in spite of reducing the abundance of bacteria in the effluents, wastewater treatment, fulfilling the legal recommendations (e.g. Directive 91/271/EEC on Urban Waste Water Treatment and following amendments), leads to the continuous release of antibiotic resistant bacteria and antibiotic resistance genes into the environment (Vaz-Moreira *et al.*, 2014; Manaia *et al.*, 2016). Unfortunately, the risks posed by these high loads of ARBs and ARGs are still poorly understood (Czekalski *et al.*, 2014; Li *et al.*, 2016; Osinska *et al.*, 2016; Proia *et al.*, 2016; Li *et al.*, 2018).

The arguments raised above have stimulated the study of occurrence and potential control of antibiotic resistance in UWTPs. Most abundant classes of ARGs were found in raw influents and final effluents, accumulating in activated sludge during secondary/biological treatment or reaching the UWTP surrounding environment (Karkman *et al.*, 2018). Not surprisingly, these genes are frequently detected in or statistically associated to bacterial genera and species known to have association with the human and animal body (Narciso-da-Rocha *et al.*, 2014; Narciso-da-Rocha *et al.*, 2018). In addition, the presence of mobile genetic elements (MGEs), involved in horizontal gene transfer (HGT), particularly, plasmids and phages, as well as, genetic recombination elements, both genes encoding recombination enzymes and site-specific recombination regions in the wastewater metagenome, highlights the potential for the propagation of antibiotic resistance genes, within and across different environmental compartments (Dolejska and Papagiannitsis, 2018). Of note, is the fact that this core wastewater resistome (totality of resistance genes) and mobilome (the totality of elements involved in HGT and genetic recombination) is continuously evolving by the rapid incorporation of genes emerging in clinical settings (Karkman *et al.*, 2018).

In face of these evidences, efforts have been directed towards the improvement of wastewater

treatment, mainly on the development of advanced treatment options, such as chlorination, ozonation or UV, or combined disinfection approaches (Dodd, 2012; Giannakis *et al.*, 2018). In addition, the reactivation and regrowth of bacteria after disinfection, particularly of fast growing bacteria, also may need attention in order to minimize the proliferation of ARB or ARG after disinfection and water storage (Sousa *et al.*, 2017).

ANTIBIOTIC RESISTANCE GENES IN AQUACULTURE

As a source of fish and shellfish, the significance of aquaculture has increased remarkably in the last 50 years (Watts *et al.*, 2017) and the aquaculture production already surpasses the catch of the wild growing aquatic species. The term aquaculture can refer to a large spectrum of activities. The extensive aquaculture relies on native species which are helped by removing predators and competing species whereas in the intensive aquaculture the cultured species are more isolated from the environment and the food is supplied externally. On the other hand, the term covers everything between farming salmon offshore in the cold Atlantic water to tropical integrated aquaculture where excess animal feed and livestock manure are nutrients for the aquaculture. Therefore, it is not too surprising that there is no single picture of the antibiotic use in aquaculture.

Use of antibiotics in aquaculture is strictly controlled in Europe, Japan and Northern America. In those regions, the use of antibiotics is limited to therapeutic applications where only a limited number of antibiotics is approved. As an example, only five different antibiotics are authorized for use in aquaculture in the UK compared 13 different ones in China (Liu *et al.*, 2017). Some forms of aquaculture use antibiotics in a sustainable way, with the salmon production in Norway as probably the best example. Between 1987 and 2013 the antibiotic use in that production system was reduced by 99% concurrent with a 20-fold increase in the production (Norwegian Ministry of Health and Care Services 2015). The main factors behind such development are implementation of strict use of vaccines and hygienic requirements, which partly resulted from active scientific research in this field. There are

obviously other than scientific and technological challenges involved since the uses of antibiotics in aquaculture vary tremendously even with the same fish species. The reason behind that difference is still not clear but it probably includes not only lack of vaccination, but also high fish density and sub-optimal fishing practices which includes at least underdeveloped hygiene and feeding with unknown components that might contain antibiotics or other agents causing the selection pressure for antibiotic resistance. Additionally, aquaculture systems integrating the wastes from livestock production are effective regarding nutrient cycling but may have potential problems of disseminating antibiotic resistance (Cabello *et al.*, 2016).

ANTIBIOTIC RESISTANCE GENES IN SOIL

Of all the environments impacted by humans, soil undoubtedly contains the richest and most diverse populations of microorganisms (Thompson *et al.*, 2017) and DNA sequence (Rodriguez-R *et al.*, 2018). Soil bacteria carry antibiotic resistance genes that are also found in human clinical pathogens, and novel ones that are for the first time being discovered (Lau *et al.*, 2017). A key human activity of concern with respect to antimicrobial resistance that impacts the soil environment is the land application of fecal material, largely through agricultural practice (O'Connor *et al.*, 2005; Larney, 2011; Wang *et al.*, 2018). Organic amendments of animal (manures) or human (i.e. biosolids) origin are widely used as a valued source of nutrients and for crop production and organic matter for soil improvement. The use of antibiotics for prophylaxis or therapy in commercial food animal production will select for and enrich antibiotic resistant bacteria in the digestive tract, and these bacteria will end up in the manure (Zhu *et al.*, 2013). Manure will also contain residues of antibiotics that have been excreted intact in the urine or feces (Pope *et al.*, 2009). The microbial and chemical composition of raw manures will vary with the commodity and the medications used. Likewise, biosolids contain residues of antibiotics and other potential co-selective agents such as biocides (Sabourin *et al.*, 2012). Treatment practices that reduce the abundance of antibiotic-resistant bacteria and destroy antibiotic

residues will reduce soil loading rates of these contaminants and are therefore desirable (Lau *et al.*, 2017; Tien *et al.*, 2017).

Another increasingly important practice of concern with respect to potentiating antibiotic resistance in soils is irrigation with reclaimed wastewater (Christou *et al.*, 2017). Irrigation with nutrient-rich raw or poorly treated sewage is sometimes practiced in lower income countries (Thebo *et al.*, 2017). Turfgrass in green spaces in urban or periurban settings may also be irrigated with reclaimed wastewater (Wang *et al.*, 2014). Depending on the degree of treatment, reclaimed wastewater will contain antibiotic resistant bacteria and residues of pharmaceuticals including antibiotics (Pan and Chu, 2018).

Soil contamination with metals and organic chemicals other than antibiotics that co-select for antibiotic resistance are of concern. Contamination of soil with copper and zinc from mining activities or the application of manure from animals that consume feed supplemented with the metals can co-select for genetic elements that confer resistance to both metals and antibiotics (Poole, 2017). Copper is also commonly spread on fruits and vegetables as a pesticide against bacterial and fungal plant pathogens. Interestingly, some herbicides decrease the sensitivity of Gram negative bacteria to some antibiotics, possibly through upregulating efflux (Kurenbach *et al.*, 2015). Likewise, there is a concern that the widespread use of fungicides in crop production will select for resistance that will end up eroding the efficacy of fungicides used to treat human fungal or yeast infections (Jensen, 2016). Fungicides are also entrained into soil through the application of biosolids (Chen *et al.*, 2013).

Given the chemical, biological and physical complexity of manures and biosolids it is extremely difficult to determine which specific agent or classes of agents interact with soil microorganisms to promote antibiotic resistance. Laboratory evidence suggests that in the presence of antibiotics, viruses (bacteriophage) disseminate antibiotic resistance genes more rapidly in manured soil than in the absence of antibiotics (Ross and Topp, 2015).

Soil can also affect the growth inhibition and selection pressure of antibiotics on the microbes by

sorption of the antibiotic reducing their bioavailability, which is determined by the chemical properties of the antibiotic and the soil matrix. In some cases soil reduces the growth inhibition of pathogens by certain antibiotics and for other antibiotics it does not (Chander *et al.*, 2005; Subbiah *et al.*, 2011). More recent mechanistic work shows that soils and their associated cations and soil organic constituents affects the bioavailability of tetracycline as measured by a bioreporter linked to ARG expression (Chen *et al.*, 2017) and that this can also be detected at the clay mineral surface indicating bacterial biofilms can sense the sorbed antibiotic thereby indicating that sorbed antibiotics can also provide selective pressure for resistance (Zhang *et al.*, 2018).

ANTIBIOTICS IN THE ATMOSPHERE

Due to its emerging threat to public health, the existence of ARGs in the atmosphere, especially urban air, has given rise to more attention. Comprehensive knowledge of the types and abundance of ARGs in the air and whether they are associated with potential pathogens can provide new guidance for assessment of air quality.

The relative abundance profiles of ARGs are spatially and temporally distributed in ambient air of various cities. On heavy air pollution days, the increase of airborne particles is conductive to suspending of microbes by providing more adhesion sites (Hu *et al.*, 2018). There was a higher richness with 64 ARG types in bacterial biota in Beijing smog than in other environments, such as pharmaceutically polluted environments, wastewater/sludge, animals and other terrestrial sources (Pal *et al.*, 2016). All the studies above indicate that airborne transmission plays a crucial role in environmental distribution and exposure of antimicrobial resistance compared with other routes. Evidences support that such a risk is attributed to diverse antibiotic usage in different cities. For non-urban environments, studies downwind of cattle feed yards have shown that airborne particulate matter promoted the transmission of microbial biota harboring antimicrobial resistance genes (McEachran *et al.*, 2015). In addition, the dispersal patterns of particulate matter-borne ARGs were also impacted by

physicochemical factors, meteorological parameters and bacterial communities (Hu *et al.*, 2018). For example, pathogens in clinical ambient air may be immune to multiple drugs, threating inhabitants as well as people outside hospitals via airborne spread (Huang *et al.*, 2012).

Unfortunately, knowledge on airborne ARGs in geographically, culturally, and economically different cities on a global scale is lacking. Their health impact is still unknown. On the other hand, considerable attention is paid on particulate matter mass concentration, e.g. PM 2.5, while biological parameters such as ARGs or ARB is yet to be adequately evaluated (Li *et al*., 2018).

TRANSMISSION OF ANTIBIOTIC RESISTANCE GENES IN THE ENVIRONMENT

Two major factors determine the fate of antibiotic resistance genes in the environment: dispersal mechanisms and selection. The dissemination of resistance genes is attributed to physical and biological forces throughout various environments. Physical forces drive the dispersal of antibiotic resistance genes, such as wind and waters, including erosion and leaching, but so do wild animals, e.g. birds, deer, raccoons. Major routes of human exposures are from contaminated foods, e.g. vegetables with exposure to manures or reclaimed wastewater, or meats contaminated with fecal waste from slaughter (Fig. 1). Less likely sources are from drinking water, which is highly source dependent and air. Pets can also be a source and a recipient from their owner, and thereby cycle the AMR (Fig. 1). Proximity to human activities provides an enriched source of ARGs for potential dispersal. For example, human proximity is closely related to the antibiotic resistance profiles of the gut bacteria of wild mammals, whose habitats can be affected by antibiotics (Allen *et al.*, 2010).

The selection pressure exerted on environmental bacteria can be of two types: selection for the microbes that are carrying ARGs that is independent of the resistance trait, e.g. for particular carbon sources, faster growth rate, resistances to stressors like pH, and selection for ARG trait which can be the antibiotic itself or co-selection for resistances that are genetically linked to another or multiple ARGs. The type of selective agent (chemical speciation and concentrations), co-exposure to other

selective agents (exposure period) and under what environmental conditions are essential for different bacterial growth (Baquero *et al.*, 2009; Larsson *et al.*, 2018). An overview of the characteristics, variability and abundance of ARGs and mobile genetic elements, and their transmission efficiencies in various circumstances is necessary to better differentiate the ambient sources of antibiotics and ARGs into the environment (Hunter *et al.*, 2008; Zhu *et al.*, 2017; Larsson *et al.*, 2018). Such transfer and selection patterns of genes and bacteria are important guides to distinguish the most urgent exposure and risk reduction practices.

CONCLUSIONS AND ONE HEALTH PERSPECTIVES

Humans profoundly impact the environment, in particular through agricultural practice. There is a need to better understand what specific practices represent what risk with respect to AMR in soil, water, air, and human health. This needs to be done considering the realities of how agriculture and aquaculture is undertaken in high, middle and lower-income settings. There is also a need to better understand how the fate and impacts of microbial and chemical contaminants that reach soil and water vary with climate, particularly extremes of temperature and rainfall (severe storms). Importantly, changes in food animal production practices that reduce antimicrobial use will reduce selection for and transmission of antibiotic-resistant bacteria. One practice that has long been questioned is the use of sub-therapeutic doses for growth promotion in animal production. It has been widely practiced because it does speed time to market and hence lower cost of food, but it has been a targeted as a non-essential practice that promotes AMR. Progress is being made though as this practice has been banned in the EU since January 2006 and in the U.S. since January 2017. This could only be accomplished in concert with changes in production systems that minimizes disease and insures farmer profitability. Currently the vaccination is an economically viable practice especially with high value species (e.g. salmon) in some developed countries. Development of alternatives to antibiotics, like vaccines, is needed but they must be cost competitive to have an impact on antibiotic use. This is an important area for research.

The relevance of ARB and ARGs as environmental contaminants is now accepted and raises particular concerns in different domains, with some examples: i) the current state of art provides limited evidences that permit an objective and quantitative assessment of the human health risks posed by the occurrence of ARB in the environment; ii) for this reason, it is difficult to define threshold values for the maximum admissible levels of ARB and ARGs in treated wastewater, in sludge and manure to be used in agriculture; iii) the water stress and the scarcity of natural resources demands the adoption of circular economy practices, where water reuse, sludge and manure application are pivotal, albeit the risks that may persist with the accumulation and spread of resistance; iv) while poor sanitary conditions in some world regions favor the dissemination of ARB and ARGs, coupled with growing tourism may spread the resistance burden to regions with adequate sanitary conditions. Establishment of adequate risk assessment frameworks, policy development and implementation, definition of circular economy good practices and insurance of adequate sanitation are priorities to control the antibiotic resistance burden at a global scale.

The One Health framework was developed in recognition of the inter-domain system in which antibiotic resistance resides and circulates (Fig. 1). Among the three domains, human health is the spotlight, due to multidrug-resistance genes have been prevalent in several important pathogens. To achieve the goal of One Health, veterinarians, doctors, food safety professionals, waste water treatment plant operators, environmental experts and regulators are obliged to work together. At a policy level, all counties were asked to formulate a "National Action Plan" to combat antimicrobial resistant appropriate for their country Many have done so but the challenge is now to effectively implement and monitor those plans and to collect the data to quantify the impact of practices intended to reduce the development and spread of AMR. The later is an important area for supportive research.

Environment is a key component of One Health, as it is the common medium for maintenance and transmission of ARGs. The transmission persistence and proliferation of ARGs in the environment and the mobile genetic elements that transfer these genes to create multidrug resistant pathogens is still

poorly understood. Understanding transmission barriers is a key to evaluate risks and to prevent ARGs from reaching humans. The One Health approach tuned to the countries needs offers the best hope for a risk assessment framework that yields policies and practices that protects our antibiotics effectiveness as long as possible.

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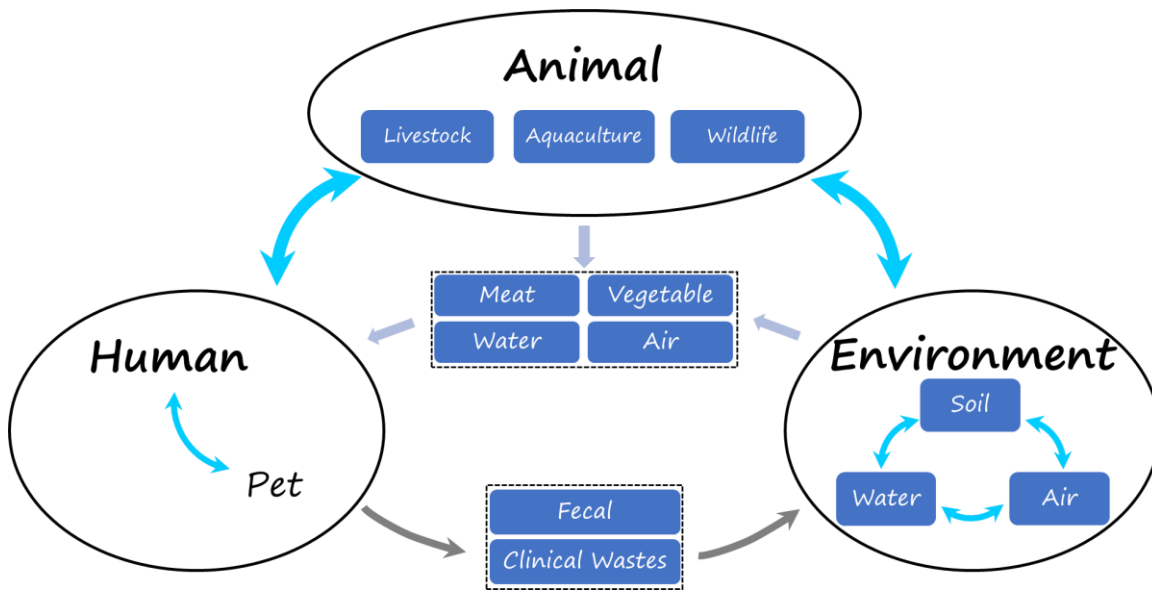
Fig. 1 The transmission of antibiotic resistance in the One Health framework with Human Health, Animals and Environment as the major domains through which antibiotic resistance genes and microbes reside, amplify and spread. Most arrows are in both directions reflecting these pathways, and the cyclic nature of the problem. Human exposure can be directly from animals and from food, water and air, but humans can also spread resistance to those domains, and especially via fecal wastes.