Antibiotic resistance and the environment —there and back again

Science & Society series on Science and Drugs

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Today, it is difficult to imagine a time when bacterial infections caused a terrible toll of disability and death. The discovery and use of antibiotics in medicine in the 20th century, together with better hygiene and vaccination programs, has drastically increased human life expectancy. In a way, however, these antibiotic ‘silver bullets’ have become victims of their own success, and most people are no longer aware of the deadly threat of bacterial infections. As a result, we have become lax and even irresponsible: We use antibiotics at every opportunity to treat mild or even viral infections, and we have used them in massive amounts in agriculture as a preventive measure against bacterial diseases, and to promote the growth of poultry, beef and pigs in animal farms.

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This complacent attitude is about to change. The past few decades have seen the rise of antibiotic-resistant bacterial strains that cause increasingly severe, difficult to treat, and sometimes even fatal infections. Multi-drug-resistant strains of *Mycobacterium tuberculosis*, *Staphylococcus aureus* and various *Enterococci* species are now nearly

untreatable with standard antibiotics and pose a growing threat to patients in hospitals and the community at large. In addition, contact with farm animals has been identified as another source for bacterial pathogens.

However, the problem does not end at the hospital entrance or the farm-yard gate; the environment is a huge source of antibiotic resistance. In fact, antibiotics and antibiotic resistance determinants are a natural phenomenon and have been present in the environment long before humans discovered and begun to use antibiotics. Microorganisms produce antibiotics to gain a growth advantage and to defend against competing organisms. Antibiotics also act as messenger molecules in microbial communities, for instance, in quorum sensing. The concentrations of these molecules are generally below the threshold for ‘antibiotic activity’, but this partly depends on how ‘antibiotic activity’ is defined. In clinical settings, it is most common to refer to minimum inhibitory concentrations (MIC) for antibiotics, above which bacterial growth inhibition is observed. The effects that antibiotics mediate in an ecosystem, however, occur at much lower concentrations. It has also been shown that even very low concentrations of antibiotics are sufficient to provide a selective advantage for resistant over non-resistant microorganisms [1], which has led to the proposal to use minimum selective concentrations (MSC) to characterize the effect of antibiotics in the environment.

The natural environment harbors a diverse reservoir of resistance determinants, including resistance genes and the mobile

genetic elements that operate as vectors for them. Commonly, gene clusters that encode the proteins required to synthesize an antibiotic also code for self-protection mechanisms. Resistance-conferring proteins either modify the antibiotic or its target, or provide a general resistance mechanism like efflux transporters. Various resistance genes have a long phylogenetic history dating back millions of years, but resistant genotypes also arise from scratch by mutation. The dynamics of how the resistome evolves and changes are presently not well understood.

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Since the beginning of the antibiotic era in the first half of the 20th century, antibiotics and antibiotic resistance genes have been introduced to or have spread to almost every ecosystem on earth. Increasing levels of antibiotic resistance genes in agricultural soil [2] or in surface waters compared to bulk water demonstrate the role of the human use of antibiotics in the dissemination of resistance. Another important aspect is the co-selection of antibiotic resistance by the presence of heavy metals or biocides that are also anthropogenically introduced into the environment. It may either lead to co-resistance if resistance determinants for

heavy metals or biocides and antibiotics are situated on the same mobile genetic element or to cross-resistance if the same determinant reduces the susceptibility to antibiotics and metals or biocides. Resistance genes from this increasing environmental reservoir can then be transferred to pathogenic bacteria [3]. Although a direct proof of such an event does not seem feasible, Kevin J. Forsberg *et al* at Washington University School of Medicine provide evidence of an exchange of resistance genes between environmental bacteria and clinical isolates [4].

The life cycle of pharmaceutically used antibiotics does not simply end when a patient swallows a pill or when livestock are treated. In most cases, the antibiotics are excreted. The exact amount varies depending on the route of application and the species, but various estimates of active compounds being excreted in urine or feces range from 10% to more than 90%. For some highly consumed antibiotic classes, such as beta-lactams, tetracyclines, (fluoro)quinolones, phenicols and trimethoprim, excretion generally exceeds 50% of the administered dose. For sulfonamides, excretion is more variable, and for macrolides, the excreted fraction is generally lower. However, exact data are not always available. Additionally, metabolites formed in the treated organism and subsequently excreted might retain their antibiotic activity. The problem is not limited to antibiotics though. Many artificial and biologically active compounds that start their life as drugs—contraceptives, hormone replacement therapy, steroidal and non-steroidal pain killers—pass through humans and eventually find their way into the environment where they affect other organisms. The residues of contraceptives and other hormones, for instance, have been shown to act as endocrine-disrupting chemicals on wildlife.

Antibiotics and their metabolites excreted by patients go through the sewage system to waste-water treatment plants (WWTPs); yet even a three-step—mechanical, biological and chemical—treatment is not sufficient to remove all pharmaceutical residues, including antibiotics. Adriano Joss and his colleagues at the Swiss Federal Institute of Aquatic Science and Technology showed slow or no removal of antibiotic compounds in batch experiments simulating activated sludge

treatment [5]. Several studies have confirmed these results and have further shown that although some antibiotics, especially fluorochinolones, are removed from the water phase, they accumulate in sewage sludge. Antibiotics can therefore either leave the WWTP in treated water that enters rivers and lakes, or they become part of the sewage sludge and are introduced into the environment when the sludge is used as fertilizer or as filling material (Fig 1).

Excreta from domestic animals, together with wastewater from cleaning stables, end up in manure storage tanks or lagoons. Again, the manure may then be used as fertilizer or as a substrate for methane production in biogas plants. The digested residues are also used as fertilizer. The consumption of crops—especially raw vegetables from manured soils—exposes humans to microorganisms from the soil and might therefore contribute to the spread of resistance [6]. Although exposure from the excreta of companion animals is lower, it should not be neglected, because humans and companion animals often live close together.

In addition, antibiotics enter the aquatic environment directly from pharmaceutical production facilities. Emissions from industrial sites can be considerable, especially in developing countries. Antibiotics are also used in culture medium for the production of biological pharmaceuticals.

Given the extent and importance of the problem, it is rather surprising that there is a lack of reliable data about how many antibiotics are actually consumed each year. Nevertheless, efforts have been made to obtain a comprehensive picture of antibiotic sales and prescriptions to give some idea. The European Center for Disease Control (ECDC) records human use of antibacterials based on population-normalized daily doses per year [7]. The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) initiative, set up by the European Medicines Agency (EMA), publishes antibiotic consumption in the 25 EU countries and Norway [8]. However, several countries only recently set up monitoring systems to record these data. Furthermore, such highly aggregated data do not allow us to draw conclusions or establish correlations of resistance with human or veterinary health concerns or environmental hot spots.

The consumption data also do not give an accurate picture of exposure in the environment. Antibiotics partition into different environmental compartments according to their physical–chemical properties and may be further transformed by abiotic or biological processes. If an antibiotic disappears in one place, it does not necessarily mean that it is gone; it might just have been transferred to another compartment. This is illustrated by the example of ciprofloxacin—a commonly prescribed fluorochinolone in human medicine and a transformation product from enrofloxacin, which is used in veterinary medicine. Waste-water treatment removes up to 90% of ciprofloxacin by sorption to sewage sludge, but biological degradation is poor. As a result, ciprofloxacin accumulates in sewage sludge and, if the sludge is used as fertilizer, in the soil in concentrations in the low mg per kg range. In the soil, ciprofloxacin persists for more than 90 days with only minimal transformation. Although the strong adsorption to soil might reduce its bioavailability, it still elicits effects on soil microorganisms for long periods of time: The resistance gene *qnrS* was detectable in soil treated with ciprofloxacin from day 14 on [9].

Antibiotic compounds are often relatively large, complex, ionizable molecules that behave differently in the environment than model chemicals used to develop environmental fate models. Spotty data from assessing the concentrations of antibiotics in environmental media therefore yield unexpected findings. Many macrolides or pleuromutilins are metabolized to a high extent in the body and would not be expected to end up in the environment according to their pharmacokinetic properties. Nevertheless, antibiotics from almost all substance classes, including the ones mentioned above, have been detected in liquid manure at relevant concentrations (μg to mg per kg). However, there is presently no systematic monitoring of antibiotic compounds in environmental matrices such as water, soil, sediment or sewage sludge, and manure or digester residues.

The presence of antibiotics in the environment in biologically relevant concentrations has the potential to select for resistant bacteria, archaea, viruses and phages. In some cases, selection is not the only mechanism, but also the spread of already resistant microorganisms from

Figure 1. Pathway of antibiotics for human and veterinary use in the environment.

Starting with the application of antibiotic agents in human and veterinary medicine in the upper left corner, the spreading of antibiotic residues in the ecosystem is drawn as a web of exposure pathways. The antibiotic residues are symbolically represented as red dots.

WWTPs or manure. While we have a better picture of how antibiotic resistance develops and spreads in hospitals, the pathways that act via environmental matrices are not well understood. Several environmental hot spots for the development and spread of antibiotic resistance have been identified so far, however. These range from biofilms, sediment close to sewage effluents, WWTP-treated effluent and sewage sludge, or pharmaceutical production sites and aquaculture facilities, to liquid manure tanks and soil repeatedly fertilized with manure.

There are two ways in which such hot spots can increase the development and spread of antibiotic resistance. The first

involves the direct transmission of already resistant microorganisms via environmental matrices: For example, intestinal bacteria from livestock treated with antibiotics might be excreted and survive in manure storage facilities. The second involves locations where large numbers of microorganisms under favorable nutrient conditions are exposed to antibiotic concentrations that can select for resistance. In addition, manure contains metal ions from animal feed and biocides from the disinfection of stables that are implicated in co-selecting for resistance, or that could enhance mutation frequencies that may lead to the development of resistance.

To better understand the role of these environmental hot spots for resistance dissemination, we need standards for monitoring antibiotics and antibiotic resistance in environmental matrices, so as to implement measures to improve the situation. Furthermore, we need to develop experimental assays to quantify the potential of an antibiotic compound to cause the development or spread of resistance genes with potential relevance for human health in environmentally relevant matrices. Several studies have been conducted, but it is often difficult to compare the outcomes because different methodologies are applied; cultivation-based and culture-independent

techniques both have their merits. Studies that relate an observed change in resistance—quantified as numbers of mRNA, genes, mobilization events or selectable organisms—to different doses of an antibiotic could lead to a NOEC (no observable effect)-like value that could be compared to measured or predicted environmental antibiotic concentrations. The MSC concept is also promising [1]. It is obvious that there is a need for reliable methods to measure the development and spread of resistance in environmental matrices.

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On top of that, we need regulatory frameworks that can make use of these results to assess the risk posed by antibiotic resistance. Antibiotics that are used in human or veterinary medicine have to undergo an authorization procedure prior to marketing, but the environmental aspects of antimicrobial resistance are neither addressed in these procedures, nor is there a requirement to monitor antibiotics or antibiotic resistance in the environment as part of the pharmacovigilance measures. For about a decade, the authorization of new human and veterinary pharmaceuticals has required an assessment of the fate and effects of active ingredients that could end up in the environment above a certain threshold concentration. The outcome of this environmental safety assessment is only taken into account for the benefit-risk analysis of veterinary but not human pharmaceuticals. Nevertheless, it provides valuable information on the behavior of antibiotics in the environment. However, the majority of antibiotics currently in use had already been authorized before environmental assessment became part of the marketing authorization. Therefore, no data are available for many of the high-volume antibiotics. Additionally, most of the study results are not publicly available. The phenomenon of resistance dissemination would also warrant applying the so-called however-clause to antibiotics, that is,

conducting an environmental assessment irrespective of concentration thresholds. Yet, the issue of antibiotic resistance within the product-specific authorization procedures falls short of including phenomena like co-selection of antibiotic resistance caused by the presence of heavy metals or biocides in the environment. Additionally, monitoring antibiotic concentrations and levels of antibiotic resistance should be addressed by environmental legislation.

From a human health perspective and from an environmental point of view, it is a priority to reduce the consumption of antibiotics and their subsequent release into the environment. Measures taken and proposed in the clinical setting that promote the prudent use of antibiotics will also reduce the amount entering the environment. Measures such as banning the use of antibiotics as growth promoters or preventive treatments for livestock—which are already implemented in the EU and are recommended in the USA—will similarly help to reduce the amount of antibiotics used if adequate surveillance is applied. Additionally, fostering animal health by improving housing conditions and vaccination programs will also help to reduce antibiotic usage. It should be noted, however, that simply addressing the sum total of antibiotic consumption might not be an appropriate parameter to assess the desired reduction, because it does not take into account differences in effectiveness and bioavailability of the different antibiotic classes and compounds.

Another important step is to restrict the veterinary use of antibiotics that are also used in human medicine, such as pleuromutilins, colistine, tigecyclin, macrolides and lincosamides [10]. From an environmental point of view, it might seem best to avoid persistent antibiotic compounds. However, a compound may be persistent for a very long time under anaerobic conditions in a manure tank, but might finally degrade under aerobic conditions in soil. Moreover, the persistence of antibiotic resistance genes in environmental matrices might differ from that of the antibiotic itself [11].

Limiting the release of antibiotics and antibiotic-resistant microorganisms into the environment and the spread of antibiotic resistance could also be accomplished by minimizing the use of matrices from hot spots of resistance development, like sewage

sludge or manure in agricultural areas. Storage or treatment processes for these matrices that preserve nutrient resources while reducing their potential to disseminate resistance could offer a way forward. One possibility could be to optimize the process of anaerobic digestion of liquid manure, which is already used for methane/biogas production, with a focus on eliminating antibiotic residues. Methods that improve nutrient reuse—for example, phosphate recycling from sewage sludge—could also contribute to lessen the amount of antibiotics spread onto agricultural land. Incineration of sewage sludge is another measure to prevent antibiotics from entering the environment.

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Improving the efficiency of WWTPs to remove micropollutants would similarly help to lower concentrations of antibiotics in the aquatic environment. A considerable amount of research has been done, albeit with the focus on removing other pharmaceutical compounds with undesired effects on wildlife, such as contraceptives or painkillers by adding a further treatment step to the waste-water treatment process. Some countries, like Switzerland, have already adopted strategies to improve waste-water treatment to better remove micropollutants.

Better knowledge and more information on the fate of antibiotics as well as the development and spread of antibiotic resistance in the environment are required to understand the underlying processes and identify hot spots. It is crucial to integrate aspects from human medicine, animal health and environmental considerations. As the recent initiatives show, such information eventually informs regulatory policies and legislation to protect human and animal health and the environment. The growing problem of multi-drug-resistant major pathogens leaves us little time to act.

Conflict of interest

The authors declare that they have no conflict of interest.

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