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REVIEW ARTICLE

Antibiotics in the environment

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Abstract

Molecules with antibiotic properties, produced by various microbes, have been around long before mankind recognized their usefulness in preventing and treating bacterial infections. Bacteria have therefore been exposed to selection pressures from antibiotics for very long times, however, generally only on a micro-scale within the immediate vicinity of the antibiotic-producing organisms. In the twentieth century we began mass-producing antibiotics, mainly synthetic derivatives of naturally produced antibiotic molecules, but also a few entirely synthetic compounds. As a consequence, entire bacterial communities became exposed to unprecedented antibiotic selection pressures, which in turn led to the rapid resistance development we are facing today among many pathogens. We are, rightly, concerned about the direct selection pressures of antibiotics on the microbial communities that reside in or on our bodies. However, other environments, outside of our bodies, may also be exposed to antibiotics through different routes, most often unintentionally. There are concerns that increased selection pressures from antibiotics in the environment can contribute to the recruitment of resistance factors from the environmental resistome to human pathogens. This paper attempts to 1) provide a brief overview of environmental exposure routes of antibiotics, 2) provide some thoughts about our current knowledge of the associated risks for humans as well as ecosystems, and 3) indicate management options to reduce risks.

Key words: *Antibiotic resistance, bacteria, environment, pollution, risk assessment, waste-water*

Exposure routes

The development and refinement of mass-spectrometric techniques during the past 20 years have allowed us to study the flow of antibiotic residues in various complex matrices in great detail. It has now become clear that man-made antibiotics can enter the environment in many ways, from the production of active pharmaceutical ingredients, through the excretion of residues after usage or through discarding, unused medicines (1–4). Some antibiotics are easily degraded, such as penicillins, whereas others are considerably more persistent, such as fluoroquinolones and tetracyclines, thus allowing them to prevail for longer times in the environment, to spread further, and to accumulate to higher concentrations.

Direct discharges from manufacturing, either during the actual production of the active ingredient, or during formulation, can be exceedingly high. In several cases concentrations exceeding 1 mg/L have been detected in treated industrial effluents or recipient waters (5–7). This is orders of magnitude higher than the ng/L levels regularly found as a result of contamination via excretion. In contaminated river sediment, 0.9 mg ciprofloxacin per gram organic matter was found downstream from a common industrial treatment plant in Patancheru, near Hyderabad in India (8). As a consequence of the industrial pollution, ground-water over a large area has been contaminated by antibiotics in Patancheru, with well-water concentrations in the low μg/L concentration range (9). It is very rare that there are regulations

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in place specifically limiting discharges of active pharmaceutical ingredients. This is true not only in India and China that serve much of the world with bulk drugs, but also in Europe and the US. Consequently there are also reports of major industrial discharges of pharmaceuticals from these regions (10–12).

For many antibiotics, urine and feces from users can contain considerable amounts of active residues. In most high-income countries with well-developed sewage infrastructure, discharge to the environment is reduced, but microbial communities within the treatment plants can nevertheless be exposed to μg/L concentrations of selected antibiotics (2). Much of the antibiotics accumulate in sludge that subsequently may be spread on farmland with the intent to recycle nutrients. In surface waters receiving municipal waste-water, concentrations of antibiotics rarely exceed 1 μg/L, but are more regularly in the low ng/L range. Antibiotic residues have also been found in marine environments (2).

Exposure routes often differ if the antibiotic is intended for animal use. Depending on the animal species, whether companion animals or animals for meat production are treated, and depending on farming practices, urine and feces are collected, treated, and distributed to land and/or water in quite different ways (1–3,13). With the obvious exception of aquaculture, in general, direct exposure to land/soil is more common for antibiotics used to treat animals than for human use. The often high microbial activity in soil contributes to a relatively rapid removal of many antibiotics over time, although some are able to persist for years. The concentrations found in manure or manure-amended soils are often considerably higher than those found in aquatic environments (1).The same holds true for sludge generated in municipal sewage treatment plants (2,3). However, many antibiotics tend to bind strongly to particles, and only a fraction of the total amounts is probably bioactive. Assessing the bioavailable fractions of antibiotics in solid matrices is important to investigate in order to assess risks, but this is still a major challenge (14).

A portion of prescribed medicines that we buy are not used and are eventually discarded (15). Many countries do not have well-established take-back programs and still dump their household waste in landfills. In such situations, environmental exposure from unused medicines should not be neglected. On the other hand, some countries have had a solid take-back program in place for decades. Sweden is one example of this, and furthermore only approximately 1% of the Swedish household waste is deposited in landfills (16). In countries with such practices, discarded medicines are very unlikely to contribute to an appreciable extent to the environmental contamination with antibiotics.

Risks

Although the ideal antibiotic is toxic to bacteria without affecting humans/animals, reality is more complicated, and directly toxic side effects are common for several classes of antibiotics at doses used for therapy. A few, relatively persistent antibiotics have been found in drinking-water at very low ng/L levels (17). Near manufacturing discharges, ground-water contamination has led to levels up to low μg/L in drinking-water wells (9). However, based on estimated total exposure of the antibiotics, not even the last-mentioned are believed to pose a direct risk for toxicity to humans ingesting the water (18).

A series of studies linked environmental fluoroquinolone exposure to malformations in embryos of kites, a common bird of prey in Europe (19). However, when scrutinized in court it turned out that at least some of the data were fabricated, thus questioning the entire case.

Another concern is the potential effect of antibiotics on the function and services of ecosystems. Microorganisms in farmland soil are crucial for e.g. nitrogen fixation and other nutrient fluxes, and there is a potential for antibiotics to disrupt such processes. To date, however, there is no clear evidence for disturbed ecosystem services in soil communities due to antibiotic exposure given the prevailing exposure levels documented in the field. Similarly, waste-water treatment of both household and industrial discharges depends on the functionality of complex microbial ecosystems within the treatment plants, ecosystems that could be disturbed by antibiotic exposure. A crucial point is, of course, the exposure level required to affect the function of the microbial ecosystems. Again, to date, concentrations of antibiotics found in municipal sewage treatment plants do not appear to be sufficient to reduce their treatment efficiency; however, for treatment plants receiving highly contaminated waste from manufacturing of antibiotics, the exposure levels and thus the risks for disturbed function can be quite different. Many microbial ecosystems tend to show a large degree of resilience, possibly governed by their ability for horizontal gene transfer and ability to rapidly accumulate mutations. Thus, given sufficiently long exposure times, even environments exposed to very high antibiotic exposures appear to harbor a rather diverse bacterial flora.

The greatest concern about antibiotics in the environment is their potential role in promoting resistance

development in human and animal pathogens (20). Many of the mobile resistance genes we face in pathogens in the clinic today have their origin in harmless bacteria in and around us (21–24). The environmental microbiome represents a much greater diversity than those micro-organisms that thrive in or on our bodies. As such, the external environment provides a reservoir or source for resistance genes not yet encountered in pathogens. The external environment also provides unique opportunities for genetic recombination and thus the creation of novel vectors for genes already circulating in pathogens. Arenas and conditions that favor such recombination and transfer events are not clear, but an added selection pressure from antibiotics, increasing the available pool of resistance genes and selecting for bacteria acquiring resistance genes through horizontal gene transfer, is an obvious risk factor. In principle, the transfer of a novel resistance gene or resistance vector to a pathogen colonizing a human being only needs to happen once in one place on our planet, as our heavy use of antibiotics, lack of sufficient hygiene, and extensive travel habits often take care of the dissemination thereafter. Such crucial gene transfer events may take place in the external environment, in animals or inside our bodies, in one or several steps. Hence, assessing risks is a complicated endeavor (25).

A crucial question for identifying risk environments and assessing the size of risks is to define the relationship between antibiotic concentrations and the selection for resistance determinants. Using pairs of bacteria, differing only by the presence of a specific resistance mutation, and marker genes providing different colours, concentrations as low as 100 ng/L of ciprofloxacin have been shown to provide a small but measurable selective advantage to the resistant bacterium (26). This corresponds to antibiotic levels found within sewage treatment plants, and thus calls for concern. However, it is still unclear how these estimated minimal selective concentrations translate to more complex communities with many competing genotypes, and a suite of selection pressures varying over time and space. Resistant bacteria as well as selected resistance genes appear to increase or decrease in relative numbers between influents and effluents of sewage treatment plants in a rather non-predictable manner, when looking at the public literature at large rather than on isolated studies. More research is needed to clarify selective concentrations in complex microbial communities and to pinpoint combinations of antibiotics and resistance determinants that are most likely to be selected for in different environments.

In environments contaminated by manufacturing discharges, concentrations well over minimal inhibitory concentrations for a range of bacteria have been found (5–7,9), creating gradients from very high to low in the receiving environments (6,8,9). Thus, it is evident beyond any reasonable doubt that such exposure drives resistance selection. In accordance, unprecedented occurrences of multi-resistant bacteria (27,28), resistance genes (8), and mobile genetic elements associated with resistance transfer (8,27) have been documented in these environments.

A common challenge in relating antibiotic concentrations to resistance selection in the field is the co-contamination of antibiotics and fecal residues. As feces often contain antibiotic-resistant bacteria as well as antibiotic residues, it is difficult to assign with certainty increased abundances of resistance genes to the contamination by antibiotics in the field. An illustrative example is the apparent increase in the abundances of a range of resistance genes over time in a study of archived soils (29). The changes over time may be due to the accumulation of more and more selective agents in the soil, but also because of years of amendment with manure from animals that over time contains more and more resistance genes as result of generations of antibiotic use on farms and selection within the intestines of farm animals, rather than within the soil.

Another challenge for assessing risk is the combinatory effect of many selective agents. In the environment, a complicated exposure scenario is the rule rather than the exception. Several antibiotics acting via the same mechanisms are generally expected to act additively. Different classes of antibiotics may co-select for resistance to other classes of antibiotics if either the same mechanisms, i.e. a pump, provide increased tolerance to both (cross-resistance) or if several resistance genes are located within the same bacterium and are selected for together (co-resistance). Such co-selection between different antibiotics is an important driver behind multi-resistance development in pathogens. Metals and antibacterial biocides are other classes of compounds that have the ability to co-select for antibiotic resistance (30,31).

Management options

Given what is at stake, the precautionary principle can be invoked in order to reduce risks with environmental antibiotic contamination also in the absence of final evidence that it ultimately results in more infections with resistant bacterial pathogens. As stressed above, the transfer of resistance from an environmental bacterium to a human pathogen can be a one-time event. Thus, preventing or delaying this from happening is key, regardless of where it might

occur. This puts challenges to co-ordinate international actions, as well as focusing mitigations toward environments where risks are considered to be particularly high. Regulations are important, but not necessarily easy to influence in other regions or countries. Furthermore, regulations are not always followed. Overall, it is important to create awareness in all relevant sectors, and to provide incentives, primarily economic incentives, in order to motivate actions to reduce risks (13). What the technical solutions might be depends heavily on the exposure route. Different management options are highlighted and discussed in a recent review by Pruden et al. (13). Importantly, not only discharges of antibiotics need to be controlled, but also antibiotic-resistant bacteria that might develop and become enriched within e.g. industrial processes. Even releases of pathogenic bacteria containing integrons, capable of capturing and expressing arrays of genes, may conceivably accelerate the development of resistance by providing increased possibilities to probe the environmental resistome for novel resistance genes not yet encountered in the clinic (27).

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