fact sheet

July 2015

Spread of antibiotic resistance in the aquatic environment

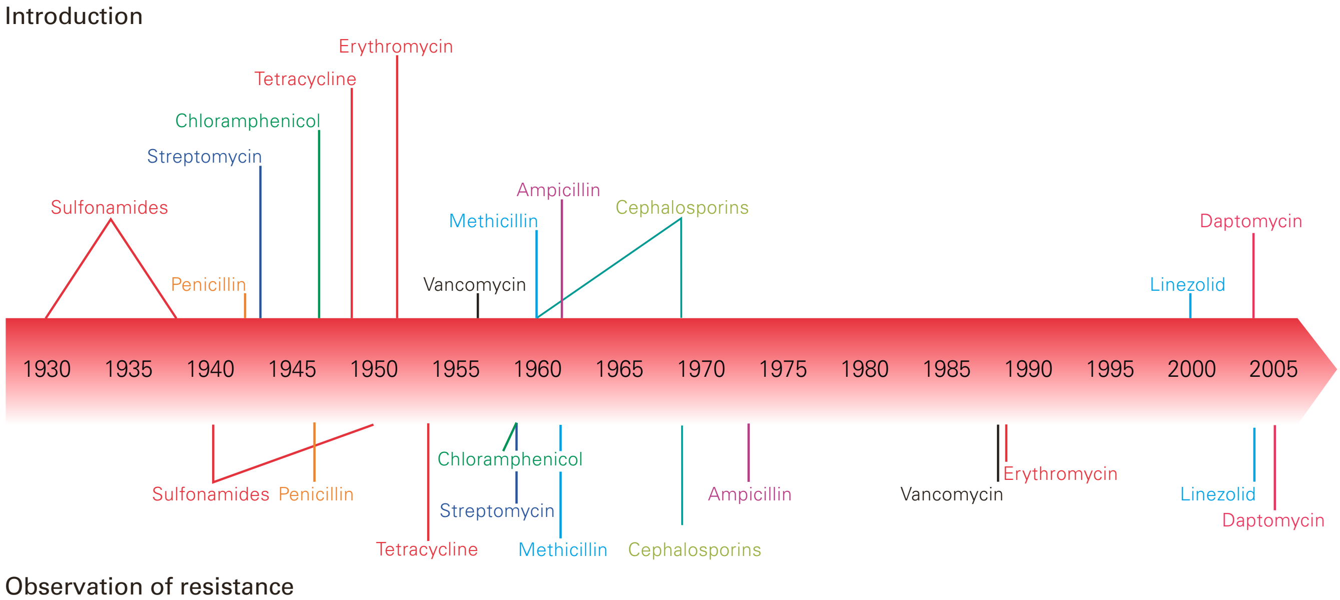
Antibiotic resistance genes are increasingly seen as emerging environmental contaminants. This factsheet provides a non-exhaustive overview, focusing in particular on antibiotic resistance in freshwater systems.

What are antibiotics?

Antibiotics, which allow bacterial infections to be effectively controlled, are among the most important types of drugs used in human and veterinary medicine. Their history goes back to the early twentieth century, when the first synthetic antibacterial agents (sulphonamides) were introduced. However, many antibiotics in current use are derived from natural substances; the most familiar example is penicillin, which is obtained from moulds of the genus Penicillium. Over the decades, numerous new classes and new chemical variants of existing substances were developed, and today's antibacterial armamentarium includes tetracyclines, quinolones, beta-lactams, macrolides and aminoglycosides. More recently – largely as a result of changes in the commercial environment – very few new antibiotics have been developed. In Germany, the use of antibiotics currently amounts to 600–700 tonnes per year in human medicine and over 1700 tonnes in veterinary medicine. In Switzerland, the corresponding figures for 2013 are around 35 tonnes and 53 tonnes [1], Despite the relatively low per capita use, Switzerland is notable for substantial regional differences: for example, outpatient antibiotic consumption was found to be three times higher in Canton Geneva than in Appenzell Rhodes [2].

Growth of antibiotic resistance

With the ever-increasing use of antibiotics, resistance soon developed: certain bacterial strains acquired the ability to survive and grow even in the presence of antibacterial drugs. The first observations of resistant pathogens tended to be reported just a few years after a new antibiotic had been introduced. Initially, given the variety of substances available, this was not a major concern. Over the years, however, increasing



Timeline of introduction (top) and first observation of resistance (bottom) for various antibiotics. Only a small number of antibiotics have been introduced since the 1970s [adapted from Clatworthy et al. (2007); Nature Chemical Biology, Vol. 3].

eawag aquatic research

numbers of resistant pathogens emerged. The incidence of antibiotic resistant infections rose, as did the number of multidrug-resistant pathogens. Today, certain pathogens are resistant to virtually all currently approved antibiotics [3]. From 2001 to 2006, in order to analyse the situation in Switzerland, a National Research Programme on "Antibiotic resistance" was conducted (NFP 49) The Final Report concluded that, while it continues to be underestimated in Switzerland, the antibiotic resistance problem is steadily progressing, and that further action is required. At the end of 2014, a draft "Strategy to combat antibiotic resistance" was issued by the federal authorities. This calls for cross-sectoral monitoring of resistance and of antibiotic use in human and veterinary medicine, in agriculture and in the environment [4]. The final version, approved by the Federal Council, is due to be published by the end of 2015. Furthermore the Federal Council has approved a new National Research Programme on antimicrobial resistence in June 2015.

How do bacteria become resistant?

The use of antibiotics exerts a massive selection pressure for the bacteria exposed to these substances. A bacterium which, as a result of genetic adaptation, shows improved survival in the presence of an antibiotic can continue to reproduce, passing on its acquired resistance to subsequent generations. At the same time, bacteria lacking this trait are destroyed by the antibiotic. Such adaptation may take various forms:

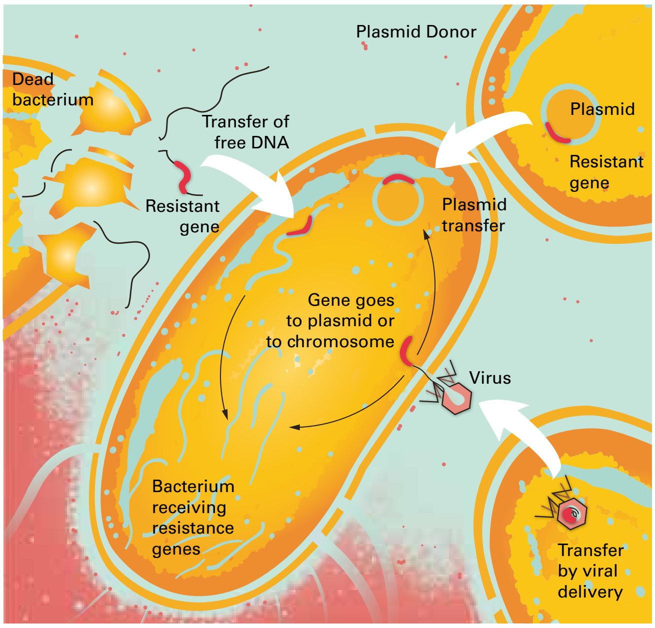
- Antibiotics attack specific molecular structures in the bacte rial cell. If these structures are altered by spontaneous mutations, the antibiotic can no longer locate its target.
- Existing mechanisms protecting against foreign substances are modified so that they also become effective against the antibiotic.
- Resistance genes are absorbed from other microorganisms. Free DNA can be taken up from the environment and incorporated into the genome. Small, ring-shaped fragments of DNA (plasmids) can be actively transferred from cell to cell. Bacterial viruses (bacteriophages) can act as vectors (see diagram).

Research has shown that many resistance factors derive from harmless environmental bacteria, where the genes in question may originally have served quite different functions. In addition, certain antibiotics function as natural protective agents in the microbial world. In the course of evolution, protective mechanisms have been developed by the organisms concerned – and also by the potential targets. Resistance genes can therefore even be detected at sites not subject to any human influences.

Various contributory factors

The likelihood of resistant bacteria evolving can be sharply increased by improper use of antibiotics, e.g. if therapy is discontinued prematurely (which frequently occurs with self-medication). Taking antibiotics when they are not indicated (e.g. in viral infections) also promotes the development of resistance. The spread of resistant pathogens can be facilitated by poor sanitation – a growing problem especially in less developed regions.

Certain antibiotics used in veterinary medicine are, if not chemically identical, at least closely related to those used in



Gene transfer mechanisms [adapted from "Horizontal gene transfer", Frolich (2006); lecture notes Microbiology, Community College of Rhode Island].

human medicine. In animal husbandry, antibiotics are also employed for preventive purposes or even, in the absence of medical need, as growth promoters – a practice now prohibited in Switzerland and the EU. Since some types of bacteria can jump from animals to humans, this practice has presumably also contributed to the rise in antibiotic resistance.

Dissemination in the environment

The genetic diversity of environmental bacteria represents a natural reservoir of resistance genes. In addition, large amounts of resistant bacteria enter wastewater with faeces. In agriculture, as a result of grazing and manure-spreading, they end up in the soil, surface waters and possibly also groundwater. These "releases" of resistant bacteria and resistance genes pose a number of risks:

- Resistance genes and resistant pathogens can be further disseminated.
- If resistance genes accumulate in the environment, they are more likely to be acquired by pathogens of concern.
- Bacterial strains can acquire or develop new forms of resistance in the environment.

For processes of this kind, urban wastewater systems, animal husbandry operations and pharmaceutical industry effluents are regarded as hotspots. During biological treatment at wastewater treatment plants (WWTPs), resistant bacteria and other pathogens in wastewater are in close contact with sewage sludge bacteria and other microorganisms well adapted for survival in freshwater. Even if pathogens are largely eliminated at WWTPs, wastewater treatment is a site of gene exchange, which can serve as an "incubator" for antibiotic resistance. Because of the presence of antibiotics and other contaminants, the conditions arising at WWTPs facilitate the survival of resistant organisms. The same is true of surface waters contaminated with antibiotics or other substances exerting selective effects.
In comparison with other countries, levels of antibiotic resistance in Switzerland – from a medical and environmental perspective – are fairly lo [5]. However, inputs of resistance factors to the environment are measurable here too. In an

Eawag study, 21 Swiss lakes were sampled to assess whether levels of antibiotic resistance are influenced by human activities: it was shown that the abundance of sulphonamide resistance genes is related to local WWTP capacity, and also to eutrophication status [6]. In another study, resistance factors were investigated in urban wastewater from Lausanne. There was evidence that accumulation of resistance genes – and selection of multiresistant strains – may occur during wastewater treatment. Close to the wastewater discharge pipe, increased levels of resistance genes were detected in lake water and sediment [7, 8]. In a Zurich University study, 36% of the Swiss rivers and lakes sampled were found to contain multiresistant bacteria. The highest levels of contamination were found in urban and intensively farmed areas [9]. In a number of water bodies, multiresistant Enterobacteriaceae – including pathogens – were detected [10].

Little studied as yet are the effects of different methods of drinking water treatment on antibiotic resistance. In a US study published in 1981, multiply antibiotic-resistant bacteria were detected in drinking water that had undergone various treatment processes [11]. In Switzerland, there are no reported cases of pathogenic resistant bacteria being detected in drinking water. Levels of non pathogenic resistant bacteria are substantially reduced by treatment.

In contrast to the situation in Switzerland, the above-mentioned problems have already assumed serious proportions in certain countries with poor sanitation, unsafe drinking water supplies and inadequate controls of pharmaceutical production. As any resistant pathogens that may emerge can spread rapidly in our interconnected world, the problems facing these countries are of global concern.

Controlling releases as far as possible

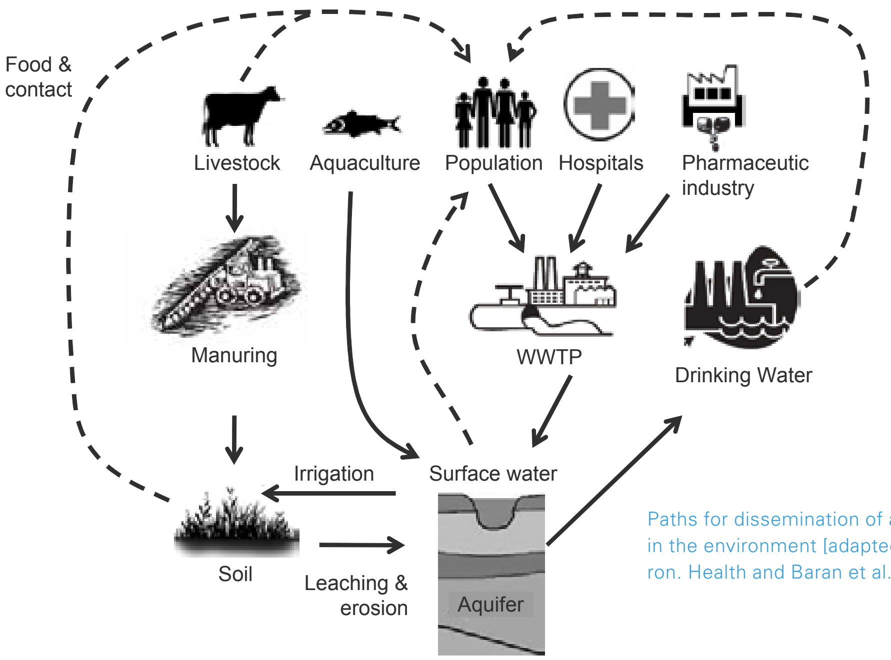
The risk of resistance evolving and spreading among pathogens can be increased by releases of resistant bacteria. While this risk cannot be precisely assessed at present, analytical methods are available for determining the contamination of environmental compartments with resistant bacteria. Limit values do not yet exist. In addition, risk assessments will vary depending on the carrier and type of resistance: if resistance is found in harmless environmental bacteria, the risk will be considered lower than for pathogens. Pathogens that survive and spread in the environment pose a greater risk than those which can only be transmitted from person to person, as shown by the example of Enterobacteriaceae [10].

It is assumed that what Switzerland has to contend with is the spread of antibiotic resistance as a long-term process rather than an acute threat. In either case, however, it would appear to be advisable to establish barriers, wherever possible, so as to control the evolving and spread of resistance – not only in clinical settings (through improved hygiene and isolation of pathogens of particular concern) but also in agriculture and in the environment.

Since, according to the current state of knowledge, surface waters are the main conduit for the spread of resistance in the environment, one possible approach involves improvements in wastewater treatment. In Switzerland, a major programme of WWTP upgrades has been launched, with the primary aim of removing micropollutants (see www.micropoll.ch). Measures such as ozonation or activated carbon treatment combined with ultrafiltration reduce not only micropollutants (including antibiotics) but also bacterial cell counts. However, the question of which methods are most suitable for eliminating antibiotic resistance is still under investigation. It has been shown that even wastewater which has undergone advanced treatment, with chemical disinfection as a final step, can still contain quantities of antibiotic resistance genes significantly higher than natural background levels [12].

Role of biocides in evolution of resistance

An important role in the evolution of resistant bacteria is played not only by antibiotics, but also by disinfectants, heavy metals and biocides – often even at low concentrations. In a recent study, sublethal concentrations of herbicides were shown to increase the expression of efflux pumps in certain bacteria, thereby promoting elimination of the toxic substances; but this



Paths for dissemination of antibiotic resistance and antibiotic residues in the environment [adapted from Kim and Aga (2007); J. Toxicol. Environ. Health and Baran et al. (2011); J. of Hazardous Materials, Vol. 196].

mechanism can also induce higher levels of antibiotic resistance in bacteria [13]. It is thus also important to avoid or control releases of such substances into the environment. Separate treatment of heavily contaminated (e.g. hospital) wastewater could be advisable, especially in cases where downstream (municipal) wastewater treatment does not guarantee effective removal of microorganisms or contaminants.

Reviewing existing barriers

Because resistance factors can again come into contact with humans, animals and foodstuffs via water and soil, existing barriers in drinking water treatment and the food industry should be reviewed and, if necessary, improved. Measures should be taken to ensure that drinking water, food and waters used for recreational purposes are not needlessly contaminated with resistance factors. In Switzerland, acute risks from antibiotic-resistant pathogens in the environment are largely excluded by existing microbiological safety regulations and monitoring programmes (e.g. for bathing waters). In developing countries, however, improvements in sanitation should be urgently supported – both through development cooperation and appropriate research efforts.

References: (Blue = Hyperlinks)

1 ARCH-Vet Bericht über den Vertrieb von Antibiotika in der Veterinärmedizin und das Antibiotika resistenzmonitoring bei Nutztieren in der Schweiz: Gesamtbericht 2013 [pdf, FSVO]

2 Filippini M. et al (2006): Socioeconomic determinants of regional differences in outpatient antibiotic consumption: Evidence from Switzerland; Health Policy 78 [pdf, Bern University]

3 Cantas L. et al (2013): A brief multi-disciplinary review on antimicrobial resistance in medicine and its linkage to the global environmental microbiota. Frontiers in Microbiology 4. doi: 10.3389/fmicb.2013.00096

4 Draft "Strategie gegen Antibiotikaresistenzen" www.bag.admin.ch/themen/medizin/14226/index.html?lang=de

5 Bürgmann H. (2014): Eintrag von Antibiotika und Antibiotikaresistenzen in Wassersysteme der Schweiz. Ein Überblick über die Lage in Bezug auf Antibiotikaresistenzen und ihre Ausbreitung in die Umwelt. Prävention und Gesundheitsförderung, Volume 9, Issue 3, pp 185-190. doi: 10.1007/s11553-014-0444-3

6 Czekalski N. et al. (2015): Does human activity impact the natural antibiotic resistance background? Abundance of antibiotic resistance genes in 21 Swiss lakes. Environment International 81:45–55; doi: 10.1016/j.envint.2015.04.005

7 Czekalski N. et al. (2012): Increased levels of multiresistant bacteria and resistance genes after wastewater treatment and their dissemination into Lake Geneva, Switzerland. Frontiers in Microbiology 3. doi: 10.3389/fmicb.2012.00106

8 Czekalski N. et al. (2014): Wastewater as a point source of antibiotic-resistance genes in the sediment of a freshwater lake. ISME Journal 8:1381–1390. doi: 10.1038/ismej.2014.8

9 Resistente Bakterien erobern die Schweiz; UZH News 16.5.2013. www.uzh.ch/news/articles/2013/resistente-bakterien-erobern-die-schweiz.html

10 Zurfluh K. et al. (2013): Characteristics of extended-spectrum β-lactamase- and carbapenemase producing Enterobacteriaceae isolates from rivers and lakes in Switzerland. Applied and Environmental Microbiology 79:3021–3026. doi: 10.1128/AEM.00054-13

11 Armstrong J.L. et al. (1981): Antibiotic-resistant bacteria in drinking water. Applied and Environmental Microbiology. [pdf, AEM]

12 LaPara T.M. et al. (2011): Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into Duluth-Superior Harbor. Environmental Science & Technology 45:9543–9549. doi: 10.1021/es202775r

13 Kurenbach B. et al. (2015): Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and glyphosate cause changes in antibiotic susceptibility in Escherichia coli and Salmonella enterica serovar Typhimurium. mBio 6 (2):e00009–15 doi: 10.1128/mBio.00009-15

Contact

Eawag, Dr. Helmut Buergmann, Surface Waters Department, Seestrasse 79, 6047 Kastanienbaum, Schweiz, +41 (0)58 765 2165, helmut.buergmann@eawag.ch

Factsheet editors: Dr. Helmut Buergmann, Dr. Nadine Czekalski, Andri Bryner

Address

Eawag, Überlandstrasse 133, P.O. Box 611, CH-8600 Dübendorf, Schweiz, +41 (0)58 765 5511, info@eawag.ch, www.eawag.ch

eawag aquatic research